TECHNICAL COMMENT

Application of Miniature Heart Rate Data Loggers for Use in Large Free-Moving Decapod Crustaceans: Method Development and Validation

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ABSTRACT

Cardiovascular responses of decapod crustaceans to environmental challenges have received extensive attention. However, nearly all of these studies have been restricted to lab-based experiments; here we describe a methodology that will enable measurement of heart rate (HR) in free-moving decapods in the field. Data storage tag heart rate and temperature loggers (DST micro-HRT; Star-Oddi) were used to record electrocardiograms (ECG) and HR in large decapod crustaceans. These loggers were originally designed for use in vertebrates and must be surgically implanted in the body cavity near the heart in order to function. We adapted these loggers for external use in large decapod crustaceans. The method involved abrading the carapace directly above the heart and placing the electrodes of the logger directly on top of the dermal tissue. The logger was then secured in place with periphery wax. This method negated some of the more intricate operations used for vertebrates. The rapid setup time of approximately 5 min suggested that animals could be easily instrumented in the field and without the use of anesthetic. The logger was calibrated by simultaneously measuring the HR changes of a West Indian spider crab *Mithrax spinosissimus* with a pulsed-Doppler flowmeter. The data gathered with the two methods showed a tight correlation during an increase in temperature. The loggers were also successfully implanted in a variety of other large species of aquatic and terrestrial decapods. The data obtained showed that the method works in a broad range of species, under different experimental conditions. In each case, the loggers comprised less than 1% of the body mass and would be suitable for use in animals >300 g. All animals survived the attachment procedures and were feeding and active after removal of the loggers. Nearly all previous cardiac measurements on decapods have been carried out in controlled laboratory settings. The use of these loggers will make significant advances in measuring HR in unrestrained, undisturbed animals in their natural environment during extended periods of time and has the potential to lead to novel findings.

Keywords: heart rate, crustacean, data logger, Star-Oddi, physiology.

Introduction

Heart rate (HR) has been measured in a wide variety of vertebrate and invertebrate taxa (e.g., deFur and Mangum 1979; Davies and Morris 1993; Acharya et al. 2006; von Borell 2007). It is used as a proxy for metabolic rate and stress levels and to quantify physiological responses to environmental and biotic variables (DeFur and Mangum 1979; Handy and Depledge 1999; Wikelski and Cooke 2006; Green 2011). There is a plethora of data on the cardiorespiratory physiology of decapod crustaceans (reviewed in McGaw and Reiber 2015). The cardiovascular system of decapod crustaceans consists of a complex series of arteries and capillary-like vessels, so much so that it is considered a partially closed rather than an open circulatory system (Reiber and McGaw 2009). Although cardiac output is influenced by changes in both HR and stroke volume, which can be modulated independent of each other, measurement of HR alone continues to provide important information on the metabolic changes, stress levels, and responses to environmental perturbations in decapod crustaceans (Stillman 2004; McGaw and Reiber 2015; McLean and Todgham 2015).

The HR of crustaceans has been measured using a variety of techniques such as impedance conversion (deFur and Mangum 1979, Stillman 2004), pulsed Doppler (Airriess et al. 1994; McGaw et al. 1994), and infrared transmitters (Depledge and Anderson 1990; Bamber and Depledge 1999; Tepolt and Somero 2014), while in transparent shrimps it can be measured with high-speed videography (Spicer 2001; Harper and Reiber 2006). Measurement of

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HR with impedance conversion and pulsed Doppler requires quite substantial operations, with the sensors protruding into the body cavity. The use of infrared transmitters is less invasive, yet the animals are still carrying probe wires that have to be attached to a recording device. In addition, the crabs often grab at and dislodge the probes (McGaw 2004), which usually requires some type of restraint (Stillman 2004; Tepolt and Somero 2014); this in itself can disrupt HR patterns (I. J. McGaw and S. J. Nancollas, unpublished observations). Despite the fact that all of these methods have their benefits and have been used to gather a plethora of interesting information, because of the electronic interface, nearly all experiments have been based in the lab. A smaller number of experiments have been carried out in the field (Bojsen et al. 1998; Styrishave et al. 2003), but the animal still has to be restrained within a cage and is still tethered to a recording instrument. Methods for measuring long-term HR in free-moving decapods in their natural environment have not been tested. This is important because a recent article suggests that simply monitoring animals in experimental apparatuses with conditions similar to those found in the field can have a significant effect on physiological responses (Simonik and Henry 2014). Indeed, the HR of *Cancer magister* is reduced and becomes more stable if there is a layer of sand in the apparatus (Florey and Kriebel 1974), and free-moving *Homarus americanus* may react differently to hypoxia compared to animals that are restrained (McMahon and Wilkens 1975). Thus, there may be limitations when extrapolating from lab studies and applying them to findings in the field.

The improvement in technology and miniaturization of equipment has led to the development of data loggers that have become small enough that they can be attached to fish, amphibians, birds, and invertebrates to allow data collection in free-moving animals (Weimerskirch et al. 2002; Campbell et al. 2005; Curtis and McGaw 2008; Clark et al. 2010). In the past 15 yr, data loggers that record heart activity have been developed and used with success to monitor HR in fish, reptiles, birds, and mammals (reviewed in Cooke et al. 2004). For optimal use, they have to be implanted internally, requiring quite an extensive operation and recovery procedure for the animal (Campbell et al. 2005; Clark et al. 2010, 2013). In addition, they are fraught with technical difficulties (Cooke et al. 2004), coupled with their high cost, which has discouraged their widespread use (Cooke et al. 2004, 2016). As a result of their fairly large size, these HR loggers have not yet been used in invertebrates. In this study we describe development of a method for using these loggers in decapod crustaceans. We deployed the loggers on several different species of crustaceans of varying sizes and in different environments in order to validate their use and determine their limitations. The strength of these loggers lies in their potential to open up a new area of research allowing one to take the animals from the lab and monitor them in the field for extended periods of time.

Material and Methods

Data storage tag heart rate and temperature loggers (DST micro-HRT; Star-Oddi, Gardabaer, Iceland) were used to record HR and electrocardiograms (ECG) in five decapod crustacean species. These loggers were originally designed for use in mammals and must be surgically implanted in the body cavity. The cylindrical ceramic device is 25.4 mm in length and 8.3 mm in diameter and weighs 3.3 g (fig. 1*A*). The sensor can store more than 40,000 paired HR-temperature measurements and has a battery life of 3.5 mo (with a sampling interval of 10 min). The sensors work by recording the electrical signal from the heart via a positive electrode and a negative electrode; the signal is grounded across a separate electrode. They can be preprogrammed to record at varying intervals; more frequent sampling will exhaust the battery and/or fill the memory more rapidly. Data collection consists of a 1–6-s measurement interval to record ECG, which is then used to calculate rate. The user may choose to store the raw ECG trace, though this substantially decreases the available memory. Otherwise, an HR value is recorded along with a quality assessment as a spreadsheet file. The data are stored in an onboard chip; thus, the loggers need to be retrieved in order to access the data. There are a number of other companies that make telemetric HR transmitters (e.g., Stellar, Data Sciences International) that are small enough to be implanted in fish; nevertheless, these are still limited in that one has to deploy a receiver in close proximity to the transmitter in order to gather data. At the time of writing, we are unaware of any other stand-alone HR logger that is waterproof and small enough to work with decapod crustaceans.

The animals used for the experiments were collected by hand from Rock Sound, Eleuthera, in the Bahamas and transferred to the Cape Eleuthera Institute, where experiments took place. In order to record an electrical signal, the logger has to be in close contact with cardiac tissue. The carapace of the animal was removed directly above the heart using a cordless Dremel tool with an abrading burr attachment. In decapod crustaceans, the location of the heart is delimited and easily identifiable in the center of the carapace (fig. 1*B*). The carapace was shaved until an opaque layer of shell was left, and this was gently removed with a pair of forceps to avoid puncturing the underlying dermis (fig. 1*C*). This was carried out while the specimen was immersed in air, and no sedation was required. (If a species was particularly aversive to hypoxia, it could be partially submerged in a seawater tray so that the gill chambers could be irrigated, preventing any respiratory distress.) The sensor was then maneuvered so the positive and negative terminals came into direct contact with the tissue. The ideal size of carapace removed was about 2 mm wider than the logger itself. This allowed the logger to sit in direct contact with the tissue but prevented side-to-side movement that could potentially disrupt contact of the electrodes with the tissue. A ring of Surgident periphery wax (Heraues Kulzer, South Bend, IN) was molded around the sensor to support it and keep it in contact with the tissue (fig. 1*D*). The wax was pressed against the sides of the logger to prevent the cylindrical unit from rotating in the groove. Wax was further molded over the top of the sensor and secured with cyanoacrylate glue. The total time for operation and securing of the sensor was approximately 5 min.

In the first experiment, the logger was tested and calibrated using the West Indian spider crab *Mithrax spinosissimus* (14.5-cm carapace width [CW], 1,365-g mass). The HR was also simultaneously recorded using a directional 545C pulsed-Doppler flow-

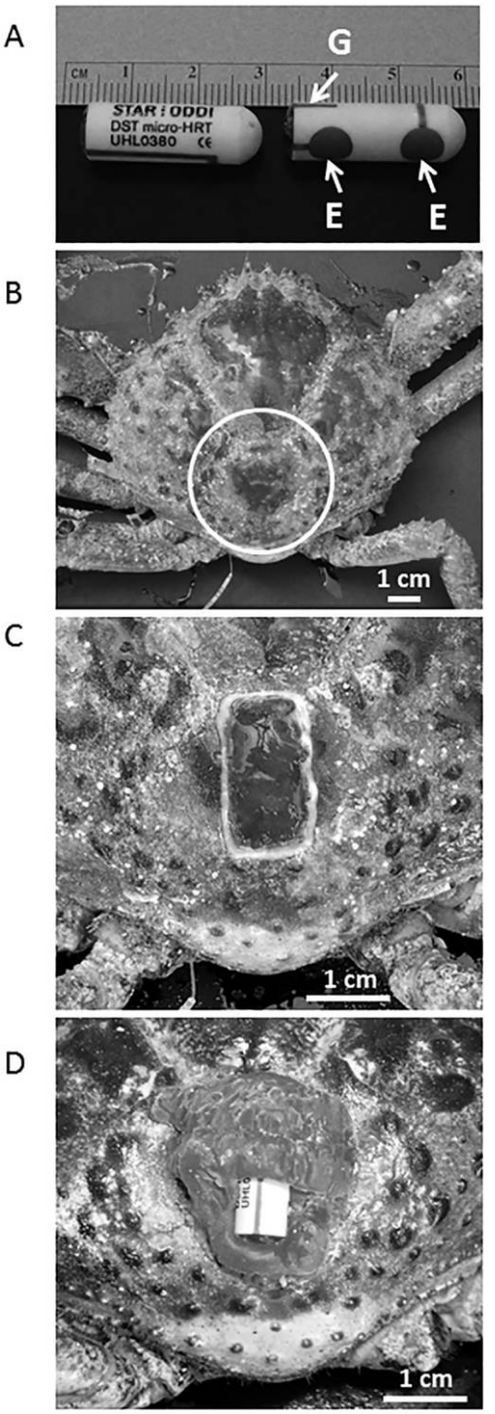

Figure 1. *A*, Photo of heart rate logger showing the two electrodes (E) and grounding electrode (G). *B*, Dorsal view of the cephalothorax of the West Indian spider crab *Mithrax spinosissimus*; in this and other decapods, the heart is clearly delineated (circle) in the posterior central area of the carapace. *C*, The area directly above the heart was abraded to reveal the underlying dermis. *D*, The heart rate logger was placed in the abraded area so that the two electrodes came into direct contact with the tissue. The logger was secured along the edges with periphery wax. Periphery wax was then molded over the top of the logger and sealed with cyanoacrylate glue.

meter (University of Iowa Bioengineering) in order to check the accuracy of the logger recordings. Once the logger was in place, a small hole was made in the first abdominal segment with the Dremel drill and covered with cyanoacrylate glue and a dental dam. Animals were then fitted with a piezoelectric crystal Doppler probe (0.5 mm^2) that was guided to lie against and record pulsatile flow in the sternal artery (Airriess et al. 1994). The time between removal of the animal from the water and fitting it with a Doppler probe was approximately 10 min. The HR logger was programmed to capture data every 10 min, and it calculated the rate from a 5-s interval. The HR from the pulsed-Doppler flowmeter trace was counted manually, and for comparison with the logger, the rate was calculated from a 5-s interval at the approximate time that the logger was programmed to record (fig. 2). After instrumentation, the crab was placed in a covered tank (90 cm diameter × 60 cm depth) in seawater of salinity of 36 ppt and 25°C and allowed to settle for 3 h. An air diffuser ensured that the water oxygenation remained above 90% saturation level, and a small aquarium heater was used to control the temperature. Recordings were carried out at 10-min intervals for a total time of 1 h at the starting temperature of 25°C. The temperature was raised by 3°C (over 15 min) and recording carried out again for a further 1 h at 28°C. This process was repeated once more with the temperature raised to 31°C for a final set of recordings. After the recording period, the logger and the Doppler probe were removed and the holes in the carapace sealed with periphery wax. The values obtained from the Doppler and the logger were compared using a two-way repeated-measures ANOVA.

In a second experiment, a smaller spider crab was used to investigate longer-term changes in HR in the laboratory. The female specimen was 10.7 cm CW and weighed 425 g. After implantation of the logger, the animal was placed in a flow-through seawater tank (80 × 40 × 50-cm depth) on a natural 12L:12D cycle; recordings were set for 40-min intervals. The crab was monitored in the apparatus for 36 h; it was then offered a meal of brown algae, and recordings were carried out for a further 36 h.

A logger was implanted in a rugose swimming crab *Callinectes exasperatus* of 170 g and 12 cm CW to determine the lower size range of animals on which they would work effectively. Recordings were made at 5-min intervals for a total time of 2 h in seawater of salinity 36 ppt and 23°C.

A black land crab *Gecarcinus ruricola* of 10.1 cm CW and 345 g was also instrumented to determine the feasibility of longer-term recording in a terrestrial environment. The logger was programmed to record HR every 30 min for 10 d. During the experiment, the crab was maintained in a land crab mesocosm. This consisted of a wooden slatted hutch (1.5 m × 1.5 m × 1.5 m). The mesocosm was equipped with PVC pipes and damp cardboard for the crab to retreat under. The crab had constant access to trays of freshwater and seawater; lettuce leaves were added to excess every other day, and the crab was allowed to feed ad lib. The mesocosm was placed under a sheltered awning so that animals were exposed to natural diurnal cycles of temperature and light.

In a final experiment, the HR loggers were tested in the field. A slipper lobster *Scyllarides aequinoctialis* of 735 g and 11.5 cm carapace length (CL) was instrumented and set out in the field on March 23, 2017. A Caribbean spiny lobster *Panulirus argus* of

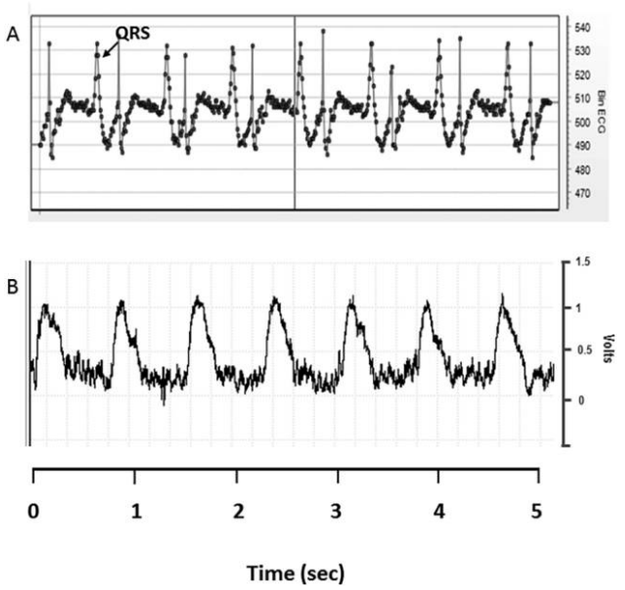

Figure 2. Raw data traces from the electrocardiogram (ECG) from the logger (*A*) are compared with heart rate recorded using the pulsed-Doppler flowmeter (*B*), which was recording pulsatile flow from the sternal artery. Both traces represent 5-s recording intervals from a male *Mithrax spinosissimus* of 1,365 g. The peaks of the ECG and pulsatile flows from the Doppler do not match exactly because the timing of data collection from the two methods could only be approximated. A color version of this figure is available online.

1,170 g and 110 mm CL was set up on March 26, 2017. The animals were placed in a wire mesh cage (150 cm × 90 cm × 75 cm, partially buried in the sand) in approximately 2 m of water. A PVC tube 50 cm in length and 20 cm in diameter was added to the cage as a shelter. The size of the cage allowed the animal to move freely, with direct contact with the substrate, while being subjected to diurnal and tidal cycles and changes in water chemistry. The animals were left in the cage for 72 h, and the loggers were programmed to record data at 30-min intervals. After retrieval of the animals, changes in HR were plotted against diurnal and tidal changes and water temperature.

Results and Discussion

There was no significant difference in the HR of *Mithrax spinosissimus* measured using either the HRT logger or the pulsed-Doppler flowmeter (two-way repeated-measures ANOVA, $F_1 = 0.4$, $P = 0.536$; figs. 2, 3) and no significant interaction term (ANOVA, $F_2 = 1.7$, $P = 0.2$). The values were not exactly the same because the total beats per minute were extrapolated from a 5-s trace (fig. 2) and the timing of the recording of each 10-min recording interval was not matched exactly between the logger and the Doppler. That being said, the Doppler does substantiate that despite a short recording period, the logger is accurately measuring HR. There was a significant increase in HR with increasing temperature (two-way repeated-measures ANOVA, $F_2 = 50.8$, $P < 0.001$), which also substantiates that the loggers were effectively measuring HR.

A logger was implanted in a smaller *M. spinosissimus* (425 g) to determine whether there was an endogenous rhythm of HR, as these crabs are nocturnally active (Winfree and Weinstein 1989; Tunberg and Creswell 1991). During the experiment, the Cape Eleuthera Institute was subjected to heavy winds associated with a cold front. The water temperature in the experimental tank dropped 8°C over 3 d (fig. 4). The HR was closely related to temperature change rather than changes associated with day/night, showing that large temperature changes will override underlying activity patterns. The crab was able to feed while fitted with the logger, suggesting that implantation was not overly stressful for the animal (fig. 4). Food intake resulted in a sharp increase in HR that remained elevated for approximately 7 h after feeding, despite the concurrent drop in temperature. This change in rate is typical compared with that measured for other decapods (McGaw 2005, 2006*b*). It is interesting to note that the time HR remained elevated was of shorter duration than what is typically observed for postprandial oxygen consumption, which can remain elevated for 12–48 h after feeding (McGaw and Curtis 2013). This is because changes in HR are associated with food handling and mechanical digestion rather than the subsequent metabolic processes (McGaw 2005, 2006*b*).

The loggers were successfully tested in the field on a slipper lobster and a spiny lobster placed in separate cages for 3 d, during which time the ECG was recorded every 30 min. The ECG traces were stored for manual counting, but this quickly filled the onboard memory of the logger, limiting recording time to just over 3 d. Future experiments can reduce the number of raw ECG traces that are stored to prolong the experiment duration. Patterns of HR were similar in direction and value to the manual count from the ECG and the internal algorithm the loggers used to calculate HR; however, they were not identical to one another (one-way *t*-test, $t_1 = 4.35$, $P < 0.001$). The algorithm calculates rate from the

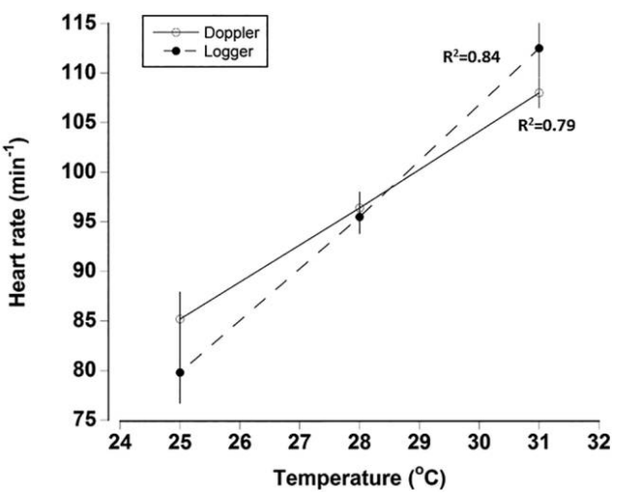

Figure 3. Changes in the heart rate of the West Indian spider crab *Mithrax spinosissimus*. Recordings were made simultaneously using the pulsed Doppler and a micro–heart rate and temperature logger during a temperature increase from 25° to 31°C. The data represent the mean ± SEM of five to eight readings at each temperature.

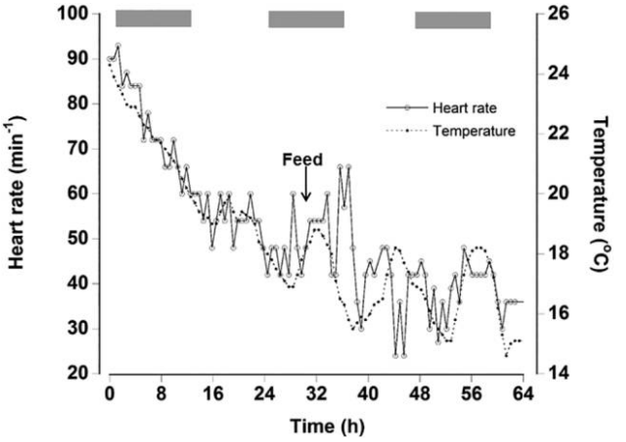

Figure 4. Changes in the heart rate of a female *Mithrax spinosissimus* of 425 g in the laboratory. Measurements were made every 40 min during a 3-d period and plotted against body temperature of the animal. The bars represent times of darkness. Food (brown algae) was introduced into the talk halfway through the experiment.

same 5-s ECG trace; for the manual count, we used the QRS peaks (which represent depolarization of the ventricle; fig. 2*A*), whereas the algorithm uses the spaces between the QRS complex to calculate rate (B. Sigurgeisson, Star-Oddi, personal communication). We did not use the interval periods for the manual count because of the difficulty in determining how partial intervals that occurred at the beginning and end of the traces contributed to the HR count. The discrepancy between the two methods likely results from extrapolating from a 5-s trace, where even one additional ECG count will result in a difference of 12 beats min^{-1} in the final count. When comparing the manual count and algorithm, 7/146 measurements for the slipper lobster and 4/146 measurements for the spiny lobster gave a count of 12 or more beats' difference (all in range of 12–15), a mismatch count of 1 ECG per 5-s trace. The other readings varied between 0 and 11 beats min^{-1}, which would be an error of less than 1 ECG per trace. The algorithm did not always accurately calculate rate; 2%–3% of the algorithm HRs from the lobsters were very high (several hundred beats per minute) or very low (<5 beats min^{-1}) readings. This in itself is a very good level of accuracy, considering that for some of the West Indian spider crabs, 20%–25% of the rates calculated from the algorithm were inaccurate. This was likely due to background noise on the ECG traces, which interfered with the ability of the algorithm to detect a clear signal. Although the onboard storage of the ECG is limiting to logger memory and the manual counting thereof is somewhat time-consuming, it does ensure that one can assess the data should the algorithm fail to produce clear data. Similar issues were found when using these loggers with sockeye salmon. In one study, 39% (26 of 67 individuals) of sockeye salmon implanted with loggers provided unusable data due to logger displacement, logger failure, internal hemorrhaging, or injuries and premature mortality (Prystay et al., forthcoming). In addition, approximately 6.5% of the calculated HRs from a given salmon were erroneous and thus discarded during analysis (T. Prystay, personal communication). However, the frequency of erroneous HR values does vary across species; for example, smallmouth bass produced considerably less (approximately 1.5%) erroneous values compared to sockeye salmon (T. Prystay, personal communication). This supports the conclusion from Cooke et al. (2016) that there are still problems that need addressing when using HRT loggers.

The slipper lobster exhibited a cycle of HR that was higher during the nocturnal high tides and lower during daylight hours (fig. 5). Slipper lobsters are primarily nocturnal, and the increased HR would likely be associated with increased locomotor activity (Spanier and Lavalli 1998). HRs declined somewhat over the 3 d; the rates were probably initially higher due to handling of the animal and operation. In contrast, even though the spiny lobster displays similar nocturnal foraging activity (Cox et al. 1997), increases in HR were not observed during the hours of darkness (fig. 6). Instead, the HR closely paralleled changes in water temperature, with maximal levels observed during the late afternoon when the ocean was warmest and lowest levels during the early morning hours. It is worthy to note that increases in HR of 10–20 beats min^{-1} were driven by temperature changes of just 1°–3°C. This may be important because it is hard to maintain exact temperatures even in controlled laboratory experiments. In the literature, many authors state that experimental temperatures were maintained at a certain level ±1°C, which could mean quite a substantial variance in rate despite what is often referred to as a stable temperature regime. The spiny lobster started to exhibit slowing or stopping of the heart after 20 h (fig. 6). Acardia is typical of resting unstressed decapods (McGaw 2004), in this case showing that almost a day was required for the animal to return to a resting state. Nevertheless, cardiac pausing behavior is not observed in all decapods: green crabs *Carcinus maenas* exhibit regular periods of acardia lasting several minutes, while the Puget Sound king crab *Lopholithodes mandtii* rarely stops the heart for more than a few seconds at a time (I. J. McGaw, unpublished observations).

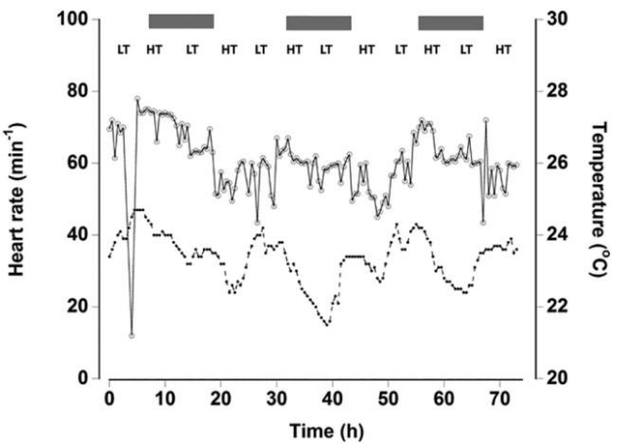

Figure 5. Changes in the heart rate (HR) of a 735-g slipper lobster *Scyllarides aequinoctialis*. After instrumentation, the lobster was held in a wire mesh cage in the shallow subtidal zone for 3 d, and the logger recorded HR every 30 min. Shaded bars indicate times of darkness. HT = high tide; LT = low tide. Body temperature is indicated by the dotted line.

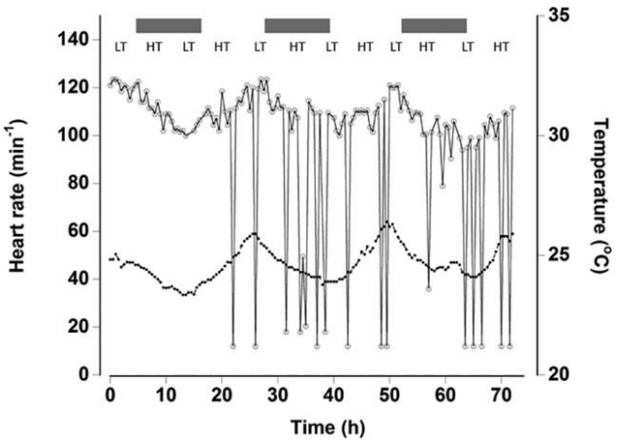

Figure 6. Changes in the heart rate (HR) of a 1,160-g spiny lobster *Panulirus argus*. After instrumentation, the lobster was held in a wire mesh cage in the shallow subtidal zone for 3 d, and the logger recorded HR every 30 min. The drops in HR represent periods of acardia, when very low or no heart beat was detected. Shaded bars indicate times of darkness. HT = high tide; LT = low tide. Body temperature is indicated by the dotted line.

The black land crab *Gecarcinus ruricola* gave the clearest ECG signal with less noise, and the algorithm miscalculated only three of a total 512 HR traces (fig. 8*C*). The clearer ECG trace in air suggests that the signal is incompletely grounded in seawater. During the first 24 h after instrumentation, HRs of *G. ruricola* were elevated, likely a combination of the stress from operation and the higher temperatures (fig. 7). This and data from the spiny lobster suggest that a settling period of at least 24 h is needed before crabs will exhibit normal heart rhythms. After this 24-h period, a clear pattern emerged: HRs rose from between 45 and 85 beats min^{-1} during the day, reaching between 110 and 150 beats min^{-1} during the hours of darkness (fig. 7). This pattern would be associated with a nocturnal increase in locomotor activity (Palmer 1971), where the crabs emerged from under the shelters and were feeding, drinking, and moving around in the mesocosm (I. J. McGaw, personal observation).

This study has demonstrated an effective way to use loggers designed for vertebrates in a variety of relatively large decapod crustaceans. A plethora of information already exists on changes in HR as a function of both physicochemical and biotic parameters (reviewed in McGaw and Reiber 2015). However, these have nearly all been carried out in lab conditions in restrained or partially restrained animals. The use of these loggers will enable measurements of HR in the natural environment. Since the loggers are somewhat costly and need to be retrieved in order to access data, the use of large cages (as used in this study) would ensure that animals can be recaptured and data downloaded. However, this in itself may be restrictive to natural behavioral reactions. Many of the larger crustaceans are territorial and show high site fidelity (Hazlett and Ritschoff 1975; Wada 1993; Lozano-Álvarez et al. 2002; Moland et al. 2011), and this would enhance recapture rates. In addition, the fitting of an acoustic transmitter (in water) or a radio telemetry transmitter (on land or in freshwater) allows one to pinpoint the location of the animal for a concentrated trapping effort (Curtis and McGaw 2008, 2012). The use of these loggers as "external" devices makes significant headway compared with fish and mammals, as no lengthy surgery is necessary and recovery time is minimal. The logger mass comprised approximately 0.2%–0.7% of the body mass, which is less than many epibiont loads that some crabs carry around, so these loggers are unlikely to hinder these large crustaceans (McGaw 2006*a*). The fact that the logger sat in a groove in the carapace and was secured with wax ensured that it stayed in place and did not move around the body cavity, which has been a problem in fish (Cooke et al. 2016). After removal of the loggers, the animals were maintained in the lab after use and monitored for at least 14 d, during which time they remained active and fed, suggesting that the animals are not unduly harmed by the procedure. The dermis of the area became thickened once exposed to seawater and would seal completely when the animal molted.

There are still several limitations for using these HR loggers in decapods. First, their use will be limited to the larger species. Although clear short-term HR and ECG readings were made from the 170-g swimming crab (fig. 8*D*), the animal was impaired by the logger and survived for only 12 h after its removal. Longer-term readings were obtained from the 345-g land crab and 425-g spider crab without any apparent detrimental effects to the crabs; an estimate of a lower size range for effective use would be 300 g, or the logger comprising approximately 1% of the body mass. Although this size range precludes many species, it does include many of the commercially important species for which many ecological data have already been collected. Second, unlike vertebrates, decapod crustaceans commonly show ventilatory and cardiac pauses. These can be quite regular and range in time from a few seconds to 5–7 min (fig. 6; McGaw 2004). The ability of the loggers to gather data for only a few seconds at a time may limit what is recorded, especially during an extended period of acardia. The loggers can be programmed to record more often, but this in turn limits the

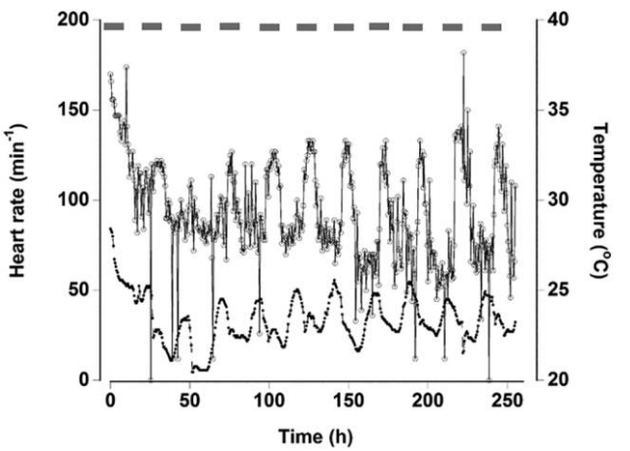

Figure 7. Changes in the heart rate of a 345-g black land crab *Gecarcinus ruricola*. After implantation of the logger, the crab was maintained in mesocosm with food and water for 10 d and was subjected to a natural diurnal cycle. Shaded bars indicate times of darkness. Body temperature is indicated by the dotted line.

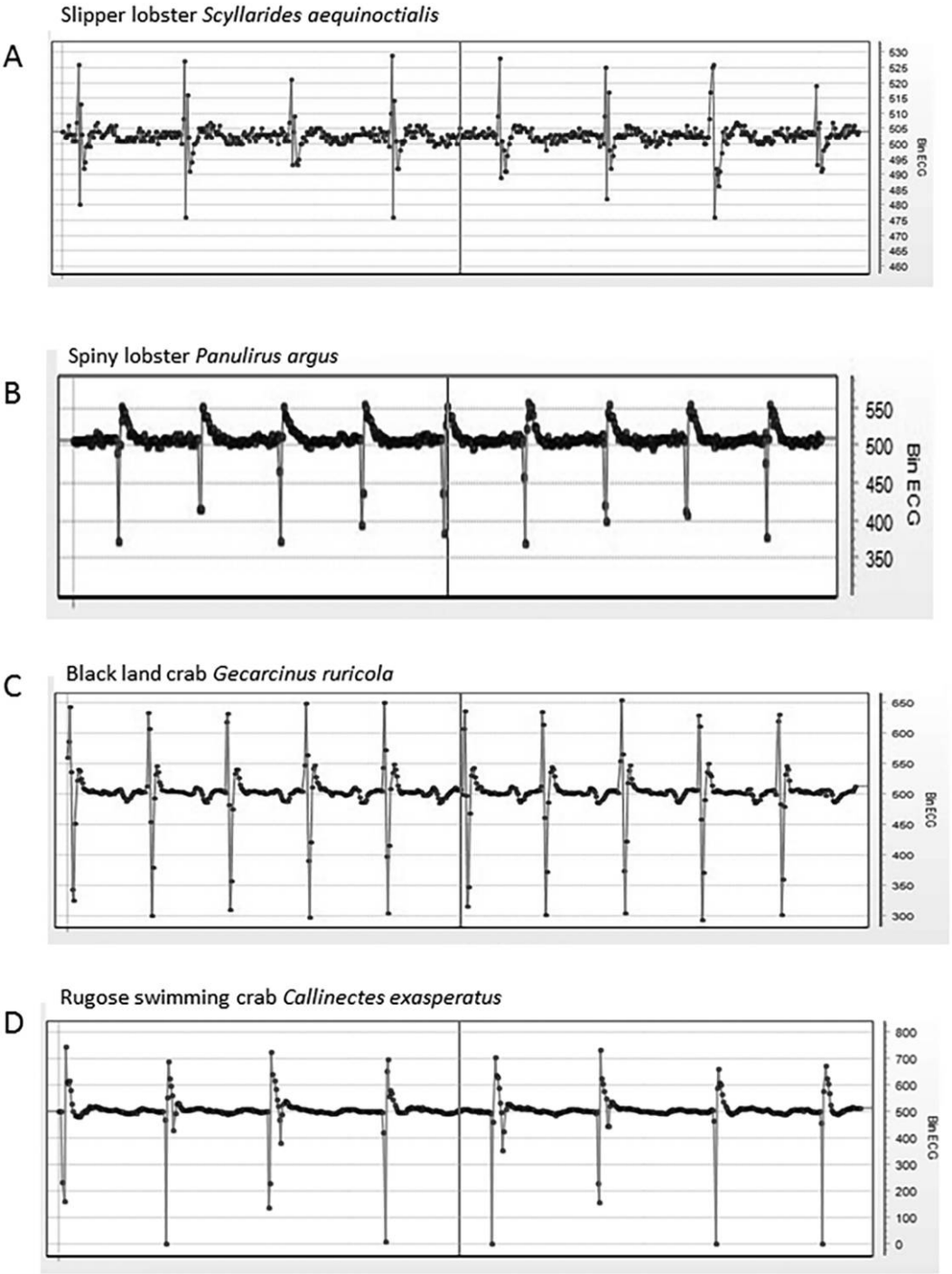

Figure 8. Five-second electrocardiogram (ECG) traces from a slipper lobster *Scyllarides aequinoctialis* of 735 g (*A*), spiny lobster *Panulirus argus* of 1,140 g (*B*), black land crab *Gecarcinus ruricola* of 405 g (*C*), and rugose swimming crab *Callinectes exasperatus* of 170 g (*D*). A color version of this figure is available online.

battery life and the amount of time that data can be gathered. Finally, although there is an extensive literature on modulation of HR in decapods, it may not be possible to concurrently record all these parameters in the field. For example, changes in temperature, depth, and salinity experienced by freely moving Dungeness crabs *Cancer magister* have been recorded using conductivity-temperature-depth tags (Star-Oddi), which are small enough to be attached directly to the animal (Curtis and McGaw 2008, 2012). However, biotic effects such as interactions with conspecifics, avoidance of predators, or feeding behavior, which all

influence HR (McGaw and Reiber 2015), will be much harder to assess. That being said, this method makes enormous strides in understanding physiological responses in free-ranging wild invertebrates. The ability to capture and instrument animals in the field and release them in 5 min is encouraging. There has been a push in recent years to monitor animals in more natural environments and even in the field (Goldstein and Pinshow 2006; Aeur et al. 2016; Treberg et al. 2016). Because lab experiments are somewhat contrived, it will be essential to validate, or otherwise, the accuracy of lab data and compare those data to data gathered under natural conditions, where the animals experience a myriad of sensory and physicochemical changes in their environment. The long battery life and high survival rate of animals after logger implantation will provide data that are currently lacking. Although there are several less costly and equally or more effective methods to measure HR in the lab (Airriess et al. 1994; Stillman 2004; Tepolt and Somero 2014), the strength of this method is the ability to take the experiment to the field. This has the potential to open up a whole new area of research examining the physiological ecology of free-moving decapods in their natural environment to understand fundamental biological mechanisms and to understand how decapods respond to anthropogenic stressors and disturbance (e.g., pollution, climate change, interactions with fisheries).

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