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# Physiological responses to hyposmotic stress in the supralittoral amphipod *Talitrus saltator* (Crustacea: Amphipoda)

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**Abstract**

We investigated the effect of hyposmotic stress upon osmotic capacity (OC), heart rate (HR) and heart rate variability (HRV) of an Atlantic population of the sandhopper *Talitrus saltator*. Strictly consecutive monitoring of cardiac activity and measurement of OC was achieved by employing a non-invasive infrared technique to monitor heart activity and direct cardiac puncture to collect the haemolymph. Body mass (range 60–160 mg) had no effect on mean HR nor the mean HRV in adult individuals of both sexes. Both OC and HR, but not HRV, were influenced by the interaction between osmotic stress and exposure time. In addition, OC and HR were coupled within a moderate range of osmotic stress, beyond which this association breaks down, and bradycardia and a positive correlation between HR and HRV occur. A possible rise in metabolic cost, indicated by HR increase, was described for hyper-osmotic regulation. This is the first assessment of the relationship OC and HR in a non-decapod crustacean, and the results indicate that, in sandhoppers, the disruptions of cardiac activity may lead to impairment in maintaining an efficient haemolymph osmotic regulation.


*Keywords:* Talitrid; Sandhopper; Osmotic capacity; Heart rate; Heart rate variability; Haemolymph; Hyperosmotic regulation; Hyposmotic stress; Environmental tolerance

## 1. Introduction

For organisms living in environments characterised by large salinity fluctuations, such as sandy beach tidal habitats, the ability to maintain the body fluid concentration relatively stable with respect to the external environment for relatively long periods of time is essential. In fact, in many semi-terrestrial and terrestrial invertebrate species of marine origin this capacity may represent the key to their success in colonizing the terrestrial environment (Little, 1990). Behavioural adaptations are also recognised as being a crucial component in the adaptation of many of these species, e.g. in the supralittoral amphipods (Morritt and Spicer, 1998). In addition, the difference in success between some related species is apparently connected to their environmental tolerance and physiological plasticity (Gaston and Spicer, 2001).

In crustaceans, the capacity to regulate the osmotic concentration of body fluids is measured through the evaluation of the osmotic capacity (OC), defined as the difference between the osmotic concentration of the body fluids (e.g. haemolymph) and the osmotic concentration of the external medium (Charmantier et al., 1984). The OC of the haemolymph represents an integrated measure of the physiological ability to compensate for the osmotic gradient that may occur between the "internal" and external environments, and has also been demonstrated to be a reliable indicator of stress status in decapod crustaceans (e.g. Lignot et al., 2000). The sandhopper *Talitrus saltator* is one of the most widely distributed and better studied European supralittoral amphipods, and represents a relevant biological model for the investigation of adaptation in sandy beach invertebrates: both in terms of behavioural (e.g. Ugolini,

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2003), and physiological (Spicer et al., 1987; Morritt and Spicer, 1998), adaptation. *Talitrus saltator* is characterised by the presence of a hypo-hyperosmotic pattern of haemolymph osmoregulation, and by a limited ability to produce hypo-osmotic urine when exposed to hypo-osmotic conditions (Morritt, 1988).

Another important physiological parameter to be taken into account as a biomarker for environmental stress responses in invertebrates is heart rate (HR) (Brown et al., 2004). Heart rate in crustaceans has been widely employed to measure metabolic activity, physical environmental stress and even the effects of social interactions (Kinne, 1960; Smith and Taylor, 1993; Reiber and McMahon, 1998; Schapker et al., 2002), although the majority of the techniques employed are invasive or involve direct observation. Recently, the use of an optocardiographic technique for the measurement of the cardiac activity in invertebrates (Depledge and Andersen, 1990) has been extended to supralittoral amphipods (Calosi et al., 2003) and has been used to demonstrate a positive cardiac response in the Mediterranean *T. saltator* exposed to hyposmotic stress (Calosi, 2004). This result, if compared with previous studies on the osmotic and ionic control capacity of the haemolymph in Atlantic individuals of the same species (Morritt, 1988, 1989), may indicate a direct involvement of cardiac activity in determining the osmoregulatory ability of *T. saltator*.

To date there has been no direct investigation into the relationship between osmotic regulation and cardiac function in amphipod crustaceans, and this gap is particularly relevant especially if compared to the situation for decapod crustaceans (Péqueux, 1995; Santos and McNamara, 1996; McMahon, 1998). Amphipods represent an interesting biological model for the study of the adaptation of arthropods to a variety of environments. Moreover, the comparative investigation of the mechanisms that underlie physiological processes may help to develop a clearer scenario for the evolutionary routes taken within the Crustacea (if not within the Arthropoda). Amongst environmental factors salinity has long been recognised as one of the major determinants of crustacean distribution (Kinne, 1963) and it is a factor known to affect their metabolism (Kinne, 1964). Therefore, it is of interest to define the osmoregulatory capacity of *T. saltator* with respect to the mechanisms that may underlie this ability. In order to determine the relationship between cardiac activity and haemolymph osmoregulation, we performed strictly consecutive measurements of both functions in adult individuals of *T. saltator*. We aimed to investigate the direction and intensity of HR, HR variability (HRV) and OC responses in talitrids exposed to hyposmotic stress: this is a condition that supralittoral amphipods occasionally experience in nature during intensive rainfalls, at certain estuarine locations and in association with ponds behind sand dunes systems. As an integral part of the study we separately tested the effect of body mass upon HR and HRV in adult individuals of *T. saltator* using gender as a covariate. It is known that body mass does not affect OC in Atlantic *T. saltator* (Calosi and Morritt pers. obs.).

## 2. Materials and methods

Adult males and non-ovigerous females of *T. saltator* were collected from Berrow beach (Somerset, England) in August 2003 and immediately transported to the laboratory at Royal Holloway (University of London) inside transparent containers with wet sand. Once in the laboratory, the animals were transferred to transparent aquaria, provided with wet sand (collected damp with sea water from the same location of talitrids) and covered by a transparent Plexiglas lid. Aquaria were kept in thermostatic and controlled room (19±1 °C) and the light regime set in phase and duration with the natural one (L:D 14:10). The sand was aerated daily by agitation, the moisture content maintained with distilled water and dry fish food provided ad libitum. Before experiments, talitrids were weighed and kept in separate numbered vials provided with a moistened (33‰) piece of paper until used in experiments. In the experiments to investigate the relationship between body mass and HR and HRV animal body mass ranged between 60–160 mg. For all subsequent experiments this factor was standardised within the range 90–150 mg. All experiments were carried out in the thermostatically controlled room at 19±1 °C with experimental animals immersed in the test medium: talitrids can respire both in air and water (Spicer and Taylor, 1987) and this medium ensures exposure to constant conditions, something which is very difficult to achieve using a sand-based medium (D Morritt pers. obs.). Artificial seawater was prepared as a solution of artificial sea salt (*Tropical Marine®*, *Tropical Marine Centre*, *Ltd.*) diluted with distilled water using a magnetic stirrer. Water osmolality was checked, before and after experiments, with a vapour pressure osmometer (*VAPRO™ 5520*, *Wescor*): duplicate samples of 10 µl of saline solution were measured on each occasion and their values averaged. Talitrids were individually transferred into experimental beakers, each provided with a needle attached to a main air-duct connected to an air pump to ensure both water circulation and continuous oxygenation. Beakers were covered with Parafilm® to reduce evaporation. After sensors were affixed to the animals (see below), talitrids were immersed in an artificial seawater solution and left to acclimatise for 48 h to standard conditions before experiments started, in order to standardize their physiological status (McDonald et al., 1977; Morritt, 1988). The standard conditions were as follows: 33‰ marine artificial water, 19±1 °C, no food (as it has been previously demonstrated that ionic regulation is not dependent on ion-rich food sources (Morritt, 1988)) and continuous aeration.