High Pressure Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713679167

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First Published:September2009

To cite this Article Robinson, Nathan J., Thatje, Sven and Osseforth, Christian(2009)'Heartbeat sensors under pressure: a new method for assessing hyperbaric physiology',High Pressure Research,29:3,422 — 430
To link to this Article: DOI: 10.1080/08957950903076398
URL: http://dx.doi.org/10.1080/08957950903076398

High Pressure Research
Vol. 29, No. 3, September 2009, 422–430

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Heartbeat sensors under pressure: a new method for assessing hyperbaric physiology

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(*Received 4 February 2009; final version received 29 May 2009*)

Non-invasive heartbeat sensors to measure the cardiac activity of crustaceans have been adapted for use under hyperbaric conditions. Able to record data continuously over long timescales, these sensors can collect high-resolution data on the physiological state of an organism up to a tested limit of 300 atm. Using this technique, heart rate was recorded in a juvenile of the sublittoral spider crab, *Maja brachydactyla* (Decapoda: Majidae), when subjected to hydrostatic pressures of 1, 50, 100, and 150 atm for periods of 30 min. Heart rate increases with pressure until 100 atm (one-way repeated measures ANOVA: $F_{(4,25)} = 154.76$, $p < 0.001$). However, the significant decrease in the mean heart rate from 137.07 bpm at 100 atm to 118.40 bpm at 150 atm (t-test: $t = 4.581$, d.f. $= 10$, $p < 0.001$) indicates a mechanistic limit in the cardiac response of this species to pressures beyond 100 atm. This method could be potentially applied to any marine invertebrate with a neurogenic heart.

Keywords: cardiac rhythmicity; hydrostatic pressure; heart rate; Crustacea; deep sea; depth tolerance

1. Introduction

Preliminary studies on the tolerances of shallow water species to the elevated pressures of the deep ocean were hampered by a lack of technology available to assess the physiological state of test organisms under hyperbaric conditions. In isolated pressure vessels, direct visual sampling or end-point measurements were commonly utilised, but these methods are limited in their application [1]. Visual sampling can only provide quantitative behavioural data in response to physiological impairment in certain circumstances, and is restricted to the study of developing ontogenetic stages or perceptibly active individuals [2,3]. End-point measurements in isolated vessels are limited to a single point of resolution and sampling requires depressurisation of the vessel, which at significant levels can cause fatalities in test organisms [4]. To this extent, end-point measurements can only measure acute, short-term physiological responses to pressure change and not the natural capacity of an organism to survive at a given pressure [5]. Developing methods for continuously monitoring the physiological state of an organism under controlled pressures was therefore integral to gain

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ISSN 0895-7959 print/ISSN 1477-2299 online

DOI: 10.1080/08957950903076398
http://www.informaworld.com

a more comprehensive understanding of the susceptibility and resilience of marine organisms to variations in depth [6,7]. When considered in conjunction with phylogenetic evidence, such methods can even help to explain the distribution of extant aquatic species with respect to depth, and how this may have changed over evolutionary time [3].

2. History of pressure chambers

During the first studies of the effects of hyperbaric conditions on life, Certes [8] and Regnard [9,10] used a simple hydraulic pump attached to an isolated pressure vessel to exert pressures up to 600 atm on a variety of marine and fresh water plants and animals. Subsequently, increasingly complex pressure chambers were built, which allowed for studies on larger organisms at higher pressures [11,12]. With the advance of technology, a few large pressure chambers were developed that could maintain organisms at both constant pressures and temperatures [13]. However, these early pressure studies were constrained to isolated vessels that were incapable of demonstrating long-term acclimation to hyperbaric conditions, due to the effects of oxygen depletion within the isolated medium over time as a consequence of respiration. Flow-through pressure chambers, such as the IPOCAMP™ (Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marines Profonds) [1], have solved this problem by incorporating a flow-through system into the pressure chamber design. Within these chambers, water is recirculated from an open external source and so chemical parameters within the system can be maintained and monitored. Such technological progress was crucial in developing the capacity to maintain organisms at controlled hydrostatic pressure for extended periods of time [6,7,14].

3. Use of heartbeat sensors under pressure

Cardiac activity was used in many of these experiments as a continual indicator of respiratory functioning [6,7]. In Crustacea, this was achieved by implanting silver wires directly into either side of the heart [15]. Although some small organisms remained highly sensitive to the procedure [16,17], it was experimentally proven not to dramatically affect short-term stress response in the hydrothermal vent crab, *Bythograea thermydon* [6]. Nonetheless, this invasive method required drilling through the carapace and penetration of the pericardial sinus when attaching the sensor [18].

In order to minimise the complexity of this method, Depledge and Anderson [19] instead developed the photoplethysmographic technique for non-invasive monitoring of cardiac activity in arthopods, which is utilised in the present study. Through this method an IR light-emitting diode (LED) coupled axially with a phototransistor (CNY-70 reflex-optocoupler, Vishay Semiconductor GmbH) is used to measure movements of internal organs. When placed correctly on the cardiac region, the IR light from the LED penetrates through the carapace of the organism into the visceral mass and is backscattered by any underlying tissues, which in this case should be the heart. The intensity and direction of the backscatter depends on the shape and movements of the tissue and any variation in backscatter is detected by the phototransistor. This change in current, reflecting the beating cardiac muscle, is converted into voltage, which is then passed to a voltage amplifier (Electrode Amplifier, Vernier) to increase the signal. The analogue signal is digitalised by the Vernier LabPro system (Vernier) and can then be interpreted and recorded using the Vernier LoggerPro software (Version 3.4.2, Vernier).

This method has been used previously on many invertebrates at ambient pressures [20–23], but for use under hyperbaric conditions on the European spider crab, *Maja brachydactyla*, commonly

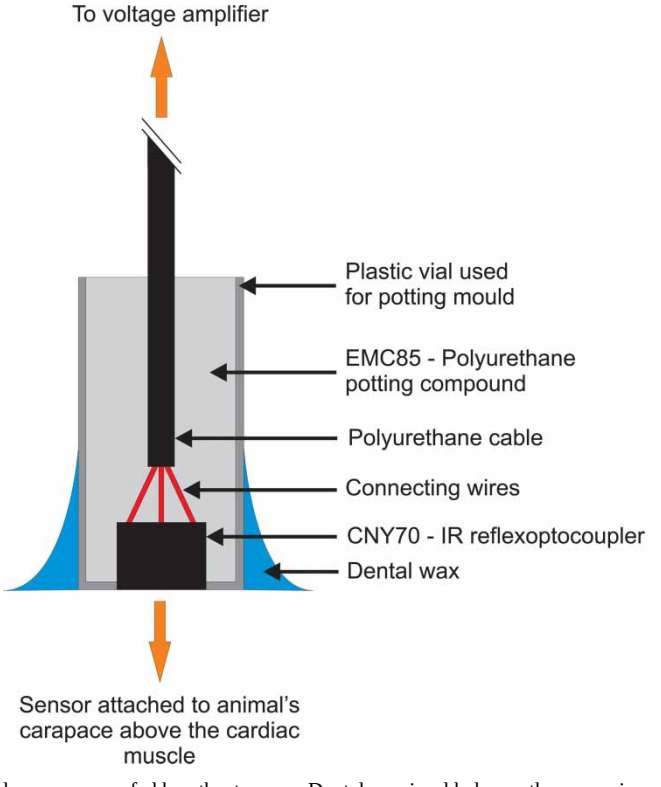

Figure 1. Water- and pressure-proofed heartbeat sensor. Dental wax is added once the sensor is correctly positioned, to hold it in place.

only found up to a depth of 100 m [24], the sensor had to be redesigned (Figure 1). First, the sensor and its connectors required waterproofing. This was achieved by encapsulating the sensor and the joining cable in a loose-fitting plastic mould. A fitted opening in the base of the mould was cut so that the face of the CNY-70 sensor was not obstructed. The mould was then filled with a polyurethane potting compound (EMC-85/10A, PDM Neptic Ltd, England) and left for 24 h at 25 °C to allow the glue to set. For further protection, the mould could remain in position after the potting compound had set. Similar use of potting compounds to water- and pressure-proof invasive cardiac sensors has also previously been detailed [25].

The IPOCAMP™ also required modification to allow for the sensor to be inserted successfully through the wall of the chamber (Figure 2). A removable plug in the shell of the IPOCAMP™ is already present, although this is a solid fitting. A new hollow plug was therefore required which extended directly into the IPOCAMP™ and through which the cables of the sensor could be passed. The new plug is designed to fit flush with the pre-built opening in the IPOCAMP™ wall and is then held in place with a second screw-fit hollow plug, which is screwed in behind the primary plug. A bulkhead connector is attached directly to the internal extrusion of the primary plug in order to join the wiring from both inside and outside the IPOCAMP™ and to seal the hollow primary plug. The bulkhead connector is then directly attached to the cable leading to the waterproofed heartbeat sensor. Further, precautionary measures are required to secure the bulkhead connector to the joining cable as this can be pulled loose by the movement of a mobile organism within the IPOCAMP™ chamber and so a plastic screw-tight casing covers the cable connection and secures it to the bulkhead connector.

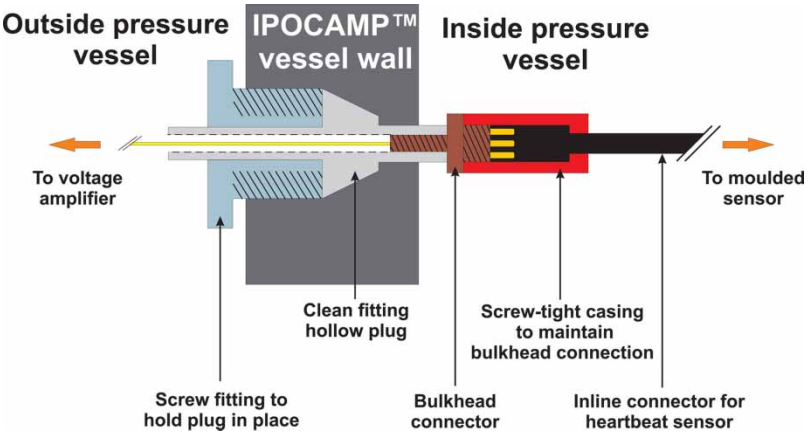

Figure 2. The connecting plug built into the wall of the IPOCAMP™ allows for the insertion of the heartbeat sensor yet retains pressure inside the vessel.

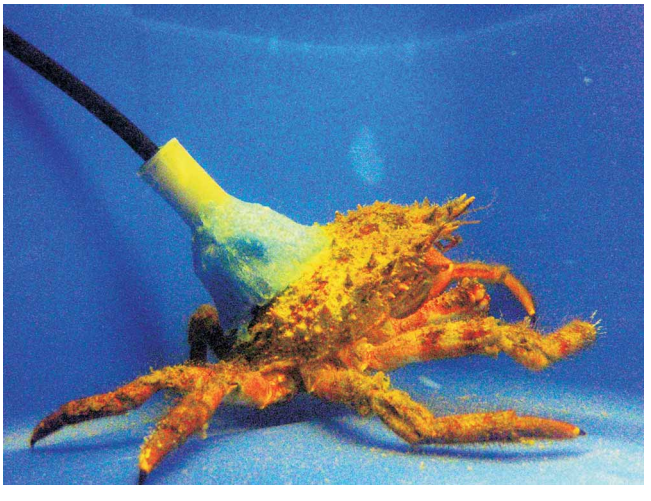

Figure 3. *Maja brachydactyla* with dorsally attached heartbeat sensor.

To fasten the sensor to the carapace of the organism, a readily malleable dental wax (periphery wax, Carmel Industries, Canada, or any similar product) is moulded around the sensor once it is in place and onto the carapace of the crab (Figure 3). The joint is further strengthened using cyanoacrylate glue (Loctite, Henkel, UK) and care is taken to make sure the glue is allowed to set before submersion.

4. Cardiac activity as a measure of stress

The cardiovascular system, of all organisms that possess one, must adequately permeate all tissues for the purpose of supplying O_2, nutrients and hormones and the removal of waste products. As environmental factors rarely remain constant, a secondary requirement is that cardiac performance must be able to acclimate to meet the variable needs of the 'host'. Cardiac activity in *Maja brachydactyla*, as with other members of the class Malacostraca, is regulated by extrinsic neuronal

and hormonal factors [26,27]. To this extent, the neurogenic hearts of decapods and isopods are analogous to those of vertebrates. Strongly linked to ventilation, cardiac activity in Crustacea is highly sensitive to both oxygen demand and ambient oxygen concentrations [28]. Thus, cardiac activity (as a proxy of respiratory capacity) can be employed as a sensitive indicator of physiological impairment [29]. Indicatively in Crustacea, changes in cardiac activity have already been observed in response to hydrostatic pressure [6,7] as well as hormone concentrations [30], mode and extent of activity [31], threat of predation [23], emersion from water [32], temperature [33] and environmental contaminants, such as copper [34].

Baited traps and oysters trawls were used to collect specimens of *Maja brachydactyla* at depths of 4–12 m from Southampton Water, UK, during November 2008. A single male with a wet weight of 195.7 g and a carapace length of 89.5 mm, measured between the central base of the rostral spines, was retained for experimentation. This individual was acclimated to laboratory conditions in a shaded tank maintained at 20 °C (±1 °C) for a period of a week. A day prior to experimentation, the heartbeat sensor was attached to the carapace of the crab, which was still able to roam freely within the tank. Heart rate was recorded under these experimental conditions on the following day to best represent the heart rate of the specimen while at rest (Figure 4(a)).

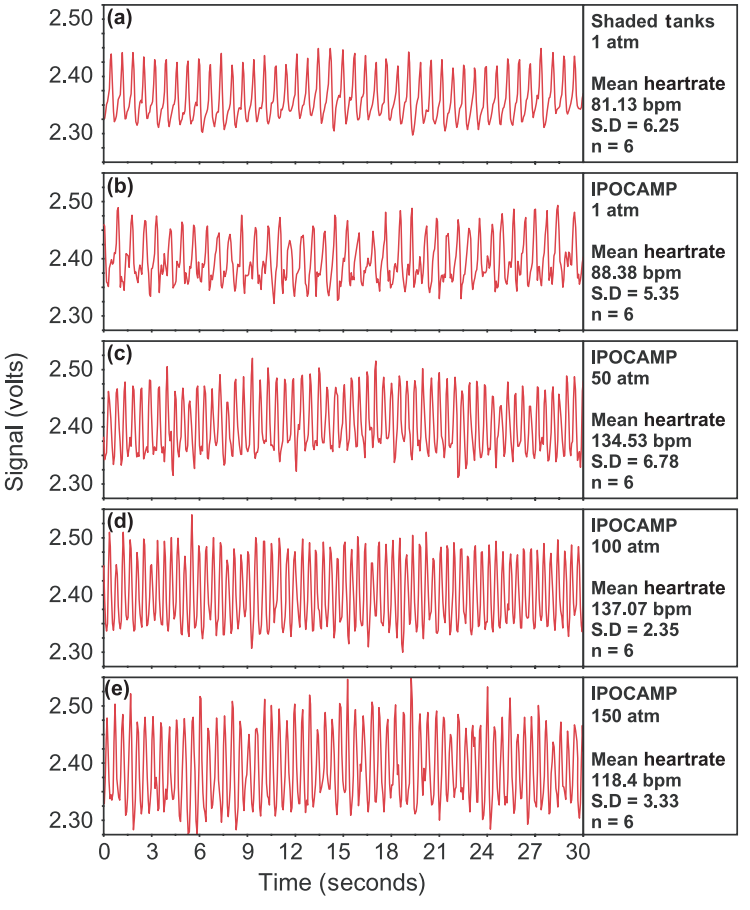

Figure 4. Cardiac response of *Maja brachydactyla* (a) at 20 °C in the shaded tank, (b) within the IPOCAMP™ at 1 atm, (c) 50 atm, (d) 100 atm, and (e) 150 atm. Mean heart rate was calculated from six randomly selected 1 min samples at each pressure.

Without adjusting the sensor, the crab was then transferred to the IPOCAMP™ and after an hour of acclimation the heart rate was once again recorded at ambient pressure (Figure 4(b)). Pressure was then increased by 10 atm every 10 min, between incremental pressures of 50, 100, and 150 atm. Each incremental pressure was maintained for 30 min (Figure 4(c–e)) and from this the heart rate was averaged from six randomly chosen 1 min samples under each investigated pressure.

In the IPOCAMP™ under ambient pressure the heart rate was observed to be 7.25 bpm faster than in the shaded tanks. Although this difference is not statistically significant (t-test: $t = -0.881$, d.f. $= 10$, $p = 0.399$), the small increase may indicate a response to handling between these measurements. Specifically, this may be attributed to the reduced acclimation time inside the IPOCAMP™ when compared with time in the shaded tank. This may explain why the cardiac signal has more frequent irregularities when inside the IPOCAMP™ than in the shaded tanks. However, it also needs to be noted that the oscillation of the pump that powers the IPOCAMP™ creates a slight pulse in water density that is vaguely evident in the signal received by the heartbeat sensor and this may explain some of these anomalies. Minor fluctuations in the cardiac activity of crustaceans have been previously observed in response to small reverberations within a water body [35].

Based on the preliminary data from a single specimen (Figure 5), a significant effect of pressure on cardiac activity is apparent (one-way repeated measures ANOVA: $F_{(4,25)} = 154.76$, $p < 0.001$). A significant increase in the heart rate of 46.15 bpm is observed when pressure is increased from 1 to 50 atm (t-test: $t = 13.089$, d.f. $= 10$, $p < 0.001$). While at this pressure the crab demonstrates normal behaviour, this large increase in cardiac activity suggests that a far greater ventilation effort is required by the specimen to maintain optimum levels of cardiovascular circulation. From 50 to 100 atm the subsequent increase in the heart rate is small at 2.54 bpm and statistically the possibility that the difference is due to random sampling variability cannot be rejected (t-test: $t = -0.867$, d.f. $= 10$, $p = 0.406$). In addition, the signal now appears distinctly regular. The standard deviation of the mean heartbeat rate is now minimal at 2.35 and this

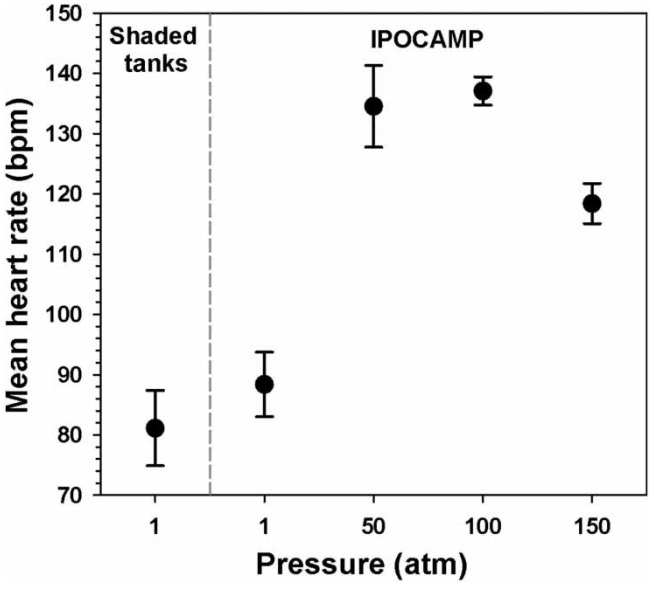

Figure 5. Effects of hyperbaric pressure on heart rate of *Maja brachydactyla* at 20 °C in the shaded tank and within the IPOCAMP™. Mean heart rate was calculated from six randomly selected 1 min samples at each pressure.

suggests that the mechanistic limits of cardiac activity are reaching the maximum level of output. This hypothesis is further supported by significant reduction in heart rate of 18.67 bpm as pressures are elevated to 150 atm (t-test: $t = 4.581$, d.f. $= 10$, $p < 0.001$). Furthermore, the standard deviation of the mean heartbeat rate remains small at 3.33 and the cardiac signal becomes more irregular. At this point, functional failure in the cardiac muscle may be occurring in response to the hyperbaric conditions. The general increase in respiratory activity with pressure coincides with that observed in *Uca pugilator* [36]. However, in response to elevated pressure, oxygen consumption returned to basal levels within 1 h and thus this data may only demonstrate an acclimation response to pressure change [36].

As the strength and form of the signal received is entirely dependant on the exact placement of the sensor, it needs to be noted that using this method the signal amplitude is never directly comparable between individuals. Changes may even be observed when reapplying the sensor to the same specimen. In addition, while heart rate does respond quickly to meet cardiovascular needs in crustaceans, the numerous factors that define the rhythm of the heart rate do not elicit an exclusive response [37]. The distinct behaviour component of heart rate should be further considered when interpreting results [27]. A locomotive factor, such as walking, may increase the heart rate yet it does not directly mean that the organism is physiologically impaired [31]. Measurements should therefore only be taken under a single behaviour, which most suitably is often resting. Although cardiac activity has been previously used to compare thermal tolerances in crustacean through Arrhenius break temperatures [38–40], a complimentary indicator of respiration is often desirable to correctly assess respiration and metabolic rates [37,41].

5. Understanding depth tolerance in Crustacea

The need for tools to measure physiological responses to environmental change over long timescales has been frequently emphasised [4,42]. The method described here allows for such assays under hyperbaric conditions and could be readily applied to a variety of sensors, such as temperature, pH, and O_2, for use under similar conditions. With minimal adjustment, this method could be applied for use on the majority of marine invertebrates with a neurogenic heart. Such sensors have been used already, at atmospheric pressure, on *Talitrus saltator* individuals less than 20 mm in length [22]. Changes in cardiac rhythm may even be used to infer physical and mechanistic limits to performance rather than physiological limits or survivability alone.

The pressure tolerances of many shallow water species are relatively unknown, even though they may play a crucial role in defining the capacity of such organisms to migrate to deeper waters [43]. These tolerances are likely to have strong implications in limiting the vertical distribution and even the geographic range of many species if colonisation is not limited by other factors, such as temperature [33]. Understanding these processes would help to infer certain evolutionary and ecological processes in the deep sea. Furthermore, as temperature of surface waters increase [44] the ability of shallow water species to migrate to deeper, cooler waters could be an important factor in understanding the impact of global warming on shallow water ecosystems [45].

Acknowledgements

This work was supported by the Marine Biodiversity and Ecosystem Functioning Network of Excellence MarBEF (contract no. GOCE-CT-2003-505446) of the FP6, and a research grant from the Total Foundation (‘Abyss2100’) to ST. This work could not have been achieved without the technical expertise and good will of Neil Jenkinson or the moral support provided by Laura Casburn and Celine Campaña. The constructive comments on a previous draft of this manuscript by two anonymous reviewers are also greatly acknowledged.

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