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PRIMARY RESEARCH PAPER

Measurement of temperature and salinity effects on oxygen consumption of *Artemia franciscana* K., measured using fibre-optic oxygen microsensors

Sandra Irwin · Vanessa Wall · John Davenport

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Abstract Oxygen consumption rates of nauplii of the brine shrimp *Artemia franciscana* Kellogg 1906 were determined over a range of salinities from 10 to 110 ppm, in temperatures from 0 to 30°C, using a multi-factorial design. The oxygen micro-sensors employed have a fast response time and are capable of accurately measuring oxygen concentrations at temperatures well below 0°C. Oxygen uptake rate ranged from 0.03 to 0.66 μmol O_2 mg^{-1} h^{-1} and was sensitive to changes in both salinity and temperature. Temperature was the dominant factor affecting oxygen consumption rates, which showed a significant increase with increasing temperature. A slight decrease was measured in oxygen consumption with increasing salinity related to differential solubility of oxygen in waters of different salinities. Thermal sensitivity of oxygen consumption determined from calculations of Q_{10}, indicated physiological adaptation of *Artemia* nauplii to the ranges of temperatures tested.

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S. Irwin (✉) · V. Wall · J. Davenport
Department of Zoology, Ecology & Plant Science
and Environmental Research Institute,
University College Cork, Distillery Fields,
North Mall, Cork,
Ireland
e-mail: s.irwin@ucc.ie

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Introduction

The brine shrimp *Artemia franciscana* is important as a model organism in ecological and physiological studies and have also been exploited as a commercial resource. Their most widespread use is in the larviculture of fish and shellfish (Shields, 2001; Sorgeloos et al., 2001) and increasingly as biological indicators of environmental contamination (Petrucci et al., 1995). Aside from an avoidance response, they are defenceless against predators and although essentially euryhaline (Thuet et al., 1968), their physiological adaptation to high salinities allows them to thrive where salinity is high enough to exclude predators (Lavens & Sorgeloos, 1996; Van Stappen, 2002). Their distribution is discontinuous throughout the world's hypersaline lakes where they experience wide fluctuations in their physicochemical environment, particularly temperature, salinity and oxygen tension. Physiological adaptations allow high survival over wide ranges of salinity (35–170%) and temperature (6–40°C) (Vanhaecke et al., 1984; Wear & Haslett, 1986). The tolerance limits of these factors are broadened for the species as a whole by the production of diapause eggs (Clegg et al., 1996).

Despite the euryhaline and eurythermal nature of *A. franciscana*, both salinity and temperature are major determinants of growth, survival and reproduction, which may show phenotypic variation according to location. A broad range of temperatures and salinities allow survival rates of more than 90% (Lavens & Sorgeloos, 1996; Van Stappen, 2002). The optimum temperature for growth has been estimated at between 20 and 28°C (Wear & Haslett, 1986), for survival between 20 and 25°C (Vanhaecke et al., 1984) and the temperature limits for reproduction are 15 and 30°C (Browne & Wanigasekera, 2000). While temperature is often reported as being more important, it interacts with salinity, each affecting the tolerance ranges of the other. While they do not reproduce at low salinities, *A. franciscana* successfully reproduce and show >90% survival at salinities as high as 180 ppm (Browne & Wanigasekera, 2000; Wear & Haslett, 1986).

Artemia franciscana are oxygen regulators and maintain respiratory independence in declining oxygen tensions (Spicer & El-Gamal, 1999; Vos et al., 1979), although this ability is reduced in both nauplii and adults at higher temperatures (Varó et al., 1991, 1998). The ability to regulate oxygen uptake develops at an early stage in the life cycle of *A. franciscana* and is concurrent with development of functional heart and gills (Spicer & El-Gamal, 1999). Metabolic adjustments are seen, however, which match changing energetic demands to variations in abiotic conditions and there is a large body of literature relating to metabolic physiology in relation to temperature and salinity, particularly of adults (De Wachter & Van Den Abbeele, 1991; Varó et al., 1998). In adults, oxygen consumption is generally seen to decrease with increasing salinity, with a variety of contradictory studies available. Positive and negative relationships between the two, as well as no observable effect, have been reported (see De Wachter & Van Den Abbeele, 1991 for review). Direct comparison between studies is complicated by the use of different strains and species of brine shrimp (*Artemia* sp.) and by the wide range of experimental designs, particularly relating to the range of salinities tested. Although fewer studies have been carried out in relation to temperature, a similar situation has arisen (Varó et al., 1991; 1993b). A significant linear relationship is reported for nauplii over the range 10–35°C (Engel & Angelovic, 1968; Varó et al., 1993b).

The aim of the present study was to use oxygen micro-sensors, capable of measuring oxygen concentrations at temperatures from –10 to 50°C, to determine the oxygen consumption rates of *A. franciscana*. The main objective was to use a multifactorial design to quantify the combined effects of temperature and salinity on oxygen uptake in *A. franciscana* and to consider the interaction between these two variables.

Methods

Hatching of *Artemia franciscana*

Cysts of the brine shrimp *Artemia franciscana* (EG grade, INVE Aquaculture NV, Belgium) were hatched in 2 l hatching cones at 28°C in filtered seawater with vigorous aeration and overhead tungsten lighting for 24 h. Temperature and light conditions remained constant during the hatching period. On completion of hatching the nauplii were separated from the shells and unhatched cysts using a siphon and rinsed for 5 min in seawater before being transferred to aerated 1.5 l tanks with overhead illumination at a density of approximately 100 ind/ml^{-1}. The hatched brine shrimp nauplii were acclimated to the appropriate experimental temperature and salinity conditions for a further 24 h prior to use in oxygen uptake experiments. Artificial seawater (ASW, Fish Antics) was prepared at six experimental salinities, 10, 30, 50, 70, 90 and 110 ppm. Temperature (0, 2, 5, 10, 15, 20, 25 and 30°C) was maintained by submersing all tanks in a constant temperature water bath (Grant Instruments, UK).

Measurement of oxygen uptake

Oxygen consumption rates (MO_2) of *A. franciscana* at 10 salinities and 8 temperatures were measured in closed 10 ml respirometers. Animals were collected directly from the acclimation tanks into the respirometry chambers, which

were then sealed under water. This technique resulted in approximately 1000 individuals in total in each respirometry chamber. It was necessary to use more nauplii at low temperatures in order to achieve a measurable decline in oxygen within the respirometer over the oxygen measurement session. The experimental arrangement was allowed to stand for 10 min before measurement commenced, as in preliminary trials the first few minutes of data were occasionally affected by minor temperature changes induced by handling of the equipment. This effect was seen not to extend past 5 min in extensive preliminary testing, and so 10 min was deemed appropriate to preclude this effect. The seawater in the respirometers was stirred gently throughout the measurement period using a submersible magnetic stirrer (Rank Brothers, UK, Ltd.) to ensure homogenous oxygen concentrations throughout the chamber. Oxygen was measured continually by means of fibre-optic oxygen microsensors (PreSens GmbH, Germany) inserted through the lid of each sealed respirometer.

These micro-optodes use a phase angle detection principle to measure the luminescence decay time of an immobilised luminophore as the oxygen dependent parameter (Klimant et al., 1995). This technique offers a relatively straightforward means of oxygen measurement when compared with traditional Clarke-type microelectrodes, that do allow fine-scale oxygen measurement, but are less sensitive, more time consuming to fabricate and more complex to operate (Holst et al., 1997). Further advantages of this method over traditional microelectrodes are that micro-optodes have a fast response time even at very low temperatures, do not consume oxygen during measurement, and are unaffected by salinity. They can be used in the range –10 to 80°C. Oxygen measures are affected by temperature, which influences the luminescence decay time as well as the luminescence intensity of the indicator due and the collisional frequency of the oxygen molecules with the indicator dye. A temperature sensor is therefore used in combination with the optodes to record temperature variations that are compensated using the data logging software. The diameter of the sensor tip is < 50 μm, and the fragile silica optical fibres are protected in a needle-type housing.

The micro-optodes were calibrated using a two point calibration prior to each run, at each experimental temperature and salinity, in aerated seawater (100% air saturation) and a solution of 0.5% $NaSO_3$ (0% oxygen). They were connected to a MICROX TX oxygen meter (PreSens, GmbH) from which data were transferred to a PC via a serial interface, capable of transmitting oxygen measures at 1 s intervals. Following the 10 min acclimation period the oxygen concentration (% air saturation) was recorded at 5 s intervals for 50 min. Four replicate measurements were carried out at each of the experimental temperatures and salinities. To prevent microbial respiration from interfering with the measurements respirometers were rinsed with alcohol prior to each measurement run. Control measurement runs were also carried out without animals in the respirometers to ensure that background oxygen consumption within the respirometer chambers was negligible. Following completion of each respirometry session, the nauplii were removed from the respirometer and counted under a binocular microscope. Although not very numerous, individuals that were not active were not included in these counts. At lower temperatures, the swimming activity of the nauplii was reduced, but recovery was rapid following restoration of ambient temperature. Survival below 0°C was too low to allow accurate measurement of oxygen consumption. For determination of dry weights of individuals of *A. franciscana*, batches of cysts were hatched and nauplii were held for 24 h, as before. The dry weight of individual 24 h nauplii was then obtained after drying for 24 h at 60°C, and was estimated at 2.0 ± 0.3 μg.

Data analysis

Oxygen consumption rates were calculated using the PO_2 of the seawater in the respirometers at the beginning and end of each measurement session. Measurements of oxygen concentrations within respirometer chambers (% air saturation) at different temperatures and salinities were

converted to dissolved oxygen concentrations using an equation of the form:

$$\ln C_o^* = \frac{a}{b} + a_0 + \frac{a_1}{T} + a_2 \ln T + a_3 T + a_4 T^2 + S(a_5 + a_6 T + a_7 T) + a_8 S^2$$

where C_o^* = dissolved oxygen concentration; T = Kelvin temperature; S = Salinity using the regression coefficients for the Weiss equation after Garcia & Gordon (1992) and Sherwood et al. (1991), valid in the range 0 to –35°C and <260 ppm. Results were expressed as dry weight specific oxygen consumption, μmol O_2 mg^{-1} h^{-1} (MO_2). A General Linear Model with temperature and salinity as crossed factors was applied to calculate the effect of these two factors on the rate of oxygen consumption by *A. franciscana* nauplii. Regression analysis was employed to determine whether relationships between variables were significant. All statistical analyses were carried out using MINITAB Release 13.32.

The temperature sensitivity of oxygen consumption was determined using the Arrhenius relationship for the increase in physiological rate with temperature (Q_{10}). Q_{10} values were calculated across three temperature ranges (0–10°C, 10–20°C and 20–30°C) using the Van't Hoff equation:

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{10/(t_2 - t_1)}$$

where k_1 and k_2 are the oxygen consumption rates at temperatures t_1 and t_2, respectively.

Results

Oxygen consumption rates of *A. franciscana* nauplii were between 0.03 and 0.66 μmol O_2 mg^{-1} h^{-1}.

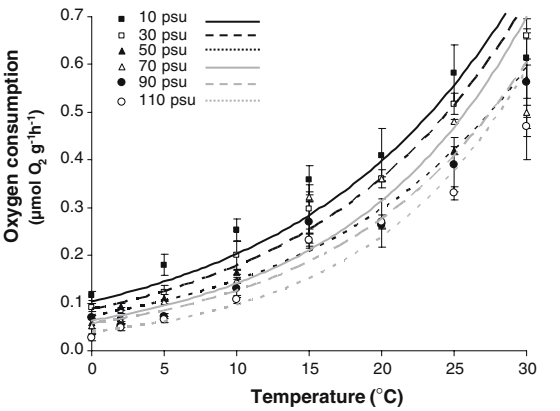

Fig. 1 Oxygen consumption rate (μmol O_2 mg^{-1} h^{-1}) of *Artemia franciscana* nauplii in relation to salinity (ppm) and temperature (°C)

The highest consumption rates were measured at 30°C at the two lowest salinities (10 and 30 ppm), with rates approaching zero at the lowest temperatures. The effects of temperature and salinity on oxygen consumption of *A. franciscana* nauplii are shown in Fig. 1. Oxygen consumption increased significantly with increasing temperature at all salinities, and a significant curvilinear relationship between the two was observed (R^2 = 0.87, 0.98, 0.98, 0.96, 0.95 and 0.95 for 10, 30, 50, 70, 90 and 110 ppm, respectively, $P < 0.05$ for all salinities). A significant effect of each factor, and a significant temperature–salinity interaction, on oxygen consumption were observed (Table 1). Oxygen consumption showed a decreasing trend with increasing salinity at all temperatures (Fig. 1).

The thermal sensitivity of oxygen consumption was determined by calculation of Q_{10} values, which were between 1.4 and 2.9 at all salinities and temperature ranges, with the exception only of the highest salinity (110 ppm) at the lowest temperature range (0–10°C), where a value of 3.8 was calculated (Table 2). Calculated Q_{10} values

Table 1 General Linear Model of the effects of salinity, temperature and the temperature–salinity interaction on oxygen consumption rate of *Artemia franciscana* nauplii

Source	Degrees of freedom	Sum of squares (SS)	F	Significance P
Temperature	7	1.82	59.8	$P < 0.05$
Salinity	1	0.17	62.5	$P < 0.05$
Interaction	7	0.06	2.1	$P < 0.05$
Error	176	0.76		

Table 2 Q_{10} in *Artemia franciscana* nauplii at different salinities

Salinity	Temperature range		
	0–10°C	10–20°C	20–30°C
10	2.2	1.6	1.2
30	2.3	1.8	1.9
50	2.7	1.9	1.5
70	2.8	2.9	1.4
90	1.8	2.0	2.6
110	3.8	2.5	2.0

decreased as temperatures rose. An increasing trend in thermal sensitivity (Q_{10}) was seen with increasing salinity, but no significant relationship was found between the two over any of the three temperature ranges ($P > 0.05$).

Discussion

Technological advances have resulted in the design and production of micro-optodes for oxygen measurement at a micro-scale in sediments and in diffusive boundary layers (Irwin & Davenport, 2002). These sensors do not consume oxygen during measurement, even at very low temperatures, and withstand freezing without damage to the sensor tip (Mock et al., 2002). They proved ideal for use in this study, and measurement below 0°C was limited by low survival of *A. franciscana* nauplii at low temperatures and not by sensor performance. These non-oxygen consuming microelectrodes, which allow compensation for temperature and salinity, are advantageous over traditional oxygen electrodes that consume oxygen and make studies such as this very difficult. They avoid the need to estimate oxygen consumption by the electrodes themselves, as is commonplace in studies using traditional Clark-type oxygen electrodes (which have substantially slowed responses at low temperature, and allow for direct measurement of oxygen consumption within respirometer chambers.

Oxygen consumption rate of *A. franciscana* nauplii in this study was between 0.03 and 0.66 μmol O_2 mg^{-1} h^{-1}, with maximum values recorded at 30°C at the lowest salinities. This observed trend is consistent with previous studies of oxygen consumption of the nauplii under a variety of conditions (Engel & Angelovic, 1968; Spicer & El-Gamal, 1999; Varó et al., 1993a). The higher end of the range of weight specific oxygen consumption rates of the nauplii measured in this study is higher than oxygen consumption rates reported for adult *Artemia* sp. (Varó et al., 1979, 1998), a difference between these life stages that is widely accepted (Van Stappen, 2002).

Oxygen uptake rate in this study was sensitive to changes in both temperature and salinity. Temperature was seen to be the more important of these two factors, and weight specific oxygen consumption rates of *A. franciscana* nauplii increased by an order of magnitude over the temperature range from 0 to 30°C. An increase in oxygen consumption with increasing temperature is consistent with previous findings at 15°C and above (Varó et al., 1993a). This present study included measures of oxygen consumption extending down to a temperature of 0°C. The plots drawn using the data from the current study, which included measurements at temperatures lower than previously considered, revealed a curvilinear relationship between oxygen consumption and temperature at all salinities tested (Fig. 1), which was linear above 5°C. This indicates that the *A. franciscana* were physiologically adapted to this range of temperatures (Pörtner, 2001). The data collected in this study at 0 and 2°C must be cautiously interpreted, as activity was compromised at these low temperatures, although normal activity was resumed upon restoration of higher temperatures (personal obs.), and *Artemia* sp. does not routinely survive when exposed to temperatures of less than 5°C for extended periods (Van Stappen, 2002).

Artemia franciscana nauplii showed a slight increasing trend in oxygen uptake under declining salinity concentrations (Fig. 1) in some experimental treatments. *Artemia* sp. is a hyper-hypoosmotic regulator and the osmolarity of body fluids varies little with external conditions (Conte, 1984). It is equipped with the necessary regulatory mechanisms to handle exposure to extreme salinity conditions. It is not impermeable to ions, and the osmolarity differential is maintained by active regulation via the salt

gland (Thuet et al., 1968). As *Artemia* sp. is isosmotic at approximately 10 ppm, the observed effect of salinity is likely to be mediated through its effect on the solubility of oxygen in seawater, which decreases with increasing salinity (De Wachter & Van Den Abbeele, 1991). *Artemia* sp. has the capacity to synthesise very efficient respiratory pigments to cope with the low oxygen concentrations typical of hypersaline waters (Wolf et al., 1989).

The thermal sensitivity of *A. franciscana* nauplii was investigated by determination of Q_{10} values, which describe the effect of lowered temperature on reaction rates and ultimately on oxygen consumption (Guppy & Withers, 1999). The decrease in oxygen consumption with decreasing temperature was less at the higher temperature ranges investigated. Temperature adaptation involves integrated responses to changes at biochemical and physiological levels. The standard metabolic rate of ectotherms ultimately sustains all biochemical reactions occurring within the organism, and under normal circumstances is exponentially related to body temperature, slowing down with decreasing temperatures. Such normal temperature dependence of metabolism is indicated by Q_{10} values such as those measured in this study (Guppy & Withers, 1999; Sokolova & Pörtner, 2003), indicating that the nauplii were adapted to these temperature and salinity conditions and no strong adjustments were made to changes in temperature. Values determined in this study were consistent with those previously reported for *Artemia* sp. nauplii (Engel & Angelovic, 1968; Varó et al., 1998). Oxygen consumption rate was seen to change less over the highest temperature range at salinities below 90 ppm, indicating a less complete compensation for temperature effects. Nonetheless, Q_{10} values in this study did not extend into the range indicative of thermal stress and reflected the eurythermal nature of this species.

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