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RESEARCH ARTICLE

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Muscling in on mussels: new insights into bivalve behaviour using vertebrate remote-sensing technology

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Introduction

The difficulty of studying marine endotherms has stimulated researchers wishing to quantify behaviour at sea to develop animal-attached remote-sensing technology that automatically records activity at all times, even when the study animal is far from land and deep underwater (see e.g. Naito 2004). Superficially, it would appear that such technology is unnecessary for more accessible animals, such as intertidal invertebrates, because they can generally be observed directly, either in the laboratory or in situ. However, problems of observation in imperfect conditions, observer bias, fatigue or simple inability to resolve behavioural events are little discussed even though they may profoundly affect the quality and interpretation of results.

In this note, we demonstrate how technology using the Hall effect, originally developed for studies on marine endotherms, may be used to elucidate and to quantify the behaviour of bivalves both in the laboratory and in the wild. To our knowledge, two other research groups have presented a remote-sensing approach to bivalves (Redpath and Davenport 1988; DeZwart et al. 1995), one of which, at least, has been used to examine the reaction of shellfish to toxins (Curtis et al. 2000), although the technology and sensors are quite different to ours. We believe that adoption of this general approach can enhance our understanding of the marine invertebrates enormously.

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Materials and methods

We used a Hall sensor and magnet system, originally proposed by Wilson et al. (2002), to study the feeding behaviour in penguins, but also used in later modifications of the same approach to study limb movement (Wilson and Liebsch 2003), respiration (Wilson et al. 2003) defecation and heart rates (Wilson et al. 2004). The system relies on a small rare earth magnet being attached to a moving element in an animal (such as on the flipper of a turtle) and a small magnetic-field strength sensor, a Hall sensor, being attached close-by on a non-moving element (such as on the carapace in the turtle example). When the Hall sensor is powered by a small archival tag set to record sensor output at a frequency of several Hertz, the movement of the element to which the magnet is attached can be logged. Suitable calibration allows the sensor output to be converted into a meaningful measure related to element position so that any movement can be precisely quantified.

In our application, we glued the magnet to one half of mussel shells and the Hall sensor to the other so that mussel gape could be quantified over time. Our experiments were with blue mussels *Mytilus edulis* and sand mussels *Astarte borealis* and took place during March and April 2004. Animals were collected from 5-m to 10-m deep sandy bottom (*A. borealis*) and from 2-m deep hard substratum (*M. edulis*) of the southern Kiel Bight, and transferred to the flow-through aquarium system of the institute within 4 h. Shells were carefully cleaned with a 400-gauge emery paper and dried before neodinium boron magnets (varying in size between 2×2×4 mm and 4×4×3 mm) were stuck to one shell half using Z-Spar A-788 glue (Kop-Coat Marine Group, USA). At the same time the Hall sensors (Siemens KSY 10—nominal size of sensor 2-mm diameter × 1-mm thick, but after encapsulation in resin sensors could be up to 8-mm diameter ×2-mm thick) were glued parallel (Fig. 1) before the mussels were replaced in running, aerated water in aquaria for

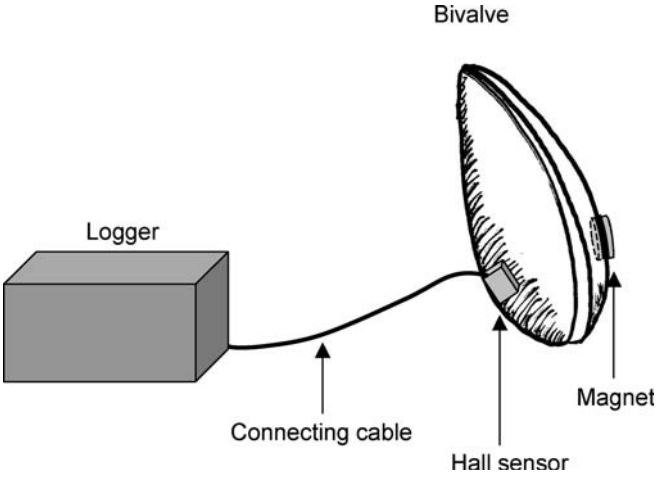

Fig. 1 Schematical diagram showing the attachment of the Hall sensor system used for determining shellfish gape angle

at least 48 h before being involved in any experiments. The Hall sensors were linked to the loggers (type IMASEN, Driesen and Kern GmbH, Bad Bramstedt, Germany) by a 0.8-mm diameter × 300-mm long, six-strand teflon-coated, waterproof cable. The loggers themselves had maximum dimensions of 72×16×33 mm and consisted of electronics and a turned titanium battery housing embedded in resin. The loggers were powered by 3.6 V lithium batteries, had a 4-mbyte flash RA memory and could be set to record at intervals up to a maximum frequency of 30 Hz. Communication with the loggers was effected by a two-pin interface, which liaised with a computer.

All devices had to be calibrated on the animals individually (cf. Wilson et al. 2002). To do this, all loggers were set to record at 5 Hz and the mussels to which systems had been attached were left in aerated aquaria. A small calibration rod, with turned diameters between 1 mm and 6 mm in steps of 1 mm (step length 1 mm), held in a clamp system, was then manoeuvred to be adjacent to the point of maximum shell gape of each of the animals and the mussels left until they began to gape. Here, the 'gape' is taken to mean the extent to which the two shell halves were apart at the point farthest from the hinge. Accordingly, gape angle (θ, in degrees) was defined as

$$\theta = 2 \times \arcsin\left(\frac{0.5\,W}{L}\right)$$

where W was the distance between the two shell halves at the maximum distance from the hinge and L was the straight-line distance between hinge and that point. Photos were then taken of the extent of shell gape together with the calibration rod at intervals ensuring that different degrees of gape were represented. Subsequently, the Hall sensor output for the corresponding time period was regressed against the mussel gape angle to derive a calibration curve. Best-fit curves (all r^2 values were 0.96 or better) were used to convert Hall sensor outputs (nominally in mV) into shell gape angles for subsequent experiments.

In order to demonstrate the general applicability of the technique for studying bivalves, we present a selection of data derived from experiments in the laboratory and in the wild (adjacent to the institute's pier in Kiel, Germany). In some of the experiments that took place in the wild, we used a multiple-channel data logger (DK 700 series, Driesen and Kern GmbH, Bad Bramstedt, Germany) to record some environmental conditions; depth, light intensity and temperature at a 10-s intervals. All parameters were logged with 16-bit resolution. 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 0.1–50,000 lux with a resolution of ca 1 lux.

Activity of mussels exposed in situ was quantified as the frequency of closing/opening events (closure defined as an angular decrease of $>1°$/s followed by reopening to pre-closure values) and as mean maximum opening angles over a 4-h period (2 h each side of noon and midnight). Differences in activity levels were compared between times of day (replicates = 3 subsequent days) and between individuals (four replicates) by two-factorial ANOVA. Other than that, in that none of the experiments was onerous or requiring special procedures, and to minimize repetition, relevant experimental information will be given together with the results, as appropriate.

Results

Calibration of gape angle using the methods presented proved unproblematic although care had to be exercised to ensure that the animals gaped perpendicularly to the camera angle to avoid parallax errors. Since very large gape angles may not be employed often, it might be difficult to assess these although they can be covered to a large extent by following the curve fit past the last calibration point. Within the ranges used, the best-fits were generally exponential or power decay functions and gave correlation coefficients in excess of 0.96.

After conversion of sensor output in mV to gape angles (°) changes in mussel gape angle over time were very apparent. Typically, blue mussels operated in the range of 0–20° (Fig. 2a) whereas sand mussels never had a shell gape in excess of 2° (Fig. 3a). Both blue and sand mussels showed similar basic behaviour, having a gape angle that varied either (i) slowly over time, where it could increase or decrease (Figs. 2b, 3b) and (ii) having a gape angle that decreased rapidly over time followed by either complete closure for an extended period (Figs. 2b, 3b) or (iii) by increasing gape angle that followed a typical logarithmic type curve (Figs. 2c, 3c). Close examination of the signal showed some

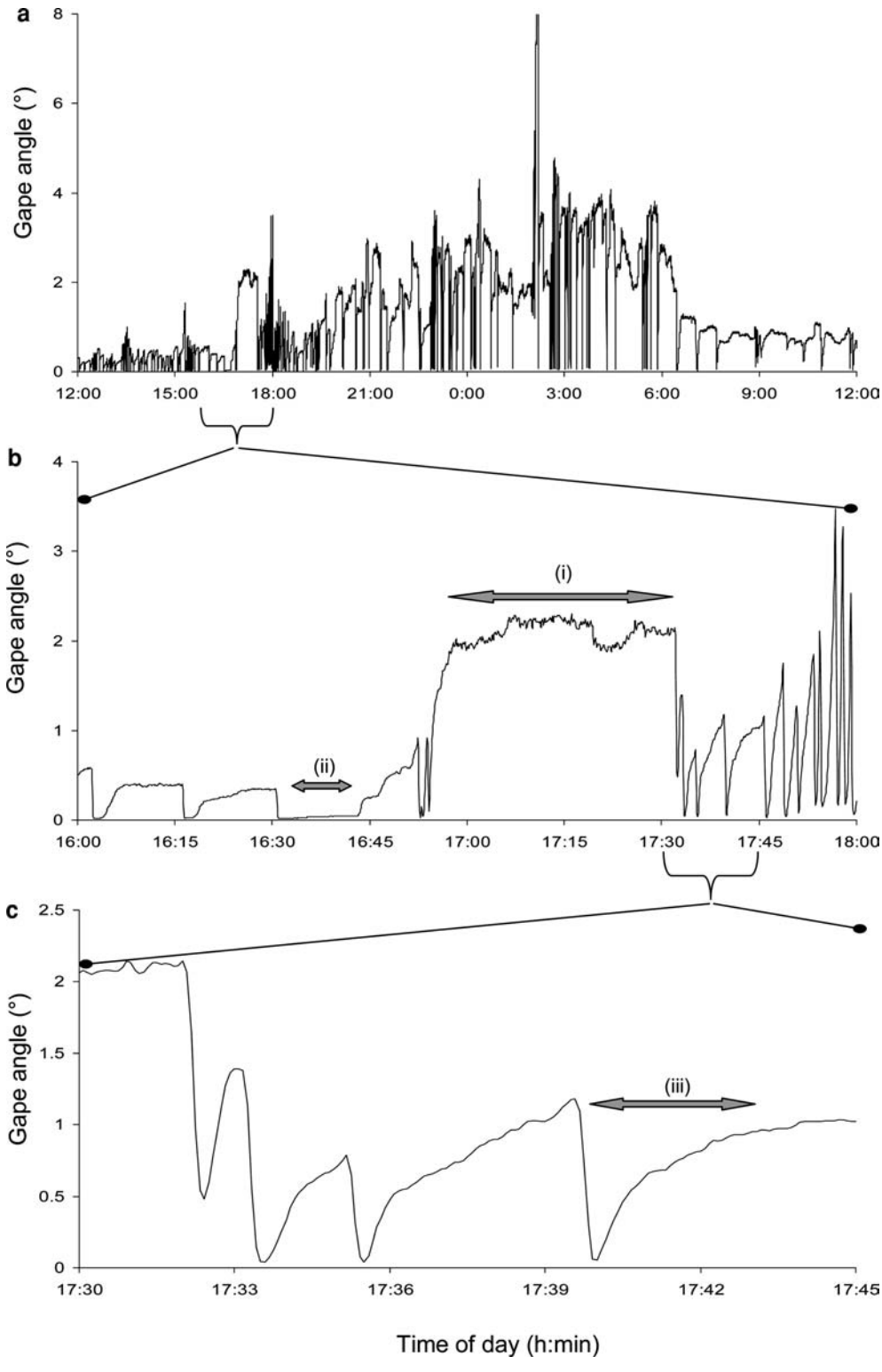

Fig. 2 Examples of changes in gape angle in a blue mussel **a** over a 24-h period **b** over a 2-h period and **c** over a 15-min period. Three major types of behaviour are highlighted: (i) slow change of gape angle over time, (ii) rapid closure leading to extended complete closure or systematic opening and (iii) rapid closure followed by slow opening approximating a log function

apparent 'noise' in all animals. This noise was systematically higher in sand mussels than in blue mussels (cf. Figs. 2, 3) which, together with assessment of the Hall sensor system output deployed on dead animals where noise was not apparent, indicates that its origin was biological. More detailed, higher frequency work will have to be conducted to ascertain the nature of this noise although some of it might be due heart beat (cf. Curtis et al. 2000).

Animals put back in the wild after extended periods in the laboratory showed an apparent diurnal rhythm in gape angle, with maximum gape angles occurring during periods with lowest light intensity and this rhythmicity became more obvious with time (Fig. 4,

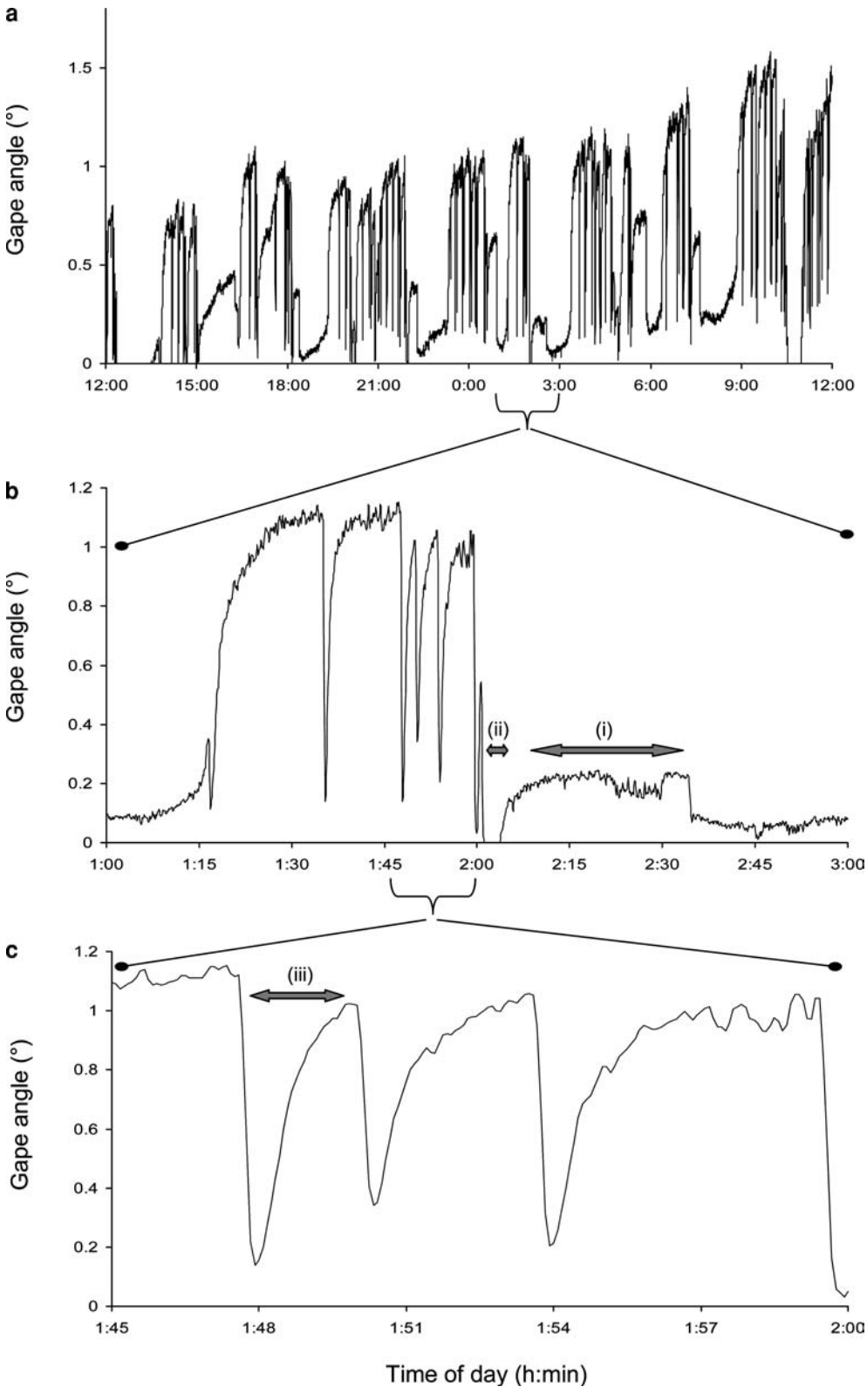

Fig. 3 Examples of changes in gape angle in a sand mussel **a** over a 24-h period **b** over a 2-h period and **c** over a 15-min period. Three major types of behaviour are highlighted: (i) slow change of gape angle over time, (ii) rapid closure leading to extended complete closure or systematic opening and (iii) rapid closure followed by slow opening approximating a log function

ANOVA; $P < 0.0001$, Fig. 5). Maximum gape angles also varied between individuals (ANOVA; $P < 0.0001$) as did the amplitude of the day/night rhythm (ANOVA; $P = 0.003$, Table 1). In addition, the increased nocturnal activity of blue mussels was reflected by a higher frequency of closing/opening events during the dark phase (day: 5.5/h SD 0.9, night: 10/h SD 3.3). Although gaping rhythm also varied between individuals (ANOVA; $P < 0.0001$, Table 2), the general increase of mussel activity in the dark was significant

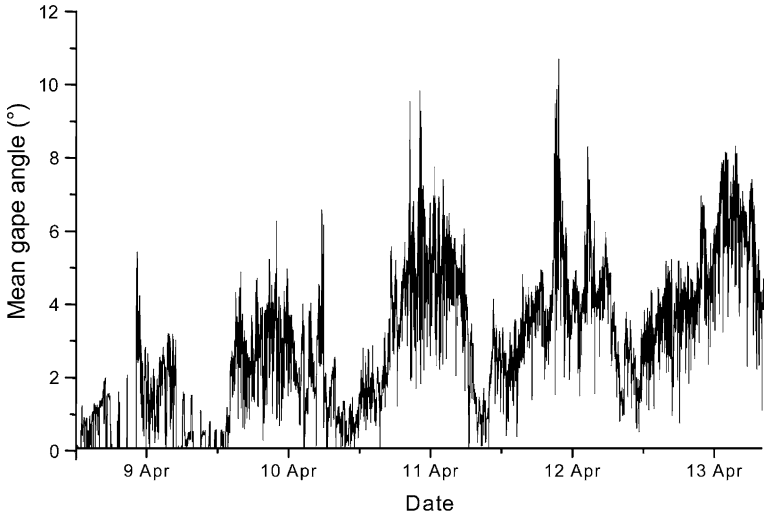

Fig. 4 Mean gape angle (resolved over 5-s intervals) for three blue mussels put in the Kiel Fiord after 10 days in the laboratory (in aerated conditions with constant Baltic seawater through-flow conditions). Note, the onset of a pronounced diel rhythm and that the extent of both the mid-night and mid-day maxima increased over time, presumably as the animals recovered from their treatment in the laboratory. Drops are due to closure events (cf. Fig. 1)

(ANOVA $P < 0.001$). This diurnal variation was much less apparent in laboratory animals exposed to a 12-h light/12-h dark regime.

Discussion

Although the use of remote-sensing techniques is becoming more prevalent in the study of marine vertebrates (Naito 2004), it currently appears to be little used on smaller, sessile invertebrates. Exceptions to this include a system for looking at the gape angle of mussels based on a strain gauge carefully connected to the shell valve (Redpath and Davenport 1988) and a high voltage, coil-based electromagnetic induction system, which also allows determination of gape angle in mussels (DeZwart et al. 1995; Curtis et al. 2000). Our work demonstrates that the highly miniaturized Hall sensor/magnet technology, to date primarily used on marine vertebrates (cf. Wilson et al. 2002; Wilson and Liebsch 2003), can be helpful in elucidating behavioural patterns in marine shellfish for work both in the laboratory and in the field. This is particularly appropriate where direct observation is difficult, such as in turbid water or where animals may be buried in the sediment (Fig. 3) and, in any event, obviates problems with animals being disturbed by procedures involving divers or workers entering the laboratory and thus possibly causing shell gap closure. Our work indicates that this latter problem may be far more prevalent than previously suspected and might, in part, explain why growth rates of blue mussels in the laboratory tend to be lower than those of animals in the wild (Famme et al. 1986; Jørgensen et al. 1990). The absence of a well-defined diurnal rhythm in gape angle in laboratory blue mussels compared to that in the wild as well as the slow return (over 2 days) to a more obvious diurnal pattern certainly implies that our study animals were substantially disturbed, even though they were treated according to standard aquaculture protocols to maximize well-being. Although 24 h rhythms have been noted for some shellfish species in the laboratory (e.g. the Manila clam *Ruditapes philippinarum*, Kim et al. 1996, 1999), our observations of its diminishment could have profound implications for the validity of studies involving these shellfish conducted in the laboratory and certainly needs further investigation.

In the wild, our blue mussels showed a marked day–night rhythm both with regard to maximum opening angle of the shell and to gaping frequency with both parameters being significantly higher during night. Although our findings are based on rather few animals equipped only for periods of a few days, if shell opening relates to filtration rate and gaping frequency to the rate of pseudo-faeces elimination, which is hypothetical still, the filtering activity of mussels and hence their impact on plankton should be substantially larger by night. Further studies should clarify this although given the inter-individual variability reported both by us and in the literature to standard conditions (Curtis et al. 2000) as well as the rapid change in behaviour with varying conditions (DeZwart et al. 1995) careful experimental protocol will have to be observed on large sample sizes.

The sudden closures of the shell gap, which occurred in all animals at all times may be related, in part, to disturbance but may also have a function related to the elimination of pseudo-faeces (Barrington 1979). If filtration activity is lower at smaller average gape angles during day pseudo-faeces must be eliminated less often, which might explain why the frequency of sudden closures was reduced by 50% during daytime. We note that extreme disturbance resulted in complete shell gap closure for extended periods but that partial closure resulted in a subsequent immediate opening of the shell according to an approximately log function. The consistency of this opening behaviour, as well as the reduction in shell-opening rate as a function of gape angle, accords with a passive opening due to the elasticity of the ligament. In contrast, muscular shell closure is much more rapid and of approximately constant rate.

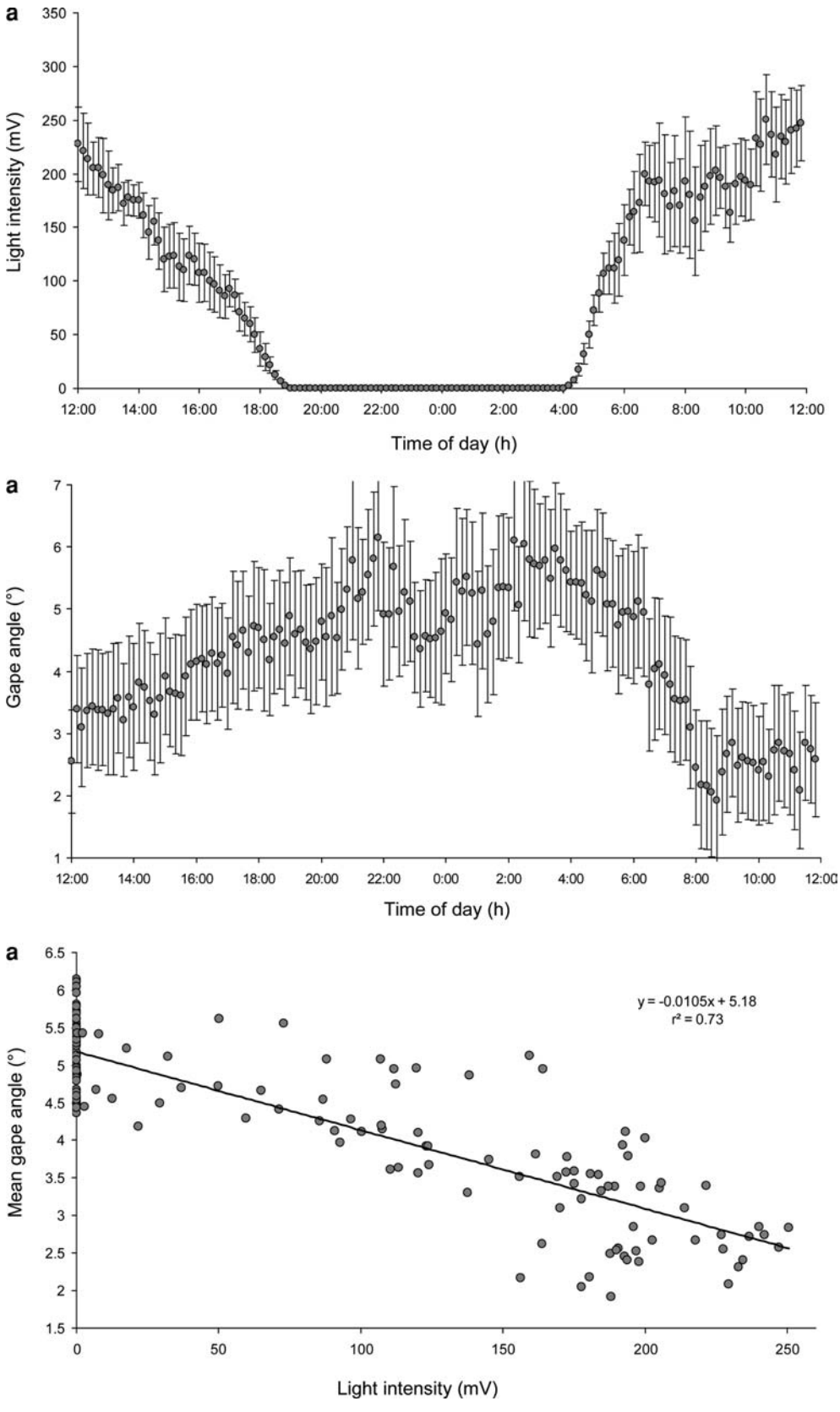

Fig. 5 **a** Mean light intensity (expressed in mV output from the photo-sensor) as a function of time of day for 4 days in April during which blue mussels in the Kiel fiord were monitored for gaping activity. *Bars* show SE. **b** Mean gape angle for four mussels over 4 days (a total of 16 mussel days) in April in the Kiel fiord. *Bars* show SE. **c** relationship between mean gape angle (data shown in **b**) and light intensity (data shown in **a**)

This means that the extent to which the shell gape angle varies over time may be accessible to mathematical modelling if the factors responsible for the incidence and degree of shell closure can be identified. Since shell gape angle is liable to be related to filtration rate, at least for smaller gape angles, this is particularly important with

Table 1 Maximum gape angle

Source of variation	SS	df	MS	*F*	*P* value	*F* crit
Individuals	249.2	3	83.1	37.5	< 0.0001	3.01
Day versus night	62.7	1	62.7	28.3	< 0.0001	4.26
Interaction	40.7	3	13.6	6.1	0.003	3.01
Within	53.2	24	2.22			

Table 2 Gape rhythm

Source of variation	SS	df	MS	*F*	*P* value	*F* crit
Individuals	350.2	3	116.7	22.5	< 0.0001	3.25
Day versus night	119.3	1	119.4	23	< 0.0001	4.49
Interaction	78.1	3	26	5	0.012	3.24
Within	83.1	16	5.2			

respect to feeding behaviour and may help explain mussel growth patterns.

The technology is not without its difficulties, however. We experienced problems attaching our magnets and sensors to the shells reliably for extended periods but note that other authors have reported better success gluing things to shells (Curtis et al. 2000). This will have to be given high priority when monitoring species in the wild exposed to high wave energies. In addition, it is highly desirable that an appropriate calibration be obtained so as to derive shell gape angle from sensor output. Here, it is important to note that magnetic field strength perceived by the Hall sensor is not linearly related to the distance between the magnet and sensor (cf. Wilson et al. 2002) so that representation of output as a direct measure of distance (or angle) is inappropriate (cf. Curtis et al. 2000). To linearise, the output the animals should be brought into the laboratory or, if the animals are to be maintained all the time outside, divers should return repeatedly to document gape angle at defined times.

None-the-less, despite these problems, we believe that the proposed use of the Hall sensor/magnet technology represents a major methodological advance in documentation of behavioural patterns in bivalves, even infaunal species, because sand and mud does not affect magnetic fields. Its advantages over existing systems are that, unlike strain gauges (Redpath and Davenport 1988), it is relatively uncritical with regards to attachment and, unlike coil-based systems (DeZwart et al. 1995), requires neither a high voltage nor the delivery of power to both halves of the bivalve shell, thus simplifying attachment and long-term stability considerably. The sensitivity of the system allows for resolution of changes in gape angles of greater than 0.01° and the highly variable frequency with which data can be gathered should allow for resolution of a variety of issues relating to shellfish: high frequency recording may allow heart beat frequency to be recorded as has been done in vertebrates (Wilson et al. 2004), which together with gape angle is useful for monitoring pollutants (deZwart et al. 1995; Curtis et al. 2000) and low-frequency recording in long-term studies may even allow researchers to determine growth rate of shellfish because oppositely mounted magnets and sensors will tend to move farther apart as the animal grows. In any event, cognisance will have to be taken of this in gape studies that span weeks rather than days. Finally, we note that systems currently on the market for recording Hall sensor outputs have memories of up to 128 mbytes, which, recording at intervals of, e.g. 1 and 5 s, gives recording lifetimes of 2 and 10 years, respectively. This should allow adequate span for determination of many parameters relevant to the lifespan of most shellfish (but see Schone et al. 2005).

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