Gaping at environmental variability: How do bivalves react to changing circumstance?

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Gaping at environmental variability: How do bivalves react to changing circumstance?

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Abstract

Documentation of behaviour involving movement with survival value is common in vertebrates, but reports are notably lacking in sessile bivalve molluscs, primarily because of the difficulty in quantifying behaviours that occur in these generally small animals, whose behaviour is characterized by minimal movement. Such movement may, however, be critical in survival and its quantification may provide insights into strategies and environmental conditions of consequence for this important animal group. Archival tag techniques based on Hall sensors and magnets working with infra-second resolution were used to record valve movements in shellfish (*Mytilus edulis, Mytilus trossulus*, *Pecten maximus, Cerastoderma edule* and *Margaritifera margaritifera*) and recommendations made with regard to correct protocol for assessing valve movement behaviours exhibited by this group. The same technology was used to examine valve movements and exhalant pumping of the blue mussel *Mytilus edulis* in the laboratory, and valve movements of blue mussels in the wild according to circumstance. Circumstances examined in the laboratory were food concentration, addition of suspended sediment and the presence of predators (as evidenced by addition of mussel homogenate to the water). Mussel activity was studied in the wild according to height of individuals in the inter-tidal, season, light, instantaneous water depth, temperature and the action and extent of waves. Bivalve gape angle varied over the diel cycle, being greater at night than during the day for both laboratory and wild animals and varied with season in the *in situ* mussels, decreasing from summer through autumn to be lowest in winter (both during the day and at night). Although all mussels in the inter-tidal closed their valves during emersion, lower-shore mussels initiated gaping later than upper-shore individuals in relation to the incoming tide. There was considerable inter-individual variance in gaping behaviour. Gape angle in the laboratory mussels correlated with extent of pumping and was, therefore, generally assumed to be a proxy for feeding behaviour. However, *M. edulis* pumping activity is complicated because exhalant pumping can occur from the top of the inhalant siphon in addition to the exhalant siphon and pumping may be a response to oxygen requirements rather than feeding. Valve adduction (reduction in shell valve gape angle) and subsequent abduction (increase in shell valve gape angle) events constituted a normal part of bivalve behaviour, occurring in the laboratory and the inter-tidal mussels although the reasons for this behaviour are complex and not fully understood. Valve adduction events further complicate the relationship between valve gape angle and exhalant pumping because maximum recorded exhalant pumping was not produced by pumping (cilia beat) but by valve adduction. The significance of the findings are discussed with respect to mussel strategies for acquiring nutrients and minimizing the likelihood of predation and highlight the need for future research.

INTRODUCTION

Blue mussels, *M. edulis*, are common in inter-tidal and shallow sub-tidal areas (Seed 1976) and are ecologically important as they form large reefs that can enhance local community diversity. Mussels also provide a critical link between benthic and pelagic systems through their filter-feeding activities (Seed 1976, Dame et al. 1991, Beadman et al. 2004). Mussels, although complex animals with fascinating biological features, are effectively self-cleaning biological pumps (Davenport et al. 2000). Thus, mussel feeding and pumping activity has been extensively studied (see Maire et al. 2007). Controversy about many aspects of bivalve behaviour, such as feeding, partly results from difficulties in accurately recording high frequency measurements of bivalve filtration activity (Maire et al. 2007). Indeed, Maire et al. (2007) highlight the importance of the determination of 'short-term' changes in valve gape and exhalant siphon area.

Mussel valve movements have been recorded for over fifty years (Lowy 1953) yet their role is not fully understood (Shick et al. 1986, Shick et al. 1988). It has been suggested that valve movements of bivalves are closely related to vital activities such as respiration, feeding and excretion (Nagai et al. 2006). Shell valve activity may be involved in enhancing perfusion of the tissues by newly re-oxygenated haemolymph (Shick et al. 1986, Shick et al. 1988). In inter-tidal mussels, air-gaping has been found in most individuals in summer, but not necessarily in winter (Shick et al. 1986). Thus, it has been suggested that the degree of air-gaping may be positively related to temperature (Shick et al. 1986). A relationship between air breathing and the continuation of cardiac activity has also been reported in mussels (Shick et al. 1986). Evaporative water loss in mussels occurs during gaping at high shore levels (Newell

1973, Widdows et al. 1979a). The apparently closely-controlled air-gaping behaviour in this species may at times promote evaporative cooling, but air breathing and cardiac activity during emersion also seem to regulate a variable aerobic component of total energy metabolism to support the costs of digestion and absorption and assimilation when food is present in the digestive system (Shick et al. 1986, Shick et al. 1988). However, our search of the literature provided no evidence of a quantified study of the relationship between mussel valve gape and air temperature.

Despite being a common model species in biological research in captivity, comparatively little is known about mussel gape behaviour in the wild. Wilson et al. (2005) reported the absence of a well-defined circadian rhythm in gape angle in laboratory mussels compared to those in the wild sub-tidal. These authors also reported that mussels put back in the wild after extended periods slowly returned (over 2 days) to a more obvious circadian pattern. Laboratory mussels may behave differently to wild conspecifics because of many factors, including disturbance by humans (Wilson et al. 2005) and not being held under circumstances closely comparable to their natural environment. These results raise many questions about the ecological variables that affect the activity patterns of mussels and the differences between laboratory and field results (c.f. Gattermann et al. 2008). Thus, laboratory data on mussels needs to be interpreted against a background of field data in order to evaluate their relevance in the natural environment.

This thesis takes the form of six manuscripts, two chapters of the thesis (Chapters 1 and 2) have already been published in a peer-reviewed journals. The remaining chapters are all submitted to peer-review journals. My co-authors, who are listed at the start of each chapter, helped me in a variety of ways: by offering advice

on experimental design, statistical analysis and constructive criticism of manuscript drafts.

As an undergraduate student with great enthusiasm for understanding more about edible shellfish (e.g. see Robson 2006, Marean et al. 2007), the PhD was designed in collaboration with my supervisors Professor Rory P Wilson and Doctor Carlos Garcia de Leaniz. An initial thrust of the PhD was to determine the appropriate recording frequency to record and define the fine- scale movements of bivalve gape and pumping behaviour (Chapter 1), because this may provide insights into strategies and environmental conditions of consequence for this important animal group. This involved adapting animal-attached logger technology used on vertebrates for use on invertebrates, specifically bivalves: mussels *Mytilus edulis* and *Mytilus trossulus*, scallops *Pecten maximus*, cockles *Cerastoderma edule* and freshwater pearl mussels *Margaritifera margaritifera*. The reasons for the selection of these species were; firstly, there is significant commercial mussel production in over 40 countries world-wide (FAO 1999) and mussels, particularly *M. edulis*, have been proposed as potent bio-indicators (e.g. Fisher et al. 1996). Thus, there is commercial interest in mussel research and funding from the UK mussel industry (Deep Dock Limited) which fulfilled a requirement for obtaining a European Social Fund PhD studentship. Second, opportunities for collaborations with researchers in the UK and abroad arose during the PhD. Those opportunities were embraced to show the general applicability of animal-attached logger technology on a range of bivalves, in the laboratory and inter-tidal and sub-tidal zones.

Once a method for determining the appropriate recording frequency to record and define the fine-scale movements of *M. edulis* valve behaviour had been established, a new method of quantifying bivalve valve behaviour according to

circumstance was developed (Chapter 2; Robson et al. 2007). Then the extent to which mussel gape angle could be used as a proxy for overall exhalant pumping rate was determined using newly-designed and constructed sensors to measure pumping rate in relation to valve gape with high temporal resolution (Chapter 3). The expectation was that since *M. edulis* is known to have a mucociliary rejection pathway out of the top of the inhalant siphon (Widdows et al. 1979b, Beninger & St Jean 1997, Beninger et al. 1999), an exhalant current coming out of the top of the inhalant siphon as well as the exhalant siphon would be found . The study found that *M. edulis* pumping activity is complicated because exhalant pumping can occur from the top of the inhalant siphon (associated with the pseudofaeces mucociliary rejection pathway) in addition to the exhalant siphon. Furthermore, the study suggested that there may not be a simple proxy for overall exhalant, or both inhalant and exhalant pumping because there is no defined barrier to exhalant pumping from the top of the inhalant siphon and it may not be assumed that inhalant pumping occurs out of the whole of the inhalant siphon area (especially when exhalant pumping occurs from the top of the inhalant siphon). However, it was concluded that gape angle could be used as a general proxy for mussel pumping activity (associated with respiration, feeding, excretion and their associated metabolic processes). The conclusion is complimentary to that of Dolmer (2000), Wilson et al. (2005) and Saurel et al. (2007) who proposed mussel gape as a single proxy for mussel activity in the wild.

Following this, and using mussel gape angle as a proxy for mussel activity, experiments to test how mussels may adapt to particular circumstances relating to food concentration and perceived risk from predators using laboratory animals were implemented (Chapter 4). With a wide variation within, and no significant difference between, the number of shell valve adduction (reduction in shell valve gape angle)

events day^{-1} across eleven different daily algal rations (ranging from 0 to 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1}), it was clear that the reasons for valve movements in the current study were not fully understood (c.f. Shick et al. 1986). However, has been suggested that it is important to record that valve adduction occurs, because it has been shown to vary according to circumstance (e.g. predation) and perhaps wellbeing (mussel health) (Robson et al. 2007).

Studies on laboratory and inter-tidal mussels were subsequently integrated to assess the relevance of valve gape studies in the laboratory to the wild (Chapter 5). It was tested whether clay (detritus) had any effect on mussel valve adduction and abduction (increase in shell valve gape angle) events during immersion and whether temperature had any effect on gape angle during emersion. It was unclear why mussels subjected to detritus adducted their valves significantly more compared to mussels without the addition of detritus at the same daily algal ration. One explanation was that mussels exposed to detritus may have more intermittent and shorter filtration periods compared to those not exposed to detritus and thus, adduct more per unit time in order to try to prevent their filtration and rejection mechanisms becoming overloaded. As suggested by Shick et al. (1986) it was found that the degree of air-gaping was positively related to temperature. As suggested by Anestis et al. (2008) this may be useful in evaluating the relevance of laboratory bivalve thermal tolerance studies to their natural environment.

Finally, the animal-attached logger technology was adapted for longer-term deployment (over weeks) in the inter-tidal zone in the Bristol Channel, UK (Chapter 6). In order to understand the behaviours shown by bivalves to the inter-tidal environment the gape and valve movement responses of mussels occupying different levels (tidal elevations) up the inter-tidal zone were compared, and the responses

considered in relation to environmental variables (depth, aerial exposure and day/night). Despite immersion and emersion by the tide approximately twice a day and the general increase in gape angle from 0-3 m seawater depth a significant circadian rhythm was found, with gape angle generally greater in darkness in inter-tidal mussels over weeks in summer 2007, autumn 2007 and winter 2008. It was unclear what the adaptive significance of this finding might be even though this gape pattern was first noticed by Dodgson (1928).

The net result of this thesis was to demonstrate clearly just how much speculation still exists about the reasons for specific mussel behaviours e.g. to gape or not, how much to gape and when and why mussels abduct and adduct their valves. The need for future research using high temporal resolution measurements of numerous biological and environmental parameters in order to further explain the results of our work is discussed.

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Chapter 1

Optimization of valve gape and pumping measurement in bivalves[a]

Abstract

We examined the effect of sampling frequency in measurements of gape angle and exhalant pumping on our ability to determine the behaviour of bivalves. For this, we used both the IUCN Red List endangered freshwater bivalve *Margaritifera margaritifera* and non-endangered mussels (*Mytilus edulis* and *Mytilus trossulus*) scallops (*Pecten maximus*) and cockles (*Cerastoderma edule*). Increasing sampling interval led to an underestimation of the rate of bivalve gape adduction and abduction events detected, an overestimation of the mean duration between gape adduction and abduction events, and a misunderstanding in the form of the gape adduction and abduction events and exhalant pumping profile. The analyses suggest minimum appropriate sampling rates for archival tags to define gape behaviour to be 2 Hz, 7 Hz and 40 Hz in *M. margaritifera*, *C. edule* and *P. maximus,* respectively, and 18 Hz to describe the metachronal wave in exhalant pumping of *M. edulis*. Careful consideration has to be given to the selection of intervals between sampling when using a non-continuous method of recording behaviour. The results emphasize the importance of measuring fine-scale behaviour patterns in order to advance our understanding of bivalves. The potential loss of information associated with the

choice of particular sampling intervals during measurements of single parameters and the biases which can result from this choice are effectively germane to all species.

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INTRODUCTION

Research on bivalve behaviour has produced insights on how organisms cope with highly fluctuating environments (e.g. Jorgensen et al. 1988). Some of the questions being addressed were aimed at providing an overall view of behaviour in a particular bivalve species. While recording behaviour with high frequency measurements allowed questions concerning fine-scale bivalve behavioural physiology to be addressed (e.g. Trueman 1966, Hoggarth & Trueman 1967, Wilson et al. 2005). This may involve assessment of valve gape, siphon movements (changes in aperture), filtration and pumping behaviour in detail in relation to associated environmental parameters (such as depth, light, temperature, particulate matter, food availability and predator interactions) (e.g. see Ropert-Coudert & Wilson 2004). Although archival tags have elucidated some remarkable animal behaviours (e.g. see Ropert-Coudert & Wilson 2004 for review), selection of the correct temporal resolution, defined by the sampling interval, is critical to defining the quantity and form of behavioural events (Boyd 1993, Ropert-Coudert & Wilson 2004). Controversy about many aspects of bivalve behaviour, such as feeding, partly results from difficulties in accurately recording high frequency measurements of bivalve filtration activity (Maire et al. 2007). Maire et al. (2007) also highlight the importance of the determination of 'short-term' changes in valve gape and exhalant siphon area.

Mussel gape and exhalant siphon area have been examined using direct observation (e.g. Newell et al. 2001, Maire et al. 2007), a simple methodology which does not, however, lend itself to work where there is high turbidity in the water or with burrowing bivalves. In addition, the effective resolution of visual-based systems to determine changing parameters and the frequency with which observations are

conducted may profoundly affect the quality and interpretation of results (e.g. Wilson et al. 2005). The use of animal-attached remote-sensing technology, and in particular Hall sensors, to measure bivalve gape (Wilson et al. 2005, Nagai et al. 2006, Robson et al. 2007) circumvents many of these problems because many measurements of can be made per second and the animal may live in its normal substrate.

Maire et al. (2007) proposed that images acquired at a frequency of once every 15 s were sufficient to assess filtration activity precisely in *Mytilus galloprovincialis*, although bivalve gape has also been recorded at e.g. 5 Hz (Wilson et al. 2005); 2 Hz (Robson et al. 2007); 1 Hz (Nagai et al. 2006) and once every 5 minutes and 10 minutes (Riisgard et al. 2006). However, technology now exists for reliably measuring gape angle at a frequency of 32 Hz (Wilson et al. 2008).

Despite its endangered status, little is known about the behaviour of the IUCN Red List endangered freshwater bivalve *Margaritifera margaritifera* or about how to measure its well-being in captivity (but see Trueman 1966). We suggest that archival tag technology (Cooke et al. 2004, Ropert-Coudert & Wilson 2004), such as that used by (Wilson et al. 2005) on blue mussels *Mytilus edulis,* could change this by allowing identification of normal, and stressed behaviour (Robson et al. 2007). Here, we examine the effects of using different sampling frequencies on our ability to elucidate the behaviour of bivalves. The prime driver behind this work is the formalization of a methodology that is appropriate to examine gape and pumping (a general proxy for activity associated with respiration, feeding, excretion and their associated metabolic processes) behaviour of endangered freshwater pearl mussels, and non-endangered mussels (*M. edulis* and *Mytilus trossulus*), scallops (*Pecten maximus*) and cockles (*Cerastoderma edule*) without bias.

MATERIALS AND METHODS

Collection and maintenance of bivalves in experiments

All research detailed below was conducted in accordance with institutional, national and international guidelines relating to the use of bivalves in research.

M. margaritifera used in experiments were held at the Environment Agency Wales, Cynrig Hatchery, Brecon, Wales. *P. maximus* were collected from the Bay of Brest, France and transferred to a flow-through aquarium system within 2 h. Inter-tidal *M. edulis* and *C. edule* were collected from Swansea Bay and Gower coast, Wales, UK, respectively and *M. trossulus* from the coastline outside the Pacific Biological Station, Vancouver Island, Canada, at low tide and transferred to a flow-through aquarium system within 2 h.

Overall experimental design

To make valve gape measurements in millimetres relative between bivalves of different lengths, we used the methods developed by Wilson et al. (2005) modified by Robson et al. (2007) to quantify gape angle in mussels *M. margaritifera, M. edulis, M. trossulus,* scallops *P. maximus* and cockles *C. edule.* However, neither Wilson et al. (2005) or Robson et al. (2007) calibrated all possible gape angles with sensor output and extrapolated bivalve gape calibration curves beyond known limits. This caused some gape data >5° to be likely overestimated. The valve gape calibration dilemma was avoided in the current study by killing or using a muscle relaxant on the bivalves after experiments and calibrating Hall sensor output in milliVolts (mV) to gape in

degrees (°) over all gape angles (but see Nagai et al. 2006 who used the Hall sensor to measure bivalve gape without the need for calibration). We recommend calibration to ensure best possible accuracy in valve gape measurements.

Briefly, quantifying bivalve gape involved using a Hall sensor (a transducer for magnetic field strength) attached to one shell valve reacting to a magnet attached to the other shell valve. Variance in gaping extent produced a corresponding variance in the magnetic field strength perceived by the Hall sensor (c.f. Wilson et al. 2002). This was recorded by an archival tag. Since Hall sensor output is proportional to magnetic field strength and angle of impingement, the transducer output has to be calibrated by comparing shell gape angle with sensor output, over a wide variety of angles. A muscle relaxant (500 ppm buffered Tricaine methanesulfonate (MS-222) (Lellis et al. 2000)) was used on the endangered freshwater pearl mussels *M. margaritifera* (we note *M. margaritifera* were not killed) to allow calibration of all possible gape angles with sensor output. The adductor muscle(s) of bivalves *M. edulis, M. trossulus*, *P. maximus* and *C. edule* were simply severed with a knife and immediately after, gape calibration over all possible gape angles took ~ 5 mins per bivalve. Subsequently, data of sensor output versus gape angle were curve-fitted (for details see Wilson et al. 2002, Wilson & Liebsch 2003, Wilson et al. 2005, Robson et al. 2007). The curve-fit could then be used to determine any gape angle by converting the transducer output accordingly.

One type of archival logger used was a 13-channel JUV-Log, equipped with 12 Hall sensors (Honeywell, SS59E) and one temperature transducer. Two other archival loggers used were 7-channel JUV-Logs, equipped with 4 Hall sensors (Honeywell, SS59E) and also recorded light (Lux), pressure (depth) and temperature (°C). Two further 13-channel loggers had Hall sensors linked to the logger (type

IMASEN, Driesen and Kern GmbH, Bad Bramstedt, Germany) and also recorded light (Lux), pressure (depth) and temperature (°C). The 13- and 7-channel JUV archival loggers were powered by 4 x 1.2 V 10 Ah NiMH D cells and the IMASEN loggers by 2 x 3.6 V ½ AA lithium batteries. Each had a 1 Gb flash random access memory and could be set to record at intervals up to a maximum frequency of 2 Hz, 12 Hz and 30 Hz, respectively. The IMASEN and JUV-Log archival loggers had 16 and 22 bit resolution, respectively, both recording gape angle at better than 0.01°. The magnets used were 5 x 5 x 2 mm neodymium boron magnets.

Magnets and Hall sensors were glued to *M. margaritifera* and *Pecten maximus* using 5 minute epoxy adhesive (type X003, Atlas Polymers, Llantrisant, UK) and Araldite® 90 Seconds (Huntsman Advanced Materials, Basel, Switzerland), respectively. The other bivalves in saltwater aquaria had their systems attached using Aquarium Sealant (Geocel®, Plymouth, UK) and high strength epoxy adhesive (Power-Fast®+, Powers Fasteners, Inc., Brewster, NY, USA) in Atlantic and Pacific inter-tidal environments. *M. margaritifera* had been in freshwater pumped from a local river for months before experiments began. *M.* edulis and *C. edule* used in aquarium experiments were placed in an aerated flow-through aquarium system containing edible particulate matter-laden seawater from Swansea Bay, Wales, UK for at least a month before being used in aquarium experiments. *P. maximus* were placed in an aerated flow-through aquarium system containing edible particulate matter-laden seawater from the Bay of Brest, France for at least a 24 h before being used in aquarium experiments. Equipped *M. edulis* and *M. trossulus* used in inter-tidal experiments were returned to the inter-tidal within 24 h of initial collection.

Bivalve Pumping

Lengths of PVC tubing (10 mm diameter, 1.5 mm wall thickness and lengths of 300 and 25 mm respectively) were glued together using high strength epoxy adhesive to form an approximate ⊢ shape (Fig. 1). A Hall sensor was attached (using Aquarium sealant) to the outside of the 300 mm long PVC tube, 60 mm below the 25 mm length of tubing (Fig.1). A vane 60.5 mm long, 18 mm wide, 0.05 mm thick, made of translucent green Silastic® (Dow Corning, Midland, MI, USA) or transparent polyethylene, had one end attached to the ~25 mm long PVC tubing using aquarium sealant (Fig. 1). A 0.1 g (in air) neodymium boron magnet was attached at the free end of the vane using Aquarium Sealant, so that the magnet and Hall sensor were aligned (Fig. 1). Pumping sensors were kept in a fixed position in mussel tanks using PVC clamps. The study mussel was then placed in relation to the vane so that the water being exhaled (from the top 10 mm of the inhalant and whole of the exhalant siphon) caused the vane to move, bringing the magnet closer to the Hall sensor, thus causing a change in magnetic field intensity perceived by the transducer (in a manner similar to that used for determining gape angle – see above). It was imperative to keep the Hall sensors and magnets from the gape and pumping sensors sufficiently far apart so they did not interact.

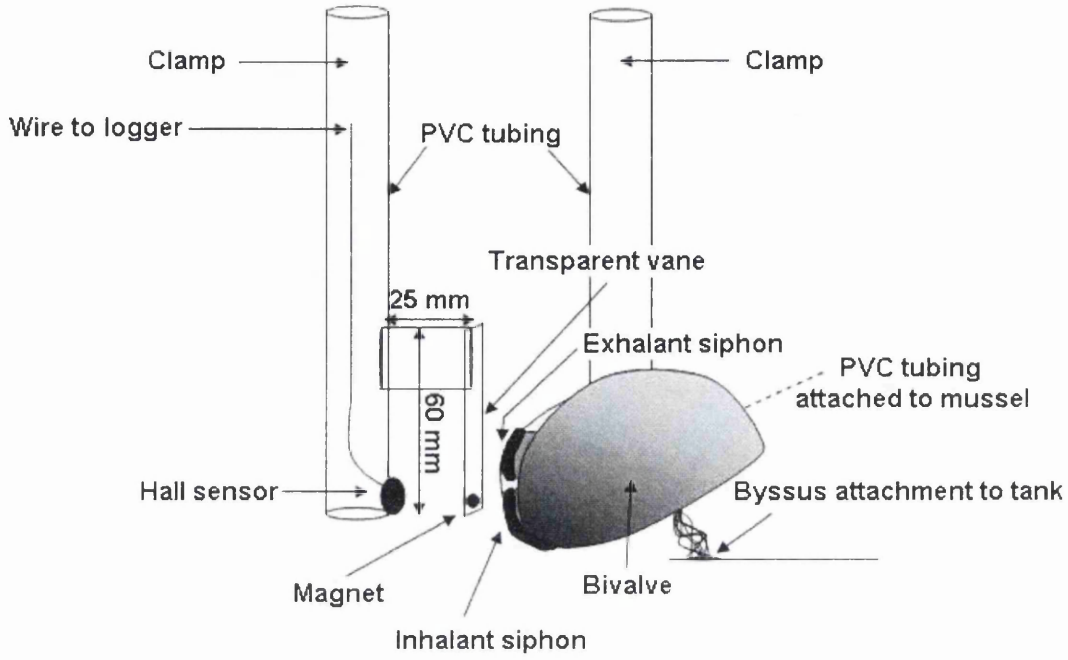

Fig. 1. Schematic diagram showing the bivalve pumping sensor for measurement of the flow of water out of the top of the inhalant and whole of the exhalant siphon (aperture). For a schematic diagram showing the attachment of the Hall sensor and magnet system used for determining bivalve gape angle see Wilson et al (2005).

In preliminary pumping experiments *M. edulis* used its foot to move the translucent green Silastic® vane out of the path of their exhalant water current and stuck it to the outside of their shell. This never occurred over 12 months of continuous pumping experiments using transparent polyethylene as the pumping sensor vane. Thus, transparent polyethylene was used as the pumping sensor vane in the current study. Sampling frequencies of 2 and 30 Hz were used to record *M. edulis* pumping. The new method for measuring pumping could not be used in strong currents because of the high sensitivity of the sensor.

Experiments

Examples of bivalve gape behaviour at various sampling frequencies in the current study were selected as representative examples from a total of 6 *M. margaritifera*, 79 *M. edulis* (gape and pumping in 48 *M. edulis*), 10 *C. edule* and 7 *P. maximus* in laboratory aquaria as well as 52 *Mytlius spp.* in the inter-tidal zone (Atlantic and Pacific). Bivalves in their natural environment fed on natural seston and bivalves in aquarium experiments fed on seston pumped from their natural environment. Experiments with bivalves took place from December 2006 until April 2008.

RESULTS

Bivalve gape

In preliminary investigations with live bivalves we made sure that our best-fit gape angle calibration curves for live animals were similar to those for sacrificed individuals. As an example, we used ANCOVA to compare two methods of gape calibration repeated in triplicate on one *M. edulis*; (1) gape calibration on the live mussel (2) gape calibration after the posterior adductor muscle was severed. Gape calibration method was the fixed factor and gape angle was the continuous variable. There was no significant effect of calibration method in the model ($F_{1,39} = 0.148$, $p = 0.702$). We reiterate that calibration of maximum gape angle was not possible in live bivalves and highlight that the majority any error in gape calibration curves was probably caused by human error (all best-fit calibration curves had r^2 values > 0.98).

All major *M. edulis* gape movements recorded at 2 Hz (0.5 s) followed the same general pattern as those recorded at 30 Hz (e.g. Fig. 2). The rate of reduction in valve gape angle (adduction) was faster than the subsequent increase in gape angle (abduction) (the latter having a roughly logarithmic form) in *M. edulis* (Figs. 2 & 3), *M. trossulus* (Fig. 4) and *M. margaritifera* (Fig. 5) with the rate decreasing near the endpoints of both adduction and abduction events. During the recording of gape at 2 Hz in the smaller and faster-moving adult *C. edule*, the rate of valve abduction did not always decrease near the endpoints of every abduction event (Fig. 6). Close inspection of *C. edule* gape data (Fig. 6) revealed that all valve adduction events occurred at a faster rate than the subsequent abduction event.

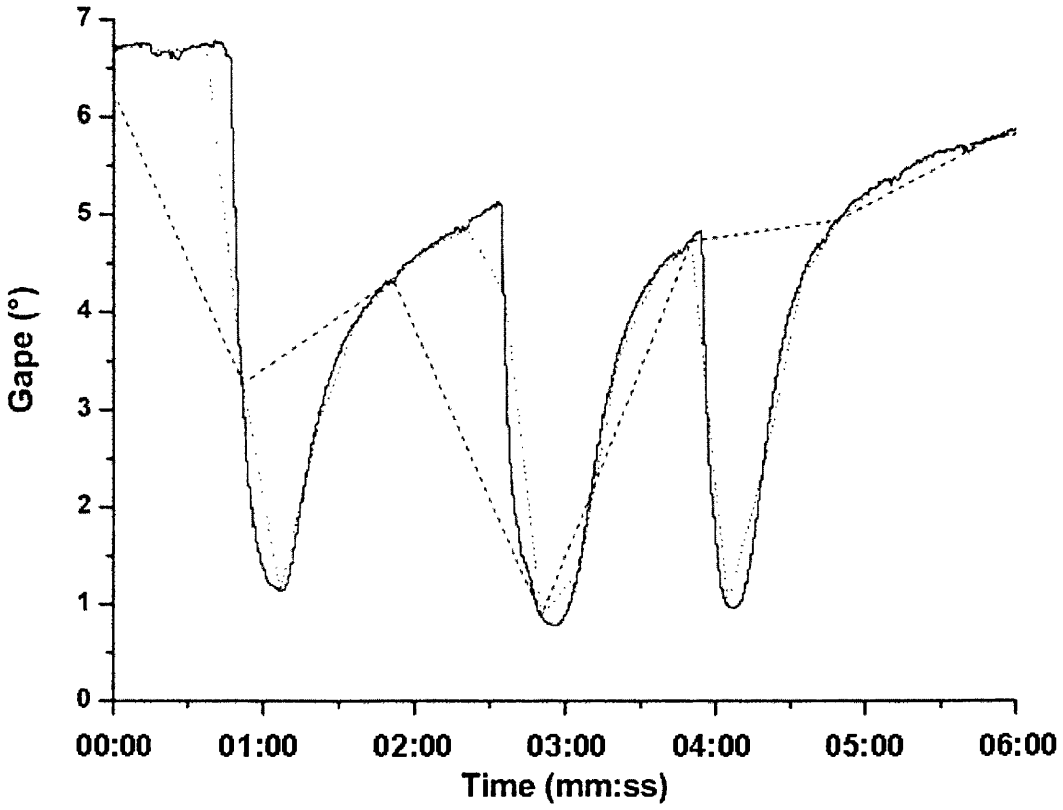

Fig. 2. *Mytilus edulis*. Example of the effect of sampling frequency on the gape (°) data from a 70 mm long mussel in an aquarium at Swansea University, UK. Sampling frequencies: 30Hz (light grey line), 2 Hz (once every 0.5 s) (black line), 0.067 Hz (once every 15 s) (dotted black line) and 0.017 Hz (once every 60 s) (dashed black line).

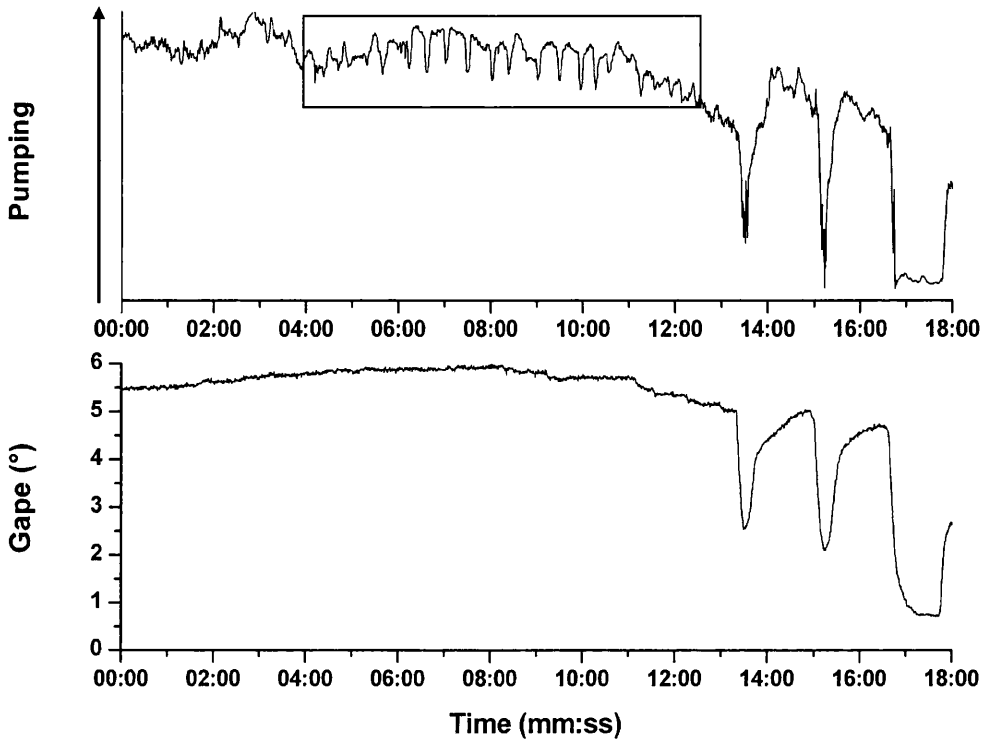

Fig. 3. *Mytilus edulis*. Detailed example of gape (°) and exhalant pumping data recorded at 2 Hz from a 72 mm mussel in a seawater aquarium at Swansea University, UK

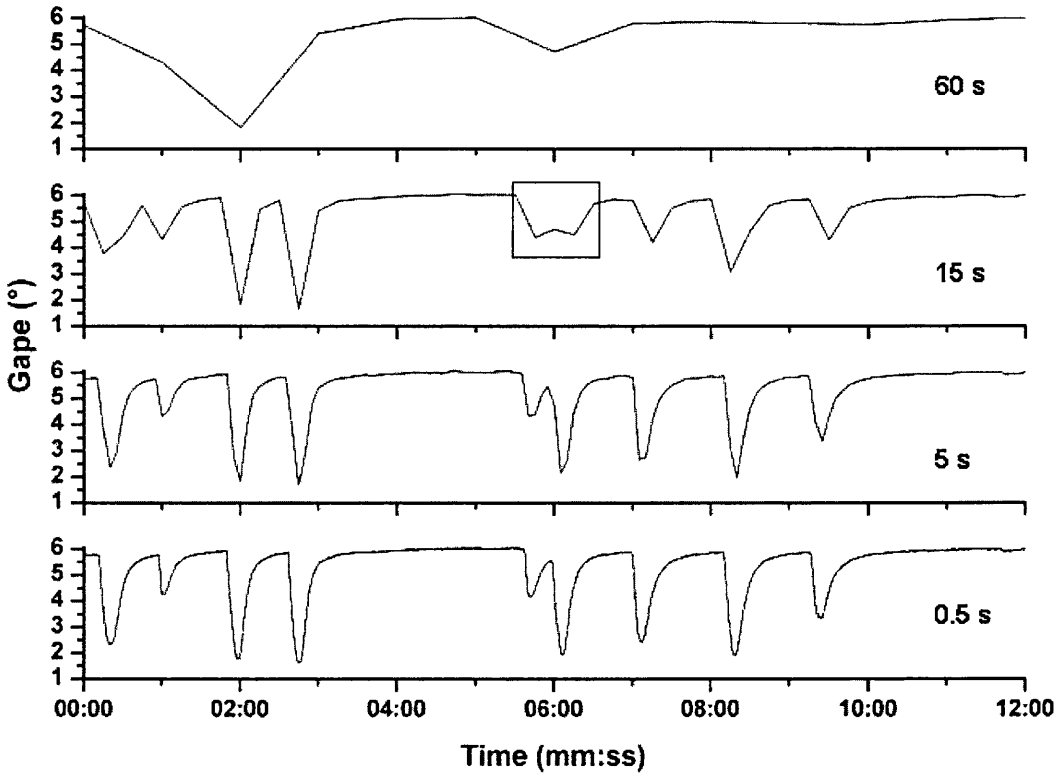

Fig. 4. *Mytilus trossulus*. Example of the effect of sampling frequency on the gape (°) data from a 55mm long mussel in the Pacific inter-tidal zone, Vancouver Island, BC, Canada. Sampling once every 0.5 s, 5 s, 15 s and 60 s. Box highlights the concatenation of adjacent gape adduction and abduction events in the data record sampling once every 15 s.

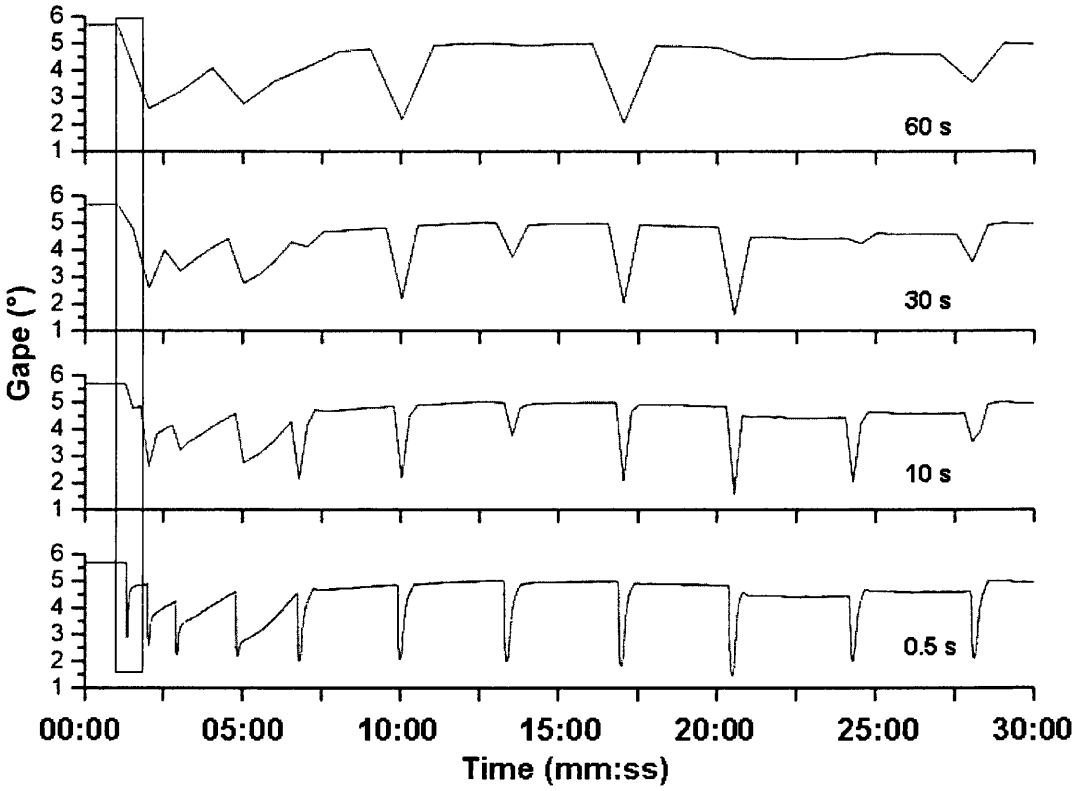

Fig. 5. *Margaritifera margaritifera*. Example of the effect of sampling frequency on the burrowing gape (°) data from a 100 mm long critically endangered freshwater pearl mussel in an aquarium. Sampling once every 0.5 s, 10 s, 30 s and 60 s. Box highlights the data loss of a valve adduction and subsequent abduction event with decreasing sampling frequency.

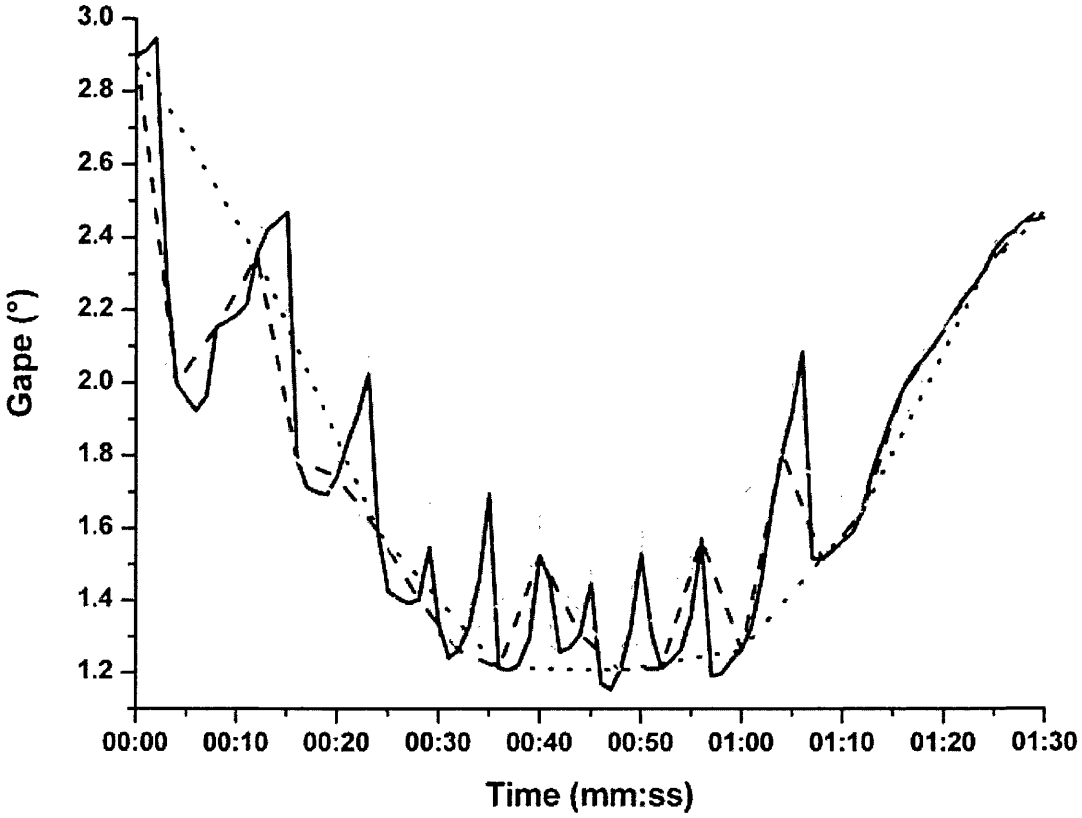

Fig. 6. *Cerastoderma edule*. Example of the effect of sampling frequency on the burrowing gape (°) data from a 30 mm long cockle in an aquarium at Swansea University, UK. Sampling once every 0.5 s (grey line), 1 s (black line), 4 s (dashed black line), 8 s (dashed grey line) and 12 s (dotted black line).

Reduction in gape sampling frequency was associated with a progressive change in the shape of the gape angle *versus* time graph in both non-burrowing and burrowing bivalves in saltwater aquaria (Figs. 2 & 6, respectively) and in wild Pacific inter-tidal marine bivalves (Fig. 4). Reducing sampling frequency below 2 Hz (intervals of 0.5 s) made valve movements appear to be faster than they actually were (Figs. 2,4-6). Accurate assessment of short-term changes in valve gape was only possible recording *M. margaritifera* gape at intervals of 0.5 s (Fig. 5) or less. Increasing the sampling interval of gape data from 0.5 s to 10 s resulted in the loss of some complete valve adduction and subsequent abduction events (e.g. Fig. 5). Visual observation of *M. margaritifera* burrowing behaviour backed up by recording gape at 0.5 s intervals (e.g. Fig. 5) highlighted the importance of valve movement for burrowing into sediment.

In one example, 45 valve adduction and subsequent abduction events over 1 h of *M. margaritifera* burrowing activity were plotted as a plateau with downwards spikes sampling at 1-5 s intervals. Increasing sampling interval ≥10 s concatenated some adjacent gape adduction and abduction events with only 10 valve adduction and subsequent abduction events detected sampling at 60 s intervals (Fig. 7). Over 1 h of burrowing activity, mean, median and minimum *M. margaritifera* gape angle (°) increased as sampling interval increased from 0.5 – 60 s (Table 1 a). Increasing sampling interval from 0.5 s to 60 s caused the interquartile range of *M. margaritifera* gape data to reduce by 0.59° and caused median gape to increase by 0.31° (Table 1 a). Maximum gape of *M. margaritifera*, and *P. maxiumus* decreased by 0.1° and 4.72° respectively, when sampling interval was reduced from 0.5 s to 60 s (Table 1 a + b, respectively). Over 1 h there was no change in mean gape ± SD but there was a reduction in maximum gape angle of *Margaritifera margaritifera* and *Mytilus edulis*

when sampling interval was increased from 0.5 – 5 s (Table 1 a and c). Also over 1 h there was no change in mean gape ± SD but there was a reduction in maximum gape angle of *Pecten maximus* when sampling frequency was decreased from 12 Hz (a sample interval of approximately 0.083 s) to once every 0.5 s (Table 1 b). However, over 1 h of *C. edule* gape data there was a change in mean gape ± SD and a decrease in maximum gape angle when sampling interval increased from 0.5 – 5 s (Table 1 d).

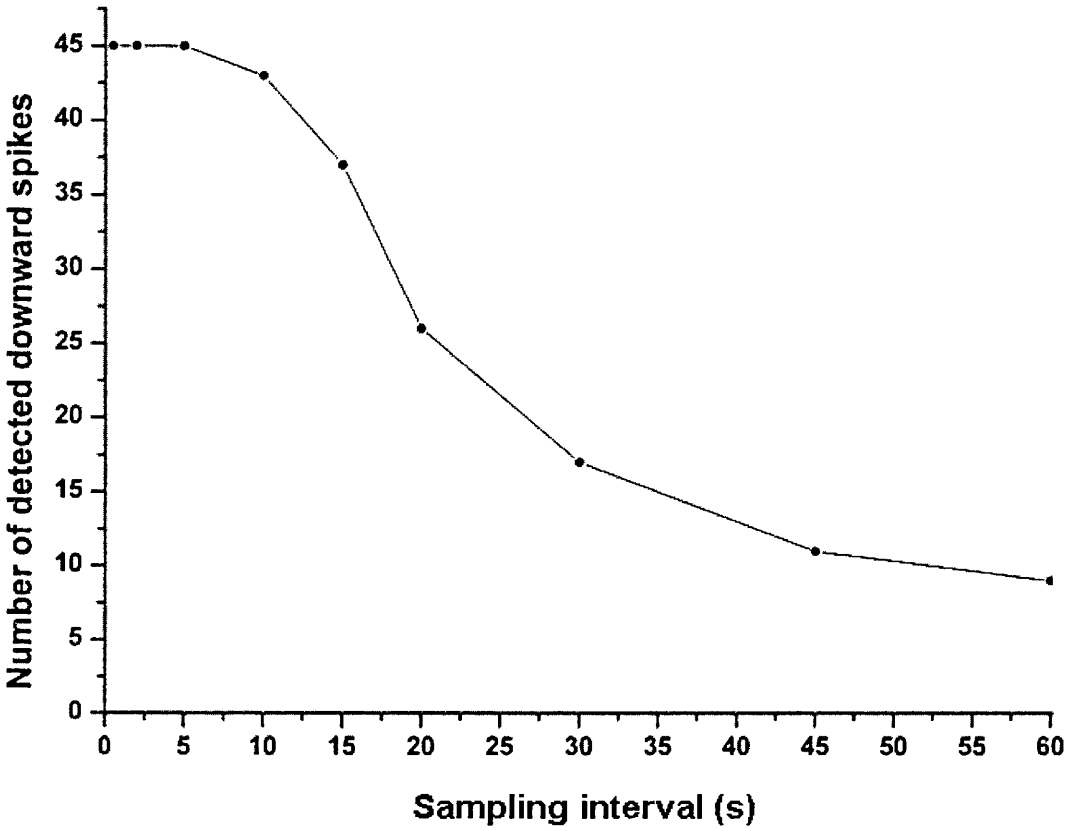

Fig. 7. *Margaritifera margaritifera*. Example of the effect of sampling interval on the number of downward spikes (i.e. valve adduction and subsequent abduction events) during 1 h of burrowing gape behaviour of a 105 mm long critically endangered freshwater pearl mussel in an aquarium

Table 1 Statistics differences describing the mean ± SD, median, maximum, minimum and interquartile range of gape (°) data at different sampling intervals over 1 h from (a) a burrowing 100 mm long freshwater pearl mussel *(Margaritifera margaritifera)* in a freshwater aquarium, (b) a 110 mm long scallop (*Pecten maximus*) in a seawater aquarium, (c) a 67 mm long *Mytilus edulis* immersed in the inter-tidal zone, Swansea Bay UK and (d) a 28 mm long cockle (*Cerastoderma edule*) in a seawater aquarium.

a

Sampling Interval (s)	Mean Gape (°) ± SD	Median Gape (°)	Maximum Gape (°)	Minimum Gape (°)	Interquartile range (°)
0.5	3.76 ± 1.34	3.70	5.90	0.79	2.35
5	3.76 ± 1.34	3.70	5.87	0.80	2.32
10	3.76 ± 1.34	3.70	5.87	0.80	2.35
15	3.76 ± 1.34	3.72	5.87	0.87	2.33
30	3.80 ± 1.31	3.75	5.87	0.87	2.39
60	3.82 ± 1.31	4.01	5.80	0.87	1.76

b

Sampling Interval (s)	Mean Gape (°) ± SD	Median Gape (°)	Maximum Gape (°)	Minimum Gape (°)	Interquartile range (°)
0.083	3.31 ± 1.58	3.44	10.81	0.97	2.76
0.5	3.31 ± 1.58	3.44	10.69	0.97	2.76
5	3.30 ± 1.57	3.53	10.03	0.98	2.76
10	3.31 ± 1.59	3.39	10.03	0.98	2.76
15	3.29 ± 1.54	3.54	8.52	0.98	2.75
30	3.27 ± 1.56	3.11	8.52	0.98	2.74
60	3.25 ± 1.53	3.02	5.97	0.98	2.83

c

Sampling Interval (s)	Mean Gape (°) ± SD	Median Gape (°)	Maximum Gape (°)	Minimum Gape (°)	Interquartile range (°)
0.5	3.28 ± 0.98	3.15	6.27	0.45	0.41
5	3.28 ± 0.98	3.15	6.24	0.48	0.40
10	3.28 ± 0.98	3.15	6.24	0.56	0.40
15	3.28 ± 0.98	3.16	6.19	0.72	0.41
30	3.28 ± 0.97	3.15	6.19	0.72	0.41
60	3.29 ± 0.94	3.16	6.09	1.37	0.35

d

Sampling Interval (s)	Mean Gape (°) ± SD	Median Gape (°)	Maximum Gape (°)	Minimum Gape (°)	Interquartile range (°)
0.5	4.57 ± 0.52	4.76	6.36	1.18	0.53
5	4.56 ± 0.54	4.75	6.18	1.19	0.54
10	4.57 ± 0.54	4.75	6.18	1.36	0.54
15	4.55 ± 0.57	4.75	5.99	1.19	0.54
30	4.53 ± 0.60	4.74	5.49	1.36	0.54
60	4.51± 0.67	4.75	5.33	1.36	0.54

Pumping

A reduction in sampling frequency of bivalve pumping behaviour was associated with a loss of definition of short-term changes in exhalant pumping (Fig. 8). At fine scale (2 Hz), *M. edulis* gape was well defined, while at the same frequency pumping was apparently rarely constant and did not appear to be fully elucidated (e.g. Fig. 8). Mussel pumping recorded at 30 Hz revealed apparent and variable 'noise' (a metachronal wave) in the pumping data of all animals (e.g. Fig. 9). We determined that the metachronal wave in the pumping data was biological in origin since it was not present when the pumping sensor was used on immersed dead mussels, or when gravity-fed water flowed out of an immersed modelled mussel exhalant siphon (made from Silastic® (Dow Corning Corporation, Michigan, USA) towards the pumping sensor.

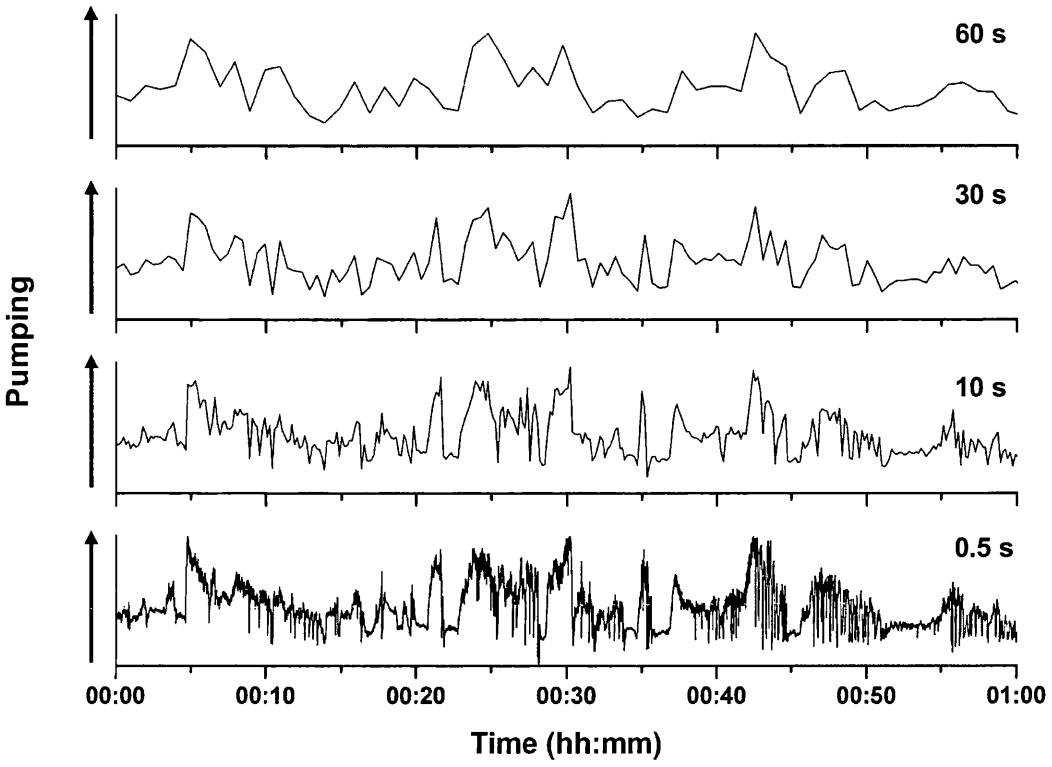

Fig. 8. *Mytilus edulis*. Example of the effect of sampling frequency on the exhalant pumping data from a 70 mm long mussel in an at Swansea University, UK. Sampling frequencies: 0.5 s, 10 s, 30 s and 60 s.

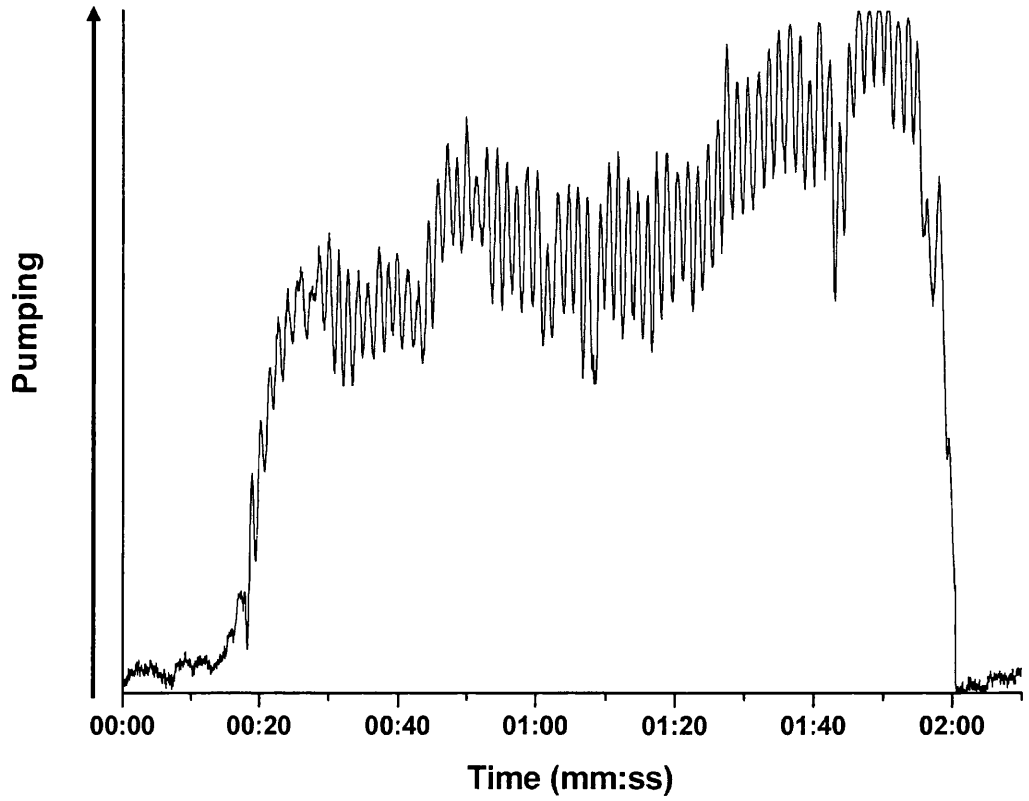

Fig. 9. *Mytilus edulis*. Example of a 75.5 mm long mussel eliminating faeces from the exhalant siphon in a seawater aquarium at Swansea University, UK. Pumping was recorded at 30 Hz with a metachronal wave evident in pumping data.

Measurements per event

Recording at 2 Hz, measurements (data points) per valve adduction and subsequent abduction event were counted for 50 events from 6 *M. margaritifera* (105 mm ± 1.4 long) and 10 *C. edule* (28.6 mm ± 1.9 long). On average, fewer measurements were made per continuous valve adduction event, compared to the subsequent abduction event in both *M. margaritifera* and *C. edule* (mean number of measurements per adduction and abduction event were 16.0 ± SD 5.7 and 44.3 ± SD 10.9 and 4.6 ± SD 1.5 and 9.1 ± SD 3.7 in *M. margaritifera* and *C. edule*, respectively), with a minimum of 10 and 3 measurements per adduction in *M. margaritifera* and *C. edule*, respectively. Complete *M. margaritifera* and *C. edule* valve adduction and subsequent abduction events had a mean number of measurements per event of 54.5 ± SD 11.5 and 14.0 ± 4.7, respectively. Recording at 12 Hz, measurements (data points) per valve adduction and subsequent abduction event were counted for 50 events from 4 *P. maximus* (107.3 mm ± 1.7 long). Mean number of measurements per adduction and abduction event were 12.1 ± SD 6.8 and 789.2 ± SD 780.2, respectively, with a minimum of 3 measurements per adduction. Complete *P. maximus* valve adduction and subsequent abduction events had a mean number of measurements per event of 1062.4 ± SD 766.1. Recording at 30 Hz, measurements per metachronal wave were counted for 50 metachronal waves from pumping data of 10 *M. edulis* (69.8 mm ± 1.6 long). A mean of 30.5 ± SD 9.4 measurements was counted per metachronal wave, with a minimum of 17 measurements per wave.

DISCUSSION

Gape

The general patterns of *M. margaritifera* valve movements recorded at 2 Hz (e.g. Fig. 5) were the same as those for non-endangered *Mytlius spp.* (e.g. Figs. 2-4) and previously described by Robson et al. (2007). Both the current study and the pioneering work by Trueman (1966) and Hoggarth & Trueman (1967) recorded *M. margaritifera* valve movements, although we found no published material on this between then and now. We believe that bivalve valve adduction and subsequent abduction events constitute a normal part of bivalve behaviour of both endangered and non-endangered bivalves, occurring in the wild sub-tidal (e.g. Wilson et al. 2005), inter-tidal (Fig. 4), simulated inter-tidal (Shick et al. 1986) and in laboratory aquariums (e.g. Figs. 2, 3, 5 & 6 and Trueman 1966, Hoggarth & Trueman 1967, Robson et al. 2007).

Adult *C. edule* are similar in size to the IUCN critically endangered little winged pearly mussel *Pegias fabula,* which rarely exceed 35 mm in length (Bogan 2002) so gape data from *C. edule* (Fig. 6) may be a good proxy for gape data from small endangered bivalves. *C. edule* data (Fig. 6) also highlight that there can be greater variability in valve movements of smaller bivalves than in the larger *M. margaritifera* (Fig. 5), indicating that recording gape of small endangered bivalves at higher frequency (i.e. > 2 Hz, see *Sampling frequency and resolution of bivalve behaviour* - below) may be appropriate (c.f. Peters 1983).

Adult *P. maximus* are similar in size (15 cm maximum shell diameter) to another marine Pectinid (scallop), the IUCN red listed *Nodipecten magnificus,* which commonly attains a size approaching 20 cm in shell diameter (Waller 2007). *P.*

maximus gape data highlight the rapid speed at which this scallop and probably *Nodipecten magnificus* can adduct. The adductor muscle(s) to shell volume ratio, or adductor muscle(s) to shell weight ratio in *P. maximus* will undoubtedly be lower than in *M. margaritifera* (although due to their endangered status *M. margaritifera* could not be sacrificed to quantify the ratios) and may account for the rapid speed of valve adduction in *P. maximus* compared to *M. margaritifera* (see *Sampling frequency and resolution of bivalve behaviour* - below).

Pumping

Although an accurate quantified measure of exhalant mussel pumping was not possible in the current study (c.f. Fdil et al. 2006), our results suggest that pumping could be considered over a fine temporal scale because we found mussel pumping (and gape) to be often highly variable, even over periods as short as one minute (c.f. Robson et al. 2007). When measuring *M. margaritifera* exhalant pumping, especially in relation to gape angle, it may be necessary to test whether an exhalant current exits from the top of the inhalant siphon, as well as the exhalant siphon. *M. edulis* has a mucociliary rejection pathway that functions via the inhalant siphon with pseudofaeces eliminated along the ventral side of the septum dividing the inhalant siphon from the exhalant siphon (Widdows et al. 1979, Beninger & St Jean 1997, Beninger et al. 1999). Along with our own observations of *M. edulis* pseudofaeces strings being eliminated in an exhalant water current out of the top of the inhalant siphon (sometimes when the exhalant siphon was closed), we found it was appropriate to measure exhalant *M. edulis* pumping out of both the top of the inhalant and whole of the exhalant siphon to determine if the mussel was pumping (active).

Biological 'noise'

More research will have to be a conducted to determine the cause of the biological 'noise' in the form of a metachronal wave of varying amplitude in *M. edulis* exhalant pumping recorded at 30 Hz (e.g. Fig. 9). Wilson et al. (2005) reported biological 'noise' in the gape data of bivalves (also present in our gape data) which was consistently higher in sand mussels (*Astarte borealis*) than *M. edulis* and suggested that some of it might be due to heart beat (c.f. Curtis et al. 2000). While there is little known about the metachronal wave in mussel pumping, it may be an important parameter to measure in bivalves; since we might expect the frequency of metachronal waves in pumping to vary according to circumstance e.g. temperature.

Sampling frequency and resolution of bivalve behaviour

The potential loss of information associated with the choice of particular sampling intervals during measurements of single parameters and the biases which can result from this choice are effectively germane to all species (c.f. Boyd 1993). The current study reveals the degree to which intervals between sampling affect our ability to identify bivalve gape adduction and abduction events and the degree of variability in bivalve pumping and therefore, ultimately, how this affects the descriptive statistics of gape and pumping behaviour. One effect of increasing sampling interval was to concatenate adjacent gape adduction and abduction events in the data record (Figs. 2, 4-7) which resulted in increased mean duration between gape adduction and abduction events and increased minimum gape angles (Table 1); this is an analogous process to

the effect of increasing sampling interval on the diving behaviour of seals (Boyd 1993).

Another effect of increasing sampling interval was substantial changes to the shape of bivalve gape adduction and abduction events (Figs. 2, 4-6) and pumping profiles (Fig. 8). Increasing sampling interval from 0.5 s – 60 s had relatively little effect on the mean gape of bivalves (Table 1). However, it was apparent that increasing sampling interval from 0.5 s - 5 s caused a reduction in maximum gape and thus a loss of definition of short-term changes in bivalve gape (Table 1).

It is essential to select the correct temporal resolution defined by sampling interval in order to detect and define fine-scale behaviour patterns. If the shape of an event is to be described via changing values in the measured parameter, then the recording frequency should be of the order of 10 measurements per event (Ropert-Coudert & Wilson 2004). Given this, our analysis of data indicates that gape should be recorded at a minimum of 2 Hz, 7 Hz and 40 Hz in *M. margaritifera*, *C. edule* and *P. maximus,* respectively, and at 18 Hz to describe the metachronal wave in exhalant pumping of *M. edulis*. Where the peak values in the measured event are important, such as peaks in bivalve pumping amplitudes (Fig 9) and the exact start and fastest part of valve adduction events, 10 measurements per event may not adequately describe these extremes. We note that some *P. maximus* valve adductions could not be defined (10 measurements per event) with any of the loggers used in the present study or 'daily diary' loggers (Wilson et al. 2008). From our experience measuring bivalve pumping, we speculate that an initial sampling frequency of 30 Hz would be required to determine the appropriate sampling frequency to measure fine-scale bivalve siphon movements (changes in aperture) of *M. margaritifera*.

An inherent problem in dealing with bivalve data measured at high sampling frequency (e.g. 2-30 Hz) over days, weeks and months is data processing time. A computer with 8 Gb RAM, 3.4 GHz Pentium 4 processor takes ~40 minutes to convert 7 million gape data points (~64.8 h and ~40.5 d of data from an archival tag channel recording at 30 and 2 Hz, respectively) from only one bivalve in mV to degrees (°), using an exponential equation in the form y=a+bexp(-x/c) in Origin® version 7.5 (OriginLab Corporation, Northampton, MA, USA). A way round this is to thin data so that curve fits can be applied to much fewer data points. However, too few data points in the time series of e.g. gape leads to poor resolution of behaviour which can lead to misinterpretation.

Temporal resolution

In the current study with a 1 GB flash memory card and the system set to record at 30 Hz on 2 channels to record bivalve gape and pumping simultaneously, the archival tag could record for ca. 70 d before the memory was full. Using 128 GB compact flash memory cards (Samsung, Seoul, South Korea) the recording times of the archival loggers could be multiplied by 128. A computer-programmed interface could stop the logger just before the memory card was full, the full memory card replaced and logger restarted within 10 minutes. Thus, high temporal resolution data can be recorded almost continuously

Conclusion

The potential loss of information associated with the choice of particular sampling intervals during measurements of single parameters and the biases which can result from this choice are effectively germane to all species. The analyses presented here demonstrate that careful consideration has to be given to the selection of intervals between sampling when using a non-continuous method of recording behaviour. We believe that, where possible, all behavioural events should be recorded because they are likely to vary according to circumstance (e.g. Wilson et al. 2005, Robson et al. 2007). Given that the minimum appropriate sampling frequency for recording fine-scale *M. margaritifera* gape and, most probably, pumping behaviour has now been established, our ongoing research can test if the breakthrough culturing *M. margaritifera* (Preston et al. 2007) can be further improved by conditioning broodstock and providing juveniles with additional food. Advances in the understanding of bivalve feeding and reproductive strategies may be gleaned by recording behaviour with high temporal and sensor resolution over a range of ecological circumstances (according to factors such as depth, light, temperature, particulate matter, food availability and predator interactions) and may aid long term survival of endangered bivalves including freshwater pearl mussels.

In summary the results of this chapter highlighted the importance of checking that the sampling frequency will record all behavioural events because some previous work may have missed important behaviour. The minimum sampling rates to define gape behaviour were found to be 2 Hz, 7 Hz and 40 Hz in *M. margaritifera*, *C. edule* and *P. maximus,* respectively, and 18 Hz to describe the metachronal wave in exhalant pumping of *M. edulis*. The potential loss of information associated with the choice of

particular sampling intervals during measurements of single parameters and the biases which can result from this choice are effectively germane to all species. *M. edulis* exhalant pumping can occur from both the top of the inhalant siphon and the exhalant siphon and the significance of this finding is discussed in detail in Chapter 3.

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Chapter 2

Mussels flexing their muscles: a new method for quantifying bivalve behaviour[b]

Abstract

A novel technique was employed to quantify how blue mussels, *Mytilus edulis*, react to predation risk in their environment by quantifying mussel gape using a Hall sensor attached to one shell valve reacting to a magnet attached to the other. Change in gape angle per second (CHIGA) versus gape angle plots resulted in a distribution with a boundary, which defined the maximum CHIGA of a mussel at all gape angles. CHIGA boundary plots for all individual mussels were similar in form. However, the CHIGA boundary increased in extent with mussel length (maximum CHIGA for mussel valve closures for mussels 2.98 and 79.6 mm long were -1.5 and $-5.5°s^{-1}$, respectively), showing that larger mussels opened and closed most rapidly. Mussel extract added to the seawater, a factor believed to signal predation, caused mussels to close significantly faster than otherwise ($P < 0.001$). This approach for assessing how mussels react to their environment indicates that mussel response to predation is graded and complex and may well indicate animal-based assessments of the trade-off between effective feeding and the likelihood of predation.

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[b]The content of this chapter has been published: Robson A, Wilson R, Garcia de Leaniz C (2007) Mussels flexing their muscles: a new method for quantifying bivalve behaviour. Marine Biology 151:1195-1204

INTRODUCTION

Animal behaviour (e.g. Scott 2004) is normally assumed to lead to maximized lifetime reproductive success (e.g. Clutton-Brock et al. 2004) and specific reactions to environmental variability constitute a part of this behaviour. Documentation of behaviour involving movement with survival value is common in vertebrates (e.g. Vilensky 1987, Bertram et al. 1997, Sandercock & Heckman 1997) but reports are notably lacking in sessile molluscs, primarily due to the difficulty of quantification of behaviours that occur in these generally small animals whose behaviour is characterized by minimal movement carried out over comparatively long time periods. Such movement may, however, be critical in survival and its quantification may provide insights into strategies and environmental conditions of consequence for this important animal group.

There is significant commercial mussel production in over 40 countries world-wide (FAO 1999) and mussels, particularly *Mytilus edulis*, have been proposed as potent bioindicators. However, this is largely based on assessment of changes in animal body composition and is thus only undertaken when the animals are sacrificed (e.g. Fisher et al. 1996). However, DeZwart et al. (1995) did employ mussels as bioindicators using remote-sensing technology, but see Wilson et al. (2005) for a review of the DeZwart et al. (1995) "mosselmonitor". We propose that appropriate assessment of behaviours denoting mussel well-being should help improve mussel cultivation and may increase the utility of mussels as bio-indicators of environmental conditions (e.g. Fisher et al. 1996). Accordingly, we show here how new sensor and logging tag methodology (Wilson et al. 2005) can be used to quantify mussel gape and

the change in gape angle per second in live blue mussels *M. edulis* in order to elucidate how these animals react to changes in their environment.

MATERIALS AND METHODS

Overall experimental design

The methods developed by Wilson et al. (2005) were used to quantify gape angle in blue mussels *M. edulis*. Briefly, this involved using a Hall sensor (a transducer for magnetic field strength) attached to one mussel valve and a small magnet attached to the other valve. Variance in gaping extent produced a corresponding variance in the magnetic field strength perceived by the Hall sensor. This was recorded by a logger. Since Hall sensor output is proportional to magnetic field strength and angle of impingement, the transducer output has to be calibrated by comparing shell gape angle with sensor output, over a wide variety of angles, and curve-fitting the data (for details see Wilson et al. 2002, Wilson & Liebsch 2003, Wilson et al. 2005). This curve-fit can then be used to determine any gape angle by converting the transducer output accordingly.

The logger used for the work was a 13-channel JUV-Log, equipped with 12 Hall-sensor (Siemens KSY 10) channels and one temperature channel, each with 22 bit resolution. The unit had a 512 Mbyte RA memory and could record at rates of up to 2 Hz. The magnets used were 5 × 5 × 2 mm neodymium boron magnets.

Collection and maintenance of mussels

Inter-tidal mussels were collected from LR SS630875 Swansea Bay, Wales, UK at low tide and transferred to a flow-through aquarium system within 2 h. Magnets and

Hall sensors were glued to mussel shells using Aquarium Sealant (Geocel, Plymouth, UK) before the mussels were replaced in an aerated flow-through aquarium system for at least a week before being used in experiments. Experiments with mussels took place from October 2005 to June 2006.

Measuring gape angle in standard conditions

In order to determine how six mussels (mussel length see Table 1) reacted to standard conditions, the logger recorded gape angle at 2 Hz for multiple periods of ~5 days. At this time the individual mussels were kept in separate aquaria. Mussels were subject to a daily regime of 13 h light and 11 h dark, water temperature 16 ± 0.3°C and each fed a mixed algal diet of ~100 million *Tetraselmis suecica* and ~1,000 million *Thalassiosira weissflogii* cells day^{-1} at 40 ± SD 3.1 cells μl^{-1}.

Table 1 Best-fit relationship between change in gape angle per second (CHIGA) ($°s^{-1}$) to define the boundary (y) and gape angle (x) (see calculation of CHIGA in Materials and methods)

Mussel length (mm)	Valve movement	Relationship
29.8	Opening	$y = (a + bx + c/x + dx^2 + e/x^2 + fx^3 + g/x^3 + hx^4)$ a 1.5888486 b −0.52408928 c −0.35121223 d 0.19131119 e 0.029613084 f −2.80E−02 g −8.63E−04 h 1.22E−03
	Closing	$y = (a + bx + cx^2 \ln x + dx^3 + ee^x)$ a 0.058736807 b −0.55896575 c 0.1513833 d −0.037638453 e 0.000702693
34.6	Opening	$y = (a + bx0.5 + cx + dx1.5 + ex^2 + fx^{2.5} + gx^3 + hx^{3.5} + ix^4 + jx^{4.5} + kx^5)$ a −3.2201826 b 28.261003 c −98.470003 d 192.8207 e −231.32861 f 1.79E + 02 g −9.21E + 01 h 3.11E + 01 i 6.66E + 006 j 8.17E−01 k −4.37E−02
	Closing	$y = (a + cx + ex^2 + gx^3 + ix^4)/(1 + bx + dx^2 + fx^3 + hx^4 + jx^5)$ a 0.03826436 b −0.30557562 c −0.3008307 d 0.054804004 e 0.063870721 f −5.85E−03 g −4.58E−03 h 2.99E−04 i 1.10E−04 j −5.27E−06
38.7	Opening	$y = (a + bx + cx^2 + dx^3 + ex^4 + fx^5 + gx^6 + hx^7 + ix^8)$ a −0.029847969 b 1.3726608 c −1.435414 d 0.91228114 e −0.37543336 f 9.52E−02 g −1.40E−02 h 1.10E−03 i −3.48E−05
	Closing	$y = (a + bx + cx^2 + dx^3 + ex^4 + fx^5)$ a 0.017766632 b −0.50999977 c −0.090428403 d 0.094381006 e −0.019634252 f 1.25E−03
44.7	Opening	$y = (a + cx + ex^2 + gx^3 + ix^4)/(1 + bx + dx^2 + fx^3 + hx^4)$ a −0.09611428 b 0.051349164 c 0.68877847 d −0.013542099 e −0.12260023 f 4.14E−04 g 7.69E−03 h 2.90E−06 i −1.65E−04
	Closing	$y = (a + bx + cx^2 + dx^3 + ex^4 + fx^5 + gx^6 + hx^7 + ix^8 + jx^9 + kx^{10}$ a 0.11750452 b −1.147895 c 1.6774392 d −1.4270188 e 0.57351823 f −1.27E−01 g 1.66E−02 h −1.31E−03 i 6.22E−05 j −1.61E−06 k 1.76E−08
54.6	Opening	$y = (a + cx + ex^2)/(1 + bx + dx^2 + fx^3)$ a −0.009530934 b 0.48840935 c 0.47804793 d −0.046097521 e −0.053146559 f −2.10E−03
	Closing	$y = (a + cx + ex^2 + gx^3 + ix^4)/(1 + bx + dx^2 + fx^3 + hx^4 + jx^5)$ a −0.017200221 b 0.36103367 c −1.278371 d −0.38897404 e 0.80373711 f 8.35E−02 g −1.72E−01 h −5.01E−03 i 1.09E−02 j 1.08E−05
79.6	Opening	$y = (a + bx + cx^2 + dx^3 + ex^4 + fx^5 + gx^6 + hx^7 + ix^8)$ a 0.03040645 b 1.0774803 c −0.35758174 d 0.069764735 e −0.00723012 f 4.21E−04 g −1.39E−05 h 2.45E−07 i −1.79E−09
	Closing	$y = (a + bx + cx^2 + dx^3 + ex^4 + fx^5 + gx^6 + hx^7 + ix^8)$ a 0.19715819 b −1.3270658 c 0.43059846 d −0.1084851 e 0.013337685 f −8.59E−04 g 2.97E−05 h −5.20E−07 i 3.63E−09

Measuring gape angle in non-standard conditions

Mussels were subject to the same daily feeding and light regime as in standard conditions. The reaction of six mussels to the chemical stimulus of an injured *M. edulis* was recorded by the logger while a damaged (shell cracked) 55 mm long conspecific was placed in a 1.5 l tank supplied with filtered, aerated seawater draining into a 50 l tank containing six mussels equipped with Hall sensors. Loggers recorded for ~4 days before and after the application of the injured mussel and the procedure was repeated six times with both fresh filtered seawater and a new injured mussel.

Calculation of CHIGA

The Hall sensor data were converted into gape angle and plotted against the change in gape angle (converted to standardized units of degrees per second, but measured over intervals of 0.5 s) to produce a characteristic pattern, which will subsequently be refer to as the CHIGA (CHange In Gape Angle per second) pattern (Fig. 1). The density of the points within the CHIGA pattern reflects the incidence of the particular conditions of change in gape angle per second as a function of gape angle, but the boundaries represent limits in the change in gape angle per second (Fig. 1). The ease with which these boundaries could be detected depended on the length of the data set and the conditions under which the mussel was held (see Fig. 4 and Results). Two non-linear curves were fitted to describe these boundaries using Table-curve (giving r^2 better than 0.99), one which corresponded to mussel opening (solid line in Fig. 1) and one for mussel closure (dashed line in Fig. 1) (see Table 1). These equations were used to predict the maximum CHIGA for any gape angle during both opening and closing events.

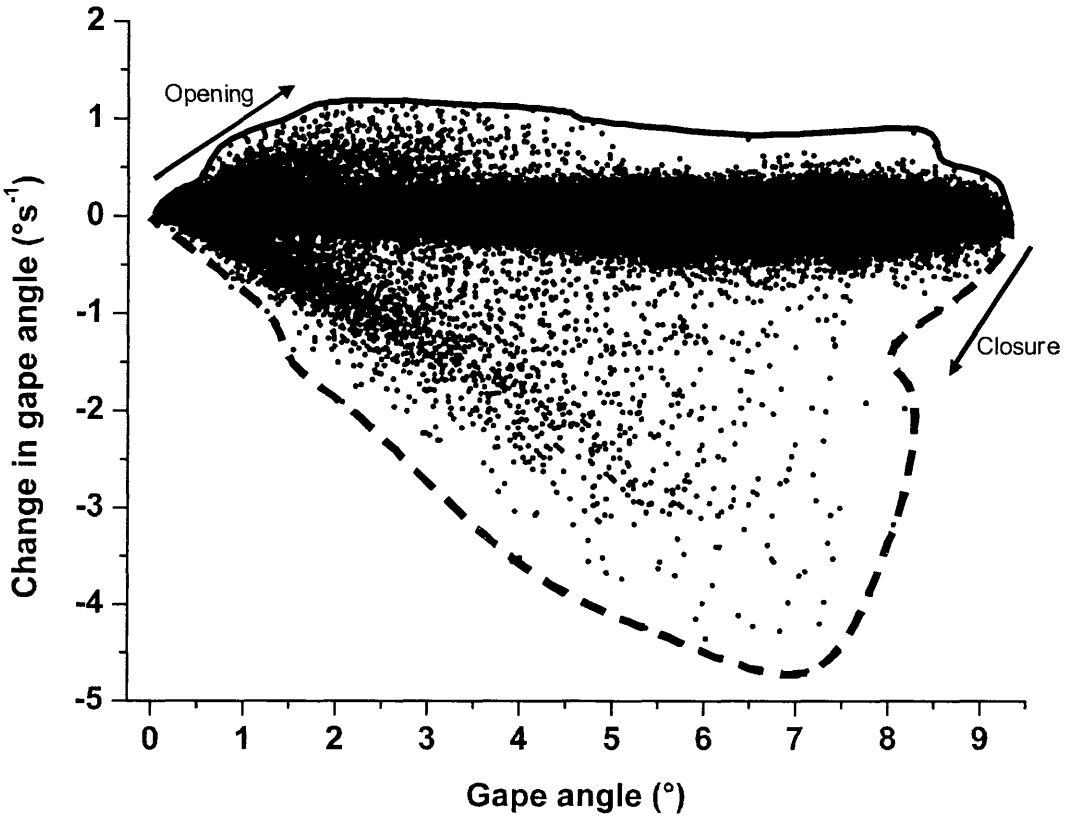

Fig. 1 Change in gape angle per second (CHIGA) versus gape angle for a single 44.7 mm long mussel using data acquired over a period of 7 days at a rate of 2 Hz. The mussel was held in standard conditions (see Measuring gape angle in standard conditions) and stressed by calibration (see Overall experimental design and see Results). The *solid line* delineates the edge contours indicating maximum CHIGA during opening, the *dashed line* that during closure

We reasoned that proximity of any particular temporal sequence of points describing a change in valve gaping during opening or closure events to the boundaries (defined by our equations in Table 1) indicated the extent to which the mussel was relaxing (abducting) or contracting (adducting) its adductor muscles in relation to the maximum rate of change achievable. Since the shell opening process is passive, mediated by the elasticity of the hinge (Ruppert et al. 2004), the most relaxed adductor muscles (anterior and posterior) results in fastest opening. Conversely, since shell gape closure is active, the faster the adductor muscles contract, the closer the CHIGA approaches the boundary defined by the equations.

Calculation of Þ

In order to work out how quickly mussel opening or closing events related to maximum rates of opening or closure, the first observed gape angle (a_n) in an opening or closure event was taken and the next angle ($a_n + 1$) predicted ($A_n + 1$) according to the boundary equation (see Table 1). The difference (D) between the next observed ($a_n + 1$) and next predicted ($A_n + 1$) angle was calculated. The process was then repeated using the next gape angle in the opening or closure sequence. This process was repeated for the entire opening or closure event and $\sum D$ calculated (an integral units $^{\circ 2}0.5\ s^{-1}$).

In order to correct for gape angle-dependent CHIGA (cf. Fig. 1), this integral × 2 (units $^{\circ 2}s^{-1}$) was subsequently divided by the total movement in degrees of the closure or opening event, to give a final value for the proximity of the closure or opening event to the maximum. This value termed Þ (units $^{\circ}s^{-1}$) (see Table 2 for a worked example of calculating Þ) is an arbitrary but relative scale. Þ permits comparison between mussels that open and close at different maximum rates. Þ avoids

the bias that bigger mussels close quicker than smaller mussels (see Results). CHIGA was recorded at 2 Hz to capture the form and accurate speed of the fastest closures.

Table 2 Worked example of the calculation of Þ for a closure event from a 44.7 mm long mussel

Observed closing 0.5 s^{-1} (°)	**Predicted maximum CHIGA ($°s^{-1}$) from equations (see Table 1)**	**Predicted next angle 0.5 s^{-1} if closing at maximum rate (°)**	**Difference between predicted next maximum angle 0.5 s^{-1} and observed next angle 0.5 s^{-1} (D)**
5	−2.097498429	3.951	−0.55
4.5	−1.998271261	3.501	−0.5
4	−1.893258608	3.053	0.053
3	−1.510913916	2.245	−0.26
2.5	−1.203356009	1.898	−0.1
2	−0.855283769		
Total closure 5–2 = 3°			$\sum D = -1.357°^2 0.5\ s^{-1}$
			Þ = $\sum D \times 2 \div$ total closure (°) = $-0.905°s^{-1}$

Statistical analysis

(Paired) *t*-tests were carried out to test for significant ($P < 0.05$) differences in valve closure rates of six mussels before and after exposure to a chemical signal from an injured conspecific.

RESULTS

Mussels were substantially disturbed during and for at least 24 h after calibration of gape angle. However, calibration of gape angle against Hall sensor output proved unproblematic although ten to fourteen calibration readings over a wide range of gape angles were needed to produce best-fit functions with correlation coefficients in excess of 0.97. Blue mussels gaped in the range of 0–11°. The vast majority of mussel gape closure (>97%) and subsequent opening (>98%) events followed a specific, recognizable pattern consisting of a relatively rapid closure followed by a slower opening, this latter having a roughly logarithmic form (Fig. 2). In the examples of mussel closures (Fig. 3a) the variance in rate of valve closure was most reduced between about 4.3 and 2.9° and valve opening (Fig. 3b) between about 1.3 and 2.3°.

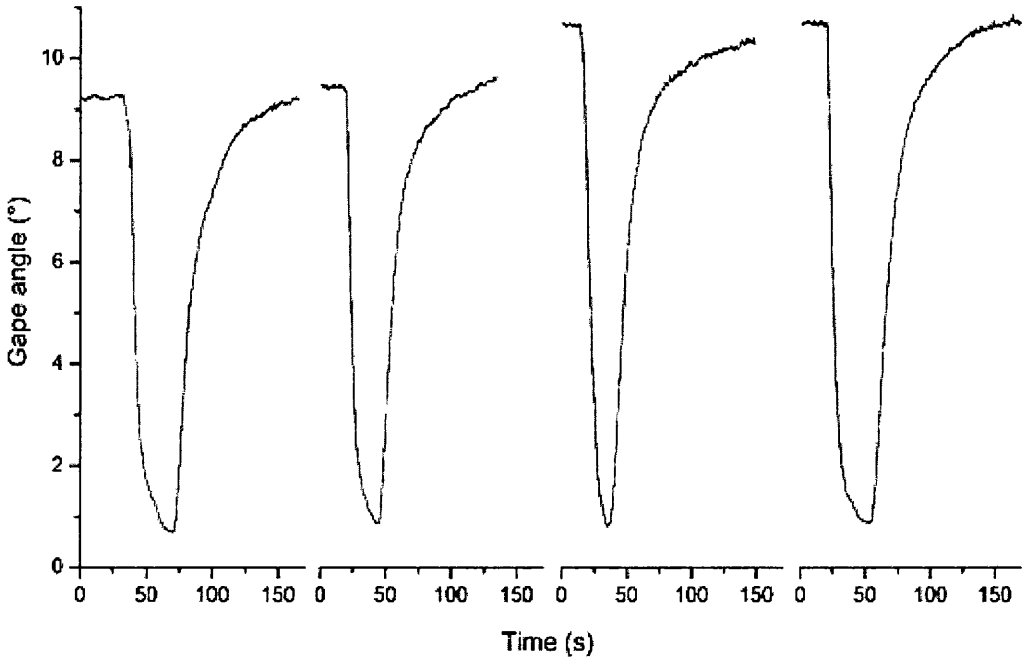

Fig. 2 Examples of the most common form of mussel valve closure and subsequent opening events. Note the faster rate of closure compared to opening with the rate decreasing near the endpoints of both closure and opening events

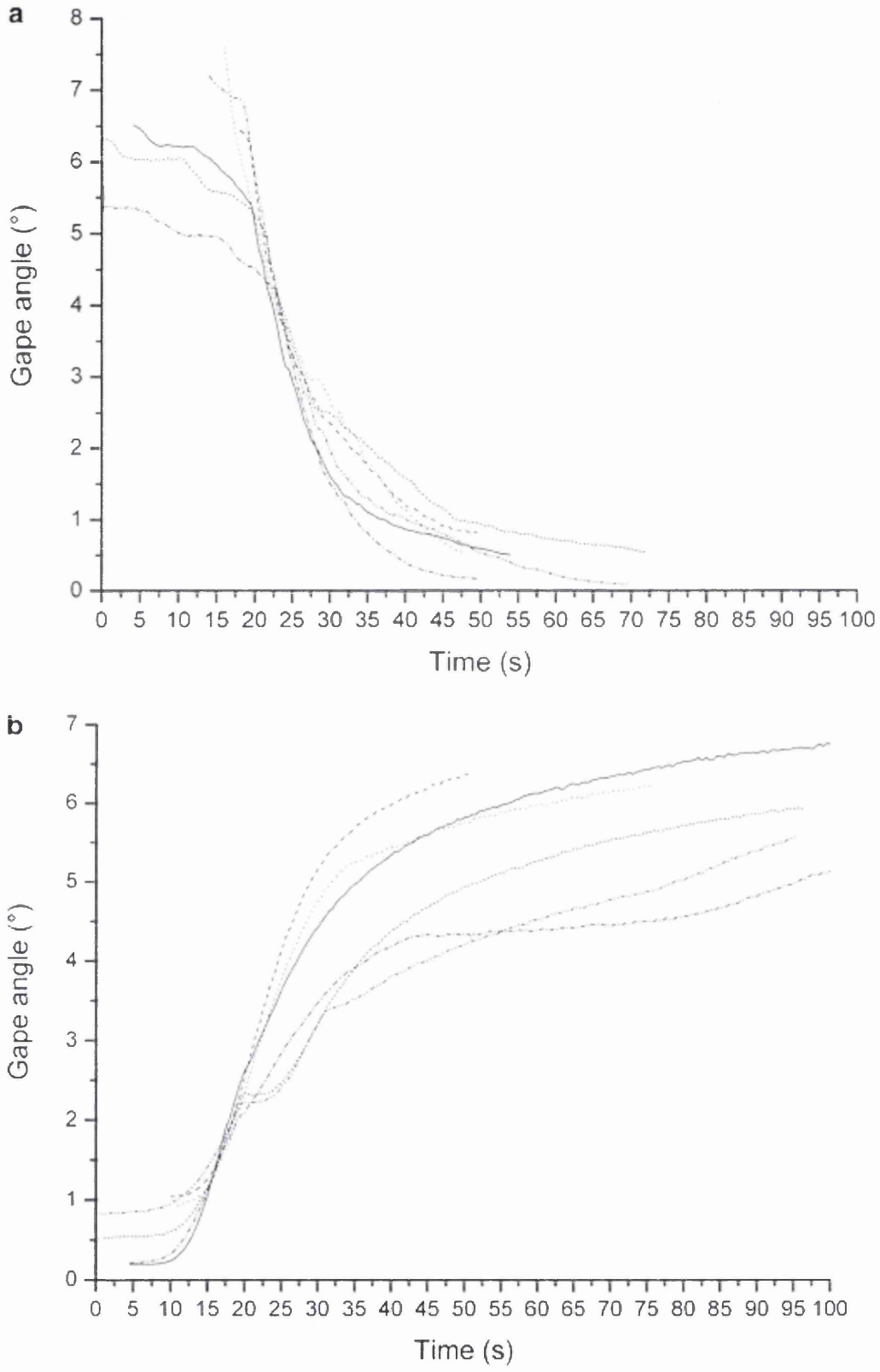

Fig. 3 Six detailed examples of the form of mussel valve closure **a** and opening **b** events from **a** 44.7 mm long mussel

The logging time required to acquire well-defined boundaries to the CHIGA pattern varied considerably between mussels. For example, the time taken to reach the boundary at a gape angle of 5° for six different mussels varied between 8 and 94 h (Fig. 4). CHIGA boundary plots for all mussels were similar in form. However, the extent of the CHIGA boundary increased with mussel length (maximum CHIGA for mussel valve closures for 29.8 and 79.6 mm long mussels were −1.5 and $-5.5°s^{-1}$, respectively, while openings were 0.5 and $1.8°s^{-1}$, respectively).

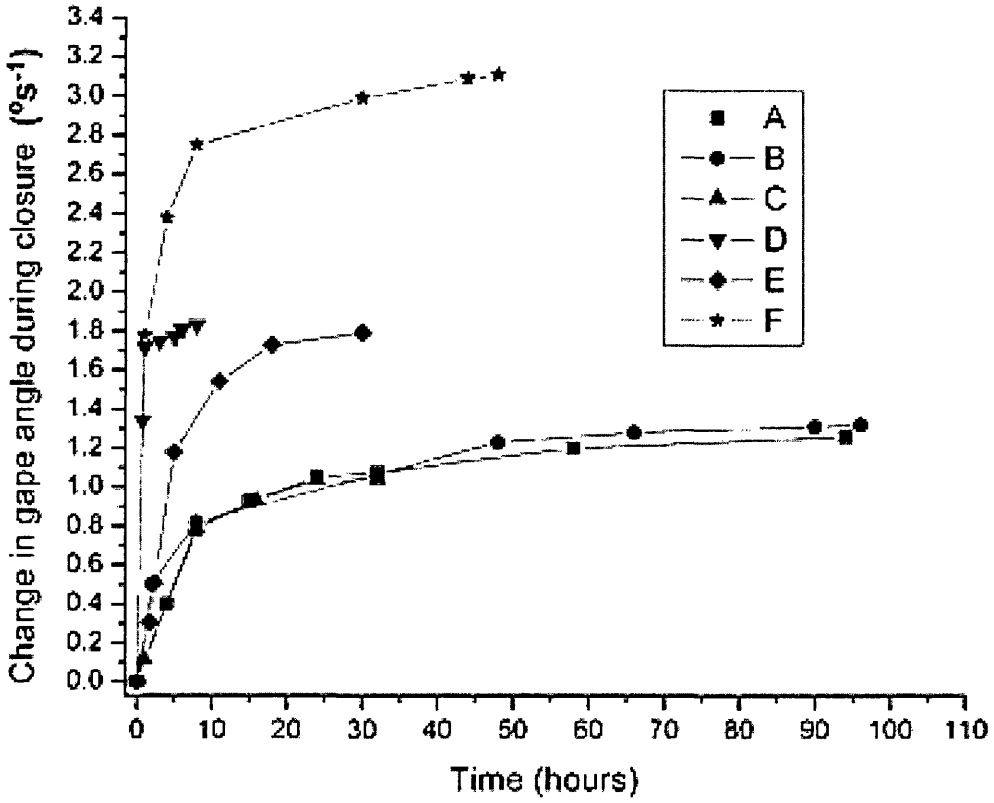

Fig. 4 Maximum recorded closure speed ($°s^{-1}$) at a gape angle of 5° as a function of time for six different individual mussels (delineated by different letters). Mussel length (mm) A 29.8, B 34.6, C 38.7, D 44.7, E 54.6, F 79.6

Contour plots of the incidence (frequency) of CHIGA versus gape angle indicated the prevalence of particular responses (e.g., Fig. 5). For instance, over 5 days the mussel in Fig. 5 was closed or nearly closed (gaping between 0 and 1°) 6% of the time and spent 89% of the time open between 6 and 11° (Fig. 6). This particular animal spent only 5% of its time gaping between 1 and 5°.

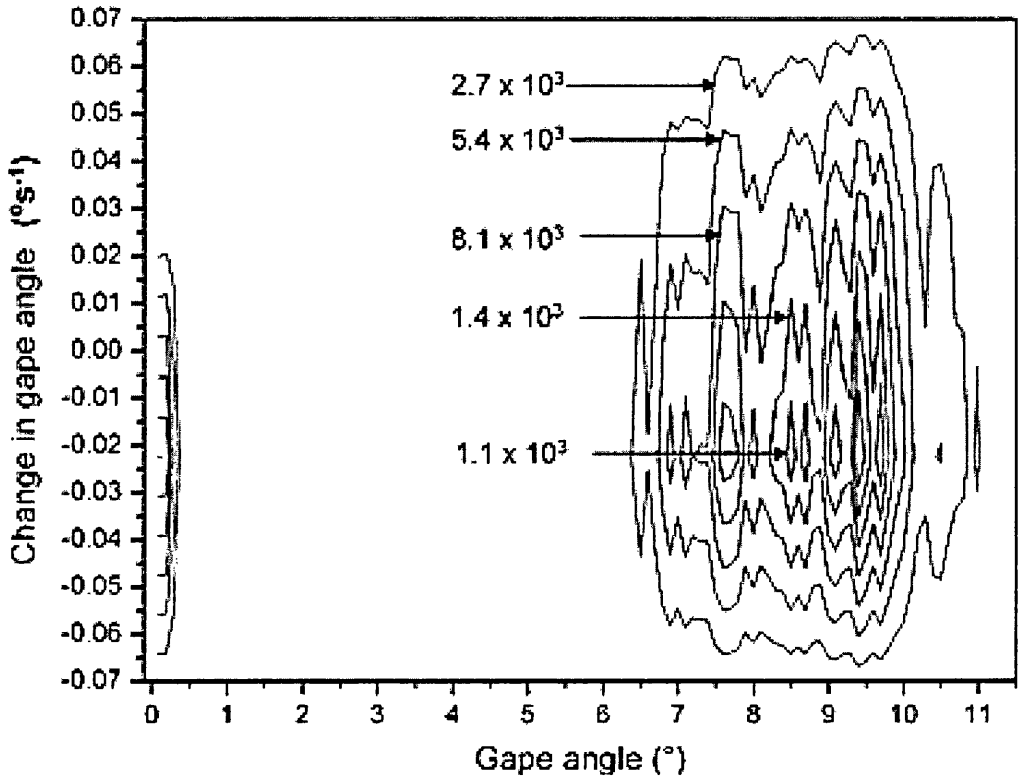

Fig. 5 Contour plot illustrating the relative incidence of CHIGA versus gape angle for a 54.6 mm long mussel over 5 days. Units of contour values is the number of observations

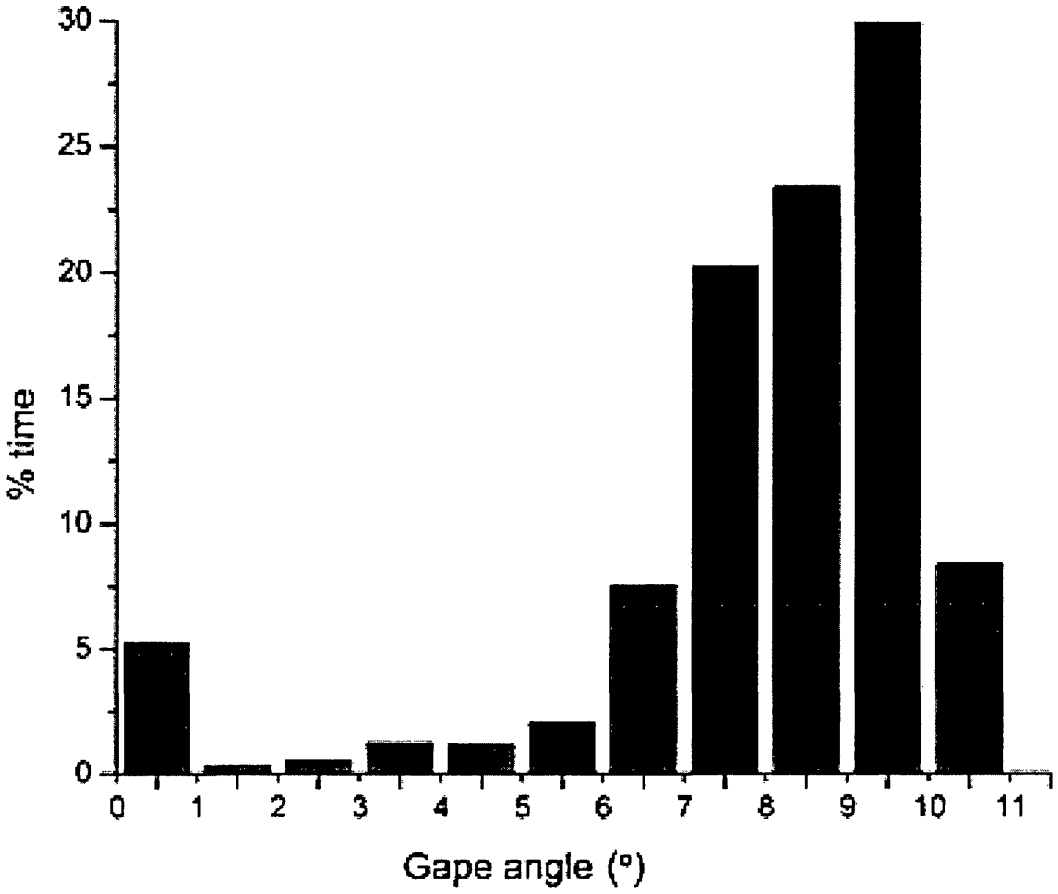

Fig. 6 Gape angle versus percentage time plot for a 54.6 mm long mussel over 5 days

From 0 to 48 h after calibration of gape angle, 18% of openings had Þ-values of ≥7.43 (Fig. 7a) and 24% of closures had Þ-values ≤−0.04°s^{-1} (Fig. 7b) (data derived from a 54.6 mm long mussel). No valve opening occurred between Þ-values of 2.59° and 7.43°s^{-1} and no valve closure between Þ-values of −0.04° and −0.186°s^{-1}.

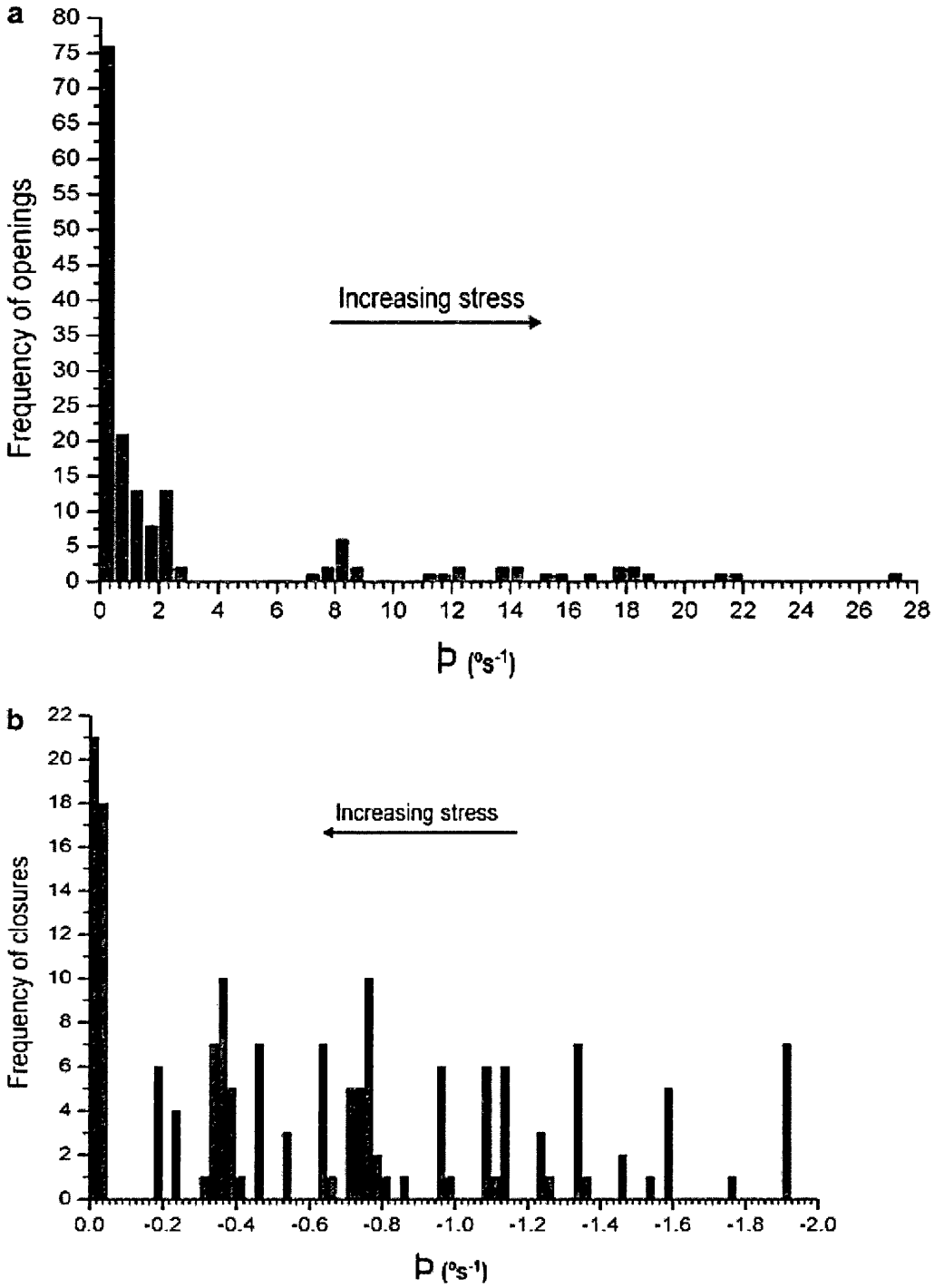

Fig. 7 Frequency of openings **a** and closures **b** versus Þ-value plot for a 54.6 mm long mussel from 0 to 48 h after calibration of gape angle

Mussels closed rapidly and almost instantaneously upon exposure to the chemical stimulus of an injured conspecific (e.g., Fig. 8). The mussels closure Þ-values were significantly higher (i.e., indicating slower closure rates) (t −10.21, $P < 0.001$) immediately prior to exposure to the seawater in which the injured conspecific was housed (mean Þ-values = $-0.772°s^{-1}$ ± SD 0.441) than immediately after (mean Þ-values = $-0.021°s^{-1}$ ± SD 0.012). In other words mussels closed slower in a predation risk free environment than in an environment where predation of conspecifics was simulated. Detailed examination of mussel response after simulated proximate predation typically showed a short period of rapid opening and closure over a small range of gape angles (0–2°) before one or several long slow openings (e.g., inset of Fig. 8). In an environment without injured conspecifics, opening Þ-value for the 34.6 mm long mussel was $0.949°s^{-1}$, while stressed opening had Þ-value of $20.531°s^{-1}$.

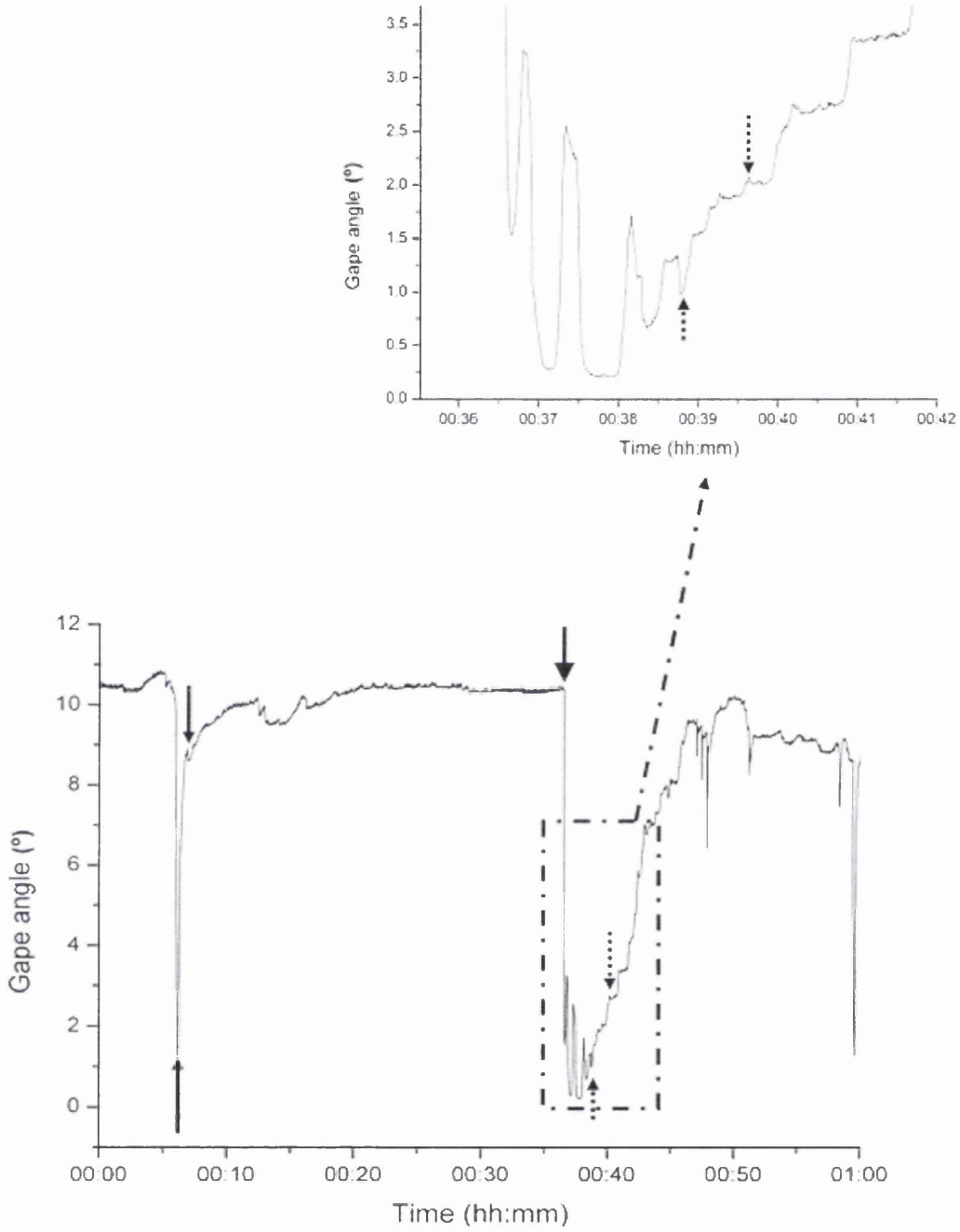

Fig. 8 Mussel gape angle versus time for 34.6 mm long mussel. The *large arrow* indicates the time when the mussel was stressed by being exposed to water in which a damaged conspecific was housed. Closure Þ-value for this was $-0.009°s^{-1}$. The inset shows greater detail of how the mussel responded to the stressor. Unstressed opening indicated by *small arrows* and stressed opening indicated by *dashed arrows*

DISCUSSION

This work takes a previously described methodology, developed to examine mussel behaviour via changes in mussel gape (Wilson et al. 2005) and proposes an analytical treatment which further enhances our ability to quantify how mussels react to their environment. The results obtained in this study concur broadly with those presented by Wilson et al. (2005). For example, the individuals used in this current study showed frequent closure events, however, their role is not fully understood (Shick et al. 1986, Shick et al. 1988). We note that mussels are also known to close as a response to suboptimal algal concentrations for efficient filtration, to prevent desiccation, in response to sudden changes in salinity and as a mechanism against predators e.g. Gosling (2003). Mussels open primarily to filter-feed, absorb oxygen and eliminate waste. Although the incidence of opening and closure events was documented to some degree by Wilson et al. (2005) these authors made no mention of the variability in the rate at which mussel gape angle changed and the present study shows how important this is in assessment of mussel behaviour.

Ruppert et al. (2004) reviews the mechanisms used by bilaterally symmetrical shellfish to close or open shell halves. The mechanism is ostensibly simple, consisting of closure brought about by contraction of the adductor muscle(s) while opening is passive, the force being derived from the elastic hinge, we speculate modulated by relaxation in the adductor muscle. Our results clearly show that opening and closure events have highly variable rates according to circumstance although, generally, the variation in the rate of valve closure and opening appears greatest at the beginning and end of the closure (Fig. 3a) or opening (Fig. 3b) event. Some variation in muscle

contraction speed in molluscs can be attributed to muscle fibre types. Ruppert et al. (2004) note that the use of "quick" muscles produces a rapid closure, but one that causes fatigue within a short period. Where mussels are to remain closed for extended periods, the use of "catch muscles" is apparently energetically more appropriate although contraction speed is slow. Our work does not allow us to determine the extent to which different muscle fibre types might be used in the contraction process but we propose that the intra-individual variability in closure and opening rates has survival value. Consequently, assessment of the rate of change of gape angle can provide a measure of an animal's assessment of the environment.

Reduced rates of gape closure, which are exemplified by high Þ-values, were greatest during low-risk periods (no stimulus in the water relating to predation) and likely to be caused mainly by contraction of "catch" muscles, especially before periods of extended closure. Þ-values were lowest (with highest rates of closure) during conditions, which might be construed as high risk (as exemplified by the presence of an injured conspecific) and is likely to be caused by contraction of "quick" muscles in response to perceived danger. This reaction is supported by the literature, which documents that mussels rapidly close their valves when threatened (Ruppert et al. 2004) by predators including eider ducks, crabs, starfish, octopus and whelks (Spencer 2002).

In general, therefore, we propose that for any given period during which multiple closure and opening events occur, the higher the percentage of opening events with Þ-values in excess of $7.43°s^{-1}$, the more stressed the mussels will have been. The lower the percentage of data with Þ-values greater than $-0.04°s^{-1}$ the more stressed the mussels will have been during closing. Consideration of all values

together should give an overall picture of how the animal perceives its environment for the period under consideration (Fig. 7). Cases where Þ-values are positive for a closure event or negative for an opening event indicate that the change in gape angle per second has exceeded the defined CHIGA boundary. This can either be treated by recalculating this boundary using a greater volume of data (see below) or by simply accepting the value as an extreme measure.

The extent to which the Þ-values recorded here can be used as standard for *M. edulis* from any environment depends critically on our ability to define the CHIGA boundary. This boundary is best approached during closure by having animals that are stressed but, conversely, best approached during opening by having animals that are maximally relaxed. Obviously, both these conditions have to occur during the calibration period. The periods of no stress and stress must be sufficiently spread apart to ensure mussels have recovered from any previous periods of stress. From this study it is recommended that CHIGA boundaries should be obtained from recording mussel gape angle over a period of >4 days with the act of calibration of gape angle against Hall sensor output acting as the induced stressor at the end of the period of data logging. Clearly, the longer the recording time, the more likely the boundary will be well defined at its maximum CHIGA (Figs. 1, 4). However, we note that CHIGA increased as mussels grew (but insignificantly over 3 weeks) so this needs to be taken into account. Further work might allow us to define standard CHIGA boundaries as a function of mussel length, which would preclude the onerous 4 day calibration period proposed above, although it is likely that boundaries may vary with a wide variety of environmental conditions including temperature, food, salinity and oxygen levels.

A primary finding of the work conducted here is that mussel gaping and closure does not represent a binary behavioural state. Rather, that both gape angle and the change in gape angle per second vary extensively and that the ability to vary these presumably has survival value. Mussels cannot feed unless they are gaping, and increased extent of gaping leads to an enhanced ability to feed (e.g. Jørgensen 1990). However, gaping mussels are more susceptible to predation so it would seem appropriate for animals to weigh up the balance of advantages of food acquisition with the likelihood of predation (c.f. Pérez-Tris et al. 2004). Mussels have a suite of sensory systems such as pallial tentacles with primary ciliary receptor cells as mechanoreceptors (Ruppert et al. 2004), pallial eyes (ocelli), cerebral eyes (cephalic eyes) and chemoreceptors, possibly including osphradia (Leonard et al. 1999, Ruppert et al. 2004), which may be used to assess environmental quality so it is appropriate that these animals display an appropriately complex behavioural response.

Precise measurement of mussel response to environmental conditions is not only useful in an animal life history strategic sense, but also has real value in a bio-indicator sense. Both Curtis et al. (2000)and DeZwart et al. (1995) proposed using mussels for examining the effects of pollution using shell movements although since this publication, no formal procedure has been developed. The system presented here allows mussel response to be measured in real time without the need to kill the bivalve. This contrasts with many other methods, which include measuring stress proteins e.g., HSP70 (e.g. Snyder et al. 2001) and immune changes (Lacoste et al. 2002) where stress cannot be measured in real time, or at the instant the stressor is applied.

The work presented here adds to the methodologies already being used to quantify mussel response to the environment. Typically, these are based on visual observation techniques (e.g. Riisgard et al. 2003) which have particular biases depending on e.g. water turbidity or parallax errors, or the use of electrical coils (e.g. Kádár et al. 2005) which give a binary output for "open" or "shut" animals. Our work shows, however, that changes in gape angle can be resolved finely and that this information gives another quantifiable dimension of animal response to the environment. Further study is required in the laboratory to determine how mussels react to particular conditions of food or predation and to examine how mussels in the wild react. Determination of Þ-values has direct commercial value as a tool to measure the performance of mussels in suspended and bottom culture in terms of mussel wellbeing and growth. Þ-values also have considerable commercial potential when used on bivalve hatchery broodstock where the environment can be manipulated to produce optimal conditions for gamete production. Þ-values used in conjunction with a technique currently being developed for measuring filtration rates using pressure micro-sensors and the current techniques for measuring bivalve heart rates (Depledge & Andersen 1990, Rovero et al. 1999) and pumping behaviour (Mouabad et al. 2001) have the potential to further our understanding of mussel behaviour.

In summary the results of this chapter highlighted that bivalve gape angle and changes in gape angle can be resolved finely and this information gives another quantifiable dimension of animal response to the environment. Bivalve gape angle and the change in gape angle per second vary extensively and the ability to vary these presumably has survival value. It was also demonstrated that bigger (longer) mussels can adduct (reduce valve gape angle) and abduct (increase valve gape angle) faster than smaller mussels. Mussel extract added to the seawater, a factor believed to signal

predation, caused mussels to close significantly faster than otherwise. But only once when the stressor was applied and mussels were gaping > 1°. Mussel response to predation was found to be graded and complex and may well indicate animal-based assessments of the trade-off between effective feeding and the likelihood of predation.

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Chapter 3

Why should mussels just sit there gaping? A laboratory assessment of blue mussel valve gape and exhalant pumping behaviour according to circumstance[C]

Abstract

Examination of blue mussel, *Mytilus edulis*, pumping activity is complicated because exhalant pumping can occur from the top of the inhalant siphon in addition to the exhalant siphon. Our results using a new vane sensor on laboratory mussels suggest that exhalant siphon area may not be the best proxy for overall exhalant pumping. Work was conducted on laboratory-held mussels over 6-8 days using pumping and gape angle sensors linked to logger-based technology and found a general positive relationship between valve gape and exhalant pumping rate. Gape angle was significantly related to pumping rate in virtually all instances but the slope of the relationship was higher in fed mussels than in food-deprived individuals ($n = 12$; $p = <0.001$) and higher in the dark than during daylight ($n = 12$; $p = <0.001$). Complete dissociations between valve gape and exhalant pumping (i.e. mussel gaping > 1° with no exhalant pumping detected) were rare (in 24 mussels, this only occurred for 0.029 % ± SD 0.028 of the time). In the laboratory, fed mussels showed a marked circadian rhythm with increased nocturnal activity, manifest as a generally greater gape angle and higher rates of exhalant pumping in periods of darkness (night). Valve adduction

events complicated the relationship between *M. edulis* gape angle and pumping because maximum recorded exhalant pumping in this study was not produced by pumping (cilia beat) but by valve adduction. Although laboratory-fed mussels were typically more active at night, their ability to adapt from natural (the wild) to unnatural environments (the laboratory) and to changes in unnatural environments and the significant intra- and inter-individual variation in gape behaviour within unnatural environments indicates that *M. edulis* is well adapted to survive as an ecologically important species in dynamic inter-tidal and sub-tidal areas providing a critical link between benthic and pelagic systems.

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[c]The content of this chapter is intended for publication: the author order will be Robson AA, Garcia de Leaniz C, Liebsch N, Halsey LG, Wilson RP.

INTRODUCTION

Mussels are common in inter-tidal and shallow sub-tidal areas (Seed 1976) and are ecologically important as they form large reefs that can enhance local community diversity. Mussels also provide a critical link between benthic and pelagic systems through their filter-feeding activities (Seed 1976, Dame et al. 1991, Beadman et al. 2004). Thus, mussel feeding and pumping activity has been extensively studied (e.g. Maire et al. 2007). Measurements based on direct observation have shown that valve gape generally increases with pumping rate in several filter-feeding bivalves including *Mytilus edulis* (Riisgard & Randlov 1981, Famme et al. 1986, Jørgensen et al. 1988, Newell et al. 1998, Newell et al. 2001, Riisgard et al. 2003, Maire et al. 2007). However, it has been suggested by Maire et al. (2007) that the correlation between valve gape and pumping rate results primarily from co-correlation between (1) valve gape and exhalant siphon area, and (2) exhalant siphon area and pumping rate. Thus, (Foster-Smith 1976) Foster-Smith (1976), Newell et al. (2001) and Maire et al. (2007) postulate that exhalant siphon area constitutes a better proxy of pumping rate than valve gape.

Research on bivalve behaviour has produced insights on how organisms cope with highly fluctuating environments (e.g. Jørgensen et al. 1988). Recently, the ability to record bivalve gape angles at high frequency has allowed more complex questions concerning fine-scale bivalve gape behaviour to be addressed (Wilson et al. 2005, Robson et al. 2007). Indeed, chapter 1 suggests that where possible, all bivalve gape and exhalant pumping behavioural events should be recorded because they are likely to vary according to circumstance (Robson et al. 2007), particularly because these may relate specifically to feeding behaviour (see above).

The purpose of this work was to determine the extent to which mussel gape angle can be used as a proxy for overall exhalant pumping rate using sensors to measure pumping rate and valve gape with high temporal resolution. We measured both exhalant pumping rate and gape angle in laboratory studies and examined how both parameters varied according to food availability and day/night conditions. The ability to measure when mussels are pumping (and possibly feeding) and understanding how mussels react in this behaviour according to circumstance paves the way for studying how these animals cope with the highly variable conditions they experience in the wild.

MATERIALS AND METHODS

Collection and maintenance of bivalves in experiments

Inter-tidal *M. edulis* were collected from LR SS630875 Swansea Bay, Wales, UK at low tide and transferred to a flow-through aquarium system within 2 h.

Overall experimental design

The methods developed by Wilson et al. (2005) were modified to quantify gape angle in blue mussels *M. edulis*. Briefly, this involved using a Hall sensor (a transducer for magnetic field strength) attached to one shell valve reacting to a magnet attached to the other shell valve. Variation in gaping extent produced a corresponding variation in the magnetic field strength perceived by the Hall sensor (c.f. Wilson et al. 2002). This was recorded by an archival tag. Since Hall sensor output is proportional to magnetic field strength and angle of impingement, the transducer output has to be calibrated by

comparing shell gape angle with sensor output, over a wide variety of angles. To do this, at the end of experiments, the posterior adductor muscle of *M. edulis* was severed with a knife to allow calibration of all possible gape angles with sensor output. Gape angle calibration took ~ 5 mins per mussel. Subsequently, data from sensor output versus gape angle were curve-fitted (for details see Wilson et al. 2002, Wilson & Liebsch 2003, Wilson et al. 2005, Robson et al. 2007). The curve-fit could then be used to determine any gape angle by converting the transducer output accordingly.

One type of archival logger used was a 13-channel JUV-Log (Juv Elektronik, Borstel, Germany), equipped with 12 Hall sensors (Honeywell, SS59E) and one temperature transducer. Three other archival loggers used were 7-channel JUV-Logs, equipped with 4 Hall sensors and also recorded light (Lux), pressure (depth) and temperature (°C). Both types of loggers were powered by 4 x 1.2 V 10 Ah NiMH D cells. The archival loggers had a 1 Gb flash RA memory and were set to record at a frequency of 2 Hz with 22 bit resolution (recording gape angle at better than 0.01°). The magnets used were 5 x 5 x 2 mm neodymium boron magnets. The parallel use of 4 JUV archival loggers allowed us to record the gape angle and exhalant pumping of 12 mussels simultaneously.

Bivalve pumping

Unless otherwise stated, all items in the experimental setup that required glue were fixed using aquarium sealant (Geocel®, Plymouth, UK). Two lengths of PVC tubing (both 10 mm diameter, with wall thicknesses of 1.5 mm and in lengths of 300 and 25 mm, respectively) were glued together using high strength epoxy adhesive (Power-Fast®+, Powers Fasteners, Inc., Brewster, NY, USA) to form an approximate ├ shape

(Fig. 1). A Hall sensor was glued to the outside of the 300 mm long PVC tube, 60 mm below the 25 mm length of tubing (Fig.1). A vane 60.5 mm long, 18 mm wide, 0.05 mm thick, made of transparent polyethylene, had one end glued to the ~25 mm long PVC tubing (Fig. 1). A 0.1 g (in air) neodymium boron magnet was glued at the free end of the vane, so that the magnet and Hall sensor were aligned (Fig. 1) thus making up a water flow sensor because pressure due to water flow on one side of the vane caused it to move closer to the Hall sensor, eliciting a change in output. Pumping sensors were kept in a fixed position in mussel tanks using PVC clamps. A study mussel was then placed in relation to the vane so that the water being exhaled (from the top 10 mm of the inhalant and whole of the exhalant siphon) caused the vane to move, bringing the magnet closer to the Hall sensor, thus causing a change in magnetic field intensity perceived by the transducer (in a manner similar to that used for determining gape angle – see above). It was imperative to keep the Hall sensors and magnets from the gape and pumping sensors sufficiently far apart so they did not interact.

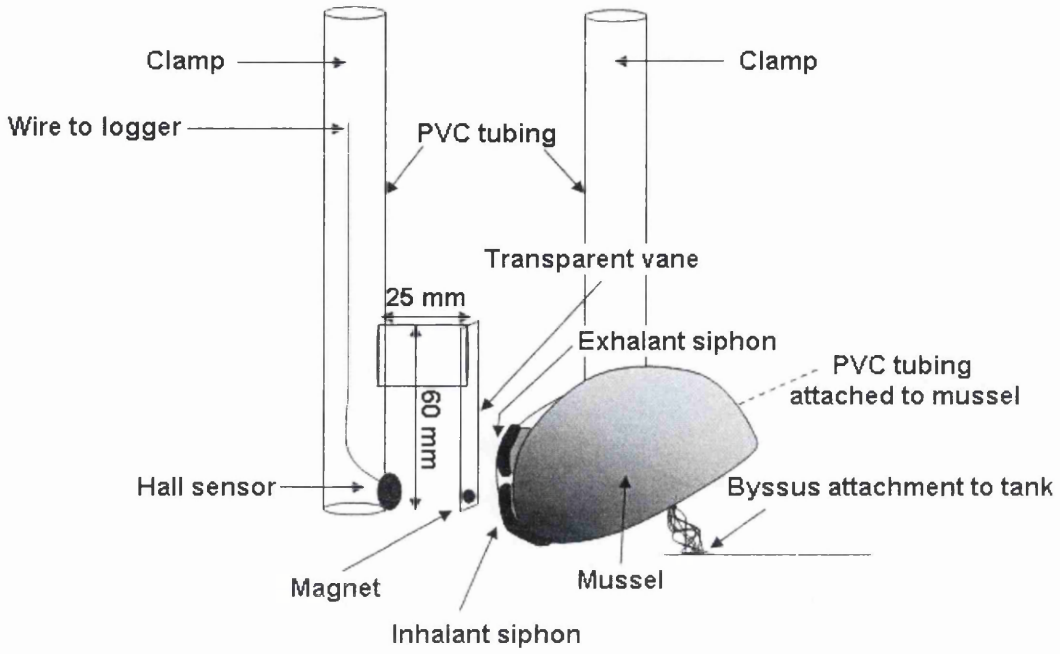

Fig. 1 Schematic diagram showing the mussel pumping sensor for measurement of the flow of water out of the top 10 mm of the inhalant and whole of the exhalant siphon.

A sampling frequency of 2 Hz was used to record simultaneously *M. edulis* pumping and gape angle*. Our method for measuring pumping could not be used in strong extraneous currents because of the high sensitivity of the new vane sensor.

Preparation of mussels for experiments

Magnets and Hall sensors were glued to *M. edulis*. Equipped *M. edulis* were placed in an aerated flow-through aquarium system containing edible seston-laden seawater from Swansea Bay, Wales, UK for at least a month before being used in aquarium experiments. All mussels in laboratory conditions were subject to natural light conditions, including moonlight from windows. All experiments with mussels took place within a 40 day period starting on 26th July 2007.

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*It was determined that 2 Hz, which results in 10 measurements per fastest valve adduction was the sampling frequency required to detect and define fine-scale gape behaviour patterns of *M. edulis* (see Ropert-Coudert & Wilson 2004).

Food-deprived and feeding experiments

Twelve mussels with an initial mean length of 76.6 mm ± SD 0.9 and mean wet weight of 47.6 g ± SD 2.84 were placed in separate, very gently-aerated tanks filled with 12 litres of 0.45 μm-filtered seawater. Six mussels were food-deprived for 96 h (4 days) and then fed intermittently at 12 pm (noon), 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at an initial concentration of approximately 250 x 10^3 cells ml^{-1} for six days (Table 1), while six mussels in the same experimental conditions were first fed for six days then food-deprived for four days (Table 1). Cell concentrations in suspension in each mussel tank were taken 10 minutes before the next algal addition and measured using a haemocytometer.

Feeds at midday and midnight

Twelve mussels with an initial mean length of 76.1 mm ± SD 1.2 and mean wet weight of 45.7 g ± SD 2.32 were placed in separate, very gently-aerated tanks filled with 12 litres of 0.45 μm filtered seawater. Mussels were initially food-deprived for 24 h before feeding. Twelve mussels were then fed at 12 pm (noon) using the same methodology and concentrations as in the above experiment for 8 days (Table 1). The same twelve mussels were then kept in edible seston laden seawater from Swansea Bay, Wales, UK for two weeks before being used in aquarium experiments where they were fed at midnight using the same methodology and concentrations as in the above experiment for 8 days (Table 1).

Table 1 Summary of experiments.

Experiment	Conditions	N
1	Food deprived for 4 days then fed at midday for 6 days	6
2	Fed at midday for 6 days then food deprived for 4 days	6
3	Food deprived for 24 h then fed at midday for 8 days	12
4	Food deprived for 24 h then fed at midnight for 8 days	12 (same mussels as in experiment 3)

Demonstration of sensitivity of transducer to varying water currents

After calibration of mussel gape angle, mussels were anaesthetised in isotonic magnesium chloride solution (100g $MgCL_2 \cdot 6H_2O$ dissolved in 1000 ml of water). After 24 h of anaesthesia, two part epoxy resin (type DR009, Atlas Polymers, Llantrisant, UK), thickened with micro bubbles, was poured into the exhalant siphon of the anesthetised mussels and allowed to solidify, then removed from the exhalant siphon along with all mussel flesh. Silastic® (Dow Corning Corporation, Michigan, USA) was poured over the epoxy resin mould of the exhalant siphons to produce models of the mussel siphons. The mould of each exhalant siphon was attached using Silastic® in approximately the same position as the original exhalant siphon in its corresponding mussel shell. Thus, artificial pumping mussels were created in order to demonstrate the general sensitivity of the vane transducer to varying water currents. Gravity-fed water flowed out of the modelled mussel exhalant siphon immersed in 12 litres of 0.45 μm filtered seawater, with different flow rates being achieved by altering the height of the 100 ml reservoir tank. Changes in mussel siphon area and direction changed the force exerted on the vane containing the magnet. We emphasize that this was only a demonstration of the sensitivity of transducer to varying water currents rather than being an attempt to calibrate the system to derive actual pumping rates from real animals.

Statistical Analysis

Percentage pumping data were converted to radians to normalise them for statistical analysis. All data were tested for auto-correlation before analysis and where

appropriate the data set was reduced before statistical analysis. For statistical analysis, night was defined as 20:30 – 05:59:59 and daylight was defined as 06:00:00 - 20:29:59. A two factor ANOVA with gape angle (°) as covariate was used to test for any difference in the slopes of gape angle *versus* pumping rate regression lines within individual mussels, when food deprived and when fed and any difference between day and night (n = 12, 4 days food deprived and 6 days fed), using SPSS v. 13.0. Differences in valve gape activity levels were compared between times of day (replicates = 8 subsequent days) and between individuals by ANCOVA. Regression analysis was used to test for any relationship between the difference in mussel gape 1h before and 1h after feeding at midday over time (n = 8 subsequent days). Differences in mean gape angle, and pumping rate between day and night and between starved and fed periods were tested for statistical significance with a Fisher's paired randomized test implemented in RUNDOM PRO (Jadwiszczak 2007) after 10,000 randomizations. Diel variation in mussel activity (gape angle) was tested by repeated measures ANOVA, using SPSS v. 13.0. Two sample t-tests were used to test for differences between means of gape angle between day and night, and paired t-tests to test for differences means of gape angle between pre-fed and post-fed mussels.

RESULTS

Gape versus pumping rate

In general, there was a positive correlation between valve gape and exhalant pumping rate (for 24 mussels, df = 602 - 858, r^2 0.833-0.972, p = <0.001, e.g. Fig. 2). Examples

of the positive relationship between *M. edulis* gape angle and exhalant pumping are shown in food-deprived mussels in Figures 3, 4, 5a and in fed mussels in Figures 2, 4, 5b, 6, and 7. However, the force of a rapid valve adduction event could elicit a water expulsion current which exceeded that caused by mussel exhalant pumping (Fig. 6). The relationship between gape angle and pumping rate had higher slopes in mussels fed at midday than in food-deprived animals (see Table 1 experiments 1 and 2), respectively ($df = 599\text{-}855$, $F = 26.78 - 337.93$, $p = <0.001$, see Table 2) (Fig. 5).

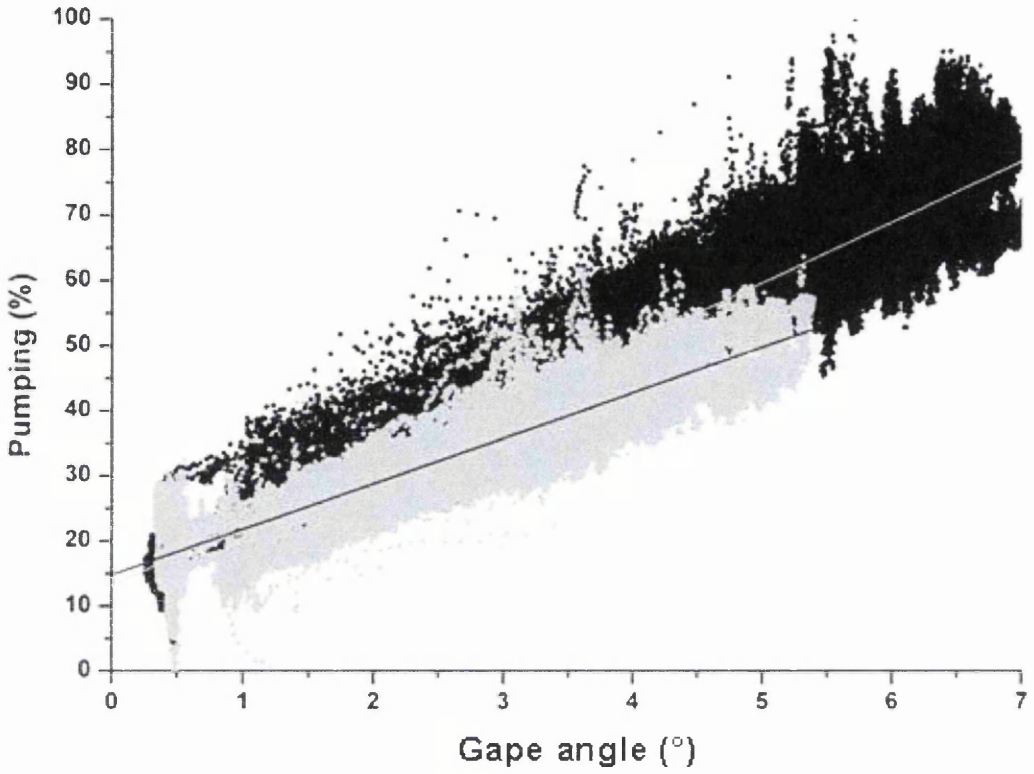

Fig. 2 Example of the relationship between valve gape and pumping rate of a 76 mm long mussel fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 12pm from 06:00:00 - 20:29:59 h in light (grey scatter plot) and 20:30 – 05:59:59 h in darkness (black scatter plot) over 6 days. All data recorded at 2 Hz. N.B. Regression statistics on non-auto-correlated data in darkness: n = 855, R^2 = 0.965, p = <0.0001 and in daylight n = 855, R^2 = 0.929, p = <0.0001.

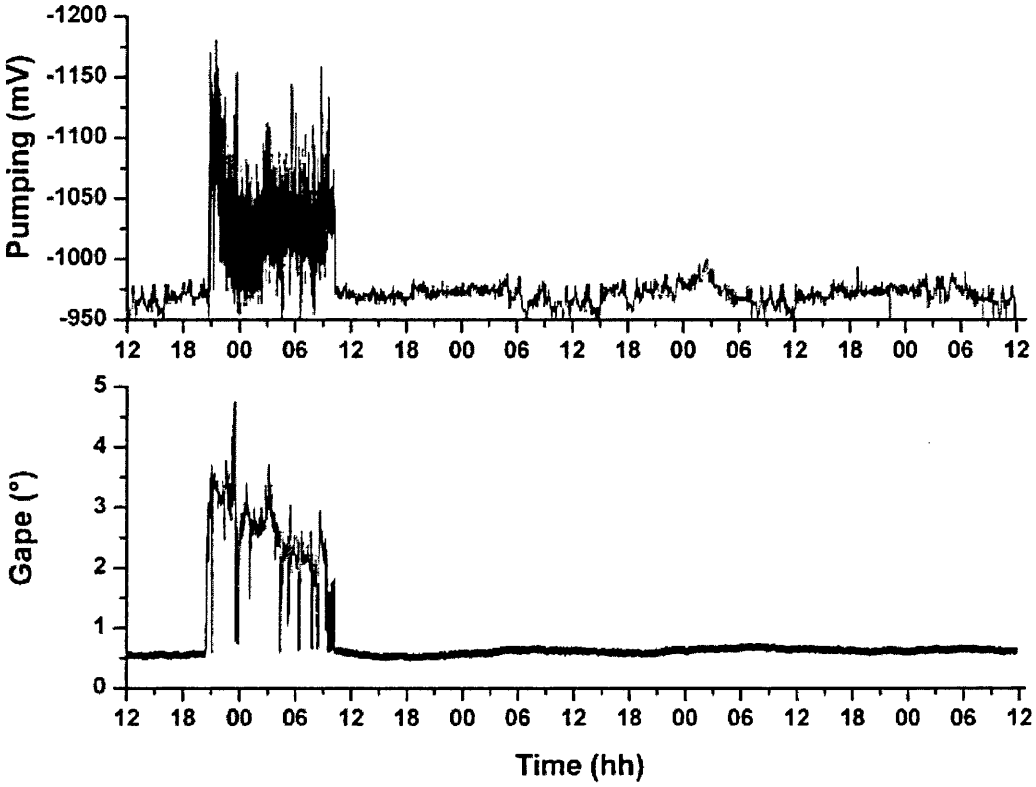

Fig. 3 Example of gape angle and pumping rate of a 76 mm long food deprived mussel recording data at 2 Hz over 4 days. Shaded areas indicate approximate periods of darkness.

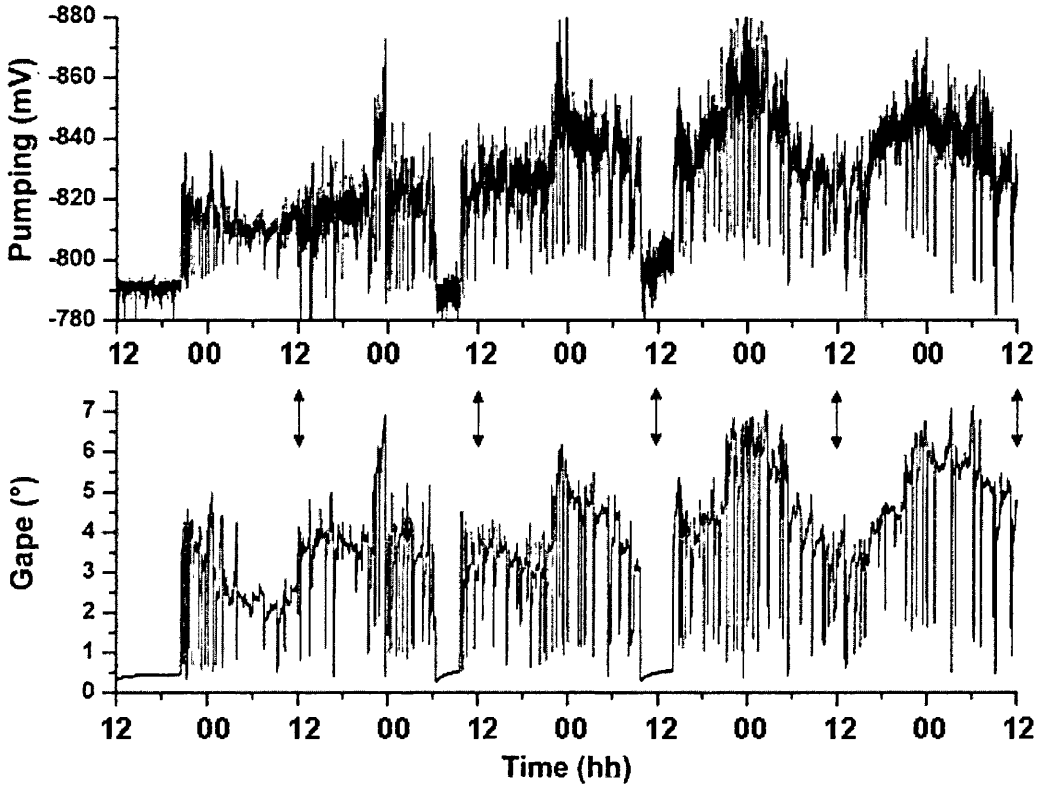

Fig. 4 Example of gape angle and pumping rate of a fed 77mm long mussel recording data at 2 Hz, over 24 hours food deprivation then fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 12pm over 4 days. Shaded areas indicate approximate periods of darkness. Double headed arrows indicate time fed.

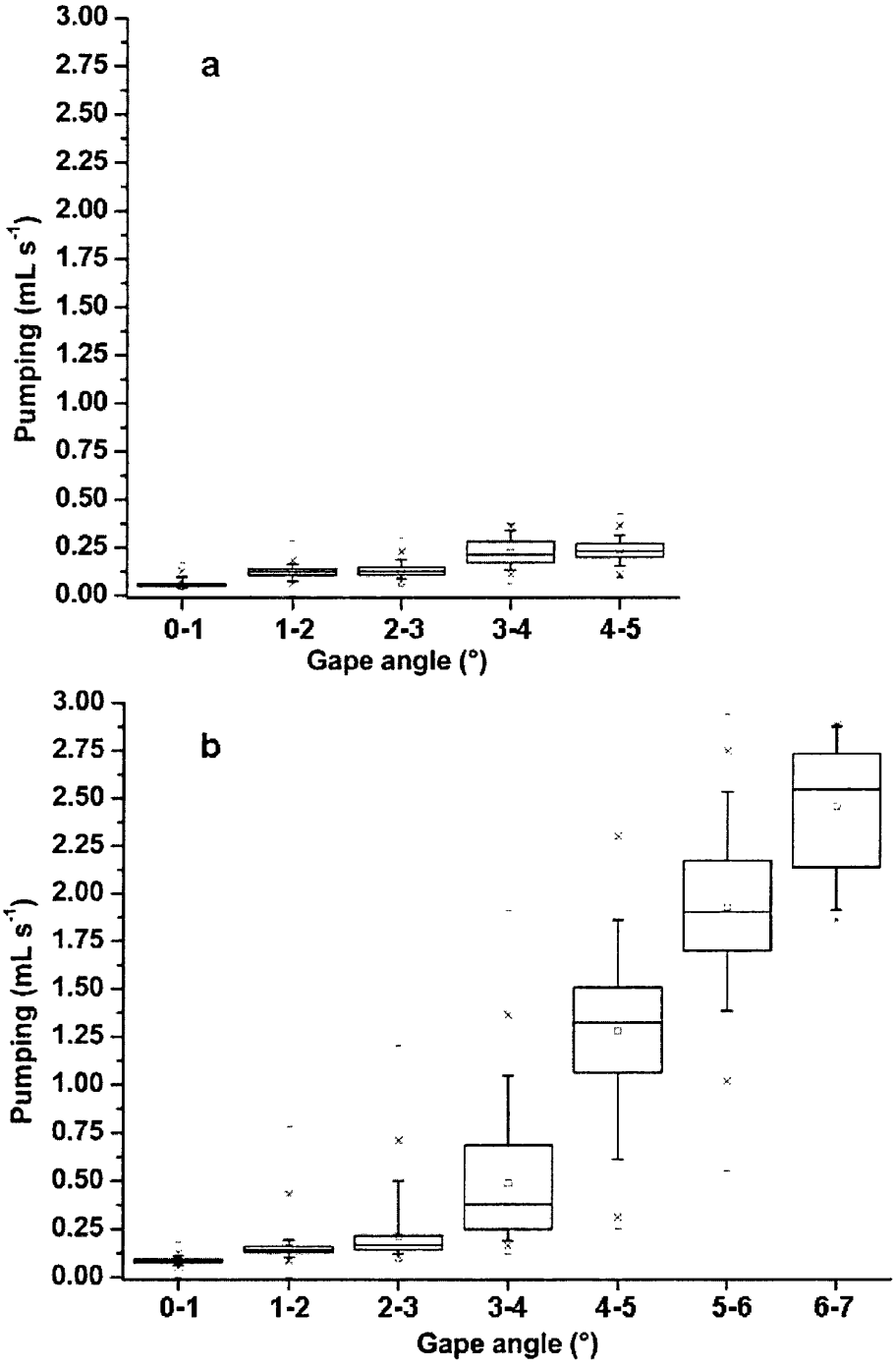

Fig. 5 Example of a frequency count of gape angle *versus* pumping rate from a 76mm long mussel recording data at 2 Hz, food deprived (a) for 4 days and fed (b) 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 12 pm for 6 days.

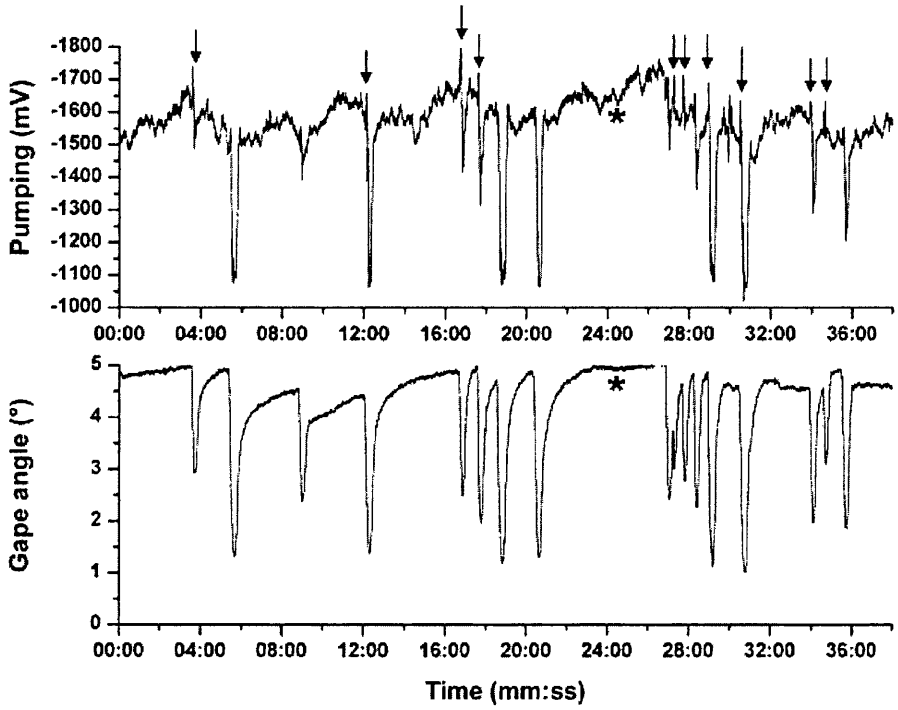

Fig. 6 Example of gape angle and pumping rate of a 75 mm long mussel fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 12pm. Data recorded at 2 Hz. Spikes in pumping data caused by the force of rapid valve adductions indicated with arrows. * indicates elimination of a faecal string from the exhalant siphon over ~ 30 s.

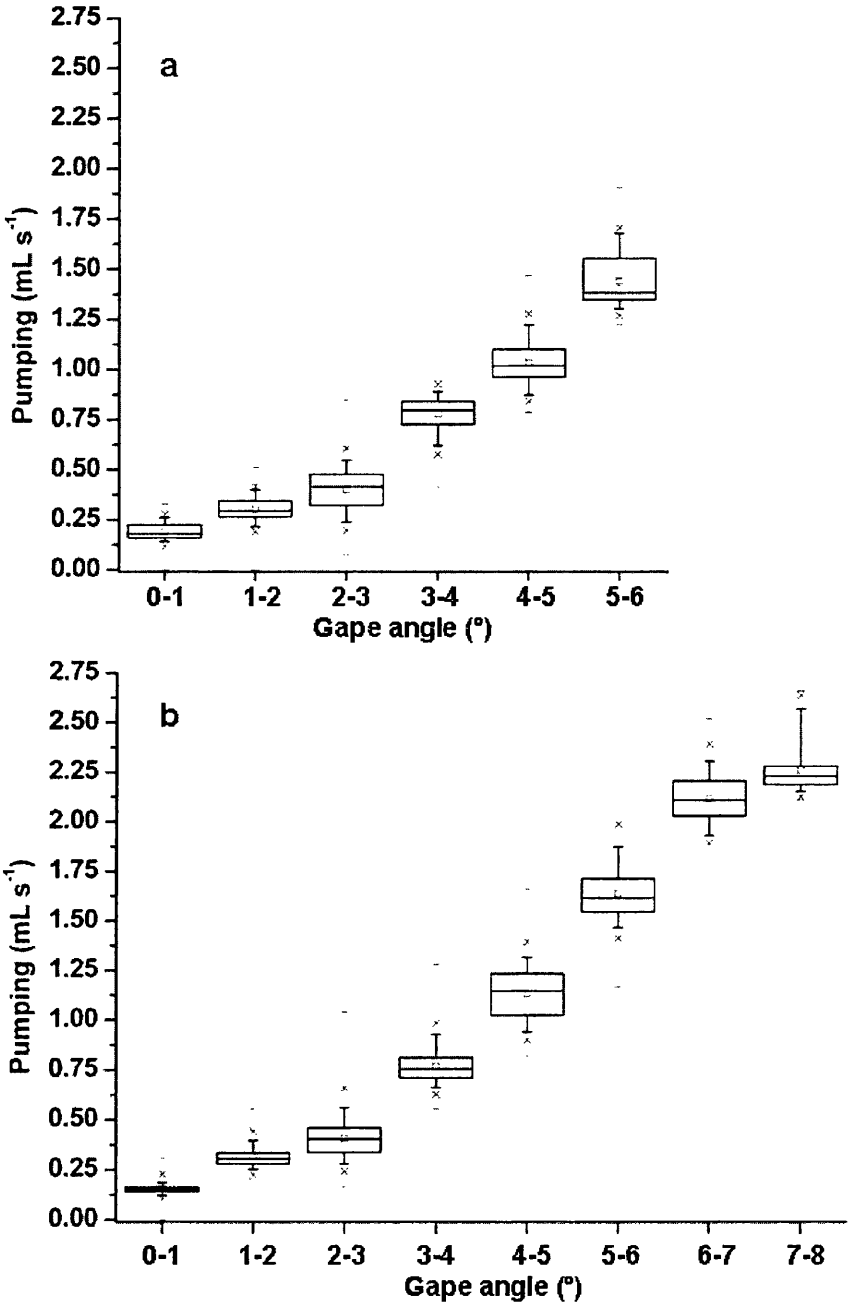

Fig 7 (a) Example of a frequency count of gape angle *versus* pumping rate for a 75 mm long mussel in daylight over 6 days. Mussel was fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 12pm (b) example of a frequency count of gape angle *versus* pumping rate for a 75 mm long mussel in darkness over 6 days. Mussel was fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 12pm. All data recorded at 2 Hz.

Table 2 Slopes from the relationship between gape angle (°) and pumping rate (radians) for 12 mussels (6 fed mussels then food-deprived and *vice versa*).

Mussel	Slope (radians/°) ± SE	
	Fed	Food-deprived
1	0.08116 ± 0.00018	0.02727 ± 0.00027
2	0.13492 ± 0.00098	0.05652 ± 0.00009
3	0.07593 ± 0.00026	0.02108 ± 0.00006
4	0.07507 ± 0.00024	0.03100 ± 0.00008
5	0.07198 ± 0.00031	0.03912 ± 0.00031
6	0.15284 ± 0.00148	0.02751 ± 0.00009
7	0.05106 ± 0.00054	0.00735 ± 0.00041
8	0.13203 ± 0.00092	0.06371 ± 0.00005
9	0.12186 ± 0.00103	0.06121 ± 0.00009
10	0.10160 ± 0.00064	0.04822 ± 0.00008
11	0.14073 ± 0.00118	0.05486 ± 0.00015
12	0.11042 ± 0.00075	0.02839 ± 0.00024

The increase in exhalant pumping rate per unit increase in gape angle for mussels fed at midday (n = 12 mussels x 6 days -see Table 1 experiments 1 and 2) was also significantly higher at night compared to in daylight (df = 599, F = 17.94 - 397.65, p = <0.001, see Table 3) (Fig. 7). In 24 *M. edulis,* complete dissociations (i.e. mussel gaping > 1° with no exhalant pumping detected) between valve gape angle and exhalant pumping > 1° gape over days were rare and amounted to only 0.029 % (± SD 0.028) of the time (e.g. Fig. 2).

Table 3 Slopes from the relationship between gape angle (°) and pumping rate (radians) at night and in daylight for 12 mussels fed at midday in darkness and daylight.

Mussel	Slope (radians/°) ± SE	
	Night	Day
1	0.08666 ± 0.00005	0.07756 ± 0.00007
2	0.26157 ± 0.00077	0.05194 ± 0.00036
3	0.08228 ± 0.00015	0.07177 ± 0.00008
4	0.07772 ± 0.00020	0.07333 ± 0.00008
5	0.08865 ± 0.00023	0.06105 ± 0.00009
6	0.17561 ± 0.00102	0.13792 ± 0.00071
7	0.09573 ± 0.00034	0.02180 ± 0.00025
8	0.20717 ± 0.00048	0.08280 ± 0.00064
9	0.20878 ± 0.00090	0.06491 ± 0.00066
10	0.15866 ± 0.00040	0.06421 ± 0.00023
11	0.18181 ± 0.00078	0.11382 ± 0.00091
12	0.14779 ± 0.00045	0.08594 ± 0.00051

Mytilus edulis initial observations of feeding behaviour

Regardless of the feeding experiment, cell concentrations in suspension were minimal 23 h and 50 mins after each algal addition (n = 24, mean cells ml^{-1} 4583 ± SD 8329). In experimental mussels (and non-experimental mussels over >2.5 years), temporal changes in exhalant siphon area were observed while valve gape remained relatively constant and at times complete dissociations between valve gape and exhalant siphon area. *M. edulis* were observed pseudofaeces strings being eliminated in an exhalant water current out of the top of the inhalant siphon, sometimes when the exhalant siphon was closed. A metachronal wave was observed in mussel faecal strings being eliminated in the exhalant current from the exhalant siphon and minimal valve movement associated with faecal elimination (e.g. Fig. 6).

Circadian activity patterns

After 24 h food deprivation, circadian rhythms became significantly more apparent in the gape angle of mussels over time after feeding ($F_{7,80}$ = 5.70, p <0.001, r = 0.998) (Fig. 4) and it could be predicted from a linear relationship (t = 9.425, p<0.001, with no significant effect of individual mussels (p = 0.543) and no interaction between mussel and day (p = 0.655) . The first addition of algal cells to mussels which had been food-deprived for 24 h induced an increase in valve gape within a few minutes and a more subtle increase in pumping rate, which reached a maximum value in the hours of darkness after the first and subsequent algal additions at midday (e.g. Fig. 4). However, after the second and subsequent additions of food for the 12 mussels fed at midday, mussel response (both gaping and pumping rate) was less immediate and

initially less pronounced, with gape remaining < 1° for > 1 h in daylight (e.g. Fig. 4). The difference (not significant – see later) in gape angle 1h before and after feeding at midday decreased significantly over time ($R^2 = 0.437$, n = 96 (pairs), $F_{11,94} = 73.003$, p = <0.0001).

There was a strong diel pattern in activity of fed mussels (12 fed at midday and the same 12 fed at midnight), with mean gape angle peaking at 2300 h for mussels fed at midday ($F_{3.73,41.04} = 65.305$, $P < 0.001$) and at 0300 h for mussels fed at midnight ($F_{3.48,38.30} = 241.766$, $P < 0.001$), corresponding to 11 h and 3 h after mussels were fed, respectively (Fig. 8). Between-subject-effects were also highly significant (mean gape of mussels fed at midday $F_{1,11} = 3687.821$, $P < 0.001$, mean gape of mussels fed at midnight $F_{1,11} = 10530.13$, $P < 0.001$), indicating that there was a significant variation in the way individual mussels adjusted their gape angle over the course of the day.

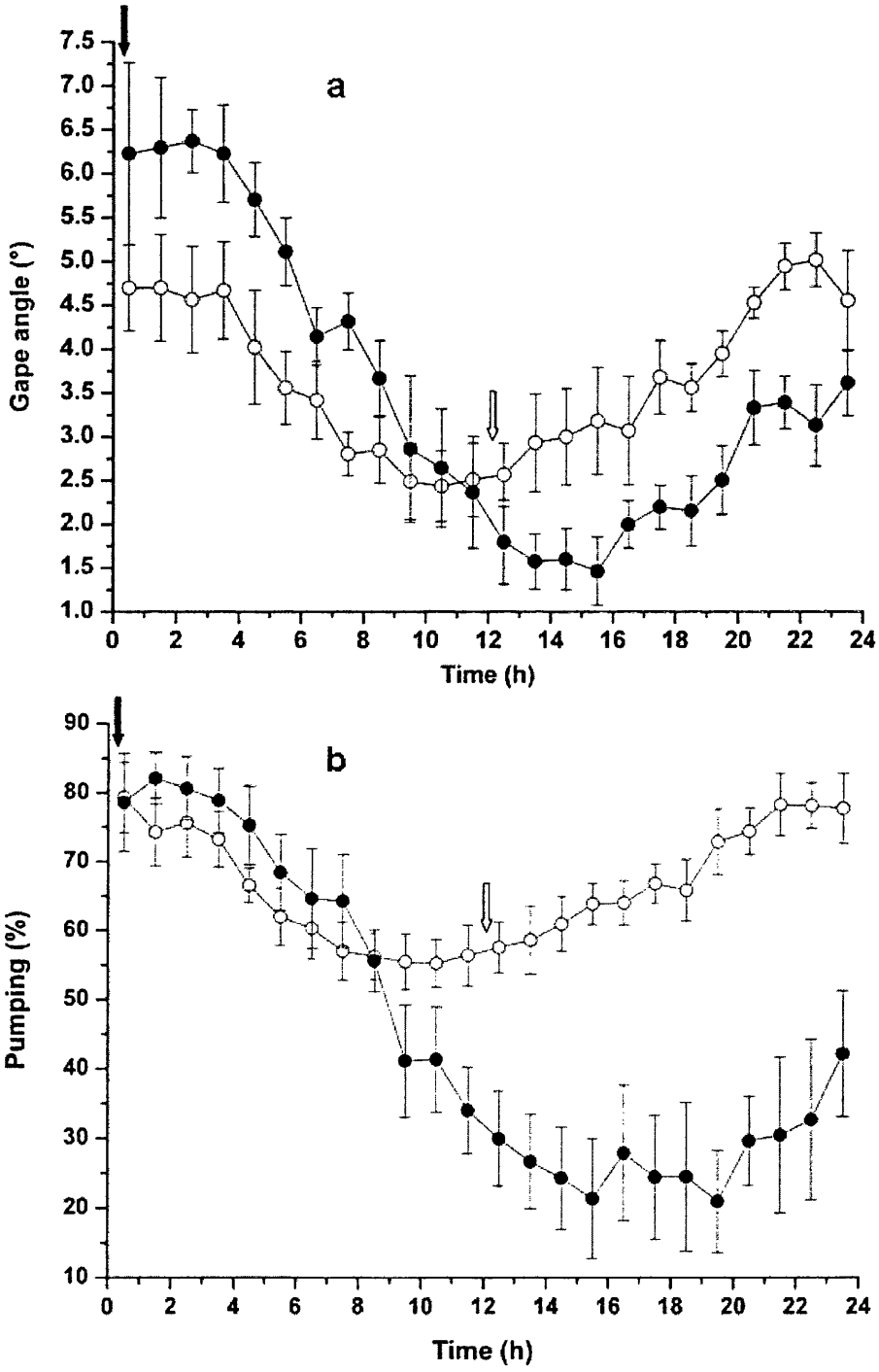

Fig. 8 Mean gape angle (a) and pumping rate (b) of 12 mussels fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at either midday (clear arrow) or midnight (black arrow) (mean length 76.1 mm ± SD 1.2) over 8 days (80 mussel days). Clear circles indicate mean ± SD of 12 mussels fed at midday. Black circles indicate mean ± SD of 12 mussels fed at midnight. Shaded areas indicate approximate periods of darkness.

Variation in mussel activity between day and night

In paired comparisons of fed mussels (12 fed at midday and the same 12 fed at midnight), mean gape angle and mean pumping rate were significantly higher during the night than during the day (Fig. 9; Fisher's randomized paired comparison test; gape angle $P = 0.0001$, pumping intensity $P = 0.0001$).

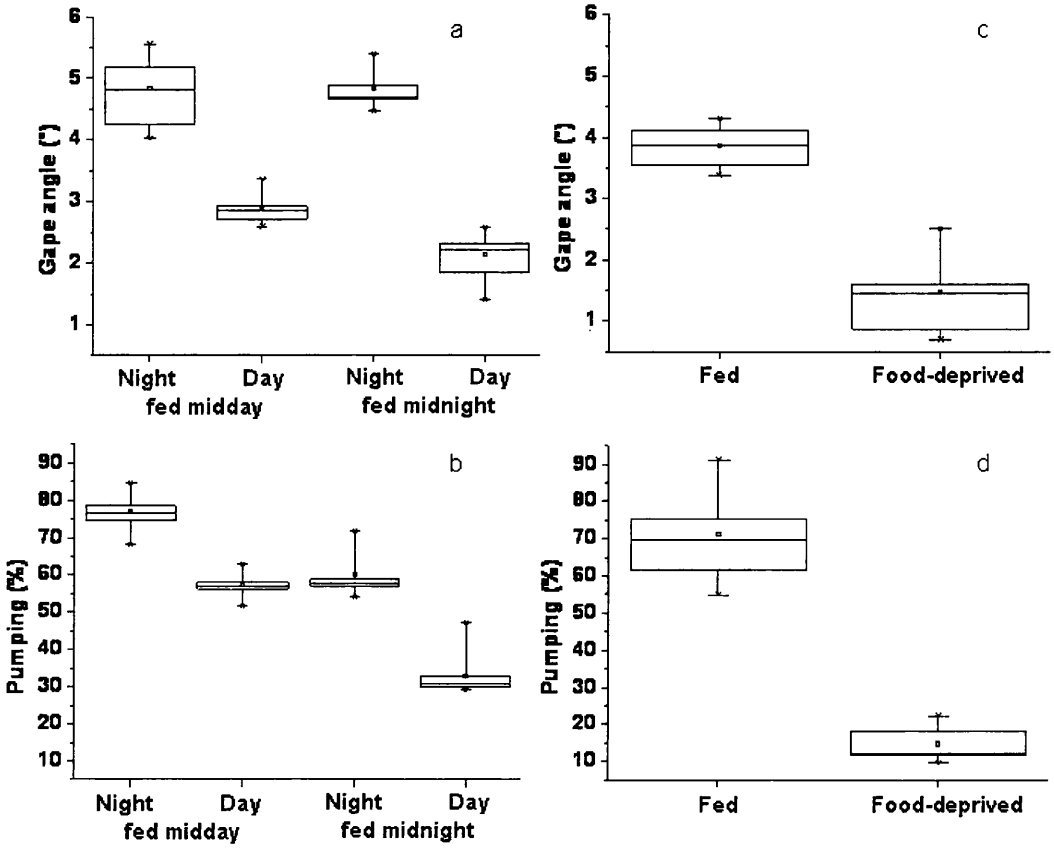

Fig. 9 Box-plots of gape angle (a) and pumping rate (b) of mussels in daytime and night time fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} over 8 days at either midday or midnight. Box-plots of gape angle (c) and pumping rate (d) of mussels fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} for 6 days and food- deprived for 4 days.

The time at which mussels were fed influenced the extent of the change in gape angle and pumping between the pre-fed and post-fed states. In mussels fed at midnight there was a less apparent increase in gape angle and exhalant pumping rate during hours of darkness before midnight than in mussels fed at midday. For mussels fed at midday (in daylight) there was no change between the mean gape angle of individual mussels between the hours before and after they were fed (n=12 mussels, mean gape width before and after feeding 2.51° and 2.56°, respectively, t=0.37, P = 0.72).

However, for mussels fed at midnight (in darkness), there were highly significant increases in mean gape angle between the pre-fed and post-fed states. For individual mussels, the mean gape width increased from 3.61° to 6.22° (n=12 mussels, t=7.82, P<0.0001).

At 12:00 there was no significant difference in the gape angle of mussels fed during the daytime *versus* nighttime (mean values of 2.51° and 2.34°, respectively, t_{19}=0.65, P=0.52). However, at 13:00 the daytime-fed mussels had significantly wider gapes than nighttime-fed mussels due to a decline in gape angle throughout the day for the nighttime-fed mussels (mean values 2.56° and 1.80°, respectively, t_{14}=11.67, P<0.0001).

Food deprivation

Within 3 hours of the start of food deprivation experiments, mussels began to eliminate faeces. Mussels responded to 96 h of food deprivation in two distinct ways (1) Six mussels gaped > 1°, which was associated with an increase in exhalant pumping well below maximum (e.g. Fig. 5a) but only over the first 24 h of food deprivation and mainly (77.6 % ± 8.6 of first 24 h) in the hours of darkness (e.g. Fig.

3) or (2) they showed periods of erratic gaping with associated exhalant pumping between periods of gaping < 1° for >2 h at levels well below maximum (e.g. Fig. 4a). Mean gape angle and mean pumping intensity were significantly higher when mussels were fed than when mussels were food-deprived (Fig. 9; Fisher's randomized paired comparison test; gape angle $P = 0.0007$ and pumping intensity $P = 0.0017$). Food-deprived mussels (n = 12) spent 70.2 % ± SD 24.6 of the 96 h recording time gaping < 1° but only 0.12 % ± SD 0.05 not gaping (0°).

DISCUSSION

Pumping sensor

The new pumping sensor was simple, but effective and allowed mussel exhalant pumping to be recorded at high temporal resolution in tandem with gape angle. It was not possible to calibrate this sensor for absolute flow rates, but the relative flow rates measured by the system proved most helpful in elucidated general trends between rates of pumping and gape within individuals.

What does gape angle tell us about pumping and feeding?

The results indicated that where mussels gape at angles >1°, they are likely to be pumping because dissociations between valve gape angle and exhalant pumping >1° were rare in the study. Mussels pump for a variety of reasons, including to acquire oxygen (respire) and food, expel (pseudo)faeces and for other metabolic processes associated with feeding and excretion.

Applying the principles of physics to biological systems (e.g. Alexander 2003) may help explain the behaviour and bodily structure cells and organisms. In the case of mussel pumping, Hagen-Poiseulle's physical law describes slow, viscous, incompressible flow of a fluid through a constant circular cross-section and notes that the rate of flow depends on the radius of the cylinder to the fourth power:

$$\Delta P = \frac{8\mu LQ}{\pi r^4}$$

Where:

ΔP is the pressure change

L is the cylinder length

μ is the dynamic viscosity of the fluid

Q is the volumetric flow rate

r is the radius of the cylinder

π is the mathematical constant (approximately 3.1416).

Thus, clearly, if there is no gap between the shell valves a mussel cannot pump water out of or into the external environment and at low gape angles, the effective radii of the inhalant and exhalant siphons will be limited. Thus, according to Hagen-Poiseulle's law, it is more energy efficient for a mussel to gape so as not to restrict the radius of the siphons when pumping water. Conversely however, when gaping, the delicate tissues/organs of mussels are susceptible to foreign matter (including male pea crabs e.g. *Pinnotheres pisum*, relatively large suspended sediment e.g. pebbles and macroalgae) touching, blocking, entering, irritating and harming the mussel tissues/organs and reducing their feeding efficiency. We speculate that there is a

compromise between mussels gaping, to feed and protect themselves from certain predators (e.g. dogwhelks*) and not gaping, to prevent potentially harmful foreign matter entering the mussel.

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*It is notable that, *M. edulis* sometimes gape to protrude their foot from the shell to defend themselves from predatory dogwhelks (*Nucella lapillus*); several mussels working together have been found to immobilise the gastropod with byssus threads (Seed & Suchanek 1992).

Food-deprived mussels

Advances in technology allowing pumping data to be recorded at high temporal resolution on food-deprived mussels indicate a more complex picture than the pioneering work reported by Bayne et al. (1976b). Firstly, and a generic feature of this work, there was substantial individual variation in response to conditions. Thus, some mussels, food-deprived over 4 days, pumped at very low levels, or did not pump for periods of > 24 h which is consistent with the findings of Davenport & Woolmington (1982). However, food deprivation also resulted in irregular pumping and gaping over several days (4 days in the current study) at levels well below the maxima found by Davenport & Woolmington (1982). Our results also indicate greater gape angle (> 1°) and pumping activity in some food-deprived mussels, mainly during the hours of darkness, albeit only in the first 24 h of food deprivation. Food-deprived mussels rarely remained closed, but often gaped < 1° which may save energy by relaxing the adductor muscles, as tonic contraction of the adductor muscles requires energy (Lowy 1953). Mussels probably gaped <1° when food deprived to conserve energy while not filter feeding by decreasing metabolic rate as food deprivation has been found to reduce the rate of oxygen uptake and heart-beat frequency compared to fed *M. edulis* (see Widdows 1973, Famme 1980). Gaping slightly presumably allows diffusion of oxygen from the exterior to the mantle fluid through the gap between the shell valves (Davenport 1979, Davenport & Woolmington 1982). Such diffusion combined with a minor ventilation of the mantle cavity when the shell valves adduct and abduct can presumably supplement ciliary ventilation at low pumping rates (Davenport & Woolmington 1982) and may be one reason for valve movement of food-deprived

mussels in the current study. Low gape angles may also allow animals to sample the water for food, test salinity and other chemical cues, including those of predators.

Fed mussels

The results from mussels fed intermittently at midday compliment the work done by Davenport & Woolmington (1982) who also recorded that intermittently-fed *M. edulis* did not always respond to the presence of food by initiating pumping, suggesting that they may become satiated with food (Davenport & Woolmington 1982). However, from our results it is evident that when mussels were well-fed they were more active (as evidenced by a generally greater gape angle and pumping rate) in darkness even though they were fed in daylight at midday, with mussels sometimes gaping < 1° for > 1 h in daylight after the addition of food (Fig. 4).

Rates of oxygen consumption and filtration by *M. edulis* have been found to increase following feeding (Bayne & Scullard 1977) and our results from dark-fed mussels are remarkably similar to those presented by Bayne & Scullard (1977), at least in respect to filtration. Some of the observed increase in metabolic rate is probably due to the increased filtration rate (exhalant pumping rate in the current study) that is stimulated by the presence of food (the 'mechanical cost of feeding' discussed by Bayne et al. 1976a). Increases in metabolic rate associated with feeding are also presumed to be due to the specific dynamic action (SDA) of the ration, varying with the type of food (see Harper 1971, for discussion). An apparent SDA, related to ammonia excretion, following feeding has been found in *M. edulis* (Bayne & Scullard 1977). It has been suggested that pumping after a meal (when food is in the gut) is necessary in *M. edulis* to sustain the oxygen uptake corresponding to

specific dynamic action (Davenport & Woolmington 1982) (and pumping) and we speculate that this may partially explain the increased gape angle and pumping rate at night in the laboratory and the wild when *M. edulis* appear more active (c.f. Wilson et al. 2005) for prandial and/or postprandial ventilation. However, *M. edulis* has also been found to be efficient at maintaining rates of ingestion at a constant level even after a 'satiation point' is reached (Foster-Smith 1975). Material that is captured but exceeds the ingestive capacity of *M. edulis* is rejected as pseudofaeces (Foster-Smith 1975) and may be due to animals having to filter for oxygen, but being unable to avoid picking up seston in the process.

Circadian rhythms in valve gape angle and exhalant pumping rate

The positive relationship between valve gape angle and exhalant pumping rate in *M. edulis* in our results echo those previously reported in several other filter-feeding bivalves including *M. edulis* (Riisgard & Randløv 1981, Famme et al. 1986, Jørgensen et al. 1988, Newell et al. 1998, 2001, Riisgard et al. 2003, Maire et al. 2007). While we found that mussels exposed to natural light (including moonlight) showed a clear day-night rhythm with increased nocturnal activity, apparent as in general greater gape angle and increased exhalant pumping rate in darkness (e.g. Fig 3), it is unclear what the adaptive significance of this finding might be (c.f. Nielsen & Stromgren 1985). One possibility is that such day-night gape behaviour may be part of a strategy to feed while minimizing the likelihood of predation by visually-feeding predators (Ameyaw-Akumfi & Naylor 1987). However, visually-feeding predators e.g. eider ducks *Somateria spp* and humans predate on mussels regardless of whether they are gaping or not, alternatively oystercatchers *Haematopus spp* are either stabbers (stab

open gaping mussels, including when mussels are submerged in shallow water) or hammerers (break the shell by hammering) (see Goss-Custard 1996, Stillman et al. 2000). Another possibility is that such day-night gape behaviour may be linked to the circadian rhythm reported in *M. edulis* byssus thread production, with greater thread production during the night (Martella 1974). From our observations we suggest that mussels may be particularly vulnerable to predation when the foot (used as a plantar during attachment of the byssus to the substrate) is protruding from the shell because when handled, mussels adducted their valves around their foot and thus, could not fully adduct their valves.

Diel patterns of behaviour associated with energy acquisition have been documented for a wide range of species (for examples see Clarke 1978, Wilson et al. 1993, Hays 2003). For *M. edulis*, this gape pattern was first noticed by Dodgson (1928) who reported diel variation in gaping in *M. edulis*, with greater activity in darkness. Wilson et al. (2005) reported a highly significant circadian gape rhythm in wild sub-tidal *M. edulis*, with a general increase in gape angle and shell valve adduction and abduction events in the dark. The experimental conditions of constant illumination may explain why Ameyaw-Akumfi & Naylor (1987) only found a weak circadian gape rhythm in *M. edulis*, with greater duration of shell-closure during hours of expected daylight. Both this study and that of Wilson et al. (2005) found that gape (and exhalant pumping in our study) diel rhythmicity became more obvious over time, probably as mussels recovered from human disturbance (cf. Wilson et al. 2005). However, Wilson et al. (2005) reported the absence of a well-defined diurnal rhythm in gape angle in laboratory blue mussels compared to those in the wild sub-tidal. These results raise many questions about the ecological variables that affect the activity patterns of mussels and the differences between laboratory and field results

(c.f. Gattermann et al. 2008). Laboratory mussels may behave differently to wild conspecifics as a result of many factors, including disturbance by humans (Wilson et al. 2005) and not being held under circumstances closely comparable to their natural environment.

It may be no coincidence that in the laboratory experiments (using mussels previously suspended in mid-water when immersed in the inter-tidal zone) and the sub-tidal (Wilson et al 2005, mussels were suspended in mid-water when immersed - Rory Wilson pers. comm.) that greatest mussel gape (and exhalant pumping in the current study) occurs at the time of expected maximum carrying capacity of sources of energy available to mussels suspended in mid-water in coastal waters (Lagadeuc et al. 1997). Mussels can access almost all sources of energy available to them in seawater, viz. dissolved organic material, seston, microbes, phytoplankton and zooplankton (Davenport et al. 2000) and the diel vertical migration (DVM) by zooplankton is a universal feature in all oceans (Hays 2003). More importantly for inter-tidal and shallow sub-tidal marine mussels suspended in mid-water, DVM is documented in coastal waters (e.g. Lagadeuc et al. 1997), with marine copepods *Temora longicornis* and *Microsetella norvegica* located close to the surface during the night in the richest phytoplanktonic water (Lagadeuc et al. 1997). It has been found that mussels ingest most mesozooplankton present in inhaled seawater and that energy can be extracted from a diet of *Artemia sp.* indicating a degree of carnivory in *M. edulis* (Davenport et al. 2000). Laboratory mussels in the experiments may gape wider at night (and grow faster at night – see Nielsen & Stromgren 1985), due to rhythms that allow them to benefit from a mixed diet including (a greater abundance and variety of) zooplankton because in the wild, normal DVM brings zooplankton into shallower depths at night (Hays 2003). As zooplankton are larger then phytoplankton, mussels may need to

gape wider for them to pass through the inhalant siphon. Indeed, *M. edulis* have been found to occasionally ingest animals as large as 6 mm in length (Davenport et al. 2000), with Wong & Levinton (2006) also reporting *M. edulis* gaping wider when feeding on rotifers compared to microalgae. The classic model of filter-feeding on phytoplankton by bivalves may have overlooked the benthos-zooplankton trophic loop in the benthic-pelagic ecosystem (Wong & Levinton 2004). Although bivalves can derive nutrients from many food resources, it has been found that mussels have best growth performance and higher metabolic rate on a mixed diet of phytoplankton and zooplankton (Wong & Levinton 2004) and some inorganic material (Bayne et al. 1987).

Despite finding a highly significant circadian gape rhythm in laboratory, wild inter-tidal (Chapter 6) and sub-tidal mussels (Wilson et al. 2005), mussels in the experiments also responded to changes in their environment with significant adaptation by changing gape and pumping behaviour according to circumstance e.g. increasing and decreasing gape and pumping activity when regularly fed and then food-deprived, respectively and *vice versa*. This pattern of feeding when food is prevalent and not when it is absent is entirely in accordance with general models of optimality in foraging (e.g. Stephens et al. 2007). In order to further explain the results, complete high temporal resolution energy budgets for *M. edulis* that combine information on their gape angle, siphon areas (inhalant and exhalant), pumping rate, (pseudo)faeces production, metabolic rate, type and energy density of prey (including zooplankton) and ingestion rates are required to assess energy balance over long periods. An ideal timescale would be over months or years, with measurements taken day and night in the laboratory. Such detailed behaviour and energy budgets combined

with the influence of predation and tidal cycle may help explain the factors driving of the observed day-night gape angle and pumping rate behaviour in mussels.

Valve movements and pumping

Valve adduction events complicate the relationship between *M. edulis* gape angle and exhalant pumping. Maximum recorded exhalant pumping in the current study was not produced by pumping (cilia beat), but by valve adduction. We did not attempt to separate the water currents produced by cilia and those produced during valve movements.

Exhalant pumping *versus* exhalant siphon area

In experimental mussels temporal changes in exhalant siphon area were observed but not quantified when valve gape remained relatively constant and at times, complete dissociations between valve gape and exhalant siphon area. This has already been noted for several filter-feeding bivalves including *Mytilus edulis* (Davenport 1979, Manley 1983, Wildish & Miyares 1990, Newell et al. 2001, Riisgard et al. 2003, Maire et al. 2007). One major question linked to the dissociations between valve gape and exhalant siphon area is their relative impact on pumping rates. Davenport (1979) stated that complete closure of the exhalant siphon made efficient pumping impossible and results in the cessation of beating of the lateral cilia. This further supports the idea that the exhalant siphon area constitutes a better proxy of pumping rate than does valve gape (e.g. Maire et al. 2007). However, since *M. edulis* is known to have a mucociliary rejection pathway out of the top of the inhalant siphon (Widdows et al.

1979, Beninger & St Jean 1997, Beninger et al. 1999), we found it appropriate to measure exhalant mussel pumping from both the top of the inhalant and whole of the exhalant siphon. Thus, our results, which showed that complete dissociations between valve gape and exhalant pumping at > 1° gape were rare, and observations made by e.g. Widdows et al. (1979) and ourselves, would suggest that although exhalant siphon area is clearly the best proxy of pumping rate out of the exhalant siphon, it may not represent exhalant pumping as a whole. There may not be a simple proxy for overall exhalant, or both inhalant and exhalant pumping because there is no defined barrier to exhalant pumping from the top of the inhalant siphon and it may not be assume that inhalant pumping occurs out of the whole of the inhalant siphon area (especially when exhalant pumping occurs from the top of the inhalant siphon).

Critique of the methods used

The method for measuring exhalant pumping could be improved by using a Hall sensor and magnet system to measure gape angle in tandem with three pumping sensors to measure inhalant pumping from the inhalant siphon, exhalant pumping from the top of the inhalant siphon and exhalant pumping from the exhalant siphon. Although an accurate quantified measure of exhalant mussel pumping was not possible in the current study, the results suggest that pumping could be considered over a fine temporal scale because often we found mussel pumping (and gape) to be highly variable, even over periods as short as one minute (c.f. Robson et al. 2007).

Although all mussels were typically more active at night, an important feature within our midday and midnight feeding experiments was the significant variation in the way individual mussels adjusted their gape angle over the course of the day. The

intra- and inter-individual variation indicates different strategies in the adaptive nature of mussels to changes in their environment. Overall, the ability of mussels to quickly adapt from natural (the wild) to unnatural (the laboratory) environments and to profound and rapid changes in unnatural environments in order to survive, and the significant intra- and inter-individual variation in gape behaviour within unnatural environments indicates that *M. edulis* is well adapted to survive as an ecologically important species in dynamic inter-tidal and sub-tidal areas, providing a critical link between benthic and pelagic systems. The nervous system of *M. edulis* (see Stefano 1990) may account for the complex and variable responses to different highly dynamic natural and unnatural environments (including successful reproduction of our mussels in the laboratory). My interest, ultimately, is how the plasticity of mussel feeding behaviour and future research may show that variabilities in feeding behaviour actually influence fitness under circumstances closely comparable to their natural environment. My findings suggest that gape angle could be used as a general proxy for mussel pumping activity (associated with respiration, feeding, excretion and their associated metabolic processes) in the wild and that a long-term (weeks, months or even years) study of this may help in this quest.

In summary the results of this chapter suggested that measuring exhalant bivalve pumping could be improved by using a Hall sensor and magnet system to measure gape angle in tandem with three pumping sensors to measure inhalant pumping from the inhalant siphon, exhalant pumping from the top of the inhalant siphon and exhalant pumping from the exhalant siphon. Pumping could be considered over a fine temporal scale because mussel pumping (and gape) was often highly variable, even over periods as short as one minute. *M. edulis* pumping activity was found to be complicated because exhalant pumping can occur from the top of the

inhalant siphon in addition to the exhalant siphon. It should be noted that maximum recorded exhalant pumping in the current study was not produced by pumping (cilia beat), but by valve adduction. Mussels showed a marked circadian rhythm with increased nocturnal activity, manifest as a generally greater gape angle and higher rates of exhalant pumping in periods of darkness (night). However, there was significant intra- and inter-individual variation in the way individual mussels adjusted their gape angle over the course of the day. Finally the results suggested that gape angle could be used as a general proxy for mussel pumping activity (associated with respiration, feeding, excretion and their associated metabolic processes) in the wild.

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Chapter 4

Mussel adaptations to varying food rations and predation risk[d]

Abstract

Blue mussels, *Mytilus edulis*, were studied in the laboratory using a Hall sensor and magnet system to record their gaping behaviour when exposed to varying food concentrations and indices of predation. Mussel response to increasing algal concentration was to increase mean gape angle in an S-shaped curve. Mussels spent most of their time gaping with inter-shell angles of <1° when food-deprived and at very low daily algal rations (0.2 x 10^7 *Thalassiosira weissflogii* cells day^{-1}) and this strategy is most likely to conserve energy while not filter-feeding. Mussel gaping <1° did not always last until the algal concentration was elevated above a lower threshold level. Food-deprived mussels probably opened to filter the water to test for food particles and/or to increase oxygen uptake. Mean gape angle decreased in a backward S-shaped curve as the amount of mussel homogenate (an indication of predation) increased in the seawater. The variance in actual speed ($°s^{-1}$) and relative speed Thorn (Þ) of mussel valve adduction (decrease in valve gape angle) and abduction (increasing in valve gape angle) events was highly variable. 14 mussels fed 0 and 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} executed minima and maxima of 3-113 and 6-160 valve adductions day^{-1}, respectively. There was no significant difference between the number of shell valve adduction events per day across daily algal rations. In order to identify the reasons for every mussel valve adduction and abduction event,

many more parameters of mussels and their environment need measuring at high temporal resolution. Our approach for assessing how mussels react to their environment indicates that mussel gape response to predation was graded and complex and indicates some form of mussel assessment of the trade-off between effective feeding and/or respiration and the likelihood of predation.

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[d]The content of this chapter is intended for publication: the author order will be Robson AA, Garcia de Leaniz C, Halsey LG, Wilson RP.

INTRODUCTION

Adult bivalves are mostly sessile and therefore not expected to display the same wide suite of behaviours envisaged for motile species. However, workers showed even 80 years ago, that there was diel variation in the gape angle of the shell valves of blue mussels *Mytilus edulis* (Dodgson 1928), something that has been reiterated more recently (Ameyaw-Akumfi & Naylor 1987, Wilson et al. 2005) with the added complexity that the patterns were less apparent, or even absent, in laboratory animals (Ameyaw-Akumfi & Naylor 1987, Wilson et al. 2005). Beyond this, other workers have now shown that the valve movement is, in fact, remarkably intricate, with gape angles and the incidence, speed and duration of adduction (decrease in valve gape angle) and abduction (increase in valve gape angle) events varying greatly according to circumstance (Shick et al. 1986, Curtis et al. 2000, Nagai et al. 2006, Robson et al. 2007). The implication is that these relatively simple animals (but see Stefano 1990) somehow exhibit appropriate complex responses according to their needs and the conditions apparent in their environment.

There are several factors believed to modulate gape angle and to initiate valve adduction and abduction events. For example, animals are considered to gape more or less according to their respiratory needs and to gape more to aid feeding (Nagai et al. 2006). In fact, measurements based on direct observation have shown that valve gape generally increases with pumping rate in several filter-feeding bivalves including *Mytilus edulis* (Riisgard & Randlov 1981, Famme et al. 1986, Jørgensen et al. 1988, Newell et al. 1998, Newell et al. 2001, Riisgard et al. 2003, Maire et al. 2007). Newell et al. (1998) even suggested that valve gape could be used as a proxy for filtration activity (see e.g. Dolmer 2000, Wilson et al. 2005, Saurel et al. 2007) although other reports indicate that pumping activity may be better indicated by exhalant siphon

aperture (see e.g. Foster-Smith 1976, Newell et al. 2001, Maire et al. 2007). Beyond simple gape, mussel valve adduction events have been attributed to stress responses (e.g. Fdil et al. 2006, Nagai et al. 2006, Robson et al. 2007), including exposure to toxicants (Fdil et al. 2006), and predation risk (Robson et al. 2007).

Thus, primary drivers for bivalve gape angle can act to incite valve adduction or abduction and, in the wild, shellfish are expected, at times, to be exposed to both driver types simultaneously. How animals react is assumed to relate to the survival value of the response and the severity of the driver. However, given the number of parameters known to influence either valve abduction or adduction (e.g. Manley 1983, Shick et al. 1986, Shick et al. 1988, Calvo-Ugarteburu & McQuaid 1998, Curtis et al. 2000, Newell et al. 2001, Mouritsen & Poulin 2002, Riisgard et al. 2003, Fdil et al. 2006, Nagai et al. 2006, Robson et al. 2007), it is easy to see how a graded and complex response may be expected in animals exposed to varying conditions.

This work uses new archival tag methodology (Wilson et al. 2005, Robson et al. 2007) on laboratory mussels to examine how mussel valve movement relates to two opposing drivers; food concentration in the water and risk of predation. Valve gape angle is predicted to increase with increasing food ration, but decrease with perceived predation risk. The new methodology incorporates a quantification of change in gape angle per second (CHIGA) in $°s^{-1}$ (Robson et al. 2007) which enhances the descriptive powers of bivalve adduction and abduction rate, whilst avoiding the bias that bigger mussels can adduct and abduct faster than smaller ones (Robson et al. 2007).

MATERIALS AND METHODS

Overall experimental design

The methods developed by Wilson et al. (2005) were modified to quantify gape angle in blue mussels *M. edulis*. Briefly, this involved quantifying bivalve gape angle using a Hall sensor (a transducer for magnetic field strength) attached to one shell valve reacting to a magnet attached to the other shell valve. Variation in gaping extent produced a corresponding variation in the magnetic field strength perceived by the Hall sensor (c.f. Wilson et al. 2002). This was recorded by an archival tag. Since Hall sensor output is proportional to magnetic field strength and angle of impingement, the transducer output has to be calibrated by comparing shell gape angle with sensor output, over a wide variety of angles. To do this, at the end of experiments, the posterior adductor muscle of *M. edulis* was severed with a knife to allow calibration of all possible gape angles with sensor output. Gape angle calibration took ~ 5 mins per mussel. Subsequently, data from sensor output versus gape angle were curve-fitted (for details see Wilson et al. 2002, Wilson & Liebsch 2003, Wilson et al. 2005, Robson et al. 2007). The curve-fit could then be used to determine any gape angle by converting the transducer output accordingly.

The archival tag used for the work was a 13-channel JUV-Log, equipped with 12 Hall-sensor (Honeywell, SS59E) channels and 1 temperature channel, each with 22 bit resolution, recording gape angle at better than 0.01°. The unit had a 1Gbyte RA memory and was deployed during all experiments (see below) set to record at rates of 2 Hz. The magnets used were 5 x 5 x 2 mm neodymium boron magnets.

Collection and maintenance of mussels for aquarium experiments

Inter-tidal mussels were collected from Swansea Bay, Wales, UK (LR SS630875) at low tide and transferred to a flow-through aquarium system within 2 h. Magnets and Hall sensors were glued to mussel shells using Aquarium Sealant (Geocel, Plymouth, UK) before the mussels were replaced in an aerated flow-through aquarium system containing seston-laden sea water from Swansea Bay, Wales, UK for at least a week before being used in experiments. Experiments with mussels in aquaria took place from July to December 2006.

Mussels in standard conditions

14 mussels with an initial mean length of 42.53 mm ± S.D 0.28 and mean wet weight 11.03 g ± SD 0.84 were kept in separate, well-aerated tanks filled with 12 litres of 0.45 μm filtered seawater. Mussel (pseudo)faeces and seawater were removed from tanks and replaced with fresh 0.45 μm filtered seawater once ever 24 hours. Mussels were subject to a daily light regime of 13 h light 11 h dark (water temperature 16.2 °C ± 0.4), and each fed a mixed algal diet of ~100 million *Tetraselmis suecica* and ~1000 million *Thalassiosira weissflogii* cells day^{-1}.

Mussel response to varying algal ration

Mussels were subject to the same light regime as in standard conditions (see *Mussels in standard conditions* in Materials and methods). 14 mussels were food-deprived for 48 hours in individual, well-aerated tanks filled with 12 litres of 0.45 μm filtered

seawater. At this time mussels were then fed eleven different daily algal rations at random of 0, 0.2 x 10^7, 0.8 x 10^7, 2.5 x 10^7, 2.75 x 10^7, 3 x 10^7, 25 x 10^7, 60 x 10^7, 120 x 10^7, 240 x 10^7 and 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at concentrations of 0, 0.17, 0.67, 2.08, 2.29, 2.5, 20.8, 50, 100, 200, 250 cells μl^{-1}, respectively, at one moment in time. Thus, after the algal cells had been added to each tank, cells concentration slowly decreased as mussels removed algae from the water. Mussel gape angle was monitored using the Hall sensors for 24 h after the initial additional of the algae. After being in one algal concentration experiment, mussels were maintained in standard conditions for two weeks before being subjected to another algal concentration experiment.

Mussel response to varying simulated predation risk

Mussels were subject to the same light regime as in standard conditions with the archival tag recoding mussel gape angle for >24 hours. At this time 14 mussels were fed 240 x 10^7 *Thalassiosira weissflogii* day^{-1} in 12 litre tanks (an initial concentration of 200 cells μl^{-1}), while mussels were exposed at random to 0, 0.00833 x 10^{-4} $g.ml^{-1}$, 0.04167 x 10^{-4} $g.ml^{-1}$, 0.2083 x 10^{-4} $g.ml^{-1}$, 0.4167 x 10^{-4} $g.ml^{-1}$, 0.8333 x 10^{-4} $g.ml^{-1}$, 2.0833 x 10^{-4} $g.ml^{-1}$, 4.1667 x 10^{-4} $g.ml^{-1}$ and 8.3333 x 10^{-4} $g.ml^{-1}$ of fresh mussel homogenate. Mussel homogenate was made from the paper towel-dried flesh of freshly-killed mussels blended for 90 s at high speed in a Waring blender (Waring Products Division, New Hartford, Connecticut). After being exposed to one mussel homogenate concentration, mussels were maintained in standard conditions for two weeks (to try to mitigate against any possible habituation to simulated predation risk) before being subjected to another homogenate concentration.

Calculation of change in gape angle per second (CHIGA, $°s^{-1}$)

Individually for six mussels in standard conditions, gape angle data was collected over one week and plotted against the rate of change in gape angle (converted to standardized units of degrees per second, but measured over intervals of 0.5 s) to produce a characteristic pattern for each mussel which we subsequently refer to as the CHIGA (CHange In Gape Angle per second) pattern (for details see Robson et al. 2007). Two non-linear curves were fitted to describe the edge contours indicating maximum CHIGA for each of the six mussels using TableCurve (Systat Software, Inc., Richmond, CA) (in each case, $R^2 > 0.99$). One curve corresponded to mussel valve abduction (positive CHIGA) and the other to mussel valve adduction (negative CHIGA). Values of the edge contours indicating maximum CHIGA for both valve adduction and abduction were taken for every 0.5° increase in gape angle for each of the six mussels and an average taken for each CHIGA value (Fig. 1). The best-fit relationship between the mean maximum CHIGA to define the edge contours via rate of change of gape angle (y) and gape angle (x) for valve abduction and abduction followed an 8^{th} order polynomial (edge contour equations); $y = (a+bx+cx^2+dx^3+ex^4+fx^5+gx^6+hx^7+ix^8)$. Mussel valve abduction constants were: a 0.058348612, b 0.895536588, c -0.39951595, d 0.087770651, e-0.02340696, f 0.009727361, g -0.00237438, h 0.000263781, i -0.000010734. Mussel valve adduction constants were: a -0.02844173, b -0.46223492, c -0.30655783, d 0.219537855, e -0.08371628, f 0.021157231, g -0.00333171, h 0.00028829, i -0.000010194. These equations were used to predict the maximum CHIGA of the 14 mussels in the aquarium experiments for any gape angle during both abduction and adduction events.

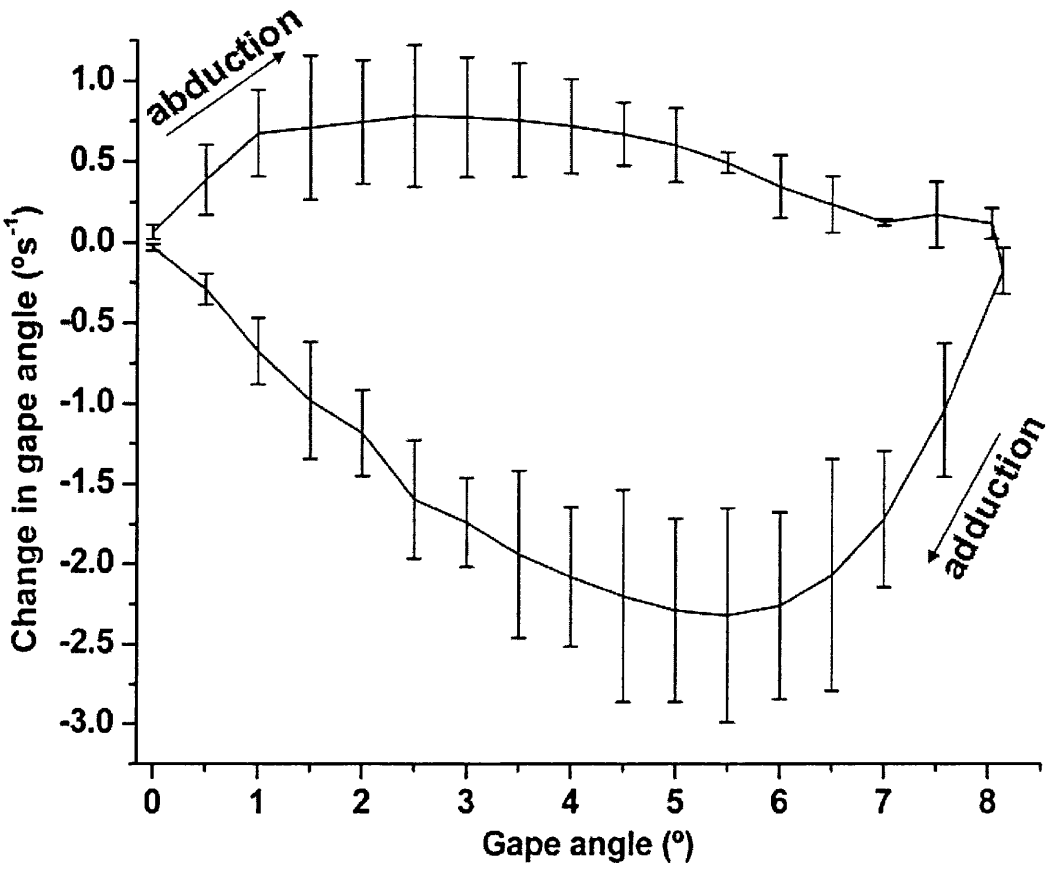

Fig. 1 Mean maximum change in gape angle per second (CHIGA) ± SD indicated by a line of best-fit around the highest and lowest y values (edge contours) at 0.5° intervals calculated from 6 mussels 42.50 mm long ± SD 0.26 using data acquired over 7 days at a rate of 2 Hz (see text for details). The mussels were held in standard conditions (see text for details). Positive *y* values delineate the mean maximum CHIGA during valve abduction, negative *y* values maximum CHIGA during valve adduction.

Calculation of the CHIGA relative to the maximum CHIGA corrected for mussel length (Thorn - Þ)

Thorn (Þ) was used to measure mussel valve behaviour (Robson et al. 2007) of the 14 mussels used in aquarium experiments. Briefly, in order to work out how the speed of mussel valve adduction or abduction events related to maximum rates of valve adduction or abduction, the first observed gape angle (a_n) in a valve adduction or abduction event was taken and the next angle ($a_n + 1$) predicted ($A_n + 1$) according to the boundary equations (see *Calculation of CHIGA*). The difference (*D*) between the next observed ($a_n + 1$) and next predicted ($A_n + 1$) angle was calculated. The process was then repeated using the next gape angle in the adduction or abduction sequence. This process was repeated for the entire adduction or abduction event and $\sum D$ calculated (an integral with units $^{\circ 2}0.5\ s^{-1}$). For full details including a worked example of calculating Þ see Robson et al. (2007).

In order to correct for gape angle-dependent CHIGA (c.f. Fig. 1), this integral x 2 (units $^{\circ 2}s^{-1}$) was subsequently divided by the total movement in degrees of the valve adduction or abduction event, to give a final value for the proximity of the adduction or abduction event to the maximum. This value, Þ (units $^{\circ}s^{-1}$), is an arbitrary, but relative, scale that allows comparison between mussels that adduct and abduct at different maximum rates.

Statistical analysis

A one-way repeated-measures ANOVA was used to test for any difference between the mean number of mussels adduction events per day in 14 mussels in response to varying daily algal ration. One-way ANOVA and post-hoc Tukey tests were used to test for any difference in mean gape angle with daily algal ration. The Mann-Whitney *U* test was used to test for any difference between the relative speed (Thorn Þ) of mussel valve abduction events after a single adduction or a repetitive adduction event. A two factor ANOVA with adduction start angle (°) as covariate was used to test for variation between the gape angle at the start of a valve adduction event and the gape angle at the end of that adduction event between 14 mussels. Linear regression was used to examine the relationship between the mean amount of valve adduction and daily algal ration

RESULTS

Mussel response to varying algal concentration

A wide variation in the number of shell valve adduction events per day was recorded within daily algal ration treatments, with food-deprived mussels and those fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} with minima and maxima of 3-113 and 6-160 adduction events per day, respectively. There was no significant effect of daily algal ration on the mean number of valve adduction events day^{-1} (mean ± SD = 42.6 ± 24.8 -$F_{10,130} = 0.98$, $p = 0.462$), equivalent to, on average, one valve adduction approximately every 34 minutes (Fig. 2a).

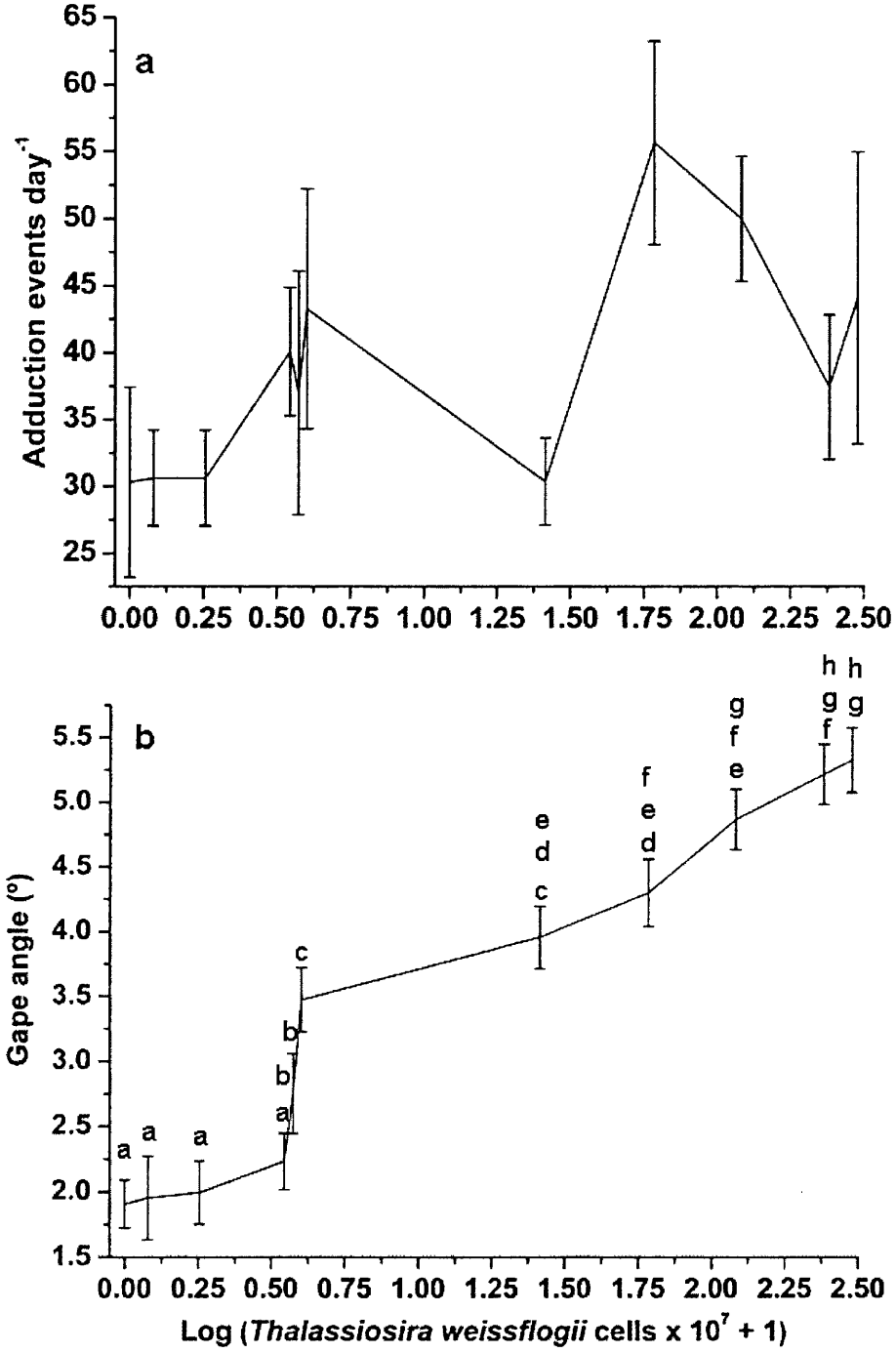

Fig. 2 (a) Shell valve adduction events per day (± SE) *versus* daily algal ration, (b) mean gape angle (± SE) *versus* daily algal ration taken from 14 mussels 42.53 mm ± S.D 0.28 long (bar daily algal ration bins not sharing a letter are significantly different).

In general, mean mussel gape for 14 mussels remained low, then increased exponentially and finally plateaued in an elongated "S"-shaped curve as algal ration increased (Fig. 2b). Mean mussel gape angle for food-deprived mussels was 1.91° ± SD 0.69 but this was not significantly different to mussels fed 0.2 x 10^7, 0.8 x 10^7, 2.5 x 10^7 *Thalassiosira weissflogii* cells day^{-1} ($P<0.05$). Mean mussel gape significantly increased as mussel daily algal ration increased from 2.5 x 10^7 to 3 x 10^7 *Thalassiosira weissflogii* cells day^{-1} ($P<0.05$). Mean mussel gape was highest at 5.32° ± SD 0.94 when animals were fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1}. Post-hoc Tukey tests indicated that there were relatively few non-significant differences ($P>0.05$) in gape angle at different daily algal rations (see Fig 2b).

Figure 3 shows details of gaping behaviour of a single mussel according to algal ration. The maximum time any individual mussel spent gaping <1° until subsequent abduction of shell valves was approximately 19 hours when food-deprived (Fig. 3). For the mussel in figure 3, mean gape angles when fed 0, 60 x 10^7 and 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} were 0.36° ± SD 0.42, 3.17° ± SD 0.86 and 6.10° ± 0.66, respectively. Figure 3 also shows how the mussel fed a daily algal ration of 2.5 x 10^7, 60 x 10^7, 120 x 10^7 and 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} initially gradually increased its maximum gape angle until it plateaued. After approximately 19 hours the maximum gape angle decreased when the animal was fed 60 x 10^7 and 120 x 10^7 *Thalassiosira weissflogii* cells day^{-1}.

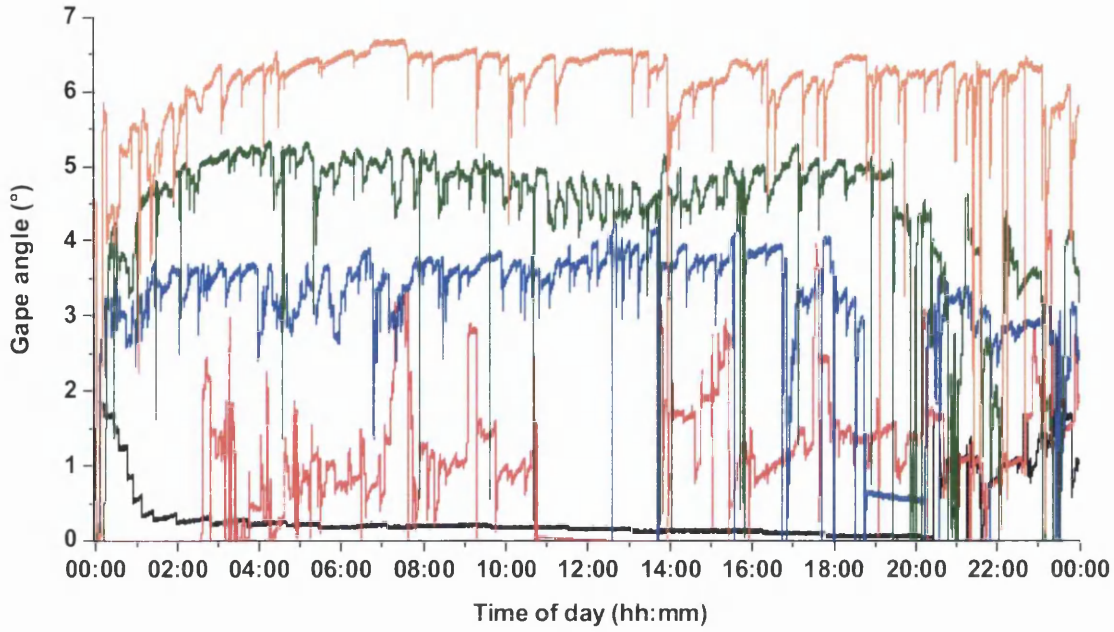

Fig. 3 Gape angle (°) *versus* daily algal ration from a single 42.32 mm long mussel. Coloured lines indicate daily algal rations of 0 (black) 2.5 x 10^7 (red), 60 x 10^7 (blue), 120 x 10^7 (green) and 300 x 10^7 (orange) *Thalassiosira weissflogii* cells day^{-1}.

Mussel valve adduction events in all algal ration experiments were defined as either a single adduction (Fig. 4) or a repetitive adduction event (Fig. 5). Repetitive valve adduction events had subsequent abduction events that were cut short (the gape angle did not level out before a subsequent adduction event) by another valve adduction. Single valve adduction events were followed by complete abduction i.e. where the gape angle stopped increasing (levelled out) and was not cut short by a subsequent adduction event. Single valve adduction events were significantly slower than repetitive adduction events (median Þ-values = -12.20 and -5.54 $^{o}s^{-1}$ respectively, Mann-Whitney *U* 100216.0, $p < 0.00001$, $n = 260$). Mussel valve abduction events after single adduction events were also significantly slower than abduction events after repetitive adduction events (median Þ-values = 24.54 and 6.48 $^{o}s^{-1}$ respectively; Mann-Whitney *U* 82040.0.0, $p < 0.00001$, $n = 260$).

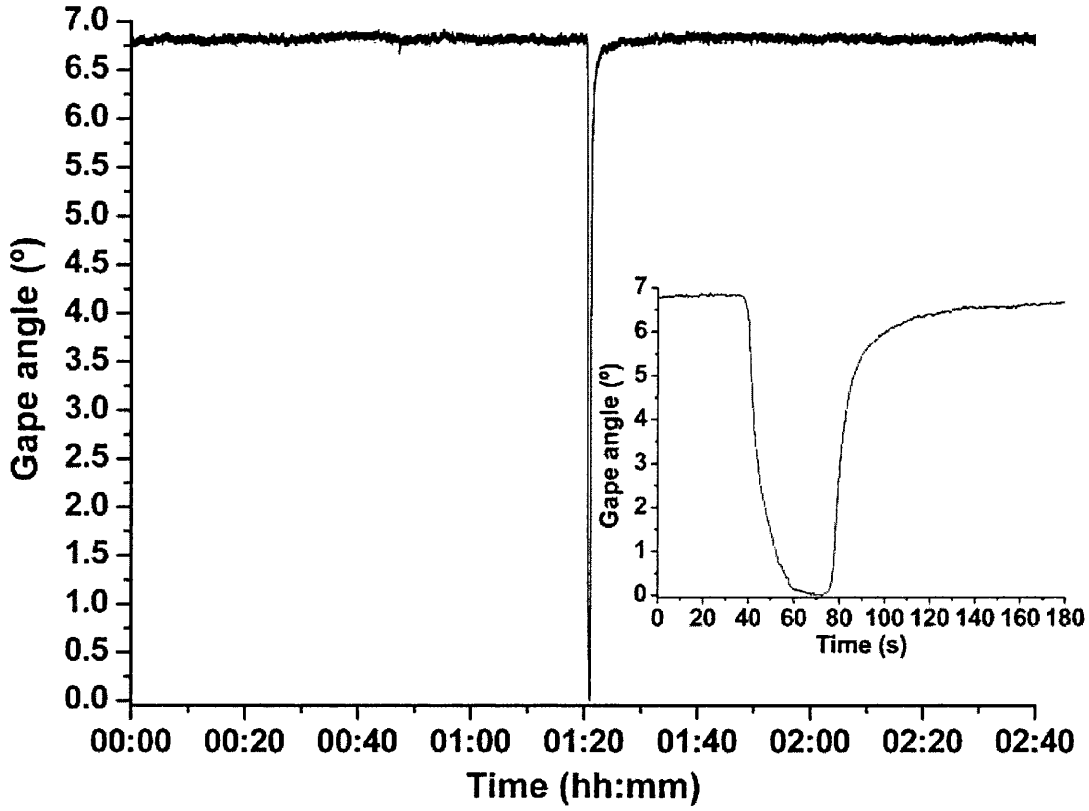

Fig. 4 A detailed example of the form of a single shell valve adduction event followed by subsequent abduction from a 42.50 mm long mussel.

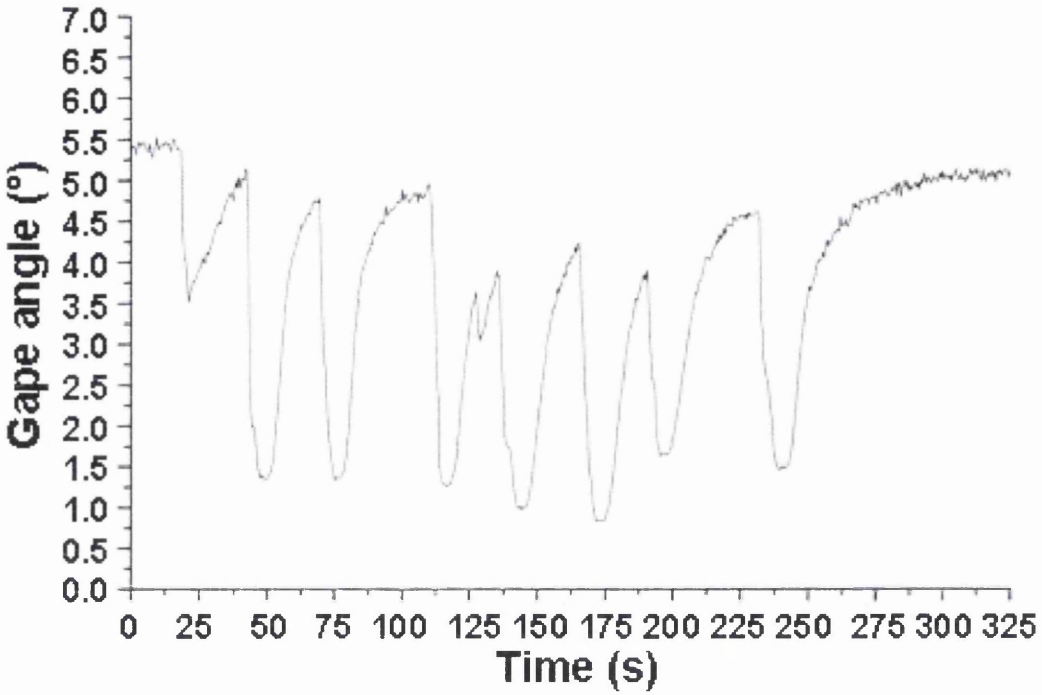

Fig. 5 A detailed examples of the form of repetitive shell valve adduction events followed by subsequent abductions from a 42.53 mm long mussel.

In general, the less a mussel gaped when a valve adduction occurred, the nearer its gape was to 0° at the end of the adduction event and *vice versa* for all 14 mussels across all daily algal rations (Table 1). Four examples of this relationship are shown in Figure 6 and there were significant differences (the intercepts of slopes were significantly different) between the minimum gape angle of 14 mussels ($F_{10,2989}$ = 3.50, $p < 0.001$). There was a significant (slopes were significantly different from zero) effect of the gape angle at the start of an adduction event on the minimum gape angle at the end of that adduction event ($F_{1,2989}$ =1414.33, $p < 0.001$). 38% (35 out of 91 intercept comparisons) of mussel minimum gape angles and 56% (51 out of 91 slopes comparisons) of slopes were significantly different from each other at the 95% significance level.

Table 1 The relationship between the minimum gape angle (°) at the end of an adduction event and the gape angle at the start of that adduction event (slope ^{o2}) from 14 mussels 42.53 mm ± S.D 0.28 long across all daily algal rations from 0 x 10^7, 0.2 x 10^7, 0.8 x 10^7, 2.5 x 10^7, 2.75 x 10^7, 3 x 10^7, 25 x 10^7, 60 x 10^7, 120 x 10^7, 240 x 10^7 and 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} at 40 ± 3.6 cells µl^{-1}.

Mussel	Intercept (°) ± SE	Slope (^{o2}) ± SE
1	-0.228 ± 0.331	0.479 ± 0.065
2	-1.249 ± 0.387	0.686 ± 0.068
3	-1.212 ± 0.304	0.760 ± 0.068
4	-1.223 ± 0.272	0.704 ± 0.059
5	-1.279 ± 0.302	0.820 ± 0.060
6	-0.330 ± 0.266	0.445 ± 0.050
7	-0.929 ± 0.333	0.694 ± 0.064
8	-1.100 ± 0.188	0.686 ± 0.048
9	0.150 ± 0.203	0.326 ± 0.038
10	-1.177 ± 0.206	0.878 ± 0.050
11	-0.900 ± 0.222	0.767 ± 0.042
12	-1.233 ± 0.272	0.790 ± 0.063
13	-1.084 ± 0.313	0.699 ± 0.057
14	-1.151 ± 0.277	0.753 ± 0.054

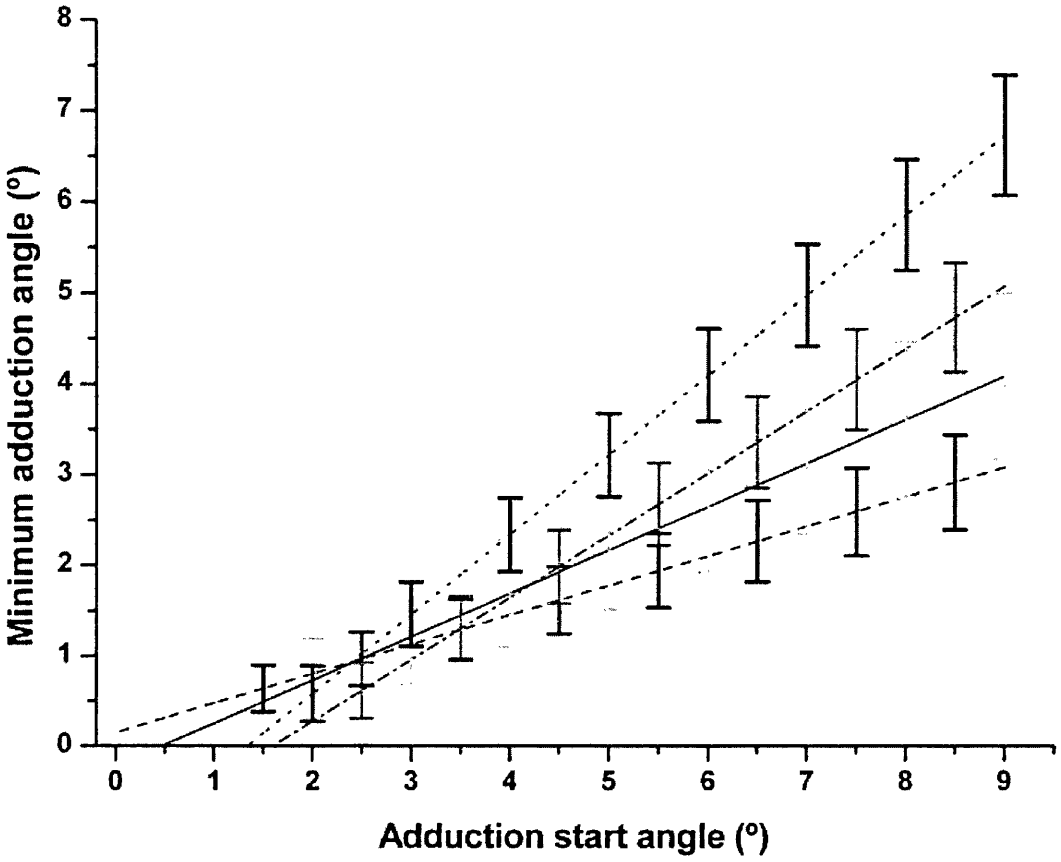

Fig. 6 Four examples of the relationship (± SD) between the gape angle (°) at the end of an adduction event (y) and the gape angle (°) at the start of that adduction event (x) averaged from 14 mussels 42.53 mm ± S.D 0.28 long across all daily algal rations from 0 x 10^7, 0.2 x 10^7, 0.8 x 10^7, 2.5 x 10^7, 2.75 x 10^7, 3 x 10^7, 25 x 10^7, 60 x 10^7, 120 x 10^7, 240 x 10^7 and 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1}.

There was little variation in the mean amount of mussel valve adduction (per adduction event) between 0 and 0.8 x 10^7 *Thalassiosira weissflogii* cells day^{-1} (1.72° ± SD 0.81 and 1.72 ° ± SD 1.10, respectively) (Fig. 7) although the mean amount of adduction significantly increased with increasing daily algal ration ($F_{1,9} = 52.65$, $p < 0.0001$, $R^2 = 0.84$, mean adduction = 1.835 + (0.3668 x log (*Thalassiosira weissflogii* cells x 10^7 day^{-1} +1)) (Fig. 7). The mean amount of adduction increased by a mean of 0.66° when mussel daily algal ration increased from 2.5 x 10^7 to 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} (derived from a change from 2.16° ± SD 1.13 to 2.82° ± SD 1.50) (Fig. 7). The mean time mussels gaped <1° decreased as daily algal ration increased in a decay type curve (Fig. 8). Maximum and minimum mean time mussels gaped <1° per day was 12.42 hours ± SD 5.97 and 0.32 hours ± SD 0.28 when fed 0.2 x 10^7 and 240 x 10^7 *Thalassiosira weissflogii* cells day^{-1}, respectively. The greatest decrease in mean time gaping <1° was 5.38 hours when daily algal ration increased from 0.8 x 10^7 to 2.5 x 10^7 *Thalassiosira weissflogii* cells day^{-1} (mean time gaping <1° was 7.16 ± SD 2.49 and 1.78 ± SD 1.16, respectively).

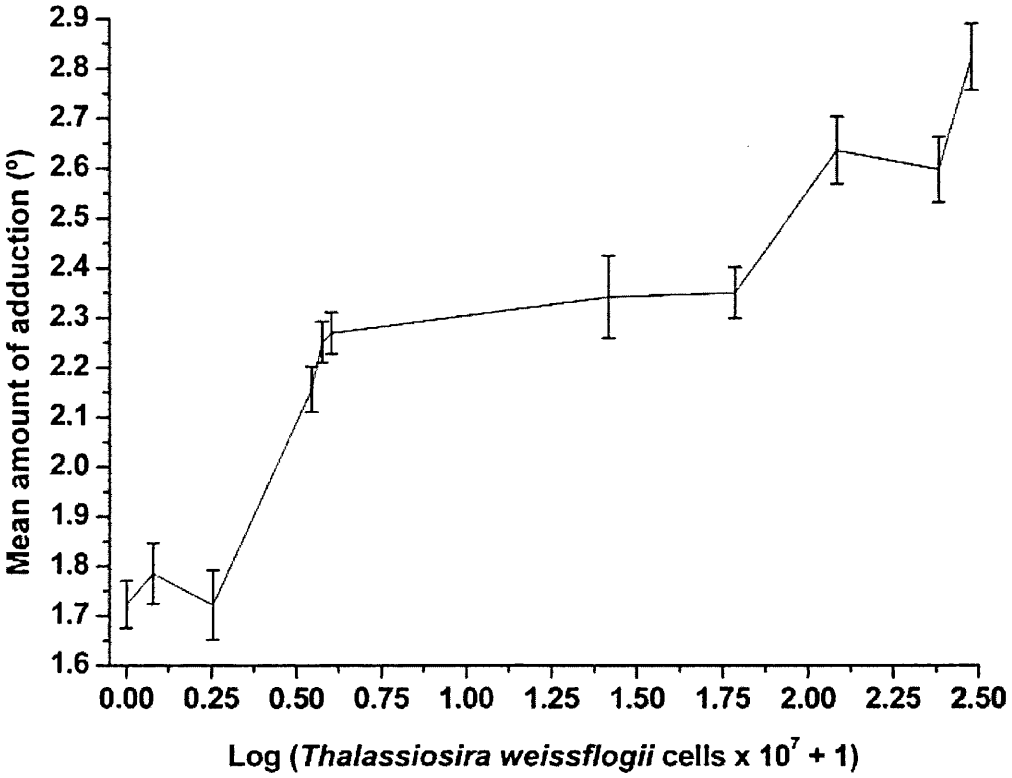

Fig. 7 Mean amount of adduction (± SE) *versus* daily algal ration.

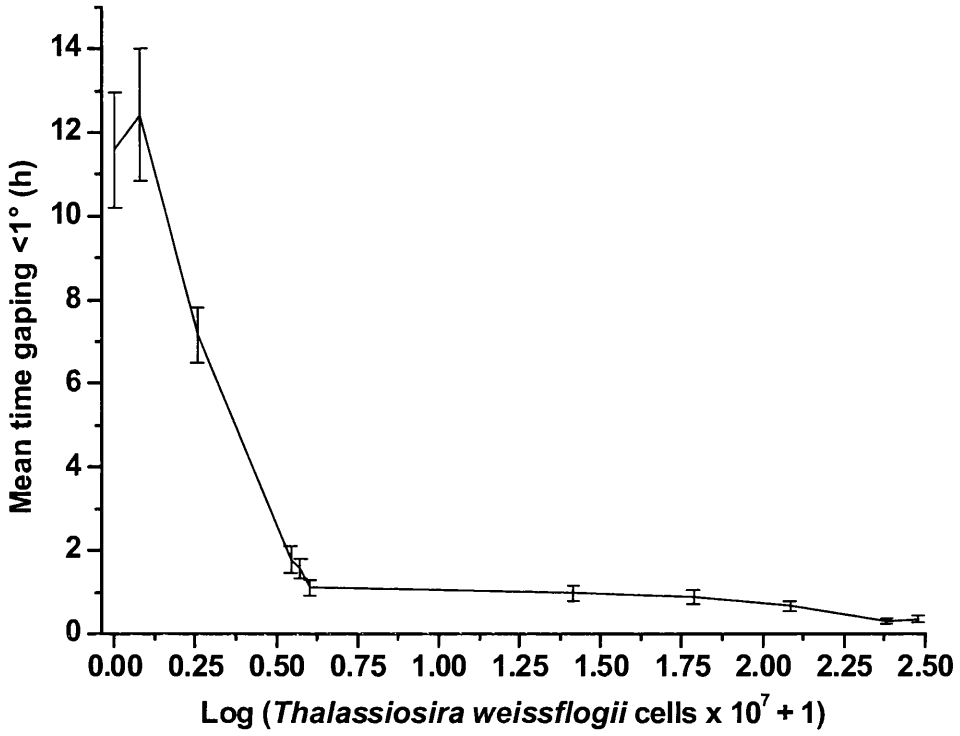

Fig. 8 Mean time spent gaping <1° (± SE) over 24 h *versus* daily algal ration.

In general, the mean time from the start of a shell valve adduction event to subsequent re-abduction (re-abduction defined as reaching 50% of the initial gape angle at the start of the adduction event) decreased as daily algal ration increased (Fig. 9). The mean maximum time mussels spent from the start of an adduction to subsequent re-abduction during adduction events was approximately 19.7 hours, (when mussels were fed daily algal rations of 0, 0.2 x 10^7 and 0.8 x 10^7 *Thalassiosira weissflogii* cells day^{-1}). Mussels food-deprived spent a mean of 81% of their time adducting until re-abducting their valve for periods ranging from 16 to 398 s. Mussels fed 240 x 10^7 *Thalassiosira weissflogii* cells day^{-1} spent a mean of 90% of their time adducting until re-abducting their valves for periods of between16 and 100 s. Mussels fed 3 x 10^7 to 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1} had the highest mean percentage time spent adducting until re-abducting of 25 s.

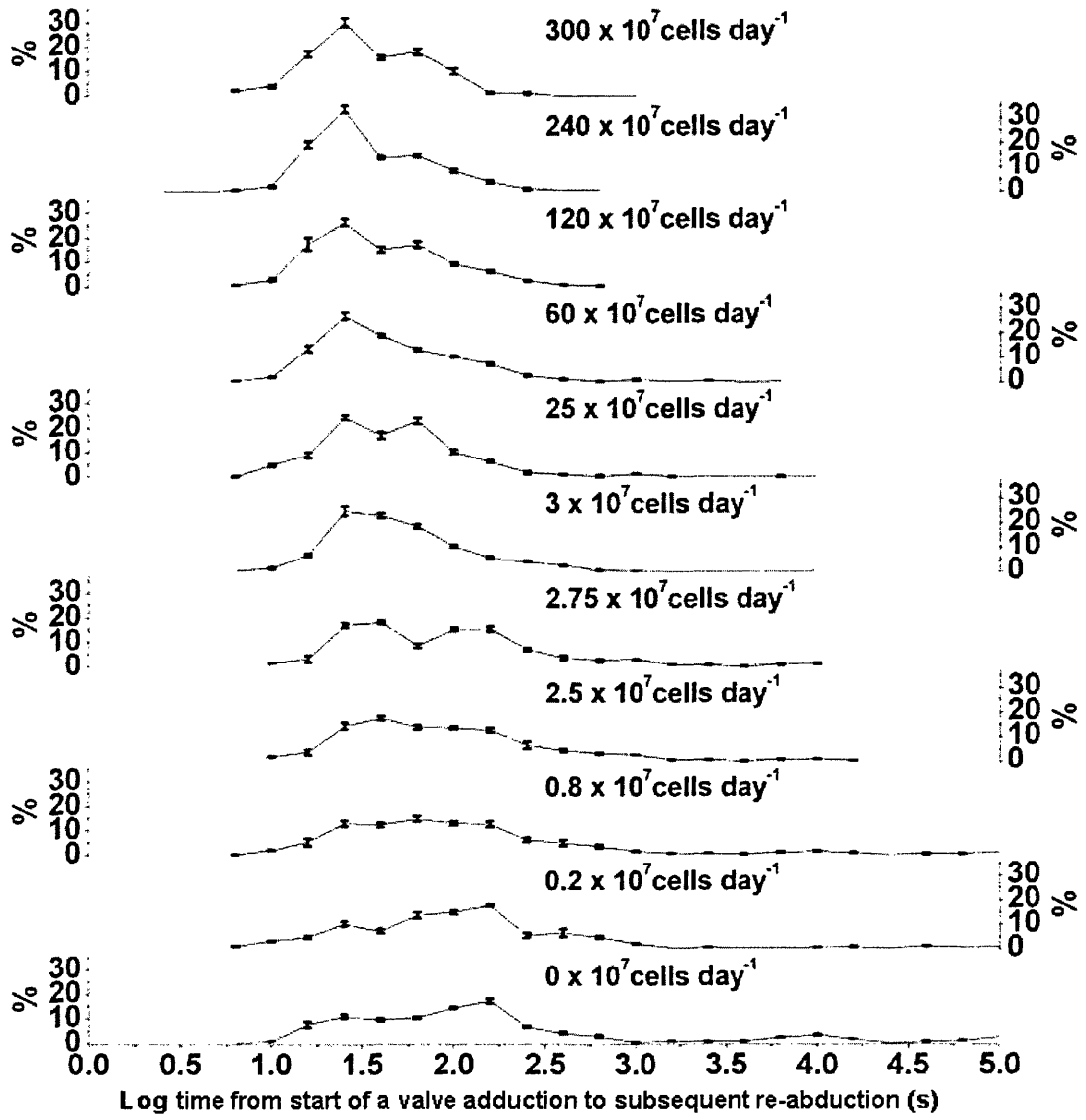

Fig. 9 Log time (± SE) from the start of a shell valve adduction event to subsequent re-abduction (re-abduction defined as reaching 50% of the initial gape angle at the start of the adduction event) *versus* percentage time plots for all daily algal rations.

Mussel response to varying simulated predation risk

Mussels fed only 240 x 10^7 *Thalassiosira weissflogii* cells day^{-1} had mean gape angles of 5.21° ± SD 0.87 but this increased to 5.38° ± SD 0.99, when 0, 0.00833 x 10^{-4} $g.ml^{-1}$ of fresh mussel homogenate was added to their water (Fig. 10, c.f. Fig. 2b). However, mean gape angle decreased in a decay type curve as the amount of mussel homogenate added to the water increased from 0.00833 x 10^{-4} $g.ml^{-1}$ to 8.3333 10^{-4} $g.ml^{-1}$. Mean gape angle decreased by 1.78° (this being the fastest rate of change at approximately 0.02° per 10^{-4} $g.ml^{-1}$) when the amount of homogenate to which mussels were exposed increased from 0.00833 x 10^{-4} $g.ml^{-1}$ to 0.04167 x 10^{-4}. Mussel mean gape angle was lowest (0.96° ± SD 0.85) when exposed to 4.1667 x 10^{-4} $g.ml^{-1}$ of homogenate. Mean gape angle decreased by 4.42° when the amount of homogenate increased from 0.0083 x 10^{-4} $g.ml^{-1}$ to 4.1667 x 10^{-4} $g.ml^{-1}$.

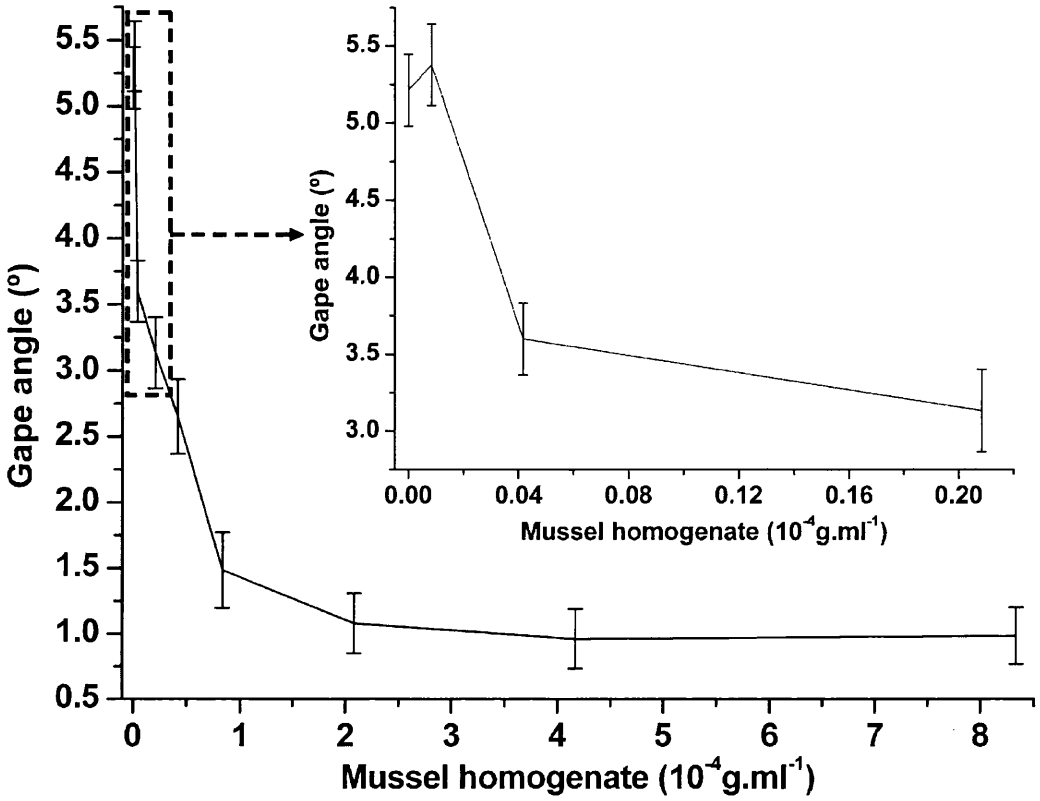

Fig. 10 Mean gape angle (± SE) *versus* mussel homogenate (10^{-4} g.ml^{-1}) when mussels were fed 240 x 10^7 *Thalassiosira weissflogii* cells day^{-1}.

DISCUSSION

Bivalve valve movements have been measured using various techniques in numerous studies including Lowy (1953), Foster-Smith (1976), Davenport & Manley (1978), Massabuau et al.(1991), DeZwart et al. (1995), Pynnonen & Huebner (1995), Kontreczky et al. (1997), Newell et al. (1998 and 2001), Curtis et al. (2000), Markich et al. (2000), Kadar et al. (2001), Tran et al.(2003a), Tran et al. (2003b), Riisgård et al. (2003), Riisgård et al. (2006), Fournier at al. (2004), Legeay et al. (2005), Borcherding (2006), Fdil et al. (2006), Juhel et al.(2006) and Nagai et al.(2006). The mussel valve gape and movement study presented here measures mussel gape angle in degrees (°) (Wilson et al. 2005, Robson et al. 2007) and change in gape angle per second (CHIGA) (Robson et al. 2007) using the principle of the Hall effect, first used to measure bivalve gape by Wilson et al. (2005), then utilised by Nagai et al. (2006) with further work by Robson et al. (2007). Nagai et al. (2006) suggested that the Hall sensor appeared to be an excellent method for measuring the valve movements of bivalves, such as pearl oysters *Pinctada spp*, in their natural state for extended periods. This study supports the conclusions of Nagai et al. (2006).

This study has highlighted considerable variance in the actual speed ($^{o}s^{-1}$) and relative speed Thorn (Þ) of mussel valve adduction and abduction events, as has already been noted by Robson et al. (2007). These different valve adduction and abduction speeds probably serve different purposes. Nagai et al. (2006) suggest that one reason for bivalve valve adduction is to reject invasion by noxious algal cells. Davenport et al. (2000) report that if the copepod *Tigriopus brevicornis* touched the inhalant siphonal tentacles of *M. edulis* there was immediate siphon closure, accompanied by some shell-valve adduction (but not if they passed through the

inhalant siphon cleanly, without touching the siphonal tentacles). In addition, bivalves have been found to adduct their valves in a defensive reaction to external stimuli such as touching or shading, or by the sudden approach of a predator, as well as in response to a deteriorating environment due to toxic red tides, oxygen deficit, or low salt concentrations (Dharmaraj 1983, Gainey & Shumway 1988, Baldwin & Kramer 1994, Rajagopal et al. 1997, Leonard et al. 1999). This is not without cost to the animals since the greater the frequency and magnitude of adduction and abduction events, the more energy the mussel must expend (c.f. Shick et al. 1986, Davenport et al. 2000). Care was taken to ensure that none of the stimuli for valve adduction mentioned above were present in the current study. It has been noted that mussels adduct at their maximum rate in response to simulated predation risk, but this maximum rate is used only once when the stressor is applied with the animals subsequently remaining at a low gape angle for extended periods of time (Robson et al. 2007).

It has been suggested that valve movements of bivalves, overall, are closely related to vital activities such as respiration, feeding and excretion (Nagai et al. 2006). Variability in the speed of valve abduction or adduction events may be related to the degree to which the mussels need to perform their vital activities; reduced gape should be tied in with reduced metabolism (see Famme 1980) while e.g. food-deprived or feeding intermittently (c.f. Davenport & Woolmington 1982). Shell valve activity may be involved in enhancing perfusion of the tissues by newly re-oxygenated haemolymph (Shick et al 1986, 1988). However, in reality, little is understood about the specific reasons for mussel valve movements that are suggested to be associated with vital activities.

The mean amount of adduction during shell valve adduction events may have significantly increased with increasing daily algal ration simply because valve gape and thus, the amount mussels could adduct their valves, generally increased as daily algal ration increased. Food-deprived mussels and those fed 300 x 10^7 *Thalassiosira weissflogii* cells day^{-1}, elicited minima and maxima of 3-113 and 6-160 valve adductions day^{-1}, respectively, which might indicate highly variable, intermittent filtration in all our experimental mussels across all experiments.

Mussels spent most of their time gaping <1° when food-deprived and at extremely low daily algal rations (0.2 x 10^7 *Thalassiosira weissflogii* cells day^{-1}). Gaping < 1° could represent a physiological adaptation to suspension feeding at extremely low phytoplankton concentrations: by reducing the water transport through the gills and mantle cavity the mussel reduces metabolic rate (see Famme 1980, Jørgensen et al. 1986a, Jørgensen et al. 1986b, Riisgard et al. 2003). However, it cannot be ruled out that mussels gaping < 1° may not be filter-feeding at all (see Thompson & Bayne 1972 who first suggested that there was a threshold concentration below which mussels do not filter at all), or intermittently and inefficiently at low phytoplankton concentrations. Despite this general response to very low concentrations of food in the water, food-deprived mussels might occasionally abduct, presumably to filter the water to test for food particles and or increase oxygen uptake associated with respiration. Mussel response to increasing algal concentration (c.f. Wilson & Seed 1974, Riisgard & Randlov 1981, Riisgård 1991, Clausen & Riisgard 1996, Dolmer 2000, Newell et al. 2001, Macdonald & Nodwell 2003, Riisgard et al. 2003) was to increase mean gape angle in an what appeared to be an S-shaped Type

III behavioural -response curve (Holling 1959a, 1959b). Type III functional responses occur in predators which increase their search activity with increasing prey density (Holling 1959a, 1959b). Although mussels clearly cannot search in the way polyphagous vertebrate predators (e.g., birds) do, this being often cited as an appropriate example of an animal adopting a type III response curves, they can increase filtration rate, which effectively increases the volume of water pumped through their filtering system, which amounts to an increased search capacity. Obviously, and in a general sense, animals should concentrate feeding at times and in areas where food concentration is highest. In the case of mussels, animals may weigh up the net energy gain and nutrient content of their food as well as its concentration against the costs of ciliary action, mucus secretion and shell-valve movements, associated with filter-feeding (c.f. Davenport et al. 2000).

Mean gape angle decreased in what appeared to be a backward S-shaped Type III behavioural response curve as the amount of mussel homogenate increased in the sea water. We speculate that above a background level (somewhere between 0.00833 x 10^{-4} $g.ml^{-1}$ and 0.04167 x 10^{-4} $g.ml^{-1}$) of mussel homogenate, the greater the concentration of stress hormones in the water the greater the threat 'perceived' by the mussel and thus the lower the gape angle. Although diminished gape angle could reduce the ability of the mussel to take up oxygen (Famme, 1980) and also limit the ability to filter-feed, caution should be exercised in interpreting any mussel responses in the study as Type III behavioural responses (Holling 1959a, 1959b) without further studies to support our findings. However, response to algal concentration appears to be nonlinear beyond a threshold concentration. Adverse environmental conditions, including very low algal concentration can induce mussels to stop feeding (cause reduction of water pumping due to closure of the valves (Riisgard & Randløv 1981,

Riisgard 1991)) to conserve energy until better conditions occur (Wilson and Seed 1974, Dolmer 2000, Riisgård et al. 2003), whereas high algal concentration may lead to reduced valve gape and a reduction in filtration rate (Riisgard & Randløv 1981, Clausen and Riisgård 1996, Macdonald and Nodwell 2003).

Although *M. eduils* is tolerant of anoxia and can survive by becoming largely anoxic (Shick et al. 1986, 1988), it cannot continue this practise indefinitely. This may explain why food-deprived mussels in this study never remained gaping <1° for more than 19 hours although mussels may have to abduct when their adductor muscles become fatigued (tonic contraction of the adductor muscles requires energy (Lowy 1953)). In fact, in order to avoid anaerobic metabolism, it is likely that bivalves avoid complete or more permanent closure of the valves (Riisgård et al (2003) which may explain why mussels in this study rarely adducted their valves fully. But inter-tidally this might lead to desiccation.

Feeding of previously food-deprived mussels has been found to increase the oxygen consumption by a factor of two, mainly due to increased valve gape associated with increased ventilation of the mantle cavity (Famme, 1980). Hence, we consider that in predation-free filtered seawater, mean mussel gape angle over 24 hours may be used as a general indicator of oxygen consumption (provided the mussels are post-absorptive) (c.f. Famme 1980). As daily algal ration increased, mean gape angle increased, indicating that mussels were not only responding by feeding more at higher concentrations but also, in a knock on effect, probably because they needed more oxygen to fuel the higher filtration rate and possibly specific dynamic action costs (Harper 1971, Bayne & Scullard 1977). In a reversal of this process, the reduction in gape angle after approximately 19 hours of mussels fed 60×10^7 and 120×10^7 *Thalassiosira weissflogii* cells day^{-1} is most likely explained by the metabolic costs of

feeding behaviour (filtration, gut passage and digestion) decreasing as feeding rate declined (c.f. Widdows & Hawkins 1989) as food concentration decreased (but see Riisgard et al. 2006).

We speculate that, in general, the time from the start of an adduction event to subsequent re-abduction decreased as daily algal ration increased because of the declining need to reduce valve gape for the purpose of reducing metabolism to conserve energy (see Famme 1980) over long periods as energy intake increased. Indeed, we suggest that a mean time spent adducting until re-abducting of 25 s when fed between 3 x 10^7 and 240 x 10^7 *Thalassiosira weissflogii* cells day^{-1} was a compromise between the need to adduct (although the reasons for adduction were not established) and feed and/or respire efficiently by reducing any restriction on the radius of the siphons when pumping water.

In order to identify the reasons for every valve adduction and abduction event, many more parameters of mussels and their environment will need measuring and at high temporal resolution. Examples of this would be; aspects of neurobiology, including muscle action potentials (Lowy 1953), video endoscopy of the gill filter-feeding and rejection tracts, oxygen uptake, haemolymph PO_2 (Shick et al 1986), heat dissipation (Shick et al 1986), mussels prey and their environment (e.g. temperature, salinity and current speed and direction) . Genetic analysis may explain the basis for individual variability in this study because genetic variability may enhance survival of mussels in general since a positive correlation between multi-locus heterozygosity (MLH) and various fitness characteristics has been documented for several species including *Mytilus* (Mitton 1994, Bayne & Hawkins 1997, Tremblay et al. 1998, Myrand et al. 2002, LeBlanc et al. 2008). A primary finding of the work conducted here, and as speculated in previous work (Robson et al. 2007) is that our approach for

assessing how mussels react to their environment indicates that mussel response to predation is graded and complex and indicates that mussels assess the trade-off between effective feeding and/or respiration and the likelihood of predation.

In summary the results of this chapter showed that mussel response to predation was graded and complex and indicated some form of mussel assessment of the trade-off between effective feeding and/or respiration and the likelihood of predation. Mussels may weigh up the net energy gain and nutrient content of their food as well as its concentration against the costs of ciliary action, mucus secretion and shell-valve movements associated with filter-feeding and the risk of predation. It was suggested that in order to identify the reasons for every mussel valve adduction and abduction event, many more parameters of mussels and their environment need measuring at high temporal resolution.

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Chapter 5

Life, stress and death: laboratory and inter-tidal field studies of the gape behaviour of the blue mussel *Mytilus edulis*[e]

Abstract

Mussels, *Mytilus edulis*, in a laboratory and the inter-tidal zone were fitted with Hall sensor systems to determine how gape angle varied in relation to a range of parameters when both immersed and emersed. In the presence of detritus (clay particles 8.3×10^{-4} $g.ml^{-1}$, ~ 4μm) immersed mussels adducted their shell valves significantly more often than in a detritus-free environment. Mussels exposed to detritus may have more intermittent and shorter filtration periods compared to those not exposed to detritus and thus, adduct more per unit time, to try to prevent their filtration and rejection mechanisms becoming overloaded. In emersed mussels, gape angle increased with air temperature in the inter-tidal zone in Swansea Bay (Wales, UK) and was described by a biphasic regression with the break point at 15°C. Gape patterns in emersed mussels are likely to be complex as individuals must balance potential exposure to desiccation and osmotic stress (e.g. following rainfall), with the need for aerial respiration and evaporative cooling. Furthermore, mussels may gape with their siphons shut and the presence of food in the gut can influence the need for aerial respiration. The transition of two inter-tidal mussels from what we consider to be normal gape behaviour, to abnormal may have been parasite

induced because parasites commonly alter the behaviour of their hosts. The high frequency of valve adduction (decrease in valve gape angle) events and high gape angles (up to 5.9°) associated with one mussel during emersion was most unusual, and we propose that fine-scale changes in gaping patterns may provide insight into mussel health. Mussel abduction and abduction (increase in valve gape angle) events can be induced by a wide variety of conditions.

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[e]The content of this chapter is intended for publication: the author order will be Robson AA, Garcia de Leaniz C, Wilson RP, Halsey LG.

INTRODUCTION

Basic tenants in biology are often conveniently studied using model species, such as the laboratory rat, *Rattus norvegicus*, and fruit flies, *Drosophila melanogaster,* which are selected for particular characteristics such as fecundity and short generation time (e.g. Connolly & Cook 1973, Meaney & Stewart 1981). The blue mussel, *Mytilus edulis*, has been commonly used in laboratory studies to examine issues such as invertebrate reaction to pollutants (e.g. Davenport & Redpath 1984, Curtis et al. 2000, Fdil et al. 2006) because of its accessibility, relaxed laws on maintenance and ease with which it can be subject to differing regimes (e.g. Widdows 1973, Foster-Smith 1975, Shick et al. 1986, Newell et al. 2001). A central premise in this is that findings will be applicable at least to mussels in the wild and, beyond that, that generalizations may be applicable to species other than mussels. Surprisingly, little is actually known about blue mussel *Mytilus edulis* gape behaviour in the wild and how this might relate to observations of the animals in captivity. Wilson et al. (2005) reported the absence of a well-defined diurnal rhythm in gape angle in laboratory mussels compared to those in the wild and that mussels put back in the wild after extended periods in the laboratory only returned to a more obvious diurnal pattern slowly (over 2 days). Wilson et al. (2005) suggest that laboratory mussels may behave differently to wild conspecifics because of many factors, including disturbance by humans and not being held under circumstances closely comparable to their natural environment. These results raise many questions about the ecological variables that affect the activity patterns of mussels generally and the differences between laboratory and field results (c.f. Gattermann et al. 2008).

One common feature of both laboratory and *in situ* mussel data is valve gaping and valve movement and these have been examined in research studying, for example, reactions to copper (Curtis et al. 2000, Fdil et al. 2006). Bivalve valve adduction (decrease in valve gape angle) and subsequent abduction (increase in valve gape angle) events have been found to constitute a normal part of bivalve behaviour, occurring in the wild sub-tidal (e.g. Wilson et al. 2005), inter-tidal (Fig. 1), simulated inter-tidal (Shick et al. 1986) and in laboratory aquaria (e.g. Lowy 1953, Trueman 1966, Hoggarth & Trueman 1967, Robson et al. 2007). Postulated reasons for mussel valve movement include abduction to filter feed, for respiration and to eliminate faeces from the exhalant siphon (e.g. Gosling 2003, e.g. Robson et al. 2007) and adduction in response to stimuli indicative of predators (Leonard et al. 1999, Robson et al. 2007) and adverse physical conditions such as low salinity and aerial exposure (see Chapter 6).

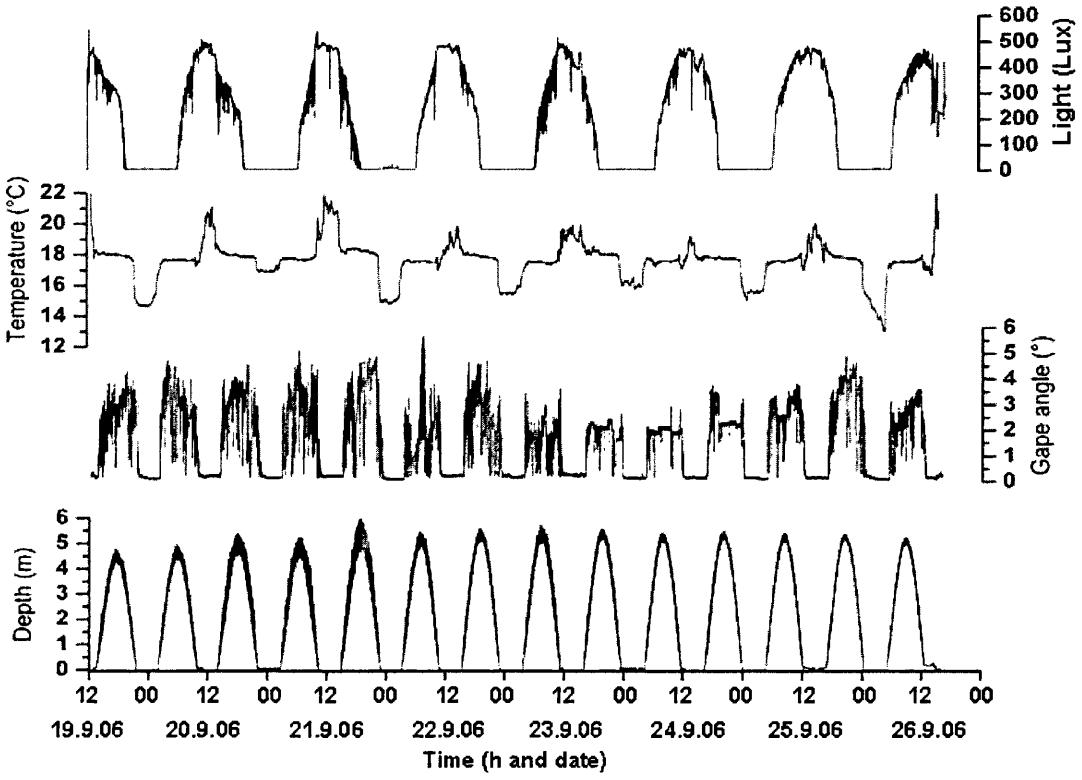

Fig. 1 Gape angle in relation to sea water depth for a 45.5 mm long inter-tidal mussel at Oxwich Bay, Swansea, UK. Variation in light intensity, shell temperature and water depth of the mussel at Oxwich Bay over 7 days (14 tidal cycles) starting on 19th September 2006. Note the light values are in Lux and the grey vertical bars indicate periods of darkness.

In the wild, mussels filter seston (including particulate organic and inorganic material, microbes, phytoplankton and zooplankton) from the seawater (e.g. Maar et al. 2007, Nielsen & Maar 2007) so that seston, including detritus, may affect mussel valve behaviour. The role of variable *Mytilus* valve adduction and abduction events underwater (for examples see Lowy 1953, Wilson et al. 2005, Robson et al. 2007) is not fully understood (Shick et al. 1986).

Valve movements also occur in mussels in air. In *M. edulis*, air-gaping has been found in most individuals in summer, but not necessarily in winter (Shick et al. 1986) which has led to the suggestion that it is a temperature-related phenomenon (Shick et al. 1986). However, gaping leads to evaporative water loss in *M. edulis* occurring at high shore levels (Newell 1973, Widdows et al. 1979a) and the closely-controlled air-gaping behaviour in this species may at times promote evaporative cooling. Air-breathing and cardiac activity during emersion (Shick et al. 1986) also seem to regulate a variable aerobic component of total energy metabolism (to support the costs of digestion and absorption and assimilation when food is present in the digestive system) which would also tend to favour gaping (Shick et al. 1986, Shick et al. 1988).

Here studies on laboratory and inter-tidal mussels were integrated to elucidate the relevance of valve gape studies in the laboratory to the wild. We tested whether clay (detritus) had any effect on mussel adduction and abduction events during immersion and whether temperature had any effect on gape angle during emersion. We also opportunistically examined the gape behaviour of two mussels dying in the inter-tidal zone.

MATERIALS AND METHODS

Experimental design

The methods developed by Wilson et al. (2005) were modified to quantify gape angle in blue mussels *M. edulis*. Briefly, this involved quantifying bivalve gape angle using a Hall sensor (a transducer for magnetic field strength) attached to one shell valve reacting to a magnet attached to the other shell valve. Variation in gaping extent produced a corresponding variation in the magnetic field strength perceived by the Hall sensor (c.f. Wilson et al. 2002). This was recorded by an archival tag. Since Hall sensor output is proportional to magnetic field strength and angle of impingement, the transducer output has to be calibrated by comparing shell gape angle with sensor output over a wide variety of angles. To do this, at the end of experiments, the posterior adductor muscle of *M. edulis* was severed with a knife to allow calibration of all possible gape angles with sensor output. Gape angle calibration took ~ 5 mins per mussel. Subsequently, data from sensor output versus gape angle were curve-fitted (for details see Wilson et al. 2002, Wilson & Liebsch 2003, Wilson et al. 2005, Robson et al. 2007). The curve-fit could then be used to determine any gape angle by converting the transducer output accordingly.

The logger used for the work was a 13-channel JUV-Log, equipped with 12 Hall-sensor (Honeywell, SS59E) channels and 1 temperature channel, each with 22 bit resolution, recording gape angle at better than 0.01°. The unit had a 1Gbyte RA memory and could record at rates of up to 2 Hz. The magnets used were 5 x 5 x 2 mm neodymium boron magnets.

Collection and maintenance of mussels for aquarium experiments

Inter-tidal mussels were collected from LR SS630875 Swansea Bay, Wales, UK at low tide and transferred to a flow-through aquarium system within 2 h. Magnets and Hall sensors were glued to mussel shells using Aquarium Sealant (Geocel, Plymouth, UK) before the mussels were replaced in an aerated flow-through aquarium system containing seston-laden sea water from Swansea Bay, Wales, UK for at least a week before being used in experiments. Experiments with mussels in aquaria took place from July to December 2006.

Mussels in standard conditions

Fourteen mussels with an initial mean length of 42.5 mm ± S.D 0.3 and mean wet weight 11.03 g ± SD 0.84 were kept in separate, well-aerated tanks filled with 12 litres of 0.45 μm filtered seawater. Mussel (pseudo)faeces and seawater were removed from tanks and replaced with fresh 0.45 μm filtered seawater once every 24 hours. Mussels were subject to a daily light regime of 13 h light 11 h dark, water temperature 16.2 °C ± 0.4 and each fed a mixed algal diet of ~100 million *Tetraselmis suecica* and ~1000 million *Thalassiosira weissflogii* cells once per day.

Mussel response to detritus

Mussels were subject to the same light regime as in standard conditions (see above). Fourteen mussels were food-deprived for 48 hours in individual well-aerated tanks filled with 12 litres of 0.45 μm filtered sea water. The logger recorded gape angle at 2 Hz for >24 hours. At the start of the experimental period seven mussels each were fed a mixture of 240 x 10^7 *Thalassiosira weissflogii* cells (200 cells μl^{-1}) and 10 g (8.3 x 10^{-4} $g.ml^{-1}$) of red "Ceramofix" particles (Eberhard Faber GmbH, Neumarkt, Germany) ~4 μm diameter (subsequently referred to as "detritus"). A further seven mussels were only fed 240 x 10^7 *Thalassiosira weissflogii* cells. As the experiment progressed, algal cell concentration decreased as mussels removed algae from the water. After being in one experiment, mussels were maintained in standard conditions for two weeks before the experiments were repeated with the same mussels. Thus, all fourteen mussels were subject to both experiments with detritus and no detritus. The number of valve adduction events day^{-1} in response to the addition of detritus at a fixed algal ration was recorded for all mussel experiments.

Data from mussels in the inter-tidal zone

Data in Figure 1 from Oxwich Bay, Swansea, UK (LR SS512854) were collected over 7 days (14 tidal cycles) starting on 19th September 2006 using a routine method (see Wilson et al 2005 for details) involving an archival logger (DK 700 series, Driesen and Kern

GmbH, Bad Bramstedt, Germany) recording gape angle (°), outer mussel shell temperature (°C), light intensity (Lux), water depth (m) and temperature (°C) at 2 Hz.

Subsequent experiments involved eight inter-tidal mussels being temporarily removed from Swansea Bay (LR SS630875) at low tide so that they could be fitted with gape angle sensing systems at Swansea University, UK. Equipped mussels were returned to the inter-tidal within 24 h of initial collection. A magnet was glued to one half of mussel shells and the Hall sensor to the other, so that mussel gape angle could be quantified over time (see Chapter X for full details). Three archival loggers were used for the work and all were 7-channel JUV-Logs (Juv Elektronik, Borstel, Germany), each equipped with 4 Hall sensors (Honeywell, SS59E) also recording light intensity (Lux), pressure (depth (m)) and temperature (°C). Depth could be resolved to an absolute accuracy of better than 1 cm, temperature to better than 0.03°C (although there was lag in the response due to the sensor being encapsulated in resin) and light intensity was measured within the range 0-40,000 Lux with a resolution of ca. 1 Lux. The loggers were powered by 4 x 1.2 V 10 Ah NiMH D cells. Each logger had a 1 Gb flash random access memory and were set to record at a frequency of 2 Hz. The JUV-Log archival loggers had 22 bit resolution recording gape angle at better than 0.01°.

Mussels each with adjacent loggers were placed in the inter-tidal zone on a specially-constructed inter-tidal apparatus (drain pipe) attached to a leg of Mumbles Pier, Swansea Bay, Wales, UK. Five mussels (length (mm) ± SD = 70.3 ± 0.8) were used in experiments lasting 16 days (from 12-28th September 2007) and three different mussels (length (mm) ± SD = 70.4 ± 1.1) were used in experiments lasting 16 days (from 29th September -15th October 2007).

Gape angle and air temperature during emersion

The relationship between gape angle and air temperature was investigated in six of the eight inter-tidal mussels in 32 emersion events (because two mussels died). Firstly, for the mid point of low tide and just before the end of low tide, gape angle was regressed against temperature in a general linear model including mussel identity as a random factor. Secondly, the two data sets were both split into two portions after visual inspection of the data indicated a possible break point at around 14°C, either side of which gape angle appeared to interact differently with temperature. To test whether the data indicate a biphasic interaction as opposed to a single, linear relationship, Burnham and Anderson's (2001) approach for model comparison was used whereby Akaike's information criterion (AIC) were generated for each model (one-line linear regression or two-line linear regression) as a measure of model fit. AICs are measures of the goodness of fit of an estimated model and an operational way of trading off the complexity of an estimated model against how well the model fits the data. The best model has the lowest AIC. Akaike weights were then calculated from the AIC values, enabling assessment of the probability that a model is the best of the candidate set, in this case the one-line versus two-line regression. For both the data for the mid point of low tide and for just before the end of low tide, Akaike weights were calculated to determine whether the one-line or two-line regression was the most suitable.

To test whether the relationship between temperature and gape angle was different during the mid point of low tide and the end of low tide, both at temperatures below 15°C and above 15°C, the relevant data were input to models including temperature, mussel ID

as a random factor, tide period and the interaction between temperature and tide period. The interaction term was removed and the model re-run if the interaction was not significant.

Further statistical analysis

In the laboratory procedures the Mann-Whitney *U* test was used to test for any difference between the numbers of mussel adduction events in response to the same algal ration with and without the addition of detritus. A Mann-Whitney *U* test was also used to test for any difference between the numbers of mussel adduction events in the detritus experiment when mussels did and did not have extended periods (>25 s) gaping <0.1°.

RESULTS

Determinants of mussel gaping behaviour

Calibration of gape angle against Hall sensor output for all mussels had an equation of the form y = a+bexp(-x/c) to produce best fit functions with correlation coefficients in excess of 0.98.

Mussels fed 240 x 10^7 *Thalassiosira weissflogii* cells once per day when 10 g (8.3 x 10^{-4} $g.ml^{-1}$) of detritus ~4 μm diameter was added to each tank adducted their valves significantly more than at the same algal ration without the addition of detritus (median number of adduction events per day = 31.5 and 175 respectively; Mann-Whitney *U* 301, p <0.00001, n = 14) (Fig. 2). The mussels could not be seen during the 24 hours when 8.3 x 10^{-4} $g.ml^{-1}$ of detritus was added to the water in which the mussels were held and 8.0 x 10^{-4} $g.ml^{-1}$ ± SD 1.4 of detritus was still in suspension after 24 hours. Thus, mussels could not been seen at the end of experiments either. When the tanks were emptied after the experiment there was no evidence that the mussels had produce faeces containing detritus (mussel faeces was green (evidence of algae) but did not contain "red" particles of detritus). The minimum and maximum number of adduction events when mussels were subjected to detritus was 161 and 372 per day respectively (equivalent to on average an adduction once every 9 and 3.9 minutes respectively). Mussels exposed to detritus that had extended periods (>25 s) gaping <0.1° adducted significantly less per day than mussels which did not gape <0.1° for extended periods (median number of adduction events per day = 150 and 301 respectively; Mann-Whitney *U* 77.0, p <0.00022, n = 7). For example, the mussel exposed to detritus in Figure 3a adducted 161 times per day and

had 3 extended periods of gaping <0.1° lasting 1h 5 mins, 2 h 10 mins and 36 mins at a gape angle less than 0.1°. The mussel in Figure 3b had no extended periods of gaping <0.1° and adducted 372 times day per day.

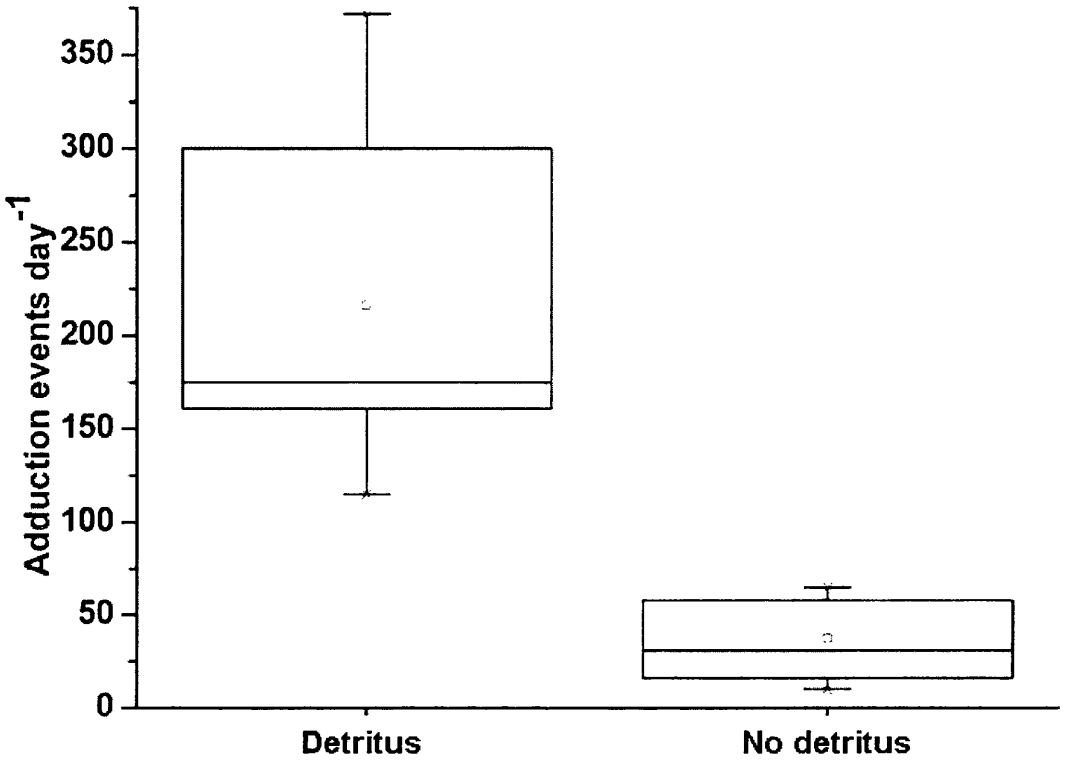

Fig. 2 Box-plots of mussel shell valve adduction events day^{-1} from mussels fed 240 x 10^7 *Thalassiosira weissflogii* cells day^{-1} with and without the addition of 10 g (8.3 x 10^{-4} $g.ml^{-1}$) of detritus ~4 μm diameter.

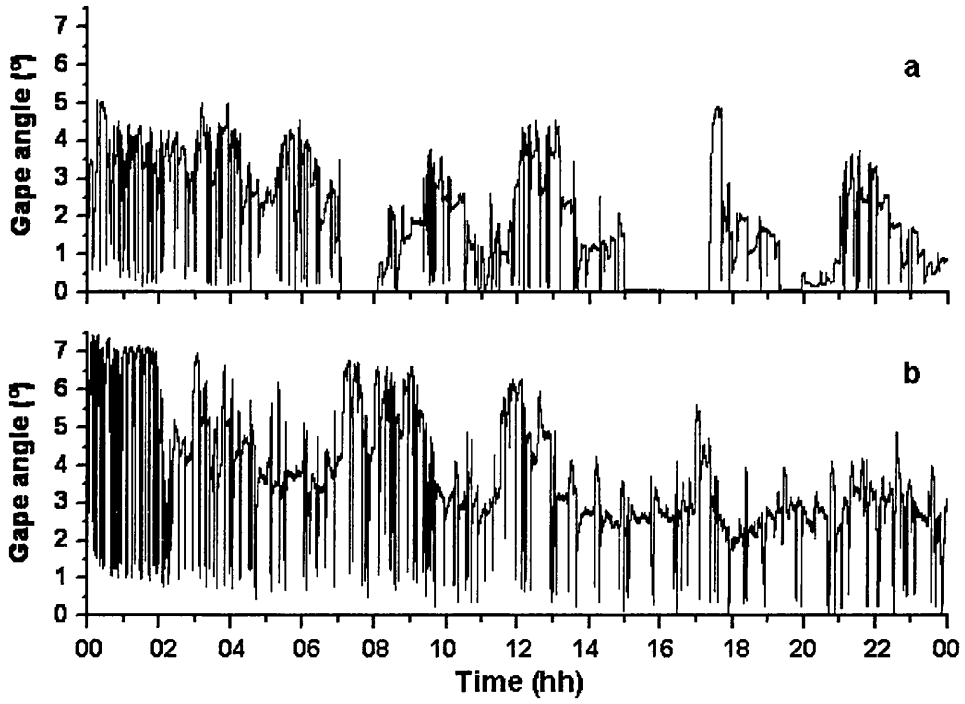

Fig. 3 Two examples of gape angle *versus* time when fed a mixture of 240 x 10^7 *Thalassiosira weissflogii* cells (200 cells μl^{-1}) and 10 g (8.3 x 10^{-4} $g.ml^{-1}$) of 240 x 10^7 of detritus ~4 μm. One example with extended closures (a) and one without (b).

Inter-tidal mussel behaviour

The temperature of immersed wild mussels was relatively constant over periods of days (c.f. Fig. 1) while the emersed state showed considerable variation (e.g. the outer mussel shell temperature was higher than when immersed during daylight, due to insolation, and lower at night (c.f. Fig. 1). Short-term complexities in temperature variation are to be expected due to local phenomena such as shallow (primarily receding) water being warmed by the sun (e.g. on 22.9.06 Fig. 4a), mussels being warmed by the sun when emersed (e.g. on 21.9.06 Fig. 4b) and evaporative cooling of the mussel shells (e.g. Fig. 4a and b).

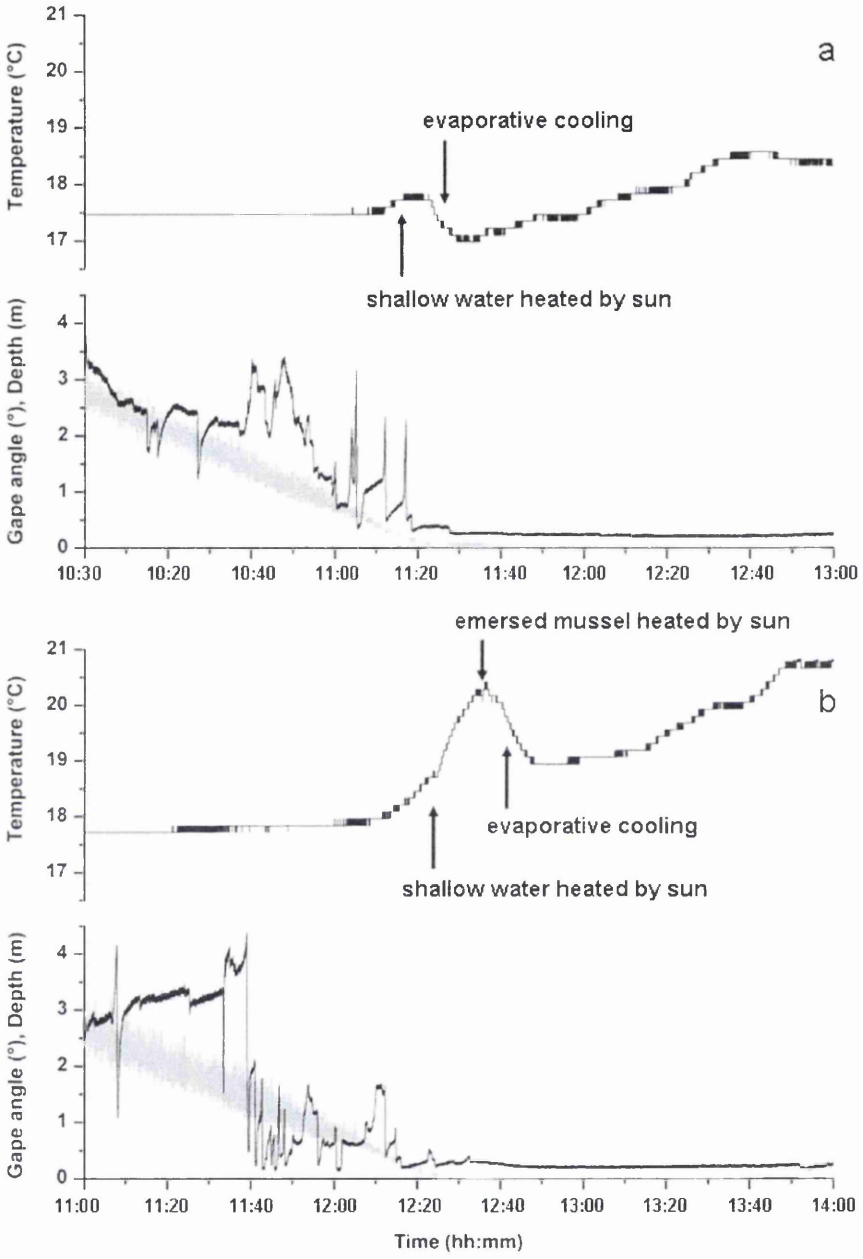

Fig. 4 Variation in gape angle (black line), shell temperature and water depth (grey line) of a mussel at Oxwich Bay, Wales, UK showing evaporative cooling of the mussel shell in daylight when the mussel was emersed at low tide on 22.9.06 (a) and 21.9.06 (b).

Two mussels died during the study, one on 5th of October 2007 (Fig. 5a) and one on 11th October (Fig. 5b). Repetitive valve adduction events were apparent in both mussels before eventual cessation of valve movement (assumed to be death), although only one of the two animals exhibited repetitive valve adduction events during emersion (Fig 5 a). Repetitive valve adduction did not start immediately the mussels were immersed by both tides in Figure 5a and the first tide in Figure 5b. Gape angle after valve adduction and subsequent abduction events generally decreased as immersion time increased in the first immersion event in Figure 5b and then generally increased in the second immersion event until a 2.2° decrease and reduction in the amount of valve movement and eventual death. Figure 6a and b shows the detailed transition from what was considered to be normal gape behaviour to abnormal gape behaviour in the mussels in Figure 5a and b, respectively.

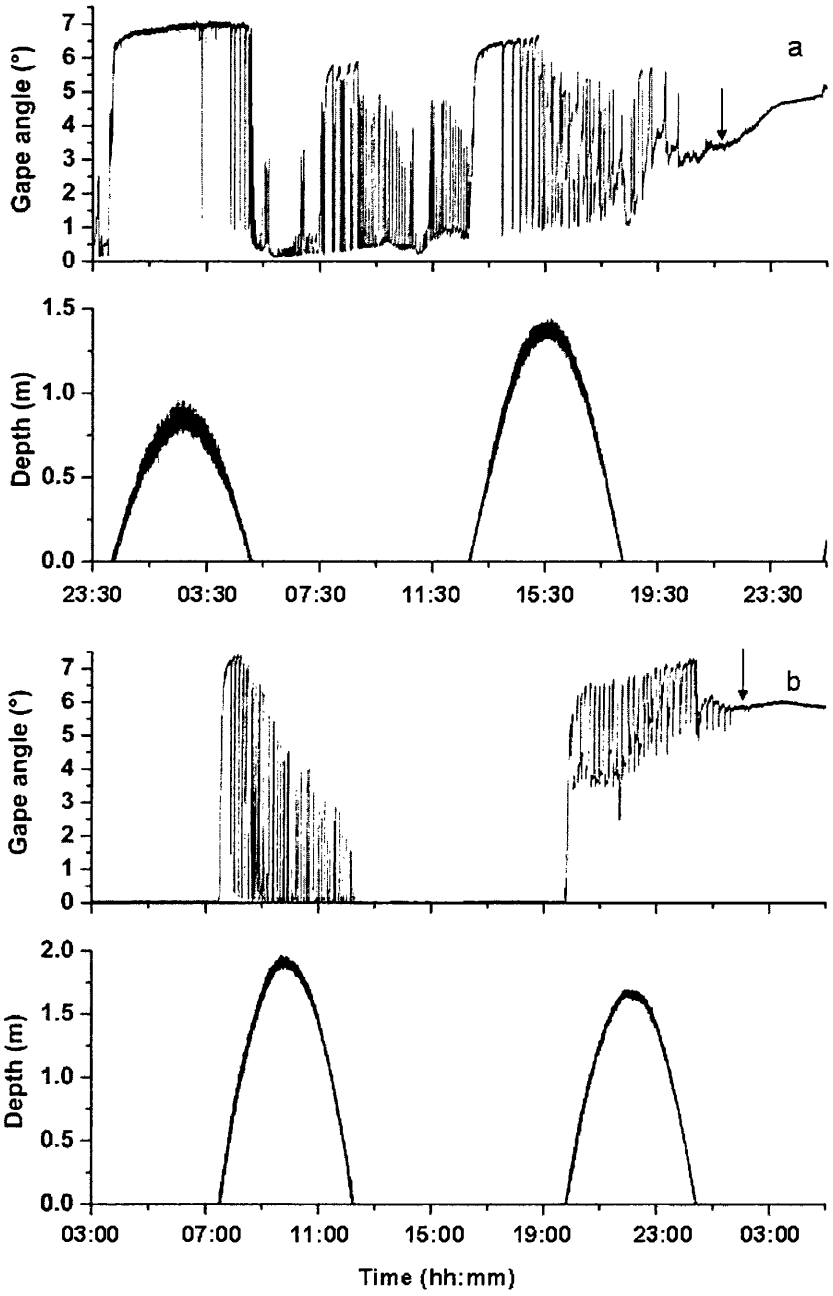

Fig. 5 The gape behaviour and death of two inter-tidal mussels in Swansea Bay, Wales, UK, one on 5th of October 2007 (a) and one on 11th October (b). Arrows indicate estimated time of death.

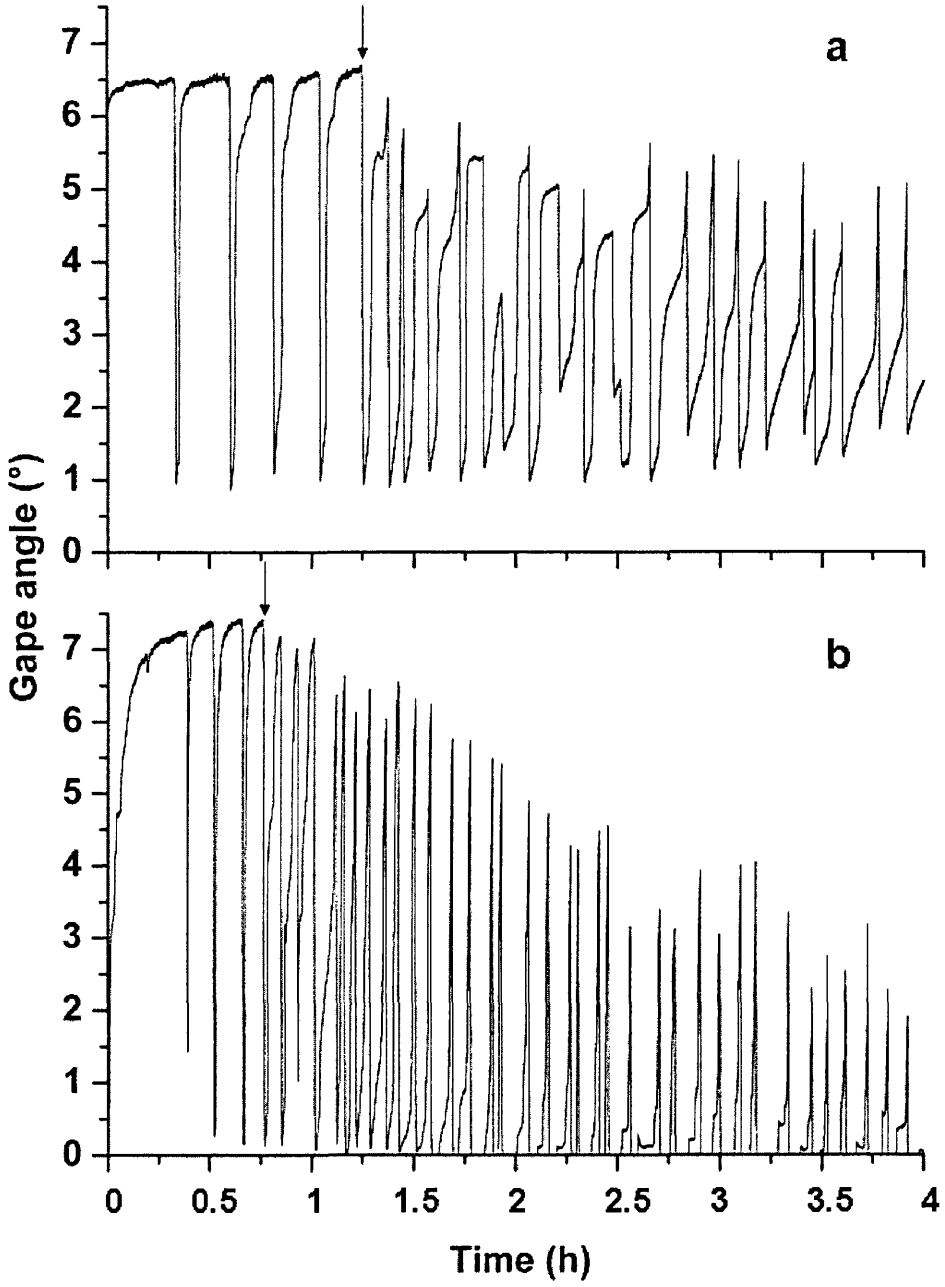

Fig. 6 Detailed transition from what appeared to be normal mussel gape behaviour to unusual gape behaviour (estimated point of transition indicated by arrow) of two inter-tidal mussels that eventually died in Swansea Bay, Wales, UK on 5th of October 2007 (a) and 11th October (b).

Gape *versus* air temperature

For both the data for the mid point of low tide and for the end of low tide, the Akaike weights strongly indicate that the two-line models are more suitable than the one-line models to describe the relationship of gape angle with air temperature (Table 1). In turn, this suggests a biphasic description for mussel gape angle and air temperature across the temperatures measured in the present study. The relationships between gape angle and air temperature at the two tidal periods are shown in Figure 7 a and b, respectively.

Table 1 Models of air temperature to describe gape angle in mussels during two tidal periods, including mussel ID as a random factor.

Model	AIC	Akaike weights	R^2
Mid-point, one line	-388.2	0.000	0.62
Mid-point, two lines	-582.8	1.000	-
End, one line	-457.0	0.000	0.70
End, two lines	-526.6	1.000	-

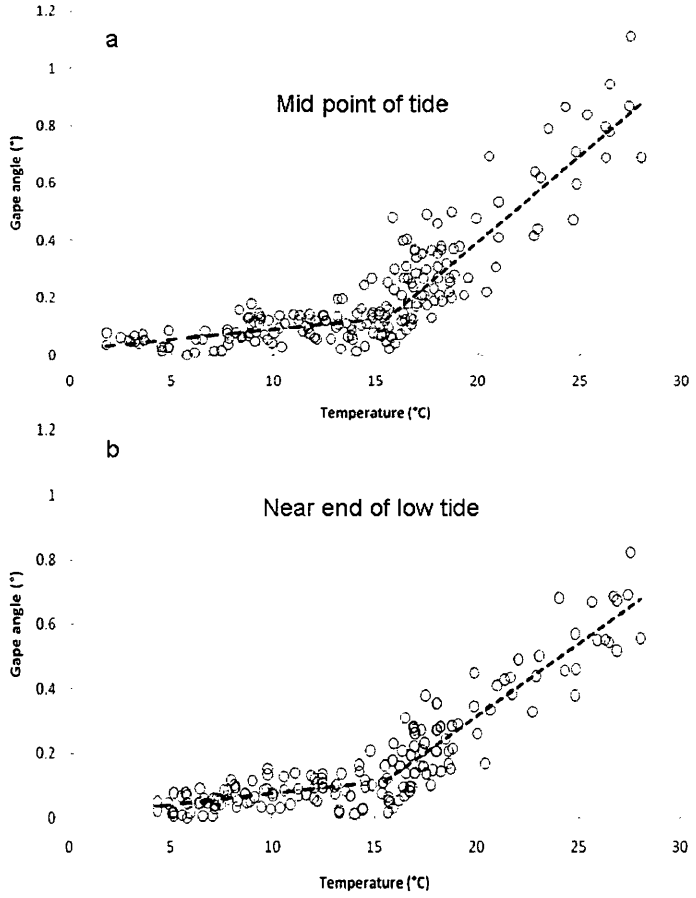

Fig. 7 (a) The interaction between mussel gape angle and air temperature at the mid-point of low tide, described by a two-line linear regression. Below about 15°C gape angle increases slowly with increasing temperature ($y = 0.007x + 0.019$; $R^2 = 0.26$; long-dash line). Above around 15°C, gape angle increases more quickly with increasing temperature ($y = 0.060x - 0.805$; $R^2 = 0.77$; short-dash line). (b) The interaction between mussel gape angle and air temperature at the end point of low tide, described by a two-line linear regression. Below about 15°C gape angle increases slowly with increasing temperature ($y = 0.007x + 0.006$; $R^2 = 0.25$; long-dash line). Above around 15°C, gape angle increases more quickly with increasing temperature ($y = 0.045x - 0.584$; $R^2 = 0.80$; short-dash line).

A model of set temperature, mussel ID as a random factor and tide period to describe gape width for all data where temperature was less than 15°C, showed all factors to be significant ($P<0.001$). However, the inclusion of the interaction between tide period and air temperature was not significant. These findings indicate that, below around 15°C, while gape angle during the mid point of low tide was significantly higher ($P<0.001$) than during the end point of low tide, tide period did not affect the relationship between sea temperature and gape angle i.e. the relationship gradient is similar during both tide periods. The same model was run for the data for temperatures about 15°C. This time, the interaction as well as all factors were significant ($P<0.001$). At temperatures above about 15°C, gape angle increased more rapidly as temperature increased during the mid-tide than during the end tide and, indeed, gape angle was greater during the mid tide.

DISCUSSION

Mussels valve movements have been recorded for over fifty years (Lowy 1953), yet their role is not fully understood (Shick et al. 1986, Shick et al. 1988). This study provides further evidence that valve adduction events constitute a normal part of *Mytilus* behaviour and that they occur in the inter-tidal zone. It is unclear why mussels subject to detritus, adducted their valves more than mussels without the addition of detritus at the same daily algal ration. Variation in the number of adduction events when mussels were subject to detritus may, in part, be related to the amount of time the mussel spent gaping <0.1° for extended periods. It has been suggested that valve movements of bivalves are closely related to vital activities such as respiration, feeding and excretion (Nagai et al. 2006).

Shell valve activity may be involved in enhancing perfusion of the tissues by newly re-oxygenated haemolymph (Shick et al. 1986, 1988). Mussels filter-feeding algae and detritus may have had to expend more energy on pre-ingestive particle sorting than mussels fed pure algae (however mussels grow better on a mixed diet including inorganic detritus (Bayne et al. 1987)). Thus, mussels exposed to detritus and algae may have had a greater oxygen demand and used frequent valve adductions to enhance perfusion of the tissues by newly re-oxygenated haemolymph (see Shick et al. 1986, 1988). Another possibility is that the mussel mucociliary rejection pathway out of the top of the inhalant siphon (Widdows et al. 1979b, Beninger & StJean 1997, Beninger et al. 1999) became overloaded with detritus because Widdows et al. (1979b) reported that both filtration rate and pseudofaeces production declined at high particle concentration. Thus, valve adduction may have been required to expel excess detritus which would reduce filtration rate. This is complimentary to the hypothesis that above suspended sediment concentration (SSC) ~100 mg L^{-1} cockles (*Cerastoderma edule*) filtration and rejection mechanisms become overloaded and cannot produce enough pseudofaeces to cope with the accumulating sediment in their mantle cavity (Ciutat et al. 2007). Another possibility is that as a means of dealing with the excess detritus, mussels in the current study may have more intermittent and shorter filtration periods compared to those not exposed to detritus and thus, adduct more per unit time, to try to prevent their filtration and rejection mechanisms becoming overloaded (see above and c.f. Widdows et al. 1979b, Riisgard & Randlov 1981, Clausen & Riisgard 1996, Macdonald & Nodwell 2003). *M. edulis* has been reported to re-suspend particles in faeces (Hildreth 1980) and one consequence of valve adduction is likely to be re-suspension of loosely-consolidated faeces and

pseudofaeces and other small benthic particulate matter. As there was little reduction in the amount of detritus suspended in the seawater after 24 h, mussels in the experiments appear to have been sorting the detritus from the algae (mussel faeces was green with no evidence of red detritus particles) and re-suspending the detritus.

M. edulis is able to respire both in air and in water (Read 1962) and may also resort to anaerobic respiration under conditions of stress (notably desiccation) (Dodgson 1928, Von Brand 1946). The ability to respire in air involves a compromise between access to air at the respiratory surface and minimizing evaporative water loss during necessary gaping (Newell 1973, Widdows et al. 1979a). This may explain why at temperatures above about 15°C, mussel gape angle significantly decreased as the time since emersion increased from the mid to near the end of low tide.

As suggested by Shick et al. (1986), we found that the degree of air gaping was positively related to temperature. Mussels in air at 15°C and below may consume little or no oxygen when emersed due to temperature-related metabolic effects (Widdows 1973) and their low valve gape angles (mostly <0.2°), will tend to favour anaerobic respiration anyway . In air temperatures above about 15°C, the increase in gape angle may be related to evaporative cooling, as well as allowing oxygen to be supplied to particular organs e.g. the digestive gland, to enable aerobic metabolism there (see Shick et al. 1986, Shick et al. 1988). Mussels in air at 15°C and below may behave in a similar manner to "winter" mussels (in low air temperatures) and those in air above about 15°C to "summer" mussels, when high temperatures may necessitate evaporative cooling to avoid hyperthermia (see Shick et al. 1986, Shick et al. 1988). The conflicting pressures of desiccation, aerial respiration, and evaporative cooling on emersed mussels are likely to

be complex because mussels may gape with their siphons shut. Changes in humidity and wind speed and direction will also have an effect on desiccation and evaporative cooling, as will the presence of food in the gut on the need for aerial respiration. Osmotic stress caused by rainfall may also have an effect on the gape angle of emersed mussels.

High temperatures have been linked to mussel mortality (e.g. Anestis et al. 2008). However, hyperthermia may not have been the cause of death of two mussels in this study. There are a wide variety of viruses, bacteria, protozoans and metazoans that infect inter-tidal invertebrates (see Sindermann 1990). Among the metazoans, trematodes are the most common parasites of inter-tidal animals e.g. castration of *M. edulis* by the trematode parasite *Prosorhynchus squamatus* (Coustau et al. 1993). By definition any parasite will damage its host to a certain degree, with effects ranging from minor metabolic changes to severe tissue destruction (see Price 1980, Lauckner 1983). When conditions approach the limit to which the host is adapted, reduced survivorship may be the rule (Mouritsen & Poulin 2002). This can be expected to apply in particular to inter-tidal species such as *M. edulis* that are exposed to wide fluctuations in food availability, mechanical stress from wave action, temperature, oxygen availability, osmotic stress from rainfall, and desiccation. Bivalves acting either as first or second intermediate host of trematodes experience reduced condition, reduced byssus thread production (in the case of *M. edulis*), and may be easier to open by predators (Lauckner 1983, Calvo-Ugarteburu & McQuaid 1998). Parasites commonly alter the behaviour of their hosts either as a side effect of infection, or as an adaptive manipulation by the parasite with the purpose of facilitating transmission (reviewed by Poulin 1994). The transition from what was consider to be normal mussel gape behaviour, to abnormal may have been parasite

induced. The atypical behaviour is similar to *M. edulis* valve gape response to copper (Curtis et al. 2000). The high frequency of valve adduction events and high gape angles (up to 5.9°) associated with one mussel during emersion was most unusual (Fig 5a). Shell valve adductions are correlated with energy (net ATP) utilization (Shick et al. 1986) and a multitude of short valve adduction events have not been found to change systematically with heart rate or heart-rate variability (Curtis et al. 2000), so it may be that over-exertion contributed to mussel mortality. It has been suggested that trematode infections enhance the activity of another bivalve *Macoma balthica* (Mouritsen 1997).

The gape data presented here allow mussel response to be measured in real time without the need to kill the bivalve. This contrasts with other methods, which include measuring stress proteins e.g. HSP70 (e.g. Snyder et al. 2001, Anestis et al. 2008) and immune changes (Lacoste et al. 2002) where stress cannot be measured in real time, or at the instant the stressor is applied. Mussel mortality is often expressed as mortality per day (e.g. Anestis et al. 2008). However, assessment of bivalve gape may allow mussel mortality to be studied in a more refined manner due to the onset of aberrant behaviour preceding death. Given the variation in the gape behaviour of the two mussels before death (e.g. atypical gaping when emersed in one mussel and not the other) gape angle may be an important parameter to measure in relation to why mussel mortality occurs (c.f. Calvo-Ugarteburu & McQuaid 1998). Although Calvo-Ugarteburu & McQuaid (1998) recorded the gape (open or closed) of mussels infected with parasites at 2 h intervals, our results suggest that gape angle could be considered over a fine temporal scale because we found mussel gape to be often highly variable, even over periods as short as one minute (c.f. Robson et al. 2007).

The data recorded in the current study demonstrate the high variability in air temperatures experienced by inter-tidal mussels in their natural environment in the sea and air and, as suggested by Anestis et al. (2008), may be useful in evaluating the relevance of laboratory bivalve thermal tolerance studies to their natural environment. It is unclear what the reasons for valve adduction and abduction events are in the current study although the explanation is liable to be complex and involve several environmental parameters. Indeed, the value of recording the fine-scale gape behaviour of bivalves is limited unless specific reasons can be attributed to each event. In order to rectify this, many more parameters of mussels and their environment need measuring at high temporal resolution. Examples of this are; aspects of neurobiology, including muscle action potentials (Lowy 1953), video endoscopy of the gill filter-feeding and pseudofaeces rejection tracts, oxygen uptake, haemolymph PO_2 (Shick et al. 1986), heat dissipation (Shick et al. 1986), mussels prey and their environment. Only when these parameters have been recorded at a high resolution may we be able to start to fully understand the complex valve behaviour of bivalves.

In summary the results of this chapter showed that immersed mussels adducted their shell valves significantly more often when exposed to detritus (8.3×10^{-4} g.ml^{-1}, ~ 4μm) than when in a detritus-free environment. It was suggested that mussels exposed to detritus may have more intermittent and shorter filtration periods compared to those not exposed to detritus and thus, adduct more per unit time, to try to prevent their filtration and rejection mechanisms becoming overloaded. The results also highlighted that inter-tidal mussels can be exposed to high variability in air temperatures in their natural environment in sea and air. When emersed gape angle increased with air temperature in

the inter-tidal zone in Swansea Bay (Wales, UK) and was described by a biphasic regression with the break point at 15°C. Mussels may balance potential exposure to desiccation and osmotic stress (e.g. following rainfall), with the need for aerial respiration and evaporative cooling. It was also suggested that mussels may gape with their siphons shut and the presence of food in the gut can influence the need for aerial respiration. Finally the transition in two inter-tidal mussels from normal gape behaviour, to abnormal may have been parasite induced because parasites commonly alter the behaviour of their hosts.

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Chapter 6

Behaving properly in an environment with limited access to food; the case of the blue mussel *Mytilus edulis* in the inter-tidal zone[f]

Abstract

Mussels, *Mytilus edulis*, in the Atlantic inter-tidal zone were fitted with Hall sensor systems to determine how gape angle varied in relation to a range of parameters when animals were both immersed and emersed. Inter-tidal mussels were in very different environments ranging from predominantly aerial (65.9% aerial) to predominantly aquatic (72.1% aquatic). In general, gape angle during immersion decreased as the time mussels were immersed increased. Tidal variations were found in the behaviour of mussels i.e. in general mussels gaped <1° when emersed and >1° when immersed. Although one mussel in winter 2008 (immersed 70.8% of the time) did not gape >1° when immersed by four non consecutive tides. Despite immersion and emersion by the tide approximately twice a day and the general increase in gape angle from 0-3 m seawater depth, a significant circadian rhythm was found, with gape angle generally greater in darkness in inter-tidal mussels. It is unclear what the adaptive significance of this finding might be. One possibility is that such day-night gape behaviour may be part of a strategy to feed while

minimizing the likelihood of predation by visually-feeding predators. In general gape angle and valve adduction rate significantly decreased from summer 2007 to winter 2008, possibly related to seasonal variations in the edible seston biomass, including zooplankton in coastal waters. Although mussels were typically more active at night, a striking feature of the inter-tidal mussel gape and adduction behaviour in the current study was the wide intra- and inter-individual variation within and between immersion events.

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[f]The content of this chapter is intended for publication: the author order will be Robson AA, Wilson RP, Garcia de Leaniz C, Forman DW, Liebsch N, Halsey LG

INTRODUCTION

Animal foraging behaviour usually involves trade-offs between food acquisition and danger because options that increase the rate of foraging gain often increase the probability of predation (c.f. Bednekoff 2007). Danger affects animal decisions in many ways (see reviews in Lima & Dill 1990, Lima 1998). For example, guppies *Poecilia reticulata* (one of the most popular freshwater aquarium fish species in the World) feed during the day and at night when no predators are present, but only during the daylight hours if predators are near (Fraser et al. 2004). Many animals restrict their feeding time in response to danger (Lima 1998) and any animal that feeds periodically, as opposed to continuously, can restrict its feeding time(s) to the safest and or most productive period, but may sometimes have to extend this feeding time into more dangerous and/or less productive periods in order to acquire nutrients during certain particular critical periods. While actively seeking food, animals often have to choose between habitats that differ in danger and productivity.

Animals living in the inter-tidal area are exposed to very particular conditions relating to food acquisition and predation pressure. Marine invertebrates in this environment can generally only acquire food when immersed and are subjected to two predator types, those that prey on them when they are immersed (such as starfish, crabs, dogwhelks and eider ducks), and those that prey on them when they are (nearly) emersed (such as oystercatchers and land-based predators) (Bumann et al. 1997). The degree to which sessile invertebrates living in the inter-tidal can maximize their fitness depends on their precise position on the shoreline because they are accordingly subject to the

complex interaction of the times and durations of immersion and emersion with all the predation risks and feeding opportunities that this entails. Thus, while mobile inter-tidal invertebrates may take steps to enhance fitness by moving, sessile animals can only modulate their behaviour.

The inter-tidal zone is an extreme and variable environment with a steep gradient of physical stresses, some of which increase while others decrease in severity further up the inter-tidal zone (Connell 1961). Inter-tidal animals are sensitive to thermal and osmotic/desiccative stress as well as a suite of predators from the land, sea and inter-tidal zone. Emersed inter-tidal animals are not only subjected to wide variations in air temperature but all weather, including precipitation (e.g. rain, hail and snow), high winds, and to extreme physical buffeting (e.g. from large waves, or substantial currents). Withstanding these severe challenges, large areas of the inter-tidal zone often are dominated by suspension feeders such as barnacles and bivalve molluscs.

Bivalves have a series of behavioural traits that enable them to maximize survival in the inter-tidal zone. Anaerobic respiration can be used during anoxia (e.g. Bayne et al. 1976, Widdows et al. 1979a) and energy acquisition may be enhanced by continuing digestion and absorption during emersion when feeding stops (Langton 1975, 1977, Robinson et al. 1981, Shick et al. 1986, Shick et al. 1988). While shell valve closure conserves water, bivalves such as mussels may gape in air, not only to promote evaporative cooling (especially in small *M. edulis* (Gnaiger 1983)), but also to allow aerobic respiration for continued digestive processes during emersion (see above - Shick et al. 1986, Shick et al. 1988). Beyond this, inter-tidally acclimated bivalves have been found to pump more or less continuously when immersed compared with a more variable

and intermittent pumping in sub-tidal bivalves (Brand & Taylor 1974, Widdows & Shick 1985) and higher shore *M. edulis* resume feeding more rapidly than lower shore *Cerastoderma edule* (Widdows & Shick 1985).

Thus, research has shown the extent to which the complexities of the inter-tidal environment appear to have led to remarkable behavioural strategies from its sessile, or minimally motile inhabitants for maximizing fitness. However, such studies have been limited in their capacity to quantify behaviours in this difficult environment. We use a new method for examining the extent of gaping in inter-tidal blue mussels according to circumstance. Increased gaping tends to be indicative of increased feeding and increased metabolic activity (Famme 1980, Jørgensen et al. 1986a, Jørgensen et al. 1986b, Riisgard et al. 2003) and is considered by some to be a useful single proxy for general mussel activity (Dolmer 2000, Wilson et al. 2005, Saurel et al. 2007). This study seeks to identify differences in gaping behaviour in individuals according to their position along the inter-tidal gradient, which will influence the amount of time that they have for feeding (the immersed state) and the extent to which they are exposed to predators of one sort or another (marine or terrestrial), the vagaries of the weather (the emersed state) and the day/night cycle. The study took place on Mumbles Pier in the Bristol Channel, UK, which has the second highest tidal range in the World (up to 15 m) and was thus an ideal site for this type of study.

MATERIALS AND METHODS

All research detailed below was conducted in accordance with institutional, national and international guidelines relating to the use of bivalves in research.

Collection and maintenance of bivalves in experiments

Inter-tidal *Mytilus edulis* were collected from Swansea Bay, Wales, UK, (LR SS630875) at low tide and transferred to a flow-through aquarium system within 2 h.

Overall experimental design

To make valve gape measurements in millimetres relative between bivalves of different lengths, the methods developed by Wilson et al. (2005) were modified to quantify gape angle in blue mussels *M. edulis* (see Robson et al. 2009). Briefly, this involved using a Hall sensor (a transducer for magnetic field strength) attached to one shell valve reacting to a magnet attached to the other shell valve. Variation in gaping extent produced a corresponding variation in the magnetic field strength perceived by the Hall sensor (c.f. Wilson et al. 2002). This was recorded by an archival tag. Since Hall sensor output is proportional to magnetic field strength and angle of impingement, the transducer output has to be calibrated by comparing shell gape angle with sensor output, over a wide variety of angles. To do this, at the end of experiments, the posterior adductor muscle of *M. edulis* was severed with a knife to allow calibration of all possible gape angles with

sensor output. Gape angle calibration took ~ 5 minutes per mussel. Subsequently, data from sensor output versus gape angle were curve-fitted (for details see Wilson et al. 2002, Wilson & Liebsch 2003, Wilson et al. 2005, Robson et al. 2007). The curve-fit could then be used to determine any gape angle by converting the transducer output accordingly.

Three archival loggers were used for the work and all were 7-channel JUV-Logs (Juv Elektronik, Borstel, Germany), equipped with 4 Hall sensors (Honeywell, SS59E) and also recorded light (Lux), pressure (depth (m)) and temperature (°C). Depth could be resolved to an absolute accuracy of better than 1 cm, temperature to better than 0.03°C (although there was lag in the response due to the sensor being encapsulated in resin) and light intensity was measured within the range 0-40,000 Lux with a resolution of ca. 1 Lux. The loggers were powered by 4 x 1.2 V 10 Ah NiMH D cells. Each logger had a 1 Gb flash random access memory and could be set to record at intervals up to a maximum frequency of 12 Hz. The JUV-Log archival loggers had 22 bit resolution recording gape angle at better than 0.01°. The magnets used were 5 x 5 x 2 mm neodymium boron magnets.

Inter-tidal apparatus

The inter-tidal apparatus (Fig. 1) was designed to accommodate 12 mussels equally spaced over 3 m vertical height (mussels being approximately 0.27 m apart). A 3.06 m long, 4 mm thick plastic drainpipe with an internal diameter of approximately 152.5 mm was cut into three lengths of approximately 1.01 m (pipe 1), 1.11 m (pipe 2) and 0.94 m (pipe 3). Two drainpipe connectors approximately 180 mm long, 16 mm thick (at the

widest point) and 178 mm internal diameter were used to connect pipes 1 to 2 and 2 to 3. Holes approximately 15 mm diameter were then drilled into the > 3 m high inter-tidal apparatus, so that the centre of the holes were placed at the following (approximate) distances from the end of the pipe; 0.03, 0.30, 0.58, 0.85 m (on pipe 1), 1.12, 1.39, 1.67, 1.94 m (on pipe 2), 2.21, 2.48, 2.76 and 3.03 m (on pipe 3) vertical height along the inter-tidal apparatus.

Then, aluminium tubing (15 mm diameter, 1.0 mm wall thickness and 45 mm long) was glued in each of twelve holes using high strength epoxy adhesive (Power-Fast®+, Powers Fasteners, Inc., Brewster, NY, USA), so that approximately 18 mm of aluminium tubing protruded from the inter-tidal apparatus. One end of aluminium tubing (13 mm diameter, 1.0 mm wall thickness and length of 45 mm) was placed 20 mm inside the tubing protruding out of the inter-tidal apparatus, encircling the 13 mm diameter tubing with duct tape to ensure a good fit inside the 15 mm diameter tubing. Holes 2.5 mm in diameter were drilled approximately 10 mm away from the end of the 15 mm tubes, through both aluminium tubes. For each of the three sections of drainpipe, the four 3 m long wires from a logger were threaded inside the drainpipe and the Hall sensors at the end of the wires were glued inside the four outwardly-protruding 13 mm diameter tubes close to the end of the tubing using high strength epoxy adhesive. The outwardly-protruding ends of the 13 mm diameter tubes were then carefully filed to ensure good contact with the mussels left valve which was then itself glued to the end of a 13 mm diameter tube using high strength epoxy adhesive.

Magnets were then stuck to the right valve of each mussels using high strength epoxy adhesive, ensuring good alignment with the Hall sensor inside the tube glued to the

mussels left valve. Holes were made in the piping to allow the loggers to be pushed in at three (roughly equidistant) points up the length of the piping on the inter-tidal apparatus. Loggers were pushed partway inside the piping so that the light, pressure and temperature sensors were protruding outside, and the end of the loggers with wires to Hall sensors were inside the drainpipe and glued in position using Marine Silicone Rubber Sealant (Dow Corning®, Midland, Michigan, USA). With the drainpipes connected, holes were drilled 35 mm from the top of the two connectors and thus also near the bottom of the associated drainpipes 2 and 3, so that a 20.5 mm long M6 brass bolt could be passed through the holes and secured with two bolts at either end to hold the battery boxes (power supply) of the associated loggers in place.

A 150 mm diameter black plastic square drain grid was used to keep the battery box and the logger inside the bottom of drainpipe, fixed using appropriately-drilled holes and cables ties passed through the holes and the drain grid. A 150 mm diameter black plastic square drain grid was also fixed to the top of the inter-tidal apparatus to ensure no objects greater than 7 mm diameter could pass inside the drainpipes. A 30 mm long M2.5 brass bolt with nut and washer secured the two tubes together with a mussel attached to each 13 mm diameter tube. Six custom-made stainless steel brackets with one curved face designed to press against a pier leg and one against the inter-tidal apparatus were fixed approximately 1.5, 2, 2.5, 3, 3.5 and 4 m from the bottom of a leg of Mumbles Pier, Swansea Bay, Wales, UK at a spring low tide using 11 mm diameter stainless steel metal banding, housings and slotted screws (Stranglehold Banding®, SEAC Ltd, Leicester, UK). The inter-tidal apparatus complete with mussels was fixed to the brackets using stainless

steel metal banding, housings and slotted screws when it was deployed at spring low tides in the inter-tidal zone.

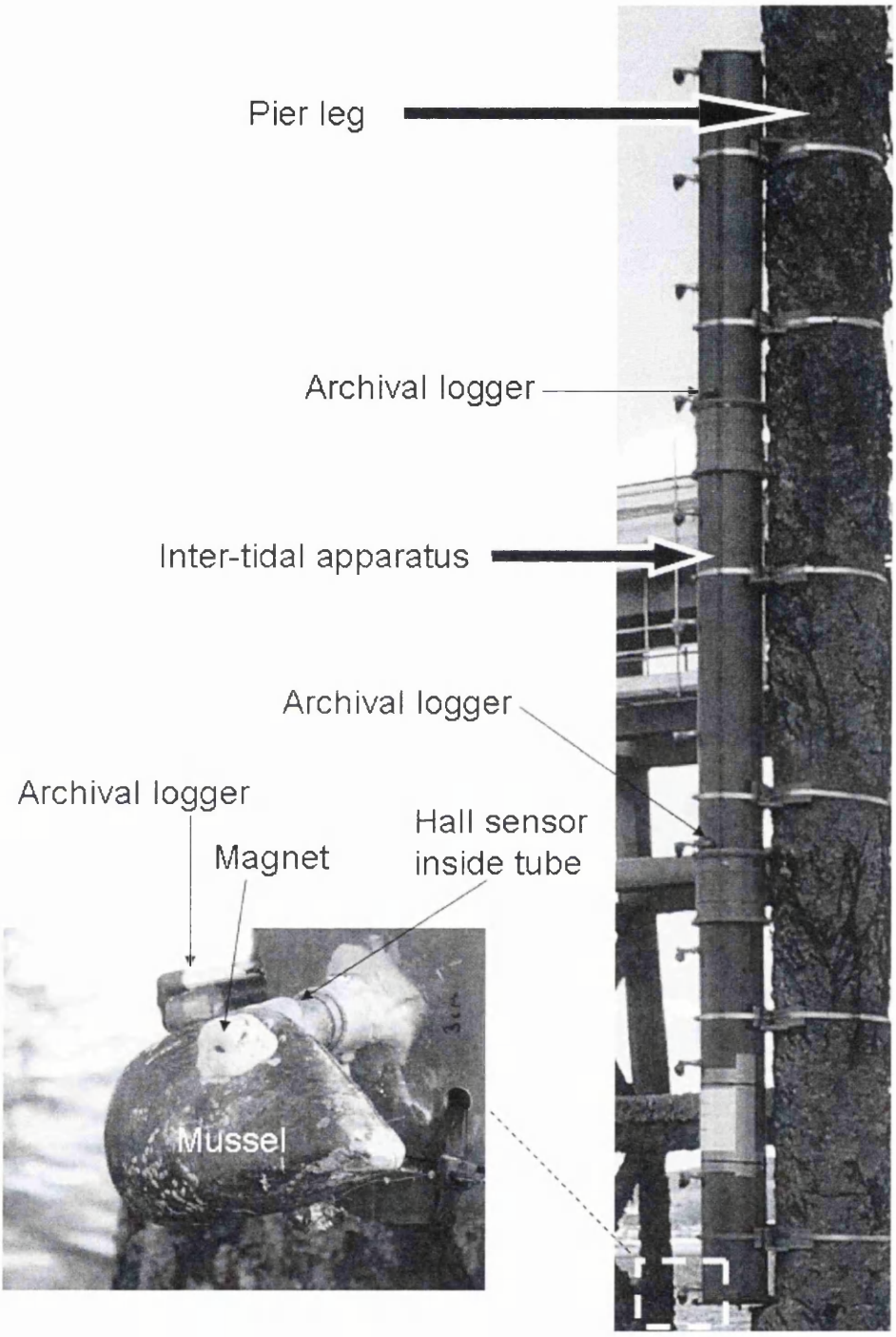

Fig. 1. The inter-tidal apparatus attached to a pier leg including mussel attachment, location of magnets and Hall sensors on mussels and location of archival loggers relative to mussels.

The 13 mm diameter tubes (containing Hall sensors) and magnets were attached to mussels and, before the animals were re-located in the inter-tidal zone, they were placed in an aerated flow-through aquarium system containing seston-laden seawater from Swansea Bay, Wales, UK. The loggers were started approximately 2 hours before deployment. A sampling frequency of 2 Hz was used to record *M. edulis* gape angle and environmental parameters. Equipped *M. edulis* were returned to the inter-tidal within 24 h of initial collection. Twelve mussels (length (mm) ± SD = 70.8 ± 1.3) were in inter-tidal experiments lasting 14 days (from 2nd -16th August 2007) in summer and twelve different mussels (length (mm) ± SD = 70.5 ± 1.0) in experiments lasting 14 days (from 12-28th September 2007) in autumn. Four mussels (length (mm) ± SD = 70.7 ± 0.5) at approximately 0.03, 0.30, 0.58 and 0.85 m up the inter-tidal apparatus were in inter-tidal experiments lasting 43 days (from 10th January - 22nd February 2008) in winter. At spring low tides, when the inter-tidal apparatus was accessible to the public, a researcher observed the inter-tidal apparatus from approximately 100 m and ensured the public did not interfere with the archival loggers or the associated mussels.

Statistics

All data were tested for auto-correlation before analysis and, where appropriate, the data set was reduced before statistical analysis. The percentage time mussels were immersed in seawater was converted to radians to normalize the data for statistical analysis. Linear regression was used to look for any relationship between immersion time and mean gape angle (°).

A repeated measures ANOVA was used on mean gape data with day (> 0.22 Lux) /night (≤ 0.22 Lux) as the repeated measure and season as the between-subjects factor to test for any difference in mussel gape behaviour between day/night and between seasons (summer 2007, autumn 2007 and winter 2008). A two sample t-test was used to test for any difference between means of shell valve adduction events between day and night. Post-hoc extraction for the Kruskal-Wallis test with Bonferroni correction was used to test for significant differences in pooled shell valve adduction data between seasons.

A Kruskal-Wallis test was used to test for any time difference between the first wave in a tidal cycle hitting the mussel and the mussels abducting >1° and the last wave in a tidal cycle hitting the mussels and the mussels adducting <1°. One-way ANOVA and post-hoc Tukey tests were used to test for any difference in mean gape angle with seawater depth. One-way ANOVA and post-hoc Tukey tests were used to test for any difference in mean wave amplitude (using a mean of fifty random wave amplitudes per tidal cycle from the depth readings of three loggers recording simultaneously = three mean wave amplitudes per tidal cycle) for each tidal cycle and the mean gape of 12 mussels when immersed by each tide.

RESULTS

Mussels and their environment

The foreshore surrounding Mumbles Pier (Swansea Bay, Wales, UK) was well used by people, particularly during spring low tides during daylight, including those in close proximity to the experimental mussels. In summer 2007, autumn 2007 and winter 2008, the maximum depth of the mussel immersed in seawater longest (67.9%, 72.1% and 70.8% (Fig. 2) of the time, respectively) was 6.51 m, 6.04 m and 6.47 m (Fig. 2), respectively. Equivalent data for the mussel least immersed in water were (40.3%, 34.1% and 59.7% of the time, respectively) 3.51 m, 3.04 m and 5.65 m. In summer 2007, autumn 2007 and winter 2008, mussel outer-shell temperature during aerial exposure ranged from 10.91-29.62°C, 6.78-30.58°C and 1.76-11.8°C (Fig. 2), respectively, while mean ± SE seawater temperature was 17.75°C ± 0.08, 16.48°C ± 0.31 and 7.43 ± 0.17 (Fig. 2), respectively. During aerial exposure light intensity was greatest in autumn 2007 (max = 1835.5 Lux) and summer (max = 1832.5 Lux) and lowest in winter (max = 495.2 Lux) (Fig. 2).

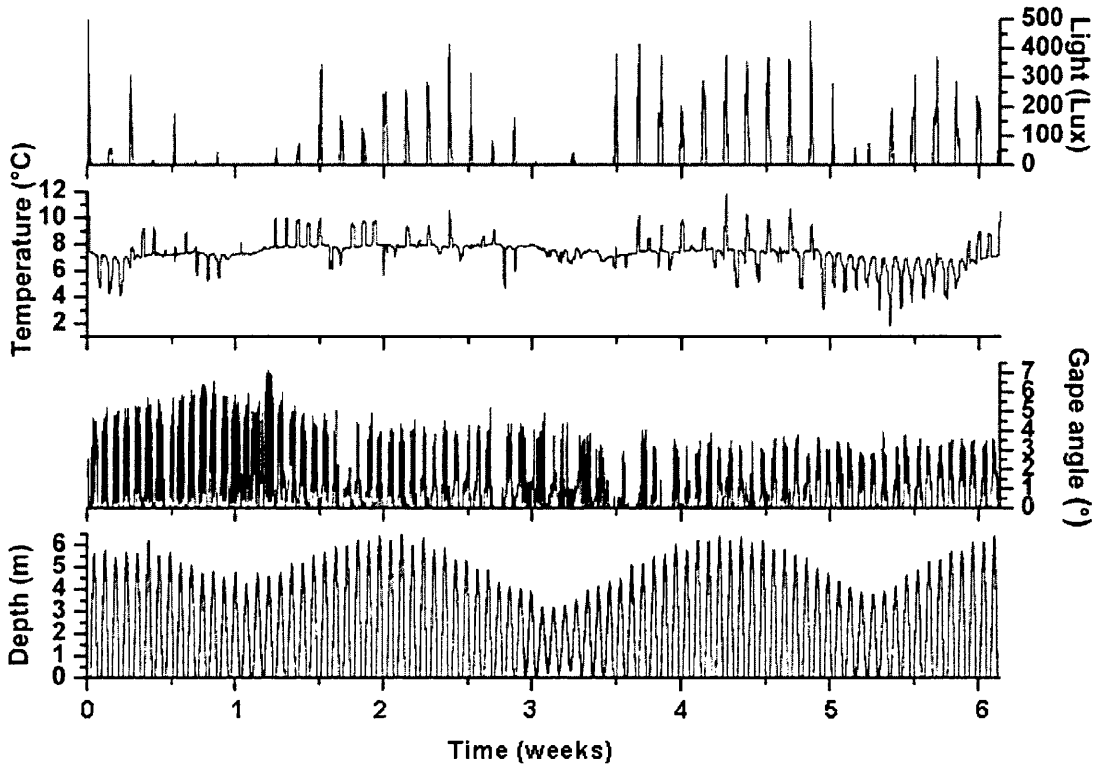

Fig. 2. *Mytilus edulis*. Gape angle of a 70.5 mm long mussel in relation to seawater depth, temperature and light intensity in the inter-tidal zone on Mumbles Pier, Wales, UK over forty three days (eighty three tidal cycles) starting on 10th January 2008.

Inter-tidal mussels gaped with a maximum range of 0-9.7°. All mussels in summer and autumn 2007 gaped > 1° at some point when immersed by every tide. However, the mussel immersed 70.8% of the time in winter 2008 did not gape > 1° when immersed during four of the 83 tidal cycles (e.g. Fig. 3). Mussels generally gaped < 1° while emersed (e.g. Figs. 2 and 3), but animals only shut their values completely (gape angle = 0^{o}) for 0.22 % ± SD 0.07 of the time.

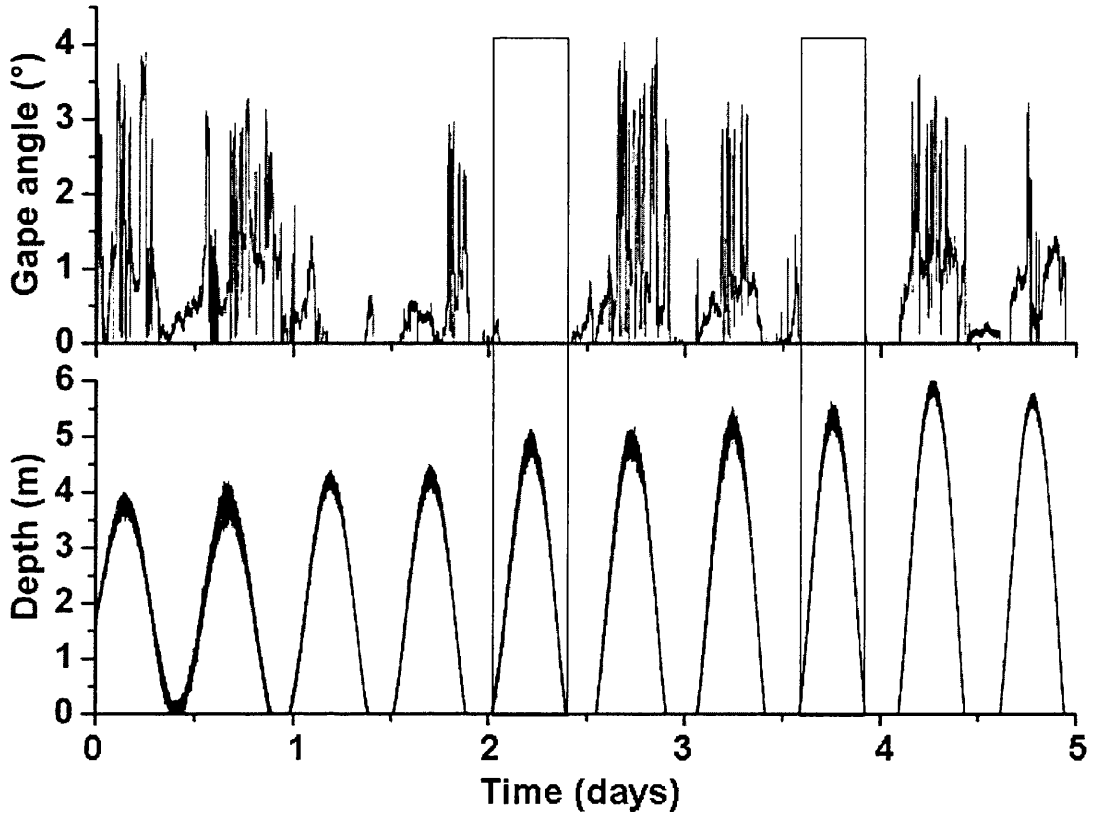

Fig. 3. *Mytilus edulis*. Example of a 70.5 mm long mussel not gaping > 1° when immersed by two tides (highlighted by rectangles) and the variation in gape behaviour from one immersion event to the next. Note the thicker the depth line, the higher the wave amplitude (i.e. rougher the sea).

Gape *versus* immersion time

In general, mean gape angle during immersion decreased significantly as the time mussels were immersed increased according to angle = 9.697 + (-5.980 x immersion time) ($F_{1, 26}$ = 28.037, $p < 0.001$ – Fig. 4) with immersion time accounting for 52% of the variation in gape angle.

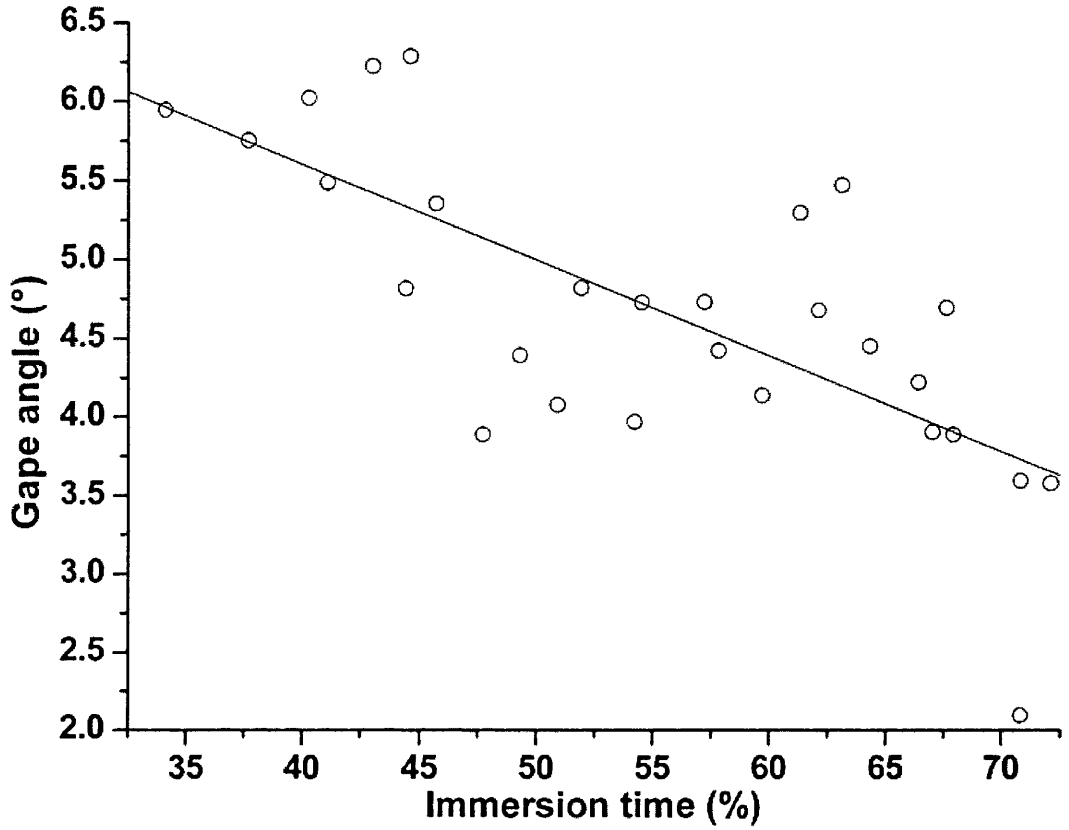

Fig. 4. *Mytilus edulis*. Mean gape angle when immersed *versus* immersion time of mussels in the inter-tidal zone on Mumbles Pier, Wales, UK. Data pooled from mussels in summer and autumn 2007 and winter 2008 (n = 28).

Day/Night and Seasonal comparisons

In general, inter-tidal mussels gaped significantly more at night ($F_{1, 25} = 267.748$, $P < 0.0001$) (Fig 5). However, there was no significant difference in the number of shell valve adduction events between day and night (mean ± SE = 3.23 h^{-1} ± 0.39 and 3.11 h^{-1} ± 0.43, respectively, $t_{27} = 0.717$, $P = 0.480$ (result for all summer, autumn and winter data combined)). There was a significant difference between certain seasons ($F_{2,25} = 7.302$, $P = 0.003$), but there was no interaction between season and day/night ($P = 0.192$). Tukey tests indicated a significant decrease in gape angle between summer 2007 and winter 2008 ($P < 0.05$) and autumn 2007 and winter 2008 ($P < 0.05$), but the decrease between summer and autumn was not significant ($P > 0.05$) (Fig 5). Shell valve adduction rate was significantly higher in summer 2007 than in winter 2008 (median number of adductions: 3.10 h^{-1} and 1.22 h^{-1}, respectively, ($U = 4.00$, $p = 0.015$, $N = 16$, $r = -0.5$) and higher in autumn 2007 (median number of adductions: 3.55 h-1) than in winter 2008 ($U = 4.00$, $p = 0.015$, $N = 16$, $r = -0.5$) but not between summer and autumn 2007 ($p = 0.843$).

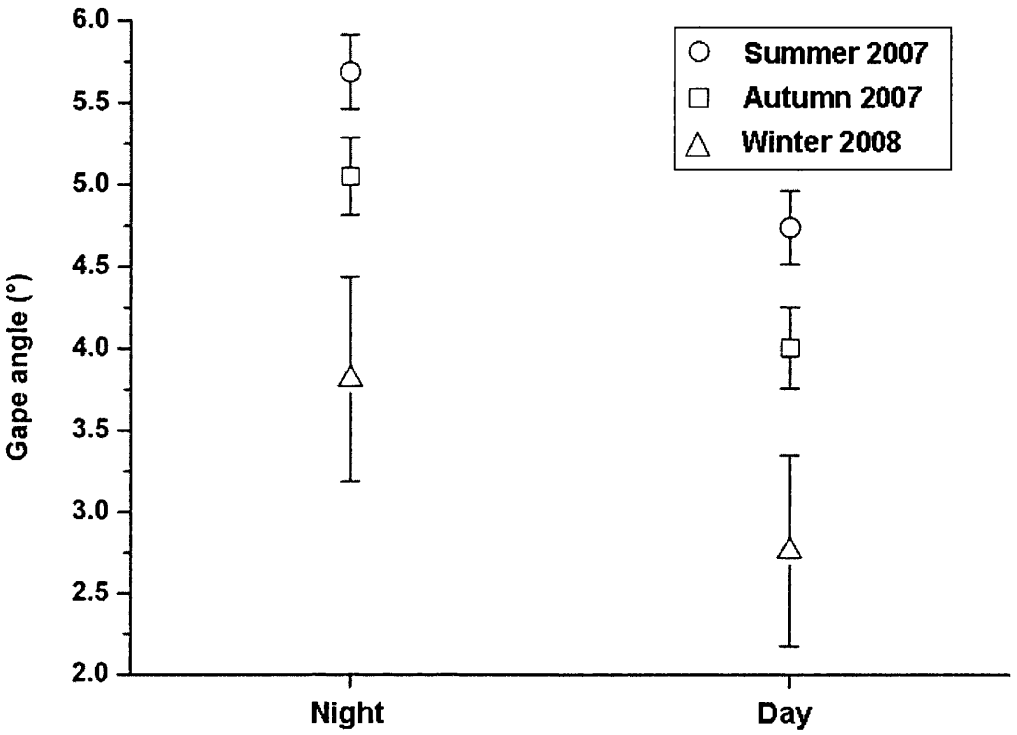

Fig. 5. *Mytilus edulis*. Gape angle (mean ± SE) when immersed in the inter-tidal zone on Mumbles Pier, Wales, UK in day and in night.

First and last wave of the tidal cycle

The amount of time mussels were immersed in seawater influenced the time difference between the first wave in a tidal cycle hitting the mussels and the mussels abducting their shell valves >1° (i.e. increasing gape angle in advance of, on, or after the first wave splash by the incoming tide). This was also true of the last wave in a tidal cycle hitting the mussels and mussels adducting <1° (i.e. decreasing gape angle for emersion in advance of, on, or after the last wave splash of the outgoing tide). There were significant differences between the time of the first wave splash hitting the mussels and mussels abducting >1° between immersion times of 70%, 57.5% and 45% ($H = 43.058$, $P < 0.0001$, $N = 168$ (pooled summer and autumn 2007 data to allow data from six mussels to be combined to give an appropriate sample size) (Fig. 6a). Mean ranks scores obtained for the above Kruskal-Wallis test indicated that the greatest time differences between the first wave in a tidal cycle hitting the mussel and the mussels abducting >1° were between 70% immersion (mean rank 90.70) and least time differences in 45% immersion (mean rank 40.83). Only mussels immersed for 57.5% or 45.0% of the time abducted >1° before the first wave splash hit them (abducting >1° up to maxima of 74 and 252 s before the first wave splash, respectively). In general, as the time mussels were immersed decreased, mussels adducted <1° significantly further from the time of the last wave splash hitting the mussels ($n = 168$ tidal cycles (84 x summer, 84 x autumn), mean ranks of 33.61, 37.02 and 78.36 indicated significant median time differences of -2 (minus indicates mussel adducted < 1° before the last wave splash), 9.5, and 252 s between immersion times of 70, 57.5 and 45.0%, respectively, $H = 49.140$, $P<0.0001$) (Fig. 6b).

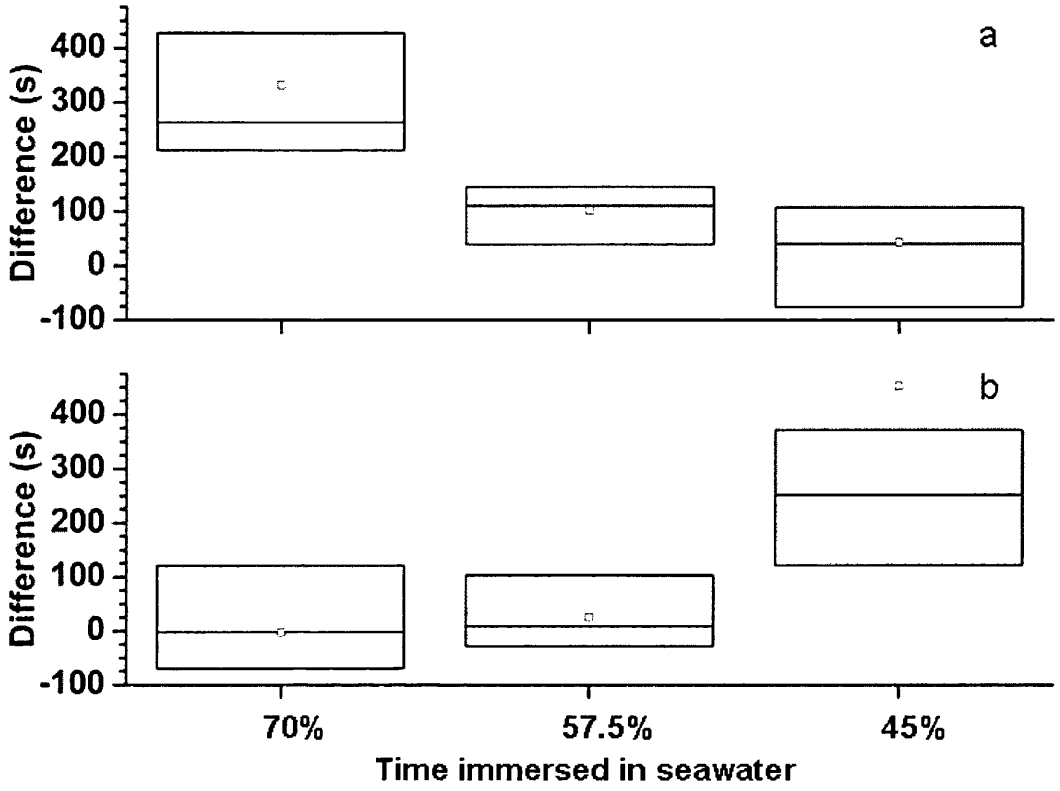

Fig. 6. *Mytilus edulis*. Box-plots (mean (square), median (line) and inter-quartile range (box) of (a) the time difference between the first wave in a tidal cycle hitting mussels and mussels abducting their shell valves >1° (i.e. increasing gape angle in advance of, on, or after the first wave splash by the incoming tide) when immersed in seawater for 70, 57.5 and 45 % of the time, (b) the time difference between the last wave in a tidal cycle hitting mussels and mussels adducting their shell valves <1° (i.e. decreasing gape angle for emersion in advance of, on, or after the last wave splash of the outgoing tide) when immersed in seawater for 70, 57.5 and 45 % of the time. N = 168 tidal cycles (84 in Summer and 84 in Autumn 2007) in the inter-tidal zone on Mumbles Pier, Wales, UK.

Gape angle *versus* seawater depth

There was high inter-individual variation in mussel valve gape behaviour in response to varying seawater depths. With some mussels, there appeared to be no relationship between gape angle and seawater depth. In general, however, mean mussel gape angle increased with depth from 0-3 m (Fig. 7a). Post-hoc Tukey tests indicated that gape angle at 0-0.5 m (mean = 3.68° ± SE 0.31) was significantly smaller than at seawater depths of 1.0-1.5 m and greater (pooled mean = 4.92° ± SE 0.13, $P < 0.05$). No other comparisons were significant. In seawater depths of 3-5.5 m the general plateau in gape angle (Fig. 7b) continued on from that shown in Fig. 7a.

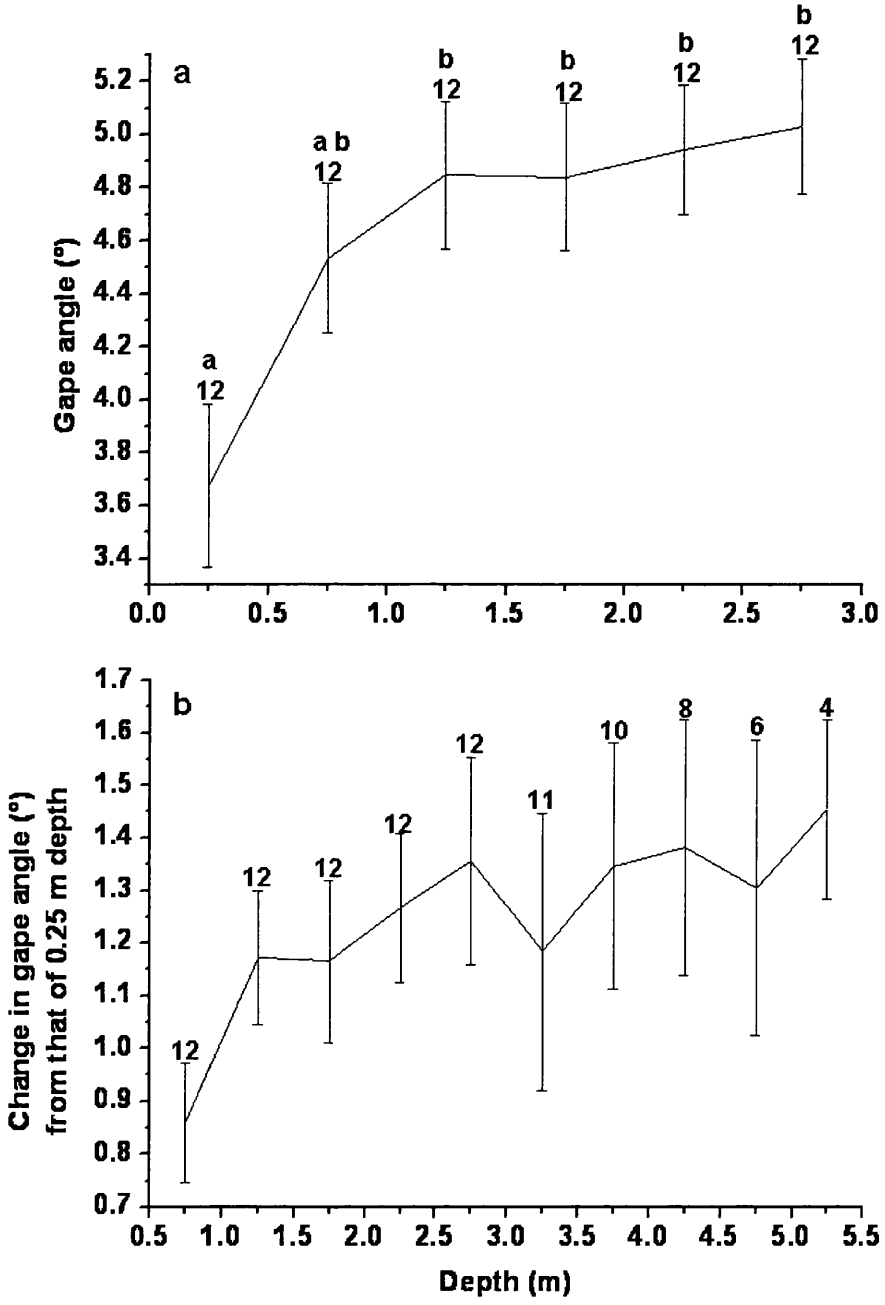

Fig. 7. *Mytilus edulis*. Pooled Summer, Autumn and Winter (a) gape (mean of means ± SE) *versus* depth (n above error, bar depth bins not sharing a letter are significantly different), (b) change in gape angle from that of 0.25 m depth bin *versus* depth (n above error bar).

Wave height *versus* gape angle

Starting on 21st September 2007 (autumn) at approximately 20:00 just prior to a period of storm, post-hoc Tukey tests indicated that the wave amplitude of the second tide (mean ± SE = 0.33 m ± 0.03) was significantly greater than those of the first and third tides (mean ± SE 0.15 m ± 0.02 and 0.11 m ± 0.02, respectively, $P<0.05$) (Fig. 8). Post-hoc Tukey tests also showed that mussel gape angle was significantly lower for mussels immersed for 47.7, 54.2, 57.8 and 50.9 % of the time in the second tide (mean ± SE = 2.86° ± 0.33) compared to the first and third (mean ± SE = 4.59° ± 0.42 and 4.41° ± 0.43, respectively, $P<0.05$) (Fig. 8). The gape angle of mussels immersed for 34.1-44.4% and 57.8-72.1% of the time was unaffected by wave height over the three tides ($P>0.05$).

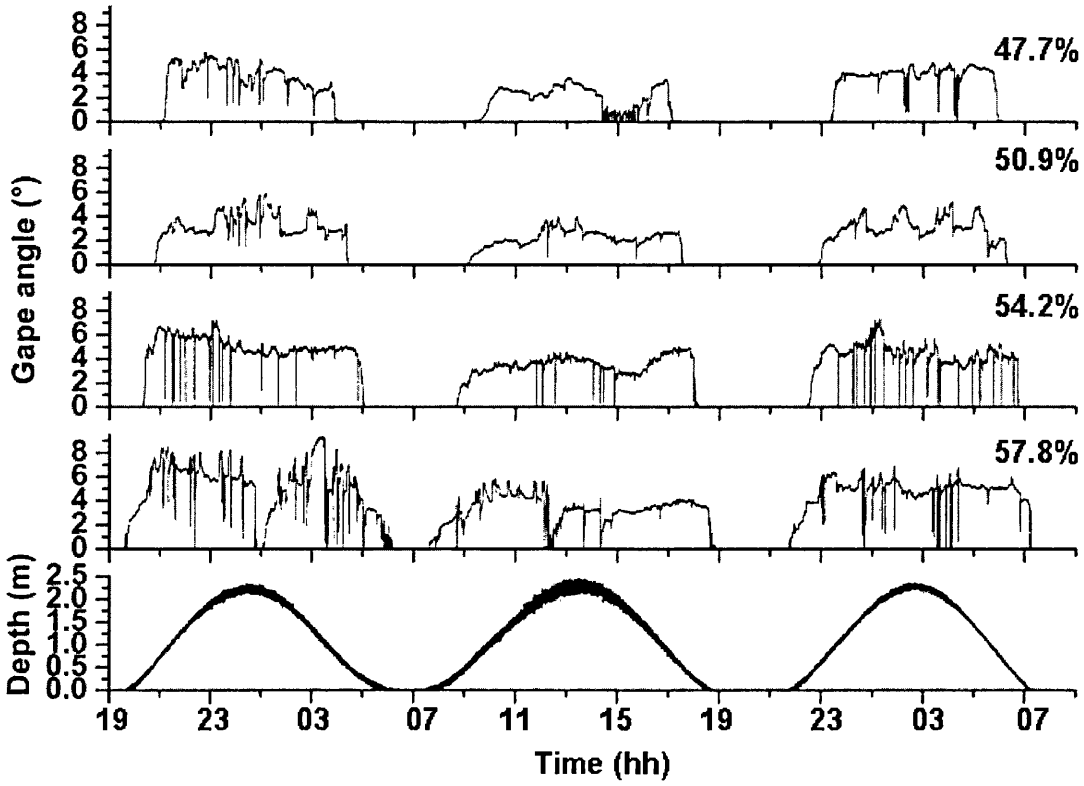

Fig. 8. *Mytilus edulis*. Gape angle versus depth for four mussels before, during and after a high wave amplitude event starting on 21st September 2007 in the inter-tidal zone on Mumbles Pier, Wales, UK. Mussel immersion times (%) written in the top right side of each mussels gape data.

DISCUSSION

The nervous system of *M. edulis* is remarkably complex for an invertebrate (Stefano 1990), with a suite of sensory systems including mechanoreceptors (e.g. LaCourse & Northrop 1977). The experimental mussels in this study probably detected the natural foraging behaviour (via vibrations perhaps) of predators such as oystercatchers *Haematopus ostralegus*, and crabs (e.g. *Carcinus maenas*) although there was no evidence of predation on experimental mussels, or rapid valve adduction associated with stress behaviour (e.g. Curtis et al. 2000, Nagai et al. 2006, Robson et al. 2007) and no animals died in this study.

Environment

The data obtained in the present study over 71 days (139 tidal cycles) demonstrate that the experimental animals on Mumbles Pier, Swansea Bay UK, *M. edulis* were subjected to an extreme environment with an outer-shell temperature range in air of 28.8°C and mean seawater temperature decreasing by 10.3°C from summer 2007 to winter 2008. This information may be useful in evaluating the relevance of laboratory bivalve thermal tolerance studies to their natural environment (Anestis et al. 2008).

Wave splash

Hunger and desiccation may be important factors for explaining why generally, as immersion time decreased (from 70, 57.5 or 45.0%), mussels gaped >1° longer before and after the first and last wave splash of a tide, respectively. Mussels emersed for longest will be the most food-deprived and possibly most dehydrated, especially on hot days (e.g. 30.58°C). Thus, their need to feed and/or rehydrate may be manifest as gaping >1° before the first wave splash to ensure they are re-hydrated and can feed as soon as possible. Complimentary to this, in laboratory experiments, mussel respiration rates have been found to begin to rise just in advance of the tide (anonymous peer pers comm). It is debatable whether wave splash acts as a stimulus for mussels to open, or whether there is an innate rhythm in mussels gaping response to the incoming tide. Mussels may also gape >1° after the last wave splash to aid aerobic digestive and absorptive processes, to enhance daily food absorption (e.g. Langton 1975, 1977, Robinson et al. 1981, Shick et al. 1988).

Immersion and emersion

Although the experimental inter-tidal mussels were only vertically spaced a maximum of 3 m apart, they were in very different environments, ranging from predominantly aerial (e.g. immersed 34.1%) to predominantly aquatic (e.g. immersed 72.1%). Immersion time significantly predicted gape angle possibly because reduction in feeding time may necessitate higher intensities of feeding to compensate although ingestion rates need to be

balanced against elements of digestive physiology (including the rate of production of digestive enzymes and gut passage times) to effect appropriate absorption efficiencies independent of immersion time (food quantity) (c.f. Navarro & Winter 1982, Bayne et al. 1987, Bayne et al. 1989). The metabolic costs of feeding behaviour (filtration, gut passage and digestion) appear to be low relative to overall metabolic rate (Widdows & Hawkins 1989), so that adjustments in feeding behaviour according to immersion time may not be heavily 'taxed' in metabolic terms (c.f. Bayne et al 1989). The current study relies on the assumption that mussel gape angle is a proxy for filtration (e.g. Newell et al. 1998, Dolmer 2000). In fact, although perhaps generally true, it is clearly an oversimplification and there may not be a simple proxy (valve gape (aperture) or siphon areas) for mussel (*M. edulis*) pumping (see Robson et al. 2009).

Immersion time only accounted for 52% of the variation in gape angle of inter-tidal mussels when immersed. The substantial inter-individual variation may augur for different feeding strategies between mussels and for other factors affecting gape angle. Two factors, the effect of current speed and current direction on mussels were seen to be complex due to eddy currents as the tide flowed passed the pier legs where the mussels were located. Although these parameters were not recorded they have been documented to have an effect on mussel feeding and growth (e.g. Sénéchal et al. 2008).

The observation that when immersed some individuals occasionally did not gape > 1° for up to a whole tidal cycle (e.g. Fig. 3) seems unusual because inter-tidally-acclimated bivalves have been reported to pump (and therefore gape) more or less continuously when immersed (Brand & Taylor 1974, Widdows & Shick 1985). Gaping < 1° while immersed or emersed is likely to be an energy-saving measure during

recuperation periods after prolonged activity, or in response to food deprivation. Various authors have noted that heart beat rate is reduced, or even stops during periods of long-term partial, or complete valve closure in *Mytilus edulis* when emersed (e.g. Woortman 1926, Helm & Trueman 1967, Coleman & Trueman 1971) and immersed (Curtis et al. 2000).

In fact, emersed mussels rarely remained fully closed, often gaping at < 1° which may save energy by relaxing the adductor muscles, as tonic contraction of the adductor muscles requires energy (Lowy 1953). More important reasons for gaping < 1° may include conserving water possibly while siphons are closed and promoting evaporative cooling when the siphons are open, and allowing air breathing for continued digestive processes during emersion (Shick et al. 1986, Shick et al. 1988).

Tidal rhythms

Tidal variations in shell gape angle of inter-tidal mussels were found, the most obvious being that, in general, mussels gaped < 1° when emersed and > 1° when immersed. A notable exception to this was provided by one mussel in winter 2008 which did not gape >1° when immersed over four different (and non-adjacent) tides. The need to feed may have been less for this mussel compared to mussels immersed for shorter periods which may have had to gape >1° to feed on low seston concentrations and/or in disadvantageously high current speeds (e.g. Sénéchal et al. 2008) in order to survive. The fact that gape angle generally increased from 0-3 m depth, with gape angle at 0-0.5 m significantly less than at depths of 1.0-1.5 m (and greater) may have been because

mussels could not feed efficiently in wave splash (a mixture of air and water) as the tide went in and out. However, since with some mussels there appeared to be no relationship between gape angle and seawater depth, it is unclear what the adaptive significance of this finding might be. One possibility is that their need to feed outweighed any sub-optimal conditions. Inter-tidal mussels may reach a gape saturation point (general plateau in gape angle) at seawater depths above ~3 m because they reach the optimal gaping degree for maximal inhalant and exhalant siphon area for filter-feeding, pseudofaeces production and other metabolic processes.

Tidally-induced variation in bivalve filtration activity (valve gape and exhalant siphon area) has been reported *in situ* in sub-tidal mussels (e.g. Newell et al. 1998, Newell et al. 2005). However, responses in valve gape and exhalant siphon opening were not directly correlated to tidally-driven variation in phytoplankton biomass in the near-bottom water (Newell et al. 1998). In the Menai Strait, Wales, UK Saurel et al. (2007) reported that chlorophyll *a* was dependent on tidal advection and, measured at 1 m above the mussel bed, regulated sub-tidal mussel feeding behaviour irrespective of the presence of predators, changes in suspended particulate matter (SPM), or flow velocity. Newell et al. (2005) reported a difference in feeding behaviour between sub-tidal benthic mussels and those in chambers supplied with surface waters. The population feeding in surface waters had high pumping rates over the whole tidal period whereas there were two peaks registered in benthic mussel exhalant siphon area, one corresponding to an increase in the *in situ* marine snow volume coincident with low tidal currents and the other peak during flood tide in response to the advection of seston-laden water (food) across the mussel bed.

Circadian rhythms

Despite immersion and emersion by the tide approximately twice a day and the general increase in gape angle from 0-3 m seawater depth, a significant circadian rhythm was found, with mussel gape angle generally greater in darkness over weeks in summer 2007, autumn 2007 and winter 2008. It is unclear what the adaptive significance of this finding might be (but see Nielsen & Stromgren 1985). One possibility is that such day-night gape behaviour may be part of a strategy to feed while minimizing the likelihood of predation by visually-feeding predators (Ameyaw-Akumfi & Naylor 1987). However, visually-feeding predators e.g. eider ducks *Somateria spp* and humans predate on mussels regardless of whether they are gaping or not, alternatively oystercatchers *Haematopus spp* are either stabbers (stab open gaping mussels, including when mussels are submerged in shallow water) or hammerers (break the shell by hammering) (see Goss-Custard 1996, Stillman et al. 2000). Another possibility is that such day-night gape behaviour may be linked to the circadian rhythm reported in *Mytilus edulis* byssus thread production, with greater thread production during the night (Martella 1974). From our observations, we suggest that mussels may be particularly vulnerable to predation when the foot (used as a plantar during attachment of the byssus to the substrate) is protruding from the shell because when handled, mussels adducted their valves around their foot and thus, could not fully close their valves.

Diel patterns of behaviour associated with energy acquisition have been documented for a wide range of species (for examples see Clarke 1978, Wilson et al.

1993, Hays 2003). For *M. edulis*, this gape pattern was first noticed by Dodgson (1928), who reported diel variation in gaping in *M. edulis*, with greater activity in darkness. The unnatural conditions of constant, or periodic illumination at night may explain why Ameyaw-Akumfi & Naylor (1987) found only a weak circadian gape rhythm in laboratory *M. edulis* and Newell et al. (2005) and Saurel et al. (2007) did not report a circadian rhythm at all in *in situ* sub-tidal mussels. No lights were used in the current study and in future studies an infra-red camera (e.g. Maire et al. 2007 but see Nielsen & Stromgren 1985) may be used to observe/measure mussel gape and siphon movements, particle size and abundance, predators and prey in a more natural environment. Without lighting, Wilson et al. (2005) reported a highly significant circadian gape rhythm in wild sub-tidal *M. edulis*, with a general increase in gape angle and shell valve adduction and abduction events in the dark. However, we found no significant difference in the number of shell valve adductions between day and night in inter-tidal mussels. Without being able to determine the reason(s) for valve adduction, it is currently unwise to speculate why they occur (but see below).

Mussels can access almost all sources of energy available to them in seawater, viz. dissolved organic material, seston, microbes, phytoplankton and zooplankton (Davenport et al. 2000, Nielsen & Maar 2007). Diel vertical migration (DVM) by zooplankton is a universal feature in all oceans (Hays 2003) so inter-tidal mussels suspended in mid-water when immersed in the current study may gape wider at night in response to DVM rhythms that allow them to benefit from a mixed diet including zooplankton (e.g. Lagadeuc et al. 1997), with marine copepods *Temora longicornis* and *Microsetella norvegica* located close to the surface during the night in the richest phytoplanktonic

coastal waters (Lagadeuc et al. 1997). As zooplankton are larger than phytoplankton, mussels may need to gape wider for them to pass through the inhalant siphon. Indeed, *M. edulis* have been found to occasionally ingest animals as large as 6 mm in length (Davenport et al. 2000), with Wong & Levinton (2006) also reporting *M. edulis* gaping wider when feeding on rotifers compared to microalgae. The classic model of filter feeding on phytoplankton by bivalves may have overlooked the benthos-zooplankton trophic loop in the benthic-pelagic ecosystem (Wong & Levinton 2004). Though bivalves can derive nutrients from many food resources, it has been found that mussels have best growth performance and higher metabolic rate on a mixed diet of phytoplankton and zooplankton (Wong & Levinton 2004) including inorganic detritus (Bayne et al. 1987). However, because of the differences between the feeding behaviour of benthic mussels and those in surfaces water chambers (Newell et al. 2005), DVM of zooplankton and marine snow may not affect benthic mussels (e.g. Newell et al. 2005, Maar et al. 2007, Nielsen & Maar 2007, Saurel et al. 2007), as it may have affected inter-tidal mussels suspended in mid-water when immersed in the current study and sub-tidal mussels (Wilson et al. 2005), which were suspended in mid-water (Rory Wilson pers. comm..). Recent *in situ* research stresses the need, when evaluating the ecological role of (benthic) suspension-feeding bivalves in shallow coastal ecosystems, to consider the heterotrophic components of the food web (Maar et al. 2007; Nielsen & Maar 2007).

Despite finding a highly significant circadian gape rhythm in inter-tidal mussels over weeks, mussels also responded to changes in their environment with significant adaptation by changing gape behaviour according to circumstance e.g. increasing and decreasing gape angle when in calm and more turbulent sea conditions respectively. The

need to feed may outweigh the likely extra energy expenditure required to pump water in storm conditions (high current speeds/wave amplitudes) for mussels immersed for shorter time periods (34.1-44.4%) compared to those immersed for longer periods (62.1-72.1%) at greater depth which may be relatively unaffected by high wave amplitudes in surface waters, or generally subject to lower flow velocities than higher up the water column (c.f. Maar et al. 2007).

Seasonal comparisons

Seasonal patterns have been found in the seston biomass in coastal waters (Widdows et al. 1979b, Maar et al. 2007) which may help explain why, in general, gape angle significantly decreased from summer 2007 to winter 2008 and from autumn 2007 to winter 2008. In winter, the seston has been found to be dominated by small particles (Widdows et al. 1979b). Thus, mussels in this study may not have needed to gape as wide to ingest small particles compared to larger heterotrophic prey which have been found to be more prominent in summer and autumn, than winter (Maar et al. 2007). However, Taghon (1981) suggested that a reduced feeding rate (therefore presumably decreased gape angle) corresponds to a decrease in food quality, as is normally experienced by UK mussels over winter months (Bayne & Widdows 1978, Widdows et al. 1979b), represents energetically optimal behaviour. The fact that mussel valve adduction rate was significantly higher in summer and autumn 2007 than in winter 2008, could be attributed to higher seawater temperatures associated with greater oxygen demand (Widdows 1973) initiating more frequent valve adductions to enhance perfusion of the tissues by newly re-

oxygenated haemolymph (see Shick et al. 1986, Shick et al. 1988). Another possibility is that in summer and autumn, when food is generally expected to be plentiful (i.e. when food material represents a relatively high proportion of total seston (see Widdows et al. 1979b)), mussels may become satiated with food and feed intermittently (c.f. Davenport & Woolmington 1982) and thus, adduct more per unit time.

The robustness of the gape statistics could have been improved by altering the immersion time (height on the inter-tidal apparatus) of individual mussels. However, in reality, access to mussels was restricted to spring low tides and substantial human disturbance may have profoundly affected mussel behaviour (c.f. Wilson et al. 2005). In the wild two tides are never the same, so this would have had to be taken into account, if mussel immersion times were altered. Further, due to emersion, the data sets had to be long enough to get sufficient data on mussels immersed in daylight and darkness for statistical analysis and thus, weeks of data were required.

Although generic patterns can be established, with all mussels being typically more active at night, a striking feature of the inter-tidal mussel gape and adduction behaviour in the current study was the wide intra- and inter-individual variation within and between immersion events. Genetic analysis may explain the basis for individual variability that may lead to an enhanced survival since a positive correlation between multi-locus heterozygosity (MLH) and various fitness characteristics has been documented for several species including *Mytilus* (Mitton 1994, Bayne & Hawkins 1997, Tremblay et al. 1998, Myrand et al. 2002, LeBlanc et al. 2008). An obvious result of this chapter is the clear demonstration of just how much speculation still exists about the

reasons for specific mussel behaviours e.g. to gape or not to gape and how much to gape and when and why mussels adduct and abduct their shell valves.

In order to further explain the results, several hypotheses need testing by future research completing high temporal resolution energy budgets for *in situ* inter-tidal *M. edulis* that combine information on their gape angle, siphon areas (inhalant and exhalant), pumping rate, (pseudo)faeces production, metabolic rate, type and energy density of prey (including zooplankton) and ingestion rates to assess energy balance over long periods. An ideal timescale would be over months or years, with high temporal and sensor resolution measurements recorded at 2 Hz, to define and quantify mussel valve adduction events. Such detailed behaviour and energy budgets combined with the influence of predation, tidal cycle, mussel orientation, current speed and direction may greatly improve the explanation of the drivers of the observed circadian gape behaviour in inter-tidal mussels in the current study and those in the sub-tidal (Wilson et al. 2005).

In summary the results of this chapter suggested that gaping was a general proxy for mussel activity associated with respiration, feeding, excretion and their associated metabolic processes. It was suggested that artificial lighting may alter mussel behaviour and highlighted that no lights were used in the current study. Despite immersion and emersion by the tide approximately twice a day and the general increase in gape angle from 0-3 m seawater depth, there was a significant circadian rhythm, with mussel gape angle generally greater in darkness over weeks in summer 2007, autumn 2007 and winter 2008. Further research will be required to determine the reason(s) for the circadian rhythm in mussel gape behaviour. The results also showed that gape angle and valve adduction rate significantly decreased from summer 2007 to winter 2008, possibly related

to seasonal variations in the edible seston biomass, including zooplankton in coastal waters. It was hypothesized that the need to feed may have outweighed the likely extra energy expenditure required to pump water in storm conditions (high current speeds/wave amplitudes) for mussels immersed for shorter time periods compared to those immersed for longer periods at greater depth which may be relatively unaffected by high wave amplitudes in surface. Finally, an obvious result of this chapter was to highlight just how much speculation still exists about the reasons for specific mussel behaviours e.g. to gape or not, how much to gape and when and why mussels adduct and abduct their shell valves.

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GENERAL DISCUSSION

Research on bivalve behaviour has produced insights on how organisms cope with highly fluctuating environments (e.g. Jorgensen et al. 1988). Some of the questions being addressed were aimed at providing an overall view of behaviour in a particular bivalve species. While recording behaviour with high frequency measurements allowed questions concerning fine-scale bivalve behavioural physiology to be addressed (e.g. Trueman 1966, Hoggarth & Trueman 1967, Wilson et al. 2005). Chapter 1 highlighted the importance of selecting an appropriate sampling frequency for measurements of single parameters (e.g. bivalve gape and exhalant pumping) to ensure all behavioural events are recorded because some previous work probably had limitations involving not recording all behavioural events (e.g. Newell et al. 1998, Newell et al. 2001, Riisgard et al. 2003, Riisgard et al. 2006). The potential loss of information associated with the choice of particular sampling intervals during measurements of single parameters and the biases which can result from this choice are effectively germane to all species (Boyd 1993, Robson et al. 2009).

Chapter 2 went on to show that bivalve gape angle and the change in gape angle per second vary extensively and the ability to vary these presumably has survival value (Robson et al. 2007). Indeed, mussel (*Mytilus edulis*) extract added to the seawater, a factor believed to signal predation, caused mussels to close significantly faster than otherwise. But only once when the stressor was applied and mussels were gaping > 1°. Mussel response to predation was graded and complex and may well indicate animal-based assessments of the trade-off between effective feeding and the likelihood of predation (Robson et al. 2007).

First noted in Chapter 1, *M. edulis* pumping activity is complicated because exhalant pumping can occur from the top of the inhalant siphon (associated with the

pseudofaeces mucociliary rejection pathway) in addition to the exhalant siphon (c.f. e.g. Newell et al. 2001, Maire et al. 2007) and this was discussed in more detail in Chapter 3. Thus, the results showed that complete dissociations between valve gape and exhalant pumping at > 1° gape were rare and suggest that although exhalant siphon area is clearly the best proxy of pumping rate out of the exhalant siphon, it may not represent exhalant pumping as a whole. There may not be a simple proxy for overall exhalant, or both inhalant and exhalant pumping because there is no defined barrier to exhalant pumping from the top of the inhalant siphon and it may not be assume that inhalant pumping occurs out of the whole of the inhalant siphon area (especially when exhalant pumping occurs from the top of the inhalant siphon).

Chapter 3 also highlighted a significant circadian rhythm in mussels with increased nocturnal activity, manifest as a generally greater gape angle and higher rates of exhalant pumping in periods of darkness (night) (c.f. Dodgson 1928, Martella 1974, Nielsen & Stromgren 1985, Wilson et al. 2005). It is apparently well known that light reduces growth in *M.* edulis (Trevelyan & Chang 1987) and it has been suggested that light probably reduces *M. edulis* growth rate by reducing ingestion rate (Nielsen & Stromgren 1985, Trevelyan & Chang 1987). However, there was significant intra- and inter-individual variation in the way individual mussels adjusted their gape angle over the course of the day. It was concluded that gape angle could be used as a general proxy for mussel pumping activity (associated with respiration, feeding, excretion and their associated metabolic processes) in the wild (c.f. Newell et al. 1998, Dolmer 2000, Wilson et al. 2005, Saurel et al. 2007).

Chapter 4 confirmed the speculation in Chapter 2 that mussel response to predation is graded and complex and indicates some form of mussel assessment of the trade-off between effective feeding and/or respiration and the likelihood of predation.

Mussels probably weigh up the net energy gain and nutrient content of their food as well as its concentration against the costs of ciliary action, mucus secretion (Davenport et al. 2000). Mussel response to increasing algal concentration (c.f. Wilson & Seed 1974, Riisgard & Randlov 1981, Riisgård 1991, Clausen & Riisgard 1996, Dolmer 2000, Newell et al. 2001, Macdonald & Nodwell 2003, Riisgard et al. 2003) was to increase mean gape angle in an S-shaped curve. Gape angle decreased in a backward S-shaped curve as the amount of mussel homogenate (an indication of predation) increased in the seawater. There was no significant difference between the number of shell valve adduction (decrease in valve gape angle) events per day across daily algal rations. In order to identify the reasons for every mussel valve adduction and abduction (increase in valve gape angle) event, many more parameters of mussels and their environment need measuring at high temporal resolution.

Chapter 5 showed that immersed mussels adducted their shell valves significantly more often when exposed to detritus (clay particles 8.3×10^{-4} g.ml^{-1}, ~ 4μm) than when in a detritus-free environment. Mussels exposed to detritus may have more intermittent and shorter filtration periods compared to those not exposed to detritus and thus, adduct more per unit time, to try to prevent their filtration and rejection mechanisms becoming overloaded (c.f. Widdows et al. 1979, Riisgard & Randlov 1981, Clausen & Riisgard 1996, Macdonald & Nodwell 2003). When emersed gape angle increased with air temperature in the inter-tidal zone in Swansea Bay (Wales, UK) and was described by a biphasic regression with the break point at 15°C (c.f. Shick et al. 1986). Mussels may balance potential exposure to desiccation and osmotic stress (e.g. following rainfall), with the need for aerial respiration and evaporative cooling. Mussels may gape with their siphons shut and the presence of food in the gut can influence the need for aerial respiration (see Shick et al. 1986,

Shick et al. 1988). The transition in two inter-tidal mussels from normal gape behaviour, to abnormal may have been parasite induced because parasites commonly alter the behaviour of their hosts (reviewed by Poulin 1994, and c.f. Mouritsen 1997).

Finally Chapter 6 revealed that in general, gape angle during immersion decreased as the time mussels were immersed increased. Tidal variations were found in the behaviour of mussels i.e. in general mussels gaped <1° when emersed and >1° when immersed (c.f. Saurel et al. 2007). Although one mussel in winter 2008 (immersed 70.8% of the time) did not gape >1° when immersed by four non consecutive tides. Despite immersion and emersion by the tide approximately twice a day and the general increase in gape angle from 0-3 m seawater depth, a significant circadian rhythm was found, with gape angle generally greater in darkness in inter-tidal mussels (n.b. artificial lighting may alter mussel behaviour. No lights were used in the current study) (c.f. Dodgson 1928, Martella 1974, Nielsen & Stromgren 1985, Trevelyan & Chang 1987, Wilson et al. 2005) . It is unclear what the adaptive significance of this finding might be. One possibility is that such day-night gape behaviour may be part of a strategy to feed while minimizing the likelihood of predation by visually-feeding predators (Ameyaw-Akumfi & Naylor 1987). However, visually-feeding predators e.g. eider ducks *Somateria spp* and humans predate on mussels regardless of whether they are gaping or not, alternatively oystercatchers *Haematopus spp* are either stabbers (stab open gaping mussels, including when mussels are submerged in shallow water) or hammerers (break the shell by hammering) (Goss-Custard 1996). Another possibility is that inter-tidal mussels suspended in mid-water when immersed may gape wider at night in response to DVM rhythms that allow them to benefit from a mixed diet including zooplankton, with marine copepods *Temora longicornis* and *Microsetella norvegica* located close to the

surface during the night in the richest phytoplanktonic coastal waters (Lagadeuc et al. 1997). As zooplankton are larger than phytoplankton, mussels may need to gape wider for them to pass through the inhalant siphon (Davenport et al. 2000, Wong & Levinton 2004).

The obvious result of thesis is just how much speculation still exists about the reasons for specific mussel behaviours e.g. to gape or not, how much to gape and when and why mussels adduct and abduct their shell valves. In order to further explain the results, several hypotheses need testing by future research completing high temporal resolution energy budgets for *in situ* inter-tidal *M. edulis* that combine information on their gape angle, siphon areas (inhalant and exhalant), pumping rate, (pseudo)faeces production, metabolic rate, type and energy density of prey (including zooplankton) and ingestion rates to assess energy balance over long periods. An ideal timescale would be over months or years, with high temporal and sensor resolution measurements recorded at a minimum of 2 Hz, to define and quantify mussel valve adduction events. Such detailed behaviour and energy budgets combined with the influence of predation, tidal cycle, mussel orientation, current speed and direction may greatly improve the explanation of the drivers of the observed circadian gape behaviour in mussels.

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