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Substratum-mediated heart rate responses of an invertebrate to predation threat

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Although the relation between behaviour and stress in humans and other vertebrates is well documented, few comparable observations exist for invertebrates. We addressed this issue by considering the impact of the physical environment on cardiac activity in invertebrates exposed to predation and nonpredation threat scenarios. We used cardiac activity as a proxy of stress in juvenile queen scallops, *Aequipecten opercularis*, under predation threat by the common starfish, *Asterias rubens*. Stress levels were monitored in juvenile queen scallops exposed, and not exposed, to predation threat on a substratum known to act as a refuge (live maerl) as well as substrata known not to possess refuge potential (dead maerl, sand and no sediment). In the vicinity of known refuges, stationary scallops under predation threat had significantly lower cardiac activity than individuals in habitats lacking refuges. Scallops not under predation threat did not show significant differences in cardiac activity. These are probably habitat-mediated physiological responses to the presence of a predator and possibly to the availability of suitable attachment substrata. These findings have implications in terms of behavioural physiology in invertebrates.

Behaviour is related to physiological processes and cardiac activity can be used as a proxy of physiological and psychological status in vertebrates. Physiological effects of high caffeine intake in humans include tachycardia (Mosqueda-Garcia et al. 1993), and psychological stimuli of social conflict in laboratory rats, *Rattus norvegicus*, have been observed to affect heart rate (HR) rhythms (Meerlo et al. 1999). Invertebrates too, show similar physiological responses; HR is positively correlated with temperature (Santini et al. 2000) and is affected by hormones (Pugliese et al. 2004). Environmental stress of anthropogenic origin also affects cardiac activity in invertebrates. For instance, exposure to high concentrations of waterborne copper increases cardiac activity in mussels, *Perna viridis* (Nicholson 2003), and decreases it in limpets, *Patella aspera* (De Pirro et al. 2001). Describing the behavioural responses of an invertebrate to a stress factor can present many difficulties, particularly when responses are cryptic or ambiguous. However, cryptic behavioural responses may be estimated through changes in a proxy such as HR.

In Bengalese finches, *Lonchura striata* var. *domestica*, HR has been used as a proxy of song perception in females and males instead of behavioural responses, which are often sexually dimorphic and not comparable (Ikebuchi et al. 2003). In addition, HR responses to social disturbance that are not manifested behaviourally have been observed in vertebrates such as mountain sheep, *Ovis canadiensis* (MacArthur et al. 1982). Aggression affects behaviour in invertebrates (Elwood et al. 1998) and motivational levels are related to those of circulating neuromodulators (Sneddon et al. 2000), which are known to affect HR directly in invertebrates (Wilkens & McMahon 1992). Therefore HR can be used as a sensitive predictor of responsiveness to various stimuli (Listerman et al. 2000; Schapker et al. 2002).

Predatory crabs, *Portunus sebae* and *Mithrax sculptus*, exposed to the ink of the sea hare *Aplysia dactylomela* show temporary pauses in heart and scaphognathite beating (Carefoot et al. 1999); similarly, introduction of a predation threat (and not the predation event itself) may be expected to alter stress levels and thus the HR of invertebrate prey. However, could reaction to refuge availability before or during predation threat serve to calm a prey organism?

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Any such change in stress levels will directly influence cardiac activity, for example via hormonal release (Sadock & Sadock 2002). During predation events themselves, prey organisms generally experience elevated HR resulting from enhanced energetic requirements associated with escape or defence tactics (Rovero et al. 1999).

Juvenile queen scallops, *Aequipecten opercularis*, are known to use maerl (a biogenic carbonate habitat composed of, and created by, marine coralline algae) preferentially as a refuge when experiencing predation threat by the common starfish, *Asterias rubens* (Kamenos et al. 2004b). Cardiac activity in the juvenile queen scallop can be recorded with noninvasive infrared phototransducer cardiography which is less likely to affect observed HR than traditionally used invasive techniques. We used this technique and study species to examine the concept of habitat-mediated stress control in marine invertebrates. We hypothesized that in the vicinity of a refuge the stress levels of invertebrates would be lower (indicated by reduced HR) than in the absence of a refuge during enhanced predation threat. As the results of our investigation may relate to a wide array of invertebrates, it was important to show that HR in scallops responded in the expected manner to well-documented external stimuli when we monitored their activity via infrared cardiography. We therefore also considered the effects of temperature and oxygen tension on HR.

METHODS

Live maerl, impacted dead maerl and sand were collected as described by Kamenos et al. (2004a). Juvenile *A. opercularis* were obtained from a commercial grower (Loch Fyne Sea Farms Ltd, Tarbert, Argyll, U.K.). The spat were collected on spat bags and grown-on in lantern nets; thus they were not preacclimatized to any particular natural substratum. Scallops were stored in flow-through tanks providing 2000 litres/h of unfiltered sea water at $12 \pm 1\,^{\circ}$C under natural photoperiod until required. Substratum and predation risk experiments were all carried out in running sea water aquaria under natural temperature and photoperiod conditions; all other experiments were carried out in a thermostatic chamber under continuous illumination conditions with continuous aeration and sea water flux. Sea water salinity was monitored with a hand-held optical refractometer. Standard conditions were salinity 35 ± 2 and temperature $11 \pm 1\,^{\circ}$C ($\pm 0.5\,^{\circ}$C in the thermostatic room). All juvenile scallops used ranged from 22 to 26 mm in shell height. Preliminary trials indicated that HR had no relation with shell height or associated wet and dry visceral masses within the size range of scallops used. Isolated rooms were used for all experiments, where ambient noise was reduced to a minimum. Recording of physical and biological parameters was remote.

Heart Rate Monitoring

We glued an infrared (IR) sensor (Temic Telefunken CNY70, Telefunken Licenses GmbH, Frankfurt am Main, Germany) with cyanoacrylic glue (Super Attack, Loctite, Henkel, München, Germany) to the upper valve of each scallop in the dextral region adjacent to the umbo, directly above the heart. The sensor was aligned with the photodiode nearest the umbo and the IR light-emitting diode (980 nm) furthest away. The signal was filtered and amplified with appropriate circuits, read by a digital oscilloscope (Fluke 123 Scopemeter, Fluke Italia s.r.l., Milan, Italy), and stored on a computer. We averaged three cardiac traces, of 60 s each, for each individual to calculate its mean HR at a given time. We did not consider amplitude because only a measure of HR is required for a neurological proxy (Sadock & Sadock 2002). After gluing the sensor to the shell, we allowed the scallops to acclimatize for 24 h before measuring HR. Only animals in a state of rest and that had shown no swimming activity in the previous 5 min were used for recordings, thus ensuring recorded HR was indicative of physiological status associated with enhanced predation threat. After the experiments, we removed the sensors without harming the scallops.

Effect of Temperature

We tested heart rate responses of juvenile queen scallops ($N = 40$) at four temperatures (7, 12, 17, $22 \pm 0.5\,^{\circ}$C) in a thermostatic room using water baths to maintain thermostatic stability. After acclimation at $12 \pm 1\,^{\circ}$C for 24 h, individuals were transferred to test aquaria and HR was recorded after a further 24 h.

Effect of Oxygen Tension

We recorded HR and oxygen tension simultaneously (Hanna Instruments – HI 9142, Hanna Instruments, Leighton Buzzard, U.K.). Respirometry was conducted in open chambers (2000 cm^3) where a stable percentage oxygen saturation ($\pm 0.75\%$ O_2) was maintained by means of a continuous dissolution of ratios of air and nitrogen. This continuous gaseous introduction also maintained permanent water circulation inside each chamber and allowed the scallops to maintain their natural filtering position. We investigated the relation between oxygen tension (% O_2) and HR in the scallops ($N = 29$) at 30-min intervals for 3 h at three oxygen tensions (55, 65, $75 \pm 0.75\%$ dissolved O_2) at a salinity of 34 and $11 \pm 1\,^{\circ}$C. Different animals were used at each oxygen tension. For each animal we determined the mean HR and mean oxygen tension over the experimental session.

Habitat-Mediated Cardiac Activity Experiment

We designed an experiment to investigate the effects that provision of a potential refuge (live maerl, impacted dead maerl, sand and control, i.e. no sediment) and predation threat (*A. rubens*) had on the HR of juvenile queen scallops. Substrata were placed in upright cylindrical flow-through chambers ($N = 30$ for each substratum/control) constructed of plastic tubing and Netlon (10 cm