CHANGES IN GAPF FREQUENCY, SIPHON ACTIVITY AND THERMAL RESPONSE IN THE FRESHWATER BIVALVES ANODONTA CYGNEA AND MARGARITIFERA FALCATA

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ABSTRACT

Physiologically-driven rhythms in bivalve molluscs are predicted to vary as a function of metabolic rate and temperature, in contrast to genetically predisposed biological clocks. These rhythms can be evaluated using long-term video monitoring techniques under controlled conditions in laboratory aquaria. The bivalves Anodonta cygnea and Margaritifera falcata were used to evaluate the effect of temperature on rhythms in gape and the formation of siphons at the mantle edge. Frequency and duration of shell closure vary with temperature in both species, but with different responses. Mean duration of intervals of valve closure decreases as temperature rises in both species, and is consistent with physiological limitation by increased biological oxygen demand. For A. cygnea, cumulative gape duration peaks at 25°C, with less time spent closed than at any other temperature, but increasing temperatures correspond to an increase in gape frequency with a strong increase observed at 31°C. In contrast, frequency of adduction and valve closure peak at 25°C in M. falcata, and continuous gapping is observed above 29.5°C. This physiological stress is consistent with evidence from sclerochronologically-calibrated stable isotope studies of shells, where growth breaks in many marine taxa coincide with maximum temperatures above 31°C as derived for δ¹⁸O carbonates. The results of this study suggest that these growth breaks may be due to physiological limitations in oxygen uptake and metabolic activity, rather than being a direct consequence of elevated temperature alone.

INTRODUCTION

Biological rhythms have been documented in bivalve molluscs for decades, and their timing and significance remains a topic of great interest. Early studies of circadian rhythms (24-h isolation cycles matching the period of Earth’s rotation) emphasized entrainment of activity by daily light variations (e.g. Pannella & MacClintock, 1968; Rhoods & Pannella, 1970). In many marine species, however, circalunidian cycles (rhythms synchronized with the lunar daily period of 24.8 h) often play a dominant role (e.g. Evans, 1972, 1975; Pannella, 1976; Richardson, 1988; Palmer, 1995). Both endogenous (e.g. Beenjes & Williams, 1983; Abell, Amegashiti, & Ochumba, 1995) and environmentally entrained rhythms have been observed, and some bivalves have been demonstrated to switch between circalunidian and circadian rhythms when transferred to controlled conditions (Kim et al., 2003). In the case of circalunidian clocks, two separate clocks appear to operate, coupled in antiphase with periods of ca. 12.4 h under normal conditions but decoupling in the laboratory (Palmer & Williams, 1986). In contrast to circadian and circalunidian rhythms, however, the influence of environmental factors on periodicities at ultradian (subdaily, infradian, intra-daily) time-scales with durations shorter than 24 h have yet to be fully evaluated.

Studies at this resolution, including measurements of gape periodicity, feeding and heart rates, are generally invasive in nature. They frequently require that the studied organism be affixed with one or more sensors and often wired to recording equipment, and are only suitable for relatively passive organisms that move infrequently. Many bivalve molluscs are ideal for such studies, and their biorhythms have been evaluated by techniques as diverse as actographs (Akberali, 1978; Kontreczky et al., 1997), electrodes (Englund, Heino & Melas, 1994), inductive proximity sensors (Miller & Payne, 1999), magnetic relays (Garcia-March et al., 2008) or electromagnetic coils and infrared optocouplers (Curtis, Williamson & Depledge, 2000). Particularly invasive studies have even required that sensors be inserted into the mantle cavity through holes drilled through the shell (e.g. Trueman & Lowe, 1971; Gordon & Carrier, 1978). However, while these techniques provide data regarding shell gape or cardiac activity, they provide no insight on siphonal activity. Beyond the problems inherent in affixing sensors, complications arise when experimental animals are studied without provision for food (e.g. Kontreczky et al., 1997; Moura et al., 2000; Curtis et al., 2000). In order to minimize the experimental impact of observation, modern workers are increasingly pursuing non-invasive video monitoring techniques both in short-term field deployments and in longer term laboratory studies (e.g. Thorin, Bourdages & Vincent, 1998; Thorin, 2000; Newell, Wildish & MacDonald, 2001; Riisgård, Kittner & Seerup, 2003; Rodland et al., 2006). While limitations remain for either setting, video techniques provide an avenue to control for potential experimental effects in more invasive studies.

One major difficulty in understanding the periodicity of behaviour, growth and activity lies in discriminating...
genetically predisposed biological clocks from physiologically controlled quasi-periodic behavioural rhythms. Clocks must be invariant with regard to environmental conditions in order to tell time with any accuracy, and thus be of any utility to the organism (Palmer, 2000). In contrast, rhythms controlled by metabolic rates and physiology should vary as a function of extrinsic factors, especially temperature. Interpretations of sub-daily growth increments in the shells of the organism depend on this distinction, and the thermal response of infradian bi-rhythms plays a key role in determining the difference between biological clocks and environmental controls.

In order to test whether infradian activity cycles respond to environmental controls, specimens of *Anodonta cygnea* (L.) and *Margaritifera falcata* (Gould) were maintained and observed in laboratory aquaria under controlled temperature conditions. Bivalve activity was recorded using a digital camera, and resulting image archives analysed for periodic siphon and adductor activity. The presence or absence of temperature effect may be useful in distinguishing endogenous 'biological clocks' and metabolic or physiological rhythms influenced by environmental conditions. Changes in the period of rhythms in siphon or gape activity resulting from temperature changes should indicate physiological effects of metabolism, whereas temperature-invariant rhythms may reflect genetically predisposed endogenous clocks.

This study also provides the opportunity to evaluate threshold transitions in behaviour regime reflecting the onset of significant physiological stress, including temperatures beyond those normally experienced under normal environmental conditions. Many marine taxa, including bivalve molluscs as well as cirripeds, all record growth breaks occurring at sclerochronologically-calibrated, $^{18}\text{O}_{\text{carbonate}}$-derived temperatures above 31°C, despite recorded environmental maxima well in excess of this value (Schöne et al., 2006a). While these recorded isotopic temperatures may simply reflect optimum growth conditions (e.g. Ansell, 1968; Tanabe, 1988; Tanabe & Oba, 1988; Schöne et al., 2002b), they might indicate a threshold temperature above which growth is inhibited in modern and fossil organisms. Thermal tolerance may be governed directly through temperature-dependent changes in enzyme activity, but can also be an indirect effect through factors such as oxygen limitation (e.g. Förster & Knust, 2007). Gape and closure frequency and duration should reflect physiological tolerance regardless of cause, but should be particularly sensitive to oxygen limitation.

**MATERIAL AND METHODS**

*Anodonta cygnea* (a common inhabitant of European and Asian ponds and lakes) was collected from the 'Große Kasse' pond (L.) and *M. falcata* (E10.27) of Oba, Germany, in December 2004 and maintained in laboratory aquaria (one 50-l aquarium from November 2005 until August 2006. Specimens of the stream-dwelling North American bivalve *Margaritifera falcata* were collected from Coghlan Creek, near Vancouver, British Columbia (ca. N49.12', W122.53') and were maintained in a 50-l aquarium from November 2005 until August 2006. Continuous image archives were constructed for two specimens (one adult, one juvenile) of *M. falcata*, and one specimen of the freshwater mussel *A. cygnea*. Aquarium temperatures were maintained for multiple days at stable levels ranging from 7.3 to 34.5°C for *M. falcata*, and from 14 to 34°C for *A. cygnea*.

Temperature in the tanks was regulated using a combination of continuous illumination by a fluorescent light in close proximity, with a refrigerating pump used to maintain stable temperature conditions (± 1°C). Results for specific temperature regimes were derived from observations during controlled temperatures maintained over 48-h intervals (see Table 1 for duration of observation of each species for given temperature ranges) with a total experimental temperature range between 7°C and 34°C. By eliminating naturally occurring cyclic variations in temperature and food supply, external factors capable of entraining biological clocks were limited. Aquaria were continuously illuminated to facilitate camera operation and to limit the influence of day–night cycles on biological rhythms, and circulation was maintained by electric pumps for constant aeration. The specimens were fed with commercially available planktonic food (Plancto® by Aquamedic for *A. cygnea*, Ultra Clam by Fauna Marin for *M. falcata*) once per day, but were fed at irregular times in order to disrupt potential entrainment by cycles of food availability.

Images were acquired continuously using a web-camera with 640 × 320 pixel resolution connected to a PC laptop via USB cable. Images were saved at 20-s intervals using custom-designed software and archived on CD-ROM. Images were analysed using the program i-analyse 1.03 (developed in conjunction with ReaSoft, USA). Gape and intervals of valve closure were identified by changes in absolute brightness records derived from images of valve margins (Rodland et al., 2006) and verified by visual observation of the image record, and the duration of these intervals measured.

**RESULTS**

Long-period valve closure cycles were observed in both *Margaritifera falcata* and *Anodonta cygnea*. Under temperature regimes reflective of life conditions for both species (<15°C for *M. falcata*, <25°C for *A. cygnea*), a quasiperiodic pattern in valve-closure frequency was observed, alternating between high- and low-activity periods every 2 days on average. As a result, all closure-frequency and duration data presented here were compiled for 48-h intervals in order to average out the range in daily values. For comparison of closure-frequency and duration, temperature values represent average values recorded over multiple days and grouped into temperature bins.

*Anodonta cygnea*

Periods of valve closure are identified most readily from absolute brightness records derived from the shell margin, occurring on average once every 48 h at temperatures below 30°C. However, valve closure occurred at increasing frequency as temperatures increased (Fig. 1). The cumulative duration of valve closure was minimal at 25°C (6.5 out of 48 h, or 13.5% of the observation interval), and increased dramatically between 30°C and 31°C (Fig. 2). In contrast, the mean duration of individual valve closure events decreased continuously at higher temperatures, with peak values observed at 15°C (Fig. 3).

*Margaritifera falcata*

Pronounced differences were observed between juvenile and adult specimens of *M. falcata*, with the juvenile gaping almost

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**Table 1. Hours of observation of Anodonta cygnea and Margaritifera falcata.**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>A. cygnea</th>
<th>M. falcata</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>168</td>
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<tr>
<td>15</td>
<td>70.6</td>
<td>216</td>
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<td>25</td>
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<td>32</td>
<td>48</td>
<td>288</td>
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<td>34</td>
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continuously unless physically disturbed. The adult specimen was observed to spend as much as 23.5 h closed, although the mean duration varied with temperature. Mean closure frequency in the adult specimen increased with temperature, reaching a peak between 25°C and 29°C (Fig. 1). However, *M. falcata* underwent a behavioural transition at 29.5°C, and valve closure was observed only in response to physical disturbance at temperatures above this threshold. The longest cumulative closure duration for each 48-h interval was observed at low temperatures for the adult (Fig. 2) but, as with *A. cygnea*, mean shell closure duration declined as temperatures increased to 34°C (Fig. 3). Results from the juvenile specimen are equivocal as gaping was nearly continuous, possibly indicating a difference in metabolic controls (e.g., feeding and growth rate) between adults and juveniles. Both specimens showed difficulty in maintaining well-defined siphons at the mantle edge at temperatures in excess of 32°C, and 34.5°C proved lethal to the adult. While the juvenile survived this extreme, it remained incapable of maintaining siphon form at lower temperatures for over a week, suggesting damage at the growing edge of the

**Figure 1.** Mean frequency of valve closure per 48-h observation interval in *Anodonta cygnea* and *Margaritifera falcata* at each temperature stage of the experiment. This also reflects the number of gaping events per observation interval; every time the valves close, they must return to a gaping position.
shell–mantle interface. Without well-defined siphons at the mantle edge, efficient inhalant and exhalant currents cannot be maintained by the animal, and both feeding and respiration may suffer.

**DISCUSSION**

Web-camera-based observational records of freshwater bivalve species were used to investigate the response of cycles in gape to changes in temperature under otherwise constant environmental conditions. Valve closure frequency and duration over 48-h intervals changed dramatically with increasing temperature. Closure frequency increased dramatically in excess of 31.5°C in *Anodonta cygnea*, while reaching maximum values between 25 and 29°C in *Margaritifera falcata*. Valve closure was observed only in this latter species when physically disturbed at temperatures above 29.5°C, and the juvenile specimen maintained gape almost continuously under all conditions, in contrast to the adult. The mean duration of each closure decreased in both species as temperature increased, consistent with elevated metabolic rates and increased oxygen stress during valve closure.

**Figure 2.** Mean values for the cumulative duration of valve closure over 48-h intervals as they vary with temperature in *Anodonta cygnea* and *Margaritifera falcata*. This is the inverse of gape duration, which is found by subtracting each value from a maximum value of 48 h.
Periods of gape and valve closure in *A. cygnea* and *M. falcata* provide different results, but are consistent with previous work. Pynnönen & Huebner (1995) reported gape intervals between 3 and 5 h and closures ranging from 5 to 20 h in a study of the influence of pH on feeding behaviour. In contrast, Kontreczky et al. (1997) documented mean closure durations of 10.5 h and gape intervals of 13.5 h in an investigation of the effects of the insecticide deltamethrin. In a study on the influence of aluminium concentrations, Kádár et al. (2001) reported mean shell gape intervals ranging between 40 and 75 h over 5 days but their results varied with season, possibly due to changes in ambient temperature as noted in this study. All three of these studies noted a decrease in gape duration relative to closure as water chemistry deviated further from control conditions. If gape duration in *A. cygnea* is linked to favourable feeding conditions, 25°C may represent an optimal temperature for this species.

**Figure 3.** Mean duration of individual valve-closure events as a function of temperature in *Anodonta cygnea* and *Margaritifera falcata* showing general decline in duration as temperatures increase.
Increased durations of valve closure occur at low algal concentrations (Riisgård et al., 2003). Extensive periods of valve gape observed for *A. cygnea* and *M. falcata* suggest that food supply was not a limiting factor on their activity, at least at temperatures below 25°C. The nearly continuous gape behaviour of the juvenile specimen of *M. falcata* could either reflect wider tolerance for varying plankton concentrations than in adults or a greater need for food due to accelerated growth during early stages of growth. Increases in valve closure for *A. cygnea* at higher temperatures are harder to interpret, but may reflect a defensive response to elevated temperature by a species normally inhabiting stagnant pools and well adapted to periods of lower oxygen availability.

Gape duration, frequency and duration of valve closure showed a dramatic change as water temperatures approached 30°C, with the adult *M. falcata* reaching peak frequencies at 25°C and abruptly ceased valve closure at temperatures in excess of 29.5°C. Valve closure insulates bivalves from environmental fluctuations, but interferes with respiration, and its duration varies according to tolerance for hypoxia. High temperatures can stress bivalves through oxygen deprivation, as solubility decreases with increased temperature while oxygen demand rises with metabolic rates. Thus, the difference in response between these species may simply reflect differing tolerance for hypoxic conditions, because both demonstrate decreasing mean closure durations as temperatures increase. *Anodonta cygnea*, common in ponds where stagnation can induce eutrophic conditions and bottom-water hypoxia, may be more tolerant than *M. falcata*, which prefers running streams.

Neither species studied here is likely to experience the upper temperature thresholds documented here in their natural habitats. *Anodonta cygnea* experience a temperature range from 5 to 25°C (Ricken et al., 2003), and appears healthy in the upper portion of this temperature range in this experiment. The natural conditions for *M. falcata* range from 4 to 15°C as reconstructed from shell-isotope records (Schöne et al., 2006b), and this may account for the greater indications of stress at elevated temperature. Disparate taxonomic groups show dramatic reduction in carbonate secretion at or above 31°C and higher temperatures appear to inhibit the production of carbonate skeletal material (e.g., Goodwin et al., 2001; Schöne et al., 2002a, 2006a); a maximum threshold of 34°C has been reported (Schöne & Gire, 2005), but it is difficult to infer ambient δ18O values from these settings. These results are consistent with evidence that the ability of skeletal carbonates to serve as paleoenvironmental archives is limited by physiological stress and temperature extremes.

As sclerochronological records gain in importance for the reconstruction of palaeoenvironmental conditions, it becomes increasingly important to understand the temporal resolution of growth increments and controls upon their formation. The results presented here are consistent with observations from marine species that elevated temperatures and correlated factors (e.g. hypoxia, disease) induce physiological stress in a variety of marine taxa (e.g. Harvell et al., 2001; Pörtner, Peck & Hirse, 2006). The results coincide with growth shutdown temperatures noted from sclerochronologically-calibrated stable-isotope studies; temperatures above this threshold cannot be reliably obtained from a variety of mollusc species (e.g. Goodwin, Schöne & Dettman, 2003; Schöne et al., 2006a).

The interactions between shell growth and periodic behaviour require further evaluation, especially for species characterized by rapid growth. Some species form growth bands at sub-diurnal intervals, including semi-diurnal tidal signatures, but finer banding is often apparent at high magnification (e.g., Pannella & Mc Clintock, 1968; Gordon & Carriker, 1978; Schöne et al., 2002a). A better understanding of ultradian growth patterns may shed further light on the physiology of shell growth, and ultimately allow the investigation of environmental records at sub-diaily resolution.

Further investigations will help to develop a greater understanding of the results presented here. Neither *A. cygnea* nor *M. falcata* experience temperatures as high as 32°C under normal conditions, and their different responses to elevated temperatures may have distinctly different causes. The role of oxygen limitation needs further study, particularly in regard to juvenile specimens and in terms of size- and metabolic-rate-dependent oxygen demand. High-resolution sclerochronological evaluation of changes in growth rate according to temperature will play a critical role in understanding this temperature threshold, but marine taxa, with their higher growth rates and more heavily calcified shells, may prove more amenable to such studies.

The relationship of the observed biological rhythms to the growth and development of shell microgrowth increments remains a topic for future investigation. If growth increment formation is influenced by intervals of valve closure, sclerochronological records may reflect a thermal influence beyond documented correlations of growth rate with temperature. The dramatic changes in activity patterns near 30°C are consistent with known cut-off temperatures for growth in marine bivalves as determined by sclerochronologically-calibrated oxygen-isotope analyses, and suggest a physiological limit to skeletal secretion. These physiological limits may reduce the range of palaeo-temperatures recorded in the shells of bivalve molluscs, but the resulting growth breaks might record the frequency of extreme temperature excursions.

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**REFERENCES**


