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RESEARCH ARTICLE

Exercise and emersion in air, and recovery in seawater in the green crab (*Carcinus maenas*): metabolic, acid–base, cardio-ventilatory and ionoregulatory responses

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ABSTRACT

In nature, the green crab exhibits emersion and terrestrial activity at low tide. Treadmill exercise in air (20–23°C) of crabs acclimated to 32 ppt seawater (13°C) revealed an inverse relationship between velocity and duration: 2.0 body lengths (BL) s^{-1} was sustainable for several minutes, and 0.25 BL s^{-1} was sustainable for long periods. Fatigue was not due to dehydration. Physiological responses over an 18 h recovery in seawater after near-exhaustive exercise (0.25 BL s^{-1}, 1 h) in air were compared with responses after quiet emersion (1 h) in air. Exercising crabs exhibited transient scaphognathite slowing and progressive increases in heart rate, whereas emersed crabs exhibited persistent inhibition of ventilation and transient heart slowing. Upon return to seawater, all these rates increased above both control and treatment levels. Post-exercise disturbances were more marked and/or longer lasting (e.g. EPOC, hyperventilation, tachycardia, metabolic acidosis, lactate elevation, ionic disturbances) than those after simple air exposure. However, an increase in net acidic equivalent excretion to the environment occurred after emersion but not after exercise. Instead, post-exercise crabs relied on carapace buffering, signalled by elevated haemolymph Ca^{2+} and Mg^{2+}. Prolonged lowering of haemolymph P_{CO_2} associated with hyperventilation also played a key role in acid–base recovery. EPOC after exercise was 3-fold greater than after emersion, sufficient to support resting $\dot{M}_{O_2}$ for >14 h. This reflected clearance of a large lactate load, likely by glycogen re-synthesis rather than oxidation. We conclude that the amphibious green crab uses a combination of aquatic and terrestrial strategies to support exercise in air, emersion in air and recovery in seawater.

KEY WORDS: Lactate clearance, EPOC, Metabolic acidosis, Heart rate, Scaphognathite rate, Carapace buffering, Osmolality

INTRODUCTION

The European green crab (*Carcinus maenas*), a model organism in decapod physiology (Leignel et al., 2014), is arguably the most successful invasive decapod throughout the world, renowned for its aggressive nature, catholic diet, and tolerance of environmental stressors, including extremes of temperature, salinity, hypoxia and air exposure (Behrens-Yamada et al., 2005; Klassen and Locke, 2007). Individuals are often stranded at low tide (Klassen and Locke, 2007; Simonik and Henry, 2014) and will emerse into air to avoid unfavourable conditions in rockpools (Taylor et al., 1973, 1977; Truchot, 1986; Zimmer and Wood, 2017). The gills contain widely spaced lamellae supported by chitin (Taylor and Butler, 1978) and cross-bridges (Johnson and Uglow, 1985) serving to prevent collapse in air. The physiological responses to air exposure have been studied extensively in this T1 amphibious crab (i.e. least terrestrial by the classification of Hartnoll, 1988). While details vary, the general pattern during short-term emersion of *C. maenas* (a few hours) is maintenance of O_2 consumption ($\dot{M}_{O_2}$) at 50–120% of aquatic rates with only moderate lactate accumulation and acid–base disturbance, and variable decreases in heart and scaphognathite rates and haemolymph oxygen levels (Ahsanullah and Newell, 1971; Newell et al., 1972; Wallace, 1972; Wheatly and Taylor, 1979; Johnson and Uglow, 1985; Depledge, 1984; Truchot, 1986; Simonik and Henry, 2014; Nancollas and McGaw, 2021).

In contrast, there is no information on the ability of this species to exercise in air, although the physiology of exercise and recovery in seawater has been investigated (Houlihan et al., 1984; Houlihan and Mathers, 1985; Hamilton and Houlihan, 1992). This member of the Family Portunidae (swimming crabs) is well adapted for aquatic exercise, exhibiting a maximum speed of about 3 body lengths (BL) s^{-1}, and a more sustainable speed (10–50 min) of about 0.4 BL s^{-1}. Exercise at the latter speed (5–10 min) caused a 5-fold increase in $\dot{M}_{O_2}$. Acid–base disturbance and lactate accumulation were moderate. Calculations suggested that exercise was largely fuelled by aerobic rather than anaerobic metabolism; excess post-exercise O_2 consumption (EPOC) lasted less than 30 min. Increases in heart (2-fold) and scaphognathite (4-fold) rates during exercise were also corrected within 30 min.

Although exercise studies on *C. maenas* are limited, there is extensive literature on true aquatic crabs exercising and recovering in seawater (e.g. McMahon et al., 1979; McDonald et al., 1979; McMahon, 1981; Taylor, 1982; Booth et al., 1982; Booth et al., 1984; Booth and McMahon, 1985; Milligan et al., 1989) and on true land crabs exercising and recovering in air (e.g. Smatresk et al., 1979; Herreid et al., 1979; Smatresk and Cameron, 1981; Wood and Randall, 1981a,b; Herreid et al., 1983; Full and Herreid, 1983; Full et al., 1985; Wheatly et al., 1986). However, we are aware of only one investigation on an amphibious intertidal crab (*Cyclograpsus lavauxi*) exercising in air and recovering in seawater (Waldron et al., 1986). Overall, these studies suggest that air-breathing crabs can perform only short-term exercise at high speeds, but can walk at slow speeds for long periods. $\dot{M}_{O_2}$ elevations during exercise are similar in air breathers and water breathers, and this may be associated with similar increases in heart and scaphognathite frequencies. However, post-exercise recovery of these rates, as well as acid–base status, appears to be slower in air breathers. Increases in haemolymph P_{CO_2} are greater during exercise in air breathers, and

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are corrected more slowly than in water breathers. The metabolic component of acidosis caused by 'lactic acid' production is similar in the two groups, but may be corrected by different mechanisms. Excretion of acidic equivalents to the external environment predominates in water breathers, and mobilization of buffer base (carbonates) from the carapace predominates in air breathers.

Against this backdrop, we investigated the exercise ability of *C. maenas* in air, and the physiological changes (cardio-ventilatory, metabolic, acid–base and ionoregulatory) that occur as a result of 60 min of sustained exercise in air and 18 h of recovery in seawater. We compared these responses with those of crabs subjected to simple quiet emersion in air and subsequent recovery in seawater for the same periods, so as to separate the effects of activity from those of air exposure alone. Our overall hypothesis was that during exercise in air, this T1 amphibious crab would exhibit similar physiological responses to those reported for true land-crabs (T3–T5), but when recovering in seawater, they would exhibit similar responses to those reported for completely aquatic crabs. We also hypothesized that disturbances associated with exercise in air would be much greater than those seen after simple emersion in air.

MATERIALS AND METHODS

Animal collection, maintenance and handling

Experiments were performed on approximately 200 male green crabs, *Carcinus maenas* (Linnaeus 1758) (40–90 g), collected by traps baited with fish fillets in areas near Seddall Island, Ecoole, Regional District of Alberni-Clayoquot, BC, Canada (49°05′00.0″N, 125°10′21.6″W). Crabs in the red phase, and crabs lacking one or more legs, were excluded. Collections were performed under Fisheries and Oceans Canada permits XR-180-2020 and XR-136-2021. At Bamfield Marine Sciences Centre (BMSC), the crabs were held for 3–6 weeks prior to experimentation, under flow-through conditions in outdoor communal 1000 l tanks served with aerated seawater (12–13°C, 32 ppt) under natural photoperiod. The crabs were provided with shelters and fed with fish fillets over a 3 day period. Feeding was alternated between tanks so that prior to removal for experiments, crabs had been fed to satiation for 3 days, then fasted for 3 days, and then for 1 additional day as they settled in their chambers (below). Experiments were approved by the BMSC Animal Care Committee.

All crabs used in experiments were allowed to settle for ≥24 h in individual 500 ml glass jars served with flowing seawater at the acclimation temperature and salinity. These jars were shielded with black plastic, had individual air-stones, and could be sealed to serve as static respirometers when flow was stopped. Temperature was maintained by an external seawater bath.

Exercise experiments in air (20–23°C) were performed using the variable-speed treadmill designed by Wood and Randall (1981a,b), which had been renovated after 40 years of storage. The treadmill had a running platform of 30 cm (width)×45 cm (length), outside walls to prevent escape, vertical bars on the walls to help the crab orient, and a cover on the front third for shade.

For exercise tests, the crab was removed from its chamber, blotted dry in a standardized fashion (but not tipped, so as to avoid loss of branchial chamber water), weighed for initial mass and measured for body length. As crabs generally run sideways, body length was taken as the distance between the first hinges of the longest left and right walking legs when the crab was standing upright. The crab was then placed on the stationary treadmill for 5 min, during which it usually explored the platform, then stopped moving. The gear was then engaged and the speed brought up to the intended velocity over a 30 s period, the end of which represented time zero of exercise. At the end of the exercise period, the weighing procedure was quickly repeated for final mass, and the crab was returned to its jar of fresh seawater, which represented time 0 h of recovery.

Emersion experiments followed an identical protocol, but without exercise. After drying and measuring the crab, it was placed in an open plastic container in air with approximately the same surface area (1020 cm^2) as the treadmill (1200 cm^2). After initial exploration, most crabs exhibited very little activity, and remained motionless. After the final drying and weighing, the crab was returned to its jar of fresh seawater, which represented time 0 h of recovery. The plastic container used for air exposure was carefully rinsed with 50 ml of fresh seawater, which was collected, and subsamples were frozen for measurement of N waste excretion.

Series 1 – exercise performance

In order to assess exercise capacity in air, crabs (N=16) were run at constant speeds ranging from 0.25 to 2.0 BL s^{-1}. Time to fatigue was noted and change in body mass was recorded. Fatigue was judged to have occurred when the crab was swept against the rear wall of the treadmill and would not resume voluntary exercise after the treadmill was stopped and restarted several times. Each crab was used only once. The air-exposure time over which the change in body mass was recorded was therefore the sum of the 5 min settling time, the 0.5 min ramping up time, and the actual exercise period up to fatigue.

Based on this series, a standard 1 h exercise period at 0.25 BL s^{-1} was employed in the subsequent series (2–4). In practice, this meant that about half of the crabs in these series continued walking voluntarily while the other half ceased voluntary exercise after 30–40 min. Nevertheless, all crabs were exercised for the full 1 h; continued activity was ensured by periodic prodding with a glass pipette.

Series 2 – respirometry and net acidic equivalent flux measurements

Oxygen consumption ($\dot{M}_{O_2}$), net acidic equivalent flux to the water, and N waste excretion to the water (the last reported in C.M.W., in preparation) were measured in crabs (N=6) at rest (2×30 min periods), during the first 2 h (8×15 min periods) after 60 min of exercise at 0.25 BL s^{-1} and at 5, 8 and 18 h after exercise (2×30 min periods each, bracketing the times). An identical protocol was used before and after 1 h of emersion (N=7). The respirometers were small, darkened, tight-fitting jars (500 ml, diameter 7.4 cm, height 11.7 cm) that essentially prevented spontaneous activity. For respirometry during each period, the jar was quickly flushed with fresh air-saturated seawater, an initial 50 ml water sample was taken, then the jar was sealed with a screw-top cap over medium-burr rubber dental dam. A final 50 ml water sample was taken at the end of the period, and then the flushing cycle was repeated. At each sampling, several filling cycles of the syringe ensured through mixing. In practice, this meant that the 8×15 min periods in the 2 h immediately after the end of exercise or emersion were actually of 9–12 min duration each (exact time noted) because of the time needed for flushing and sampling. Oxygen partial pressure (P_{O_2}) was measured immediately. The water samples were aliquoted for net acidic equivalent flux measurements (titration alkalinity and ammonia), and the remaining volume was immediately frozen at −20°C for later analyses of other N waste. A companion study (C.M.W., in preparation) reports on the nitrogenous waste metabolism of these same animals during these experiments.

As the exercise treadmill and emersion containers were open to the air, it was not possible to perform respirometry during the

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60 min periods of exercise or emersion. However, to provide an estimate of resting $\dot{M}_{O_2}$ in air, crabs (N=6) were sealed into the same darkened 500 ml jars at 20–23°C in air, and after 5 min of initial settling, the rate of decline of P_{O_2} in air was monitored continuously overnight (10–12 h) using a needle-mounted O_2 micro-optode (PreSens, Regensberg, Germany) inserted through a seal on the lid.

Series 3 – haemolymph acid–base status, metabolites, osmolality and ions

The exercise (1 h at 0.25 BL s^{-1}; N=28 crabs) and emersion (1 h; N=21 crabs) protocols were identical to those of series 2. In the exercise trials, samples were taken under control (pre-exercise) conditions and at 0, 0.5, 1, 2, 5, 8 and 18 h post-exercise, with N=6–7 samples at each time. In the emersion trials, samples were taken under control (pre-emersion) conditions and at 0, 2, 5, 8 and 18 h post-emersion, with N=6–7 samples at each time. Each crab was sampled twice, with times chosen at random. Haemolymph samples (0.5 ml) were drawn from the arthrodial membrane of the posterior walking legs of crabs while they remained underwater. Samples were drawn into an ice-cold gas-tight syringe (Hamilton, Reno, NV, USA). To minimize confounding effects, samples from crabs that struggled were rejected. Total CO_2 and pH were measured immediately; the remainder of the sample was aliquoted into four subsamples, immediately frozen in liquid nitrogen, then stored at −80°C for later analysis of metabolites.

Series 4 – ventilation and heart rate

Movements of both scaphognathites (N=6 in each treatment) and heart (N=6 in each treatment) were measured by impedance recording in separate groups of crabs subjected to the same emersion (1 h) and exercise (1 h at 0.25 BL s^{-1}) protocols as in series 3. Operations to implant impedance leads (insulated fine wires, 120 cm length) were performed 24–48 h before experiments, and the crabs were allowed to recover in their individual jars under flow-through conditions. Small holes were drilled through the dorsal carapace on either side of each scaphognathite, or on either side of the heart, with a 20 gauge needle, to allow insertion of the non-insulated tips of the wires. The wires were cemented in place using cyanoacrylate glue and strips of dental dam. Each pair was led out through Clay-Adams PE160 polyethylene tubing (Becton Dickinson, Franklin Lakes, NJ, USA) and the two scaphognathite pairs were pig-tailed together to prevent tangling. It was necessary to tape the chelipeds shut with duct tape in order to prevent the crabs from cutting the impedance leads. In the exercise series, recordings were made on crabs at rest, at 15, 30, 45 and 60 min exercise in air, immediately after return to seawater (0 h), at 5 min (0.08 h) after return to seawater, and then at 0.5, 1, 2, 5, 8 and 18 h of further recovery in seawater. In the emersion series, the sampling times were the same, except that the 0.5 and 1 h recovery times were omitted, in parallel with the haemolymph sampling times of series 3.

Analytical techniques and calculations

Recordings of heart and scaphognathite rates (min^{-1}) were made with Transmed 2991 impedance converters (Fullerton, CA, USA) outputted to a PowerLab data integrity system (ADInstruments, Colorado Springs, CO, USA), and visualized in LabChart software v.7.0 (ADInstruments). At each time point, measurements were made over approximately 2 min, and the calculated mean rates included periods of cardiac and scaphognathite pausing, and in the case of the latter, instances of unilateral ventilation where the rate was sometimes zero on one side.

P_{O_2} (torr) in water was measured using a DO 6+ galvanic oxygen electrode and meter (Oakton Instruments, Vernon Hills, IL, USA), and in air by a custom needle-mounted micro-optode system (PreSens), both calibrated to 0% and air saturation, taking the vapour pressure of water into account. O_2 consumption rate ($\dot{M}_{O_2}$) over each interval was calculated in the standard fashion:

$$\dot{M}_{O_2} = [((P_{O_2,\text{initial}} - P_{O_2,\text{final}}) \times \alpha O_2) \times V]/(M \times T), \quad (1)$$

where αO_2 (μmol l^{-1} $torr^{-1}$) is the appropriate solubility coefficient from Boutilier et al. (1984) for seawater, or Dejours (1981) for air, V (l) is the volume of the respirometer, M is the body mass (kg) and T is time (h). Tests with empty chambers demonstrated that there was no detectable blank O_2 consumption.

Excess post-exercise or post-emersion oxygen consumption (EPOC) was calculated by integrating the area under the individual $\dot{M}_{O_2}$ versus time plots, relative to the pre-treatment resting control values in seawater.

The titratable alkalinity was measured by titration of N_2-bubbled seawater samples to pH 4.00 with standardized 0.01 mol l^{-1} HCl (BDH, VWR International Ltd, Poole, UK), as described by McDonald and Wood (1981). Micro-burettes (Gilmont Instruments, Great Neck, NY, USA) and Accumet AE 150 pH meters and electrodes (Fisher Scientific, Toronto, ON, Canada) were employed. The difference (initial minus final titratable alkalinity concentrations) gives the net loss of titratable base from the crab where negative values represent titratable base loss (=titratable acid uptake), and positive values represent titratable base uptake (=titratable acid excretion). The addition of this plus the simultaneously measured total ammonia excretion (positive) yields the net acidic equivalent flux. Thus, the convention used here is that net acid excretion to the water is positive, and net base excretion to the water is negative. The resolution of these measurements is limited by short flux periods. Therefore, the four measurements in the 0–1 h period were averaged, as were the four measurements in the 1–2 h period. The two measurements in the control, 5, 8 and 18 h points were similarly averaged at each time.

Total CO_2 (TCO_2, mmol l^{-1}) in seawater and haemolymph samples was measured using a Corning 965 analyser (Corning Instruments, Corning, NY, USA) calibrated with $NaHCO_3$ standards. Water and haemolymph pH values were measured in a temperature-controlled water bath, using an MI-4156 Micro-Combination pH probe (Microelectrodes Inc., Bedford, NH, USA) and an Accumet pH meter (Fisher Scientific), calibrated with Radiometer precision buffers (Copenhagen, Denmark). Haemolymph P_{CO_2} (torr) and [HCO_3^-] (mmol l^{-1}) were calculated using rearrangements of the Henderson–Hasselbalch equation:

$$P_{CO_2} = TCO_2/[\alpha CO_2 \times (1 + \text{antilog}(\text{pH} - pK_{app}))], \quad (2)$$

$$[HCO_3^-] = TCO_2 - (\alpha CO_2 \times P_{CO_2}), \quad (3)$$

where αCO_2 is the solubility coefficient of carbon dioxide (mmol l^{-1} $torr^{-1}$) and pK_{app} is the apparent carbonic acid dissociation constant in the haemolymph at the appropriate acclimation salinity and temperature, both tabulated for *C. maenas* by Truchot (1976a).

ΔH^+_m, the metabolic load of acidic equivalents (positive values) or basic equivalents (negative values) of non-respiratory origin appearing in the haemolymph, relative to the mean acid–base status of crabs at rest under control conditions, was calculated by the equation of McDonald et al. (1980):

$$\Delta H^+_m = [HCO_3^-]_c - [HCO_3^-]_t - \beta \times (pH_c - pH_t), \quad (4)$$

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where the subscripts 'c' and 't' refer to measurements at control and various experimental times, respectively, values of haemolymph [HCO_3^-] and pH are as in Eqns 2 and 3, and β is the slope (mmol l^{-1} pH $unit^{-1}$) of the non-bicarbonate buffer (NBB) relationship for *C. maenas* haemolymph. Truchot (1976b) reported that β was a linear function of total protein concentration in the haemolymph of *C. maenas*. Total protein concentrations did not change significantly within either of the experimental treatments in the present study, but were significantly different overall between them (see Results). Therefore, different β values were used in Eqn 4 and in Davenport (1974) diagrams for the emersion (−12.47 mmol l^{-1} pH $unit^{-1}$) and exercise treatments (−9.65 mmol l^{-1} pH $unit^{-1}$), respectively, based on the regression relationship of Truchot (1976b).

Haemolymph glucose was determined enzymatically using an Infinity™ glucose hexokinase liquid stable reagent kit (Thermo Fisher, Middletown, VA, USA). Lactate in haemolymph was determined enzymatically (lactate dehydrogenase) using a 10 139 084 035 L-lactic acid kit (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). The total concentration of haemolymph protein was determined at 595 nm by the colorimetric assay of Bradford (1976) using bovine serum albumin as a standard. Haemolymph and seawater osmolality were determined with a 5100C vapor pressure osmometer and Opti-Mole™ standards (Wescor, Logan, UT, USA). A Varian AA240FS atomic absorption spectrophotometer (AAS) (Varian Medical Systems, Palo Alto, CA, USA), operated in flame mode with acetylene as the purging gas, was used to determine the concentrations of K^+, Ca^{2+} and Mg^{2+} in haemolymph samples, using 1% lanthanum chloride ($LaCl_3$) for the dilution of samples and commercial standards (Fisher Scientific).

Statistical analyses

Data are expressed as means±1 s.e.m.; *N* is the number of crabs. The resting control measurements in seawater were compared between the emersion series and the exercise series for all parameters using Student's two-tailed *t*-test. This test (paired or unpaired as appropriate) was also used for comparing some calculated physiological parameters between the two treatment groups. In order to detect significant overall effects of treatment, recovery time and their interactions, two-way ANOVA were performed on the 0–18 h recovery data for the exercise and emersion treatments. In series 2 and series 4 where the same animals were followed over time, these were repeated measures two-way ANOVA. Additionally, in series 4, a separate two-way repeated measures ANOVA was applied to the 15, 30, 45 and 60 min exercise and emersion data. One-way ANOVA (regular or repeated measures as appropriate) followed by Dunnett's *post hoc* test were then used within each treatment to identify significant differences from resting control measurements in water. Prior to these tests, all data were checked for normality of distribution and homogeneity of variance. If data failed, they were then subjected to standard transformations (log, square route, inverse). If data still failed, non-parametric alternatives were used (Mann–Whitney *U*-test, Kruskal–Wallis ANOVA on ranks followed by Dunn's *post hoc* test). A significance level of $P \leq 0.05$ was used throughout.

RESULTS

Exercise performance and mass loss

Green crabs could run on a treadmill in air at about 2.0 BL s^{-1} for several minutes, but sustainable speed declined exponentially with duration, reaching a plateau from 30–40 min onwards at about 0.25 BL s^{-1} (Fig. 1A). Based on this relationship, a standard exercise treatment of 0.25 BL s^{-1} for 1 h was chosen for subsequent experimental series. About half of the crabs ceased voluntary exercise after 30–40 min but were made to continue walking for the full 1 h. Most crabs appeared to be close to or at exhaustion by 1 h, but all were still capable of assuming a defence posture and slow walking.

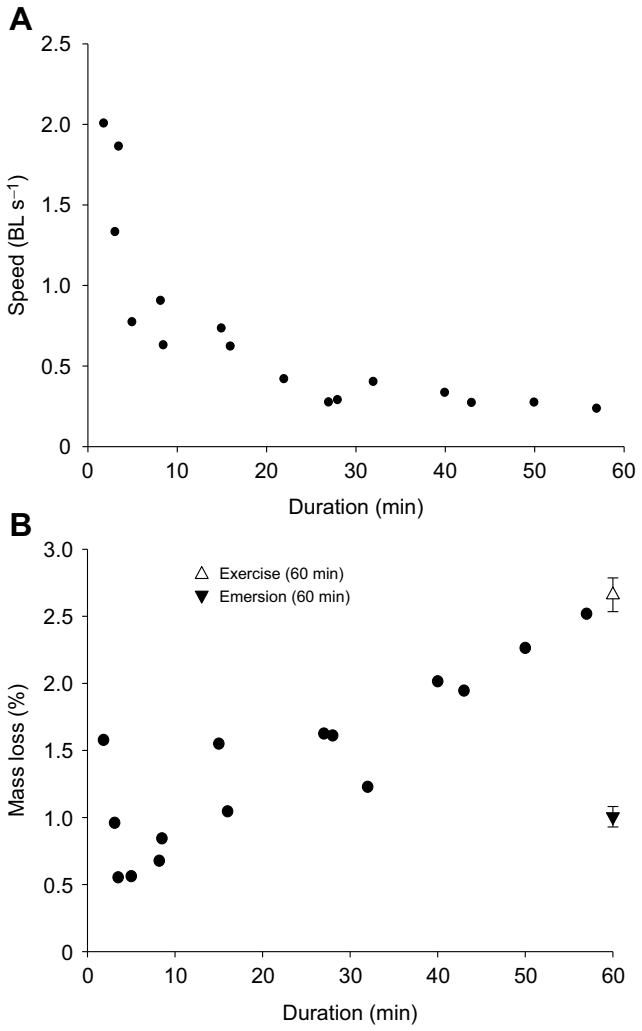

Fig. 1. Exercise performance and percentage mass loss of *C. maenas* in response to exercise or emersion (series 1). (A) Relationship between running speed in air (BL, body lengths) and voluntary running duration in individual crabs. (B) Relationship between mass loss and running duration in these same crabs. Triangles indicate mass loss (means±1 s.e.m.) after 1 h of exercise (*N*=27) and simple emersion (*N*=23) in series 2 and 3.

Mass loss was approximately a linear function of exercise duration, regardless of speed (Fig. 1B). The mean percentage decreases after 1 h of exercise (2.66±0.13%, *N*=27) and after 1 h of emersion alone (1.01±0.08%, *N*=23) from series 2 and 3 were significantly different (Fig. 1B).

Oxygen consumption

$\dot{M}_{O_2}$ for resting crabs in seawater was about 530 µmol kg^{-1} h^{-1}, and did not differ between the two treatments (Fig. 2A,B). $\dot{M}_{O_2}$ was significantly elevated after 1 h in air, with elevations of 4.4-fold after exercise and 3.9-fold after emersion, measured during the first 15 min of recovery in seawater. Thereafter $\dot{M}_{O_2}$ declined in both groups, staying significantly elevated for 8 h in the exercised crabs, and for 2 h in the emersed crabs. Two-way repeated measures

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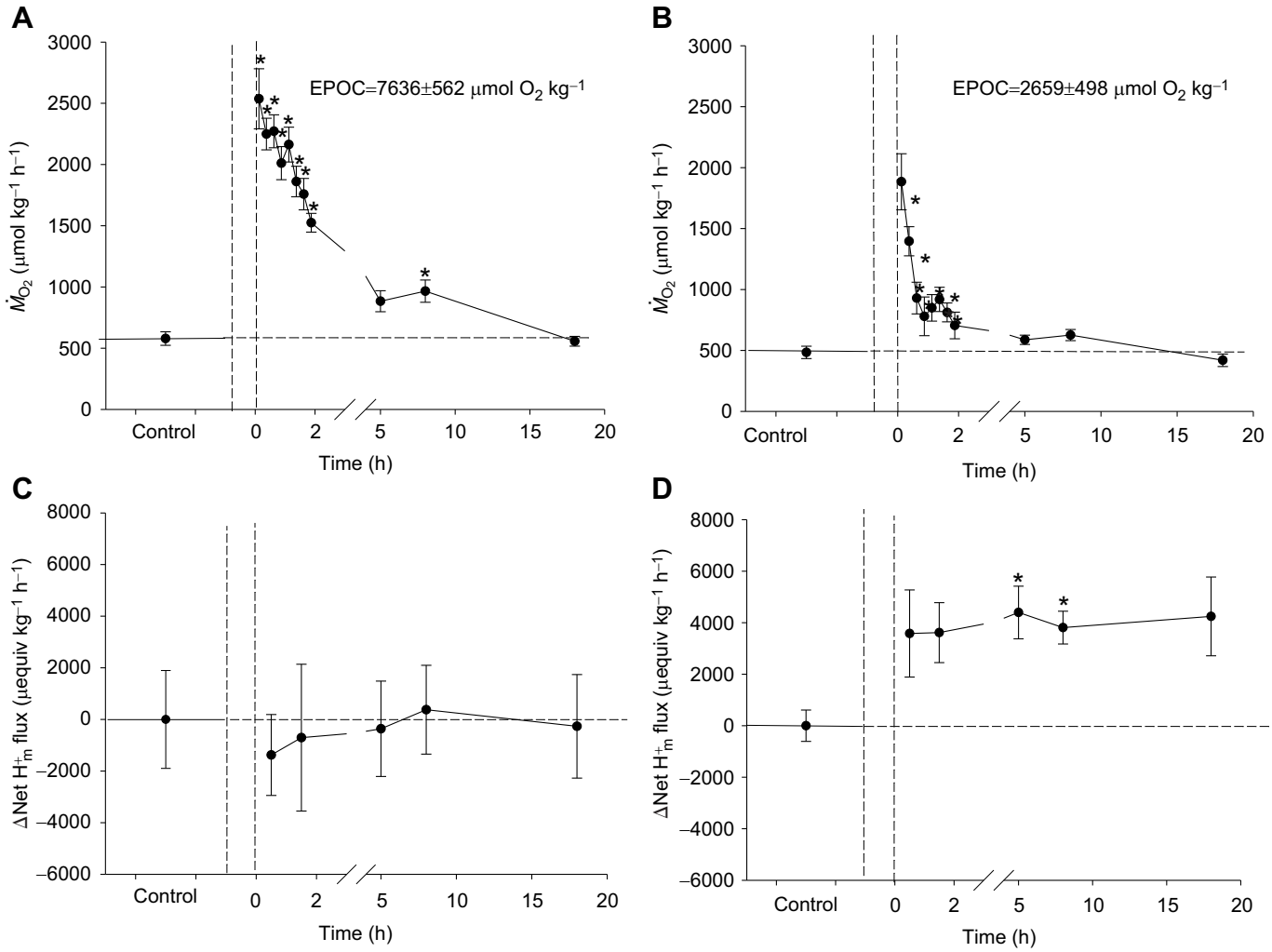

Fig. 2. Oxygen consumption and net acidic equivalent flux of *C. maenas* in response to exercise or emersion (series 2). (A,B) O_2 consumption ($\dot{M}_{O_2}$) and (C,D) net acidic equivalent excretion rates to the seawater ($\Delta netH^+_m$ flux) in response to (A,C) 1 h of exercise in air (left, *N*=6) or (B,D) 1 h of simple emersion in air (right, *N*=7), followed by 18 h recovery in seawater. The air-exposure period is indicated by dashed vertical lines, and resting control rates in seawater by dashed horizontal lines. The excess post-exercise and post-emersion O_2 consumption (EPOC) is also shown. Data are means±1 s.e.m. For $\dot{M}_{O_2}$, two-way repeated-measures ANOVA during recovery: treatment $P<0.001$, time $P<0.001$, interaction $P<0.001$. For $\Delta netH^+_m$ flux: treatment $P=0.04$, time P=n.s., interaction P=n.s. Asterisks indicate significant differences ($P\leq0.05$) from control values.

ANOVA identified significant effects of treatment, time and their interaction (all $P<0.001$), with $\dot{M}_{O_2}$ consistently higher in exercised crabs than in emersed crabs throughout recovery. EPOC was 7636±562 μmol O_2 kg^{-1} (*N*=6) after exercise and 2649±498 μmol O_2 kg^{-1} (*N*=7) after emersion, a significant difference.

$\dot{M}_{O_2}$ during the first hour of air exposure was 1450±212 μmol kg^{-1} h^{-1}, representative of values of crabs emersed for 1 h in series 2–4. During prolonged air-exposure, $\dot{M}_{O_2}$ fell to 997±198 μmol kg^{-1} h^{-1} in the final hour and averaged 1297±204 μmol kg^{-1} h^{-1} (*N*=6) over the whole 10–12 h. These rates were all significantly greater than in control crabs resting in seawater (528±31 μmol kg^{-1} h^{-1}, *N*=13). $\dot{M}_{O_2}$ was not measured during exercise in air.

Net acidic equivalent flux to the water

Net acidic equivalent flux rates under control conditions were highly variable, with 10 of the 13 crabs exhibiting net basic equivalent excretion to the water (i.e. negative acid excretion). There were no differences between the two treatments, with the overall control rate averaging −1476±990 μequiv kg^{-1} h^{-1} (*N*=13). In light of this variability, rates during recovery were expressed relative to the control rate (i.e. $\Delta netH^+_m$ flux rate) in each individual crab. By two-way repeated measures ANOVA, there were significant effects of treatment ($P=0.04$), but not of time or their interaction. After exercise, there were no significant changes during recovery (Fig. 2C). However, after emersion, $\Delta netH^+_m$ flux rate was elevated to about +3000 μequiv kg^{-1} h^{-1}, significant only at 5 and 8 h as a result of temporal variability (Fig. 2D). Nevertheless, all 7 emersed crabs excreted acidic equivalents at greater rates over the 18 h recovery period relative to their own control rates. In contrast, 4 of 6 exercised crabs excreted basic equivalents at greater rates during recovery.

Haemolymph acid–base status and metabolites

The components of acid–base status (pH 7.87±0.02, P_{CO_2} 2.67±0.19 torr and $[HCO_3^-]$ 9.27±0.40 mmol l^{-1}, *N*=14) for the venous haemolymph of resting crabs in seawater were not significantly different between the two groups (Fig. 3). Recovery patterns differed between the two treatments, with significant effects

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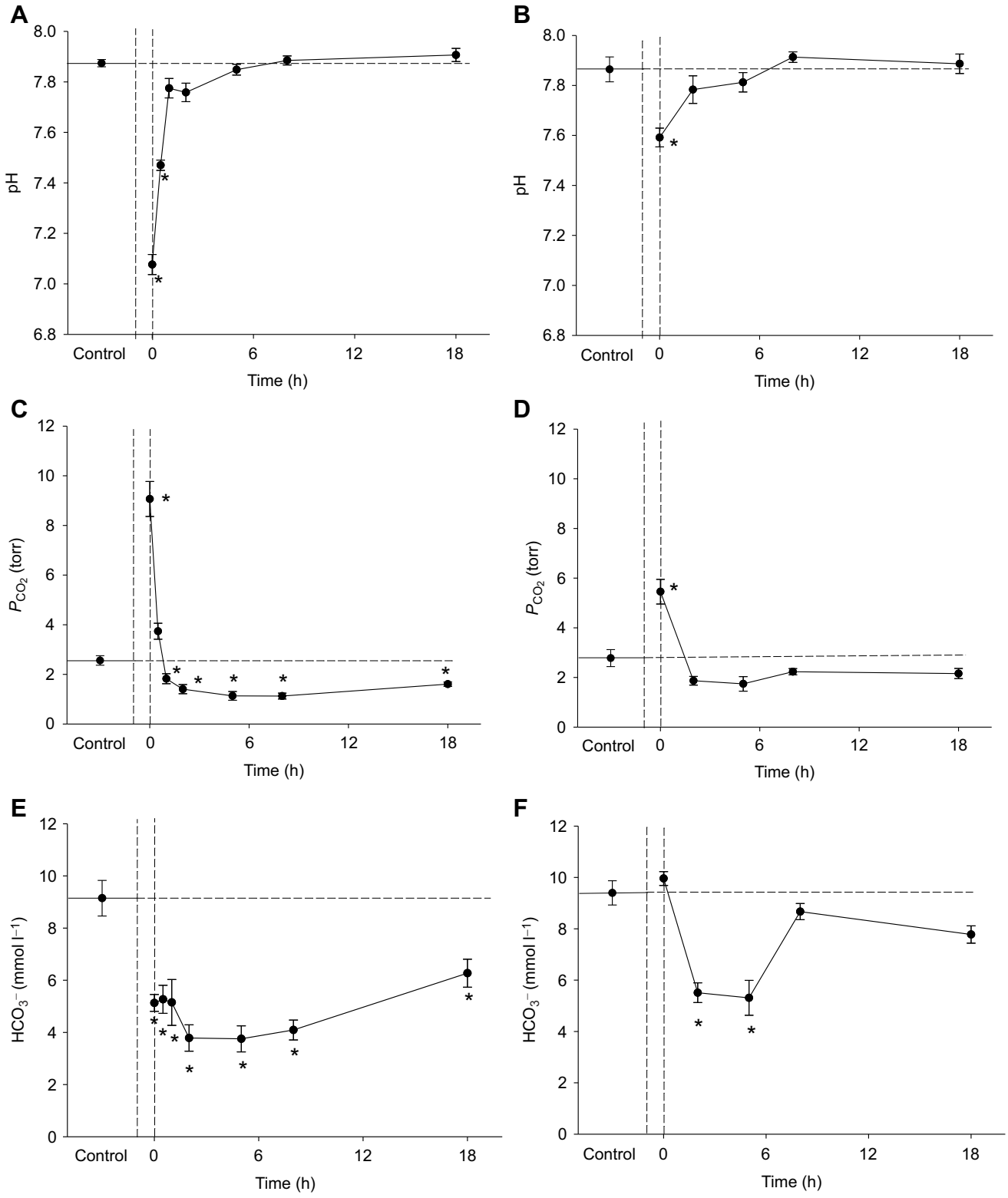

Fig. 3. Acid–base status of venous haemolymph of *C. maenas* in response to exercise or emersion (series 3). (A,B) pH, (C,D) P_{CO_2} and (E,F) $[HCO_3^-]$ in response to 1 h of exercise in air (left, *N*=6–7) or 1 h of simple emersion in air (right, *N*=6–7), followed by 18 h recovery in seawater. Asterisks indicate significant difference from control. Two-way ANOVA during recovery for all three parameters: treatment $P<0.001$, time $P<0.001$, interaction $P<0.001$. Other details as in Fig. 2.

of treatment, time and their interaction for all three acid–base parameters ($P<0.001$), and greater, longer-lasting disturbances after exercise than after emersion.

Immediately after exercise, pH dropped precipitously to about 7.1 but recovered to control levels by 1 h, and remained stable thereafter (Fig. 3A). P_{CO_2} rose greatly to approximately 9 torr but returned to

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control levels by 0.5 h, and then fell significantly below control levels throughout the remainder of the 18 h recovery (Fig. 3C). Haemolymph [HCO_3^-] fell by almost 50% immediately after exercise, and like P_{CO_2} remained significantly depressed for 18 h (Fig. 3E). After emersion, pH fell to only about 7.6, and recovery was similarly rapid (Fig. 3B). P_{CO_2} rose to about 5.5 torr, but thereafter returned to the control level for the whole recovery period (Fig. 3D). [HCO_3^-] was initially unchanged after emersion, but fell significantly by about 40% at 2 and 5 h, before returning to control concentrations for the remainder of recovery (Fig. 3F).

Haemolymph protein concentration did not change with exercise or emersion, and was not significantly different between the treatment groups under resting conditions, with an overall control mean of 52.9±3.9 g l^{-1} (N=14). However, averaged over all samples, it was significantly higher in the crabs in the emersion treatment (84.1±6.3 g l^{-1}, N=36) than in the exercise treatment (63.9±3.7 g l^{-1}, N=50), reflected in different β values (see Materials and Methods) in Figs 4 and 5A.

A Davenport (1974) diagram display of acid–base data revealed a mixed respiratory and metabolic acidosis immediately after exercise (0 h), the latter causing a sharp downward deviation from the NBB line (Fig. 4A). Thereafter, there was strong respiratory compensation as the large persistent decrease in P_{CO_2} quickly brought pH back to normal while [HCO_3^-] remained depressed. After emersion, there was a qualitatively similar but much smaller disturbance, with a modest initial deviation from the NBB line indicative of a mild mixed acidosis, again followed by respiratory compensation (Fig. 4B).

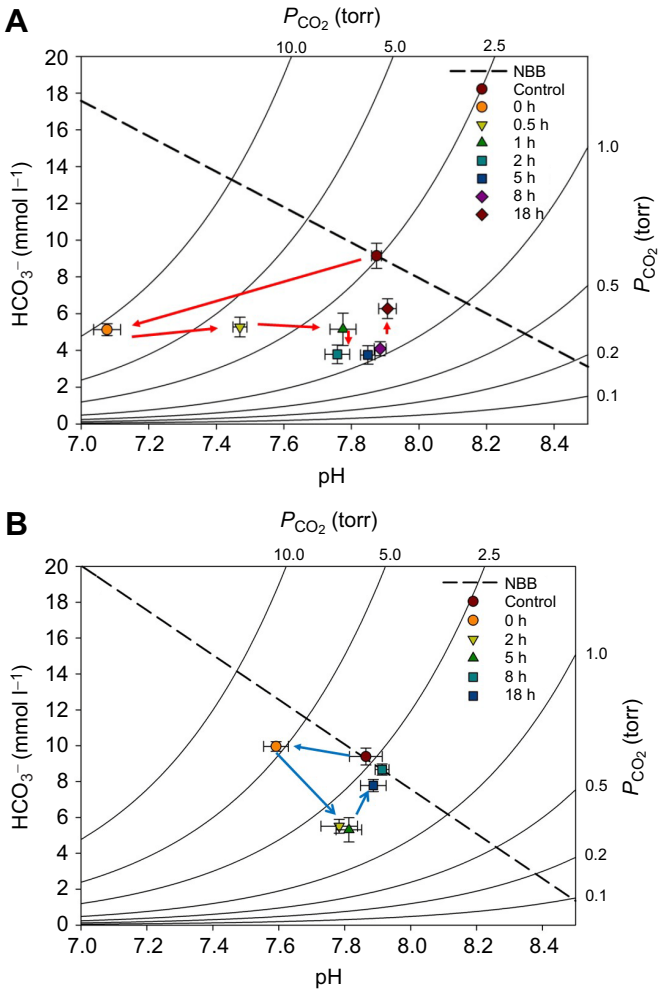

Fig. 4. Temporal change in pH, P_{CO_2} and [HCO_3^-] in the venous haemolymph of *C. maenas* in response to exercise or emersion (series 3). Davenport (1974) diagrams showing effect of (A) 1 h of exercise in air or (B) 1 h of simple emersion in air, followed by 18 h recovery in seawater (means±1 s.e.m., N=6–7). The non-bicarbonate buffer (NBB) lines (dashed) are plotted through the control points.

Haemolymph lactate levels were low under control conditions, averaging 0.66±0.18 mmol l^{-1} (N=14), and did not differ between the two groups (Fig. 5A,B). During recovery there were significant effects of treatment, time and their interaction (all P<0.001). After 1 h of exercise in air, a dramatic increase in lactate to more than 25 mmol l^{-1} was followed by a steady decline, but the elevation remained significant to 8 h, with a return to control levels at 18 h (Fig. 5A). Lactate elevation after emersion reached only 4 mmol l^{-1}, and was attenuated by 2 h of recovery with no changes thereafter (Fig. 5B).

Haemolymph ΔH^+_m exhibited very different patterns after exercise from those after emersion, with significant effects of treatment, time and their interaction (all P<0.001) (Fig. 5A,B). There were also large quantitative differences from simultaneously measured lactate concentrations. Immediately after exercise, ΔH^+_m increased significantly to about 12 mmol l^{-1}, slightly less than 50% of the lactate elevation (Fig. 5A). ΔH^+_m subsequently declined until 1 h, but remained stable and still significantly elevated at about 5 mmol l^{-1} to 8 h, despite the steady decline in lactate over this period. Control levels were re-established at 18 h. Throughout 0–5 h of recovery, ΔH^+_m remained significantly below the lactate concentration by 54–76% (Fig. 5A). In contrast, after emersion, there were only modest but significant elevations of ΔH^+_m to 3–5 mmol l^{-1} at 0–5 h, with return to control levels thereafter (Fig. 5B). A significant difference occurred at 5 h between the still elevated ΔH^+_m and lactate, which had returned to control levels.

Haemolymph glucose concentration under control conditions was 1.99±0.17 mmol l^{-1} (N=14), and did not differ between the two groups (Fig. 5C,D). During recovery, there were significant effects of treatment and time (both P<0.001), but no significant interaction. Immediately after exercise, glucose increased to about 7 mmol l^{-1}, thereafter declining to a level not significantly different from control by 5 h of recovery (Fig. 5C). A smaller elevation after emersion to about 4 mmol l^{-1} was significant only at 0 h (Fig. 5D).

Ventilation and heart rate

The rates of left and right scaphognathites invariably differed within individuals, with many instances of unilateral pausing, especially under resting conditions, but there were no consistent differences, so average rates are reported (Fig. 6A,B). Control rates in seawater averaged 115.9±5.9 beats min^{-1} (N=12) and did not differ between the two treatment groups. During the 1 h period in air, two-way repeated measures ANOVA identified significant effects of treatment, time and their interaction (all P<0.001). After 15 min of exercise, scaphognathite rate had decreased significantly by 27%, but thereafter steadily increased, returning to the control rate as exercise continued (Fig. 6A). In contrast, during simple emersion, scaphognathite rate dropped by 65% within 15 min, remaining significantly depressed to 60 min (Fig. 6B). Upon return to seawater, rates rapidly accelerated in both treatment groups to approximately 260 beats min^{-1} at 0 h and 0.08 h post-treatment. Thereafter, rates declined, reaching values not significantly different from control at 5 h post-exercise and 2 h post-emersion. During recovery, there were no significant effects of treatment or interaction, only of time (P<0.001).

Resting heart rate (83.5±8.5 beats min^{-1}, N=12) did not differ between the two groups (Fig. 6C,D). During the 1 h of air exposure,

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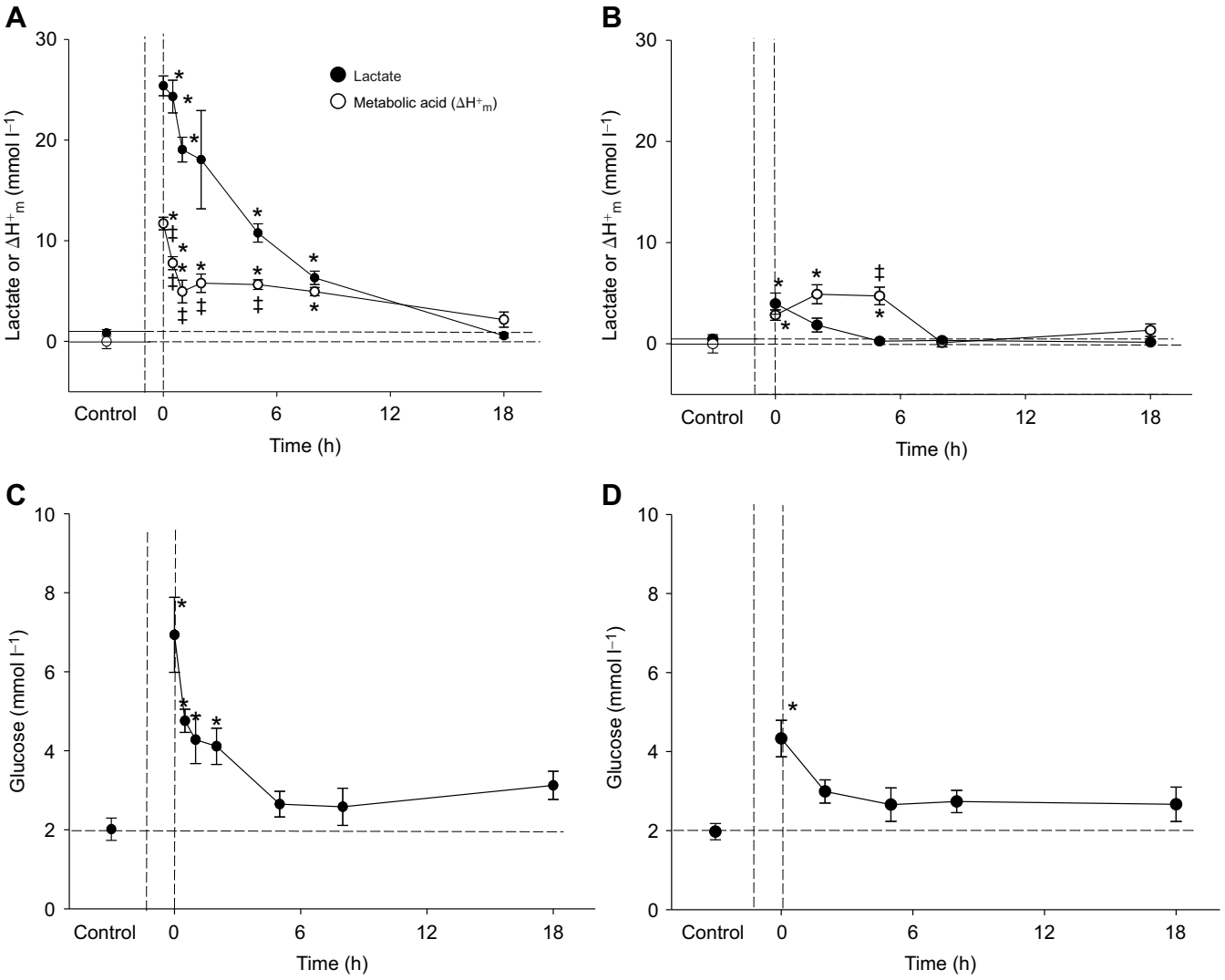

Fig. 5. Haemolymph lactate and metabolic acid levels and glucose concentration of *C. maenas* in response to exercise or emersion (series 3). (A,B) Haemolymph lactate and metabolic acid load (ΔH^+_m) and (C,D) glucose responses to 1 h of exercise in air (left, *N*=6–7) or 1 h of simple emersion in air (right, *N*=6–7), followed by 18 h recovery in seawater. Two-way ANOVA during recovery for both lactate and ΔH^+_m: treatment $P<0.001$, time $P<0.001$, interaction $P<0.001$. For glucose: treatment $P<0.001$, time $P<0.001$, interaction P=n.s. Asterisks indicate significant differences ($P\leq0.05$) from control values in each treatment group; double daggers indicate significant differences ($P\leq0.05$) between ΔH^+_m and lactate concentrations at the same time points.

two-way repeated measures ANOVA identified significant effects of treatment ($P<0.04$), time ($P<0.001$) and their interaction ($P<0.002$). Exercise was accompanied by a significant tachycardia within 15 min, and heart rate continued to increase steadily thereafter, reaching about 200 beats min^{-1} at 60 min (Fig. 6C). In contrast, the initial response to emersion alone was a significant 40% reduction at 15 min, that was progressively reversed as air exposure continued (Fig. 6D). Immediately upon return to seawater (0 h), heart rate further accelerated to about 280 beats min^{-1} in the exercised animals (Fig. 6C) and 205 beats min^{-1} in the emersed animals (Fig. 6D). Within 0.08 h, heart rate fell substantially, and by 2 h, resting rate was restored in both treatments. Only the effect of time was significant ($P<0.001$); there were no significant treatment or interaction effects during recovery.

Haemolymph osmolality and ions

Control haemolymph osmolality was identical for the two treatment groups, averaging 933.6±6.1 mOsm kg^{-1} (*N*=14) (Fig. 7A,B). During recovery, there were significant effects of treatment and time (both $P<0.001$), but no significant interaction. Immediately after exercise, osmolality increased significantly by 14%, declining slowly thereafter but remaining elevated to 5 h (Fig. 7A). After emersion, there were significant 5% and 7% increases at 0 h and 5 h, respectively (Fig. 7B). Haemolymph K^+ concentration was very uniform, averaging 12.3±0.3 mmol l^{-1} (*N*=14) under resting control conditions (Fig. 7C,D). There were no significant treatment, time or interaction effects, and no significant changes from control values.

The two major carapace cations (Ca^{2+} and Mg^{2+}) increased substantially in the haemolymph after exercise, but not after emersion (Fig. 8). Control haemolymph [Ca^{2+}] was the same in the two treatment groups, averaging 10.8±0.4 mmol l^{-1} (*N*=14) (Fig. 8A,B). Two-way ANOVA showed a significant effect of treatment only ($P<0.001$), with no time or interaction effects. At the end of exercise, [Ca^{2+}] had risen by 41% to 14.9 mmol l^{-1} and stayed significantly elevated at this level to 1 h (Fig. 8A). There were no significant changes after emersion (Fig. 8B). Haemolymph [Mg^{2+}] was about twice as high as haemolymph [Ca^{2+}] under control conditions, averaging 22.4±1.8 mmol l^{-1} (*N*=14) in the two

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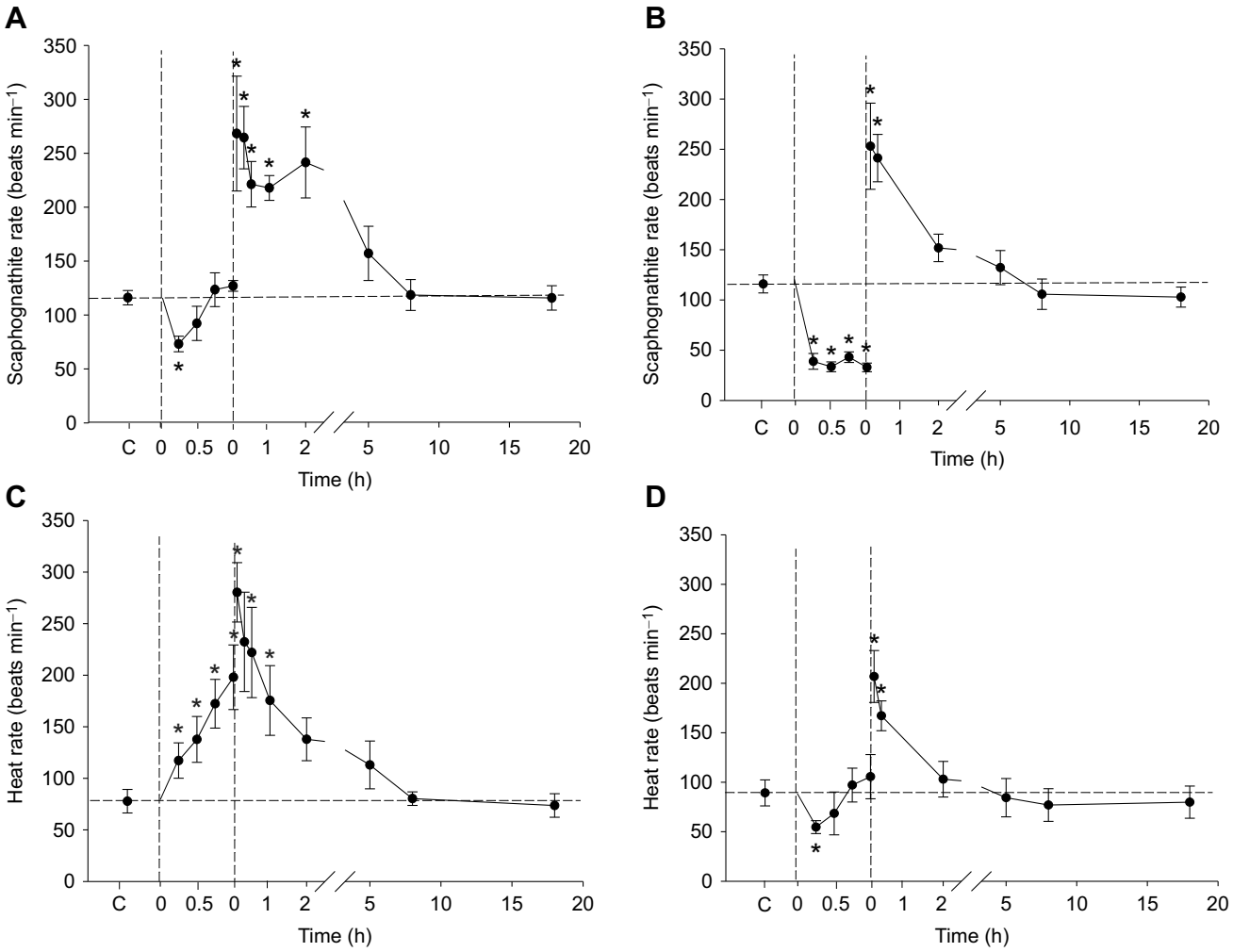

Fig. 6. Ventilation and heart rate responses of *C. maenas* to exercise or emersion (series 4). (A,B) Ventilatory (scaphognathite rate) and (C,D) cardiac (heart rate) responses to 1 h of exercise in air (left, *N*=6) or 1 h of simple emersion in air (right, *N*=6), followed by 18 h recovery in seawater. The time scale is expanded to show responses during the 1 h air (exercise/emersion) period, delimited by the dashed vertical lines. Asterisks indicate significant difference from control, C. Two-way repeated measures ANOVA during the air-exposure period of exercise or simple emersion for ventilation: treatment $P<0.001$, time $P<0.001$, interaction $P<0.001$; for the recovery period: treatment P=n.s., time $P<0.001$, interaction P=n.s. For heart rate during the air-exposure period of exercise or simple emersion: treatment $P<0.04$, time $P<0.001$, interaction $P<0.002$; for the recovery period: treatment P=n.s., time $P<0.001$, interaction P=n.s. Other details as in Fig. 2.

groups (Fig. 8C,D). Thereafter, there was a significant effect of treatment only ($P<0.002$), with no time or interaction effects, similar to the pattern with $[Ca^{2+}]$. However, $[Mg^{2+}]$ exhibited even larger absolute changes after exercise, rising 34% to 28.9 mmol l^{-1} at 0 h (Fig. 8C). Thereafter, like $[Ca^{2+}]$, it stayed high, with significant elevations at 0, 0.5 and 8 h. $[Mg^{2+}]$ did not change significantly after emersion (Fig. 8D).

DISCUSSION

Overview

Despite being classified as only T1 for terrestriality (Hartnoll, 1988), *C. maenas* exhibits an impressive capacity for terrestrial activity. We had hypothesized that during exercise in air, this species would exhibit similar physiological responses to those reported for T3–T5 land crabs, but when recovering in seawater, would exhibit similar responses to those reported for aquatic crabs. Some responses concurred with this hypothesis, whereas others did not. Overall, a more nuanced pattern was seen, reflecting the ability of this amphibious crab to 'mix and match' strategies from the two media. Future exercise studies on both more subtidal and more terrestrial crabs may cast light on the evolutionary physiology of land invasion. Our second hypothesis was supported: physiological disturbances after near-exhaustive exercise in air were greater and often longer lasting (e.g. EPOC, metabolic acidosis, lactate and ΔH^{+}_{m} elevation, ionic disturbances) than after simple air exposure alone for the same 1 h period. The one clear exception was the increase in net acidic equivalent excretion to the water that occurred after emersion but not after exercise (Fig. 2C,D). This reflected fundamentally different strategies of acid–base compensation, with the post-exercise crabs relying on carapace buffering and the post-emersion crabs employing acidic equivalent excretion to the external seawater. Depression of haemolymph P_{CO_2} associated with hyperventilation also played a key role in acid–base recovery, especially after exercise (Fig. 4).

Environmental relevance and condition of the crabs

Our protocol of acclimation to 12–13°C seawater, with control and recovery in this medium, but exercise and emersion in 20–23°C air

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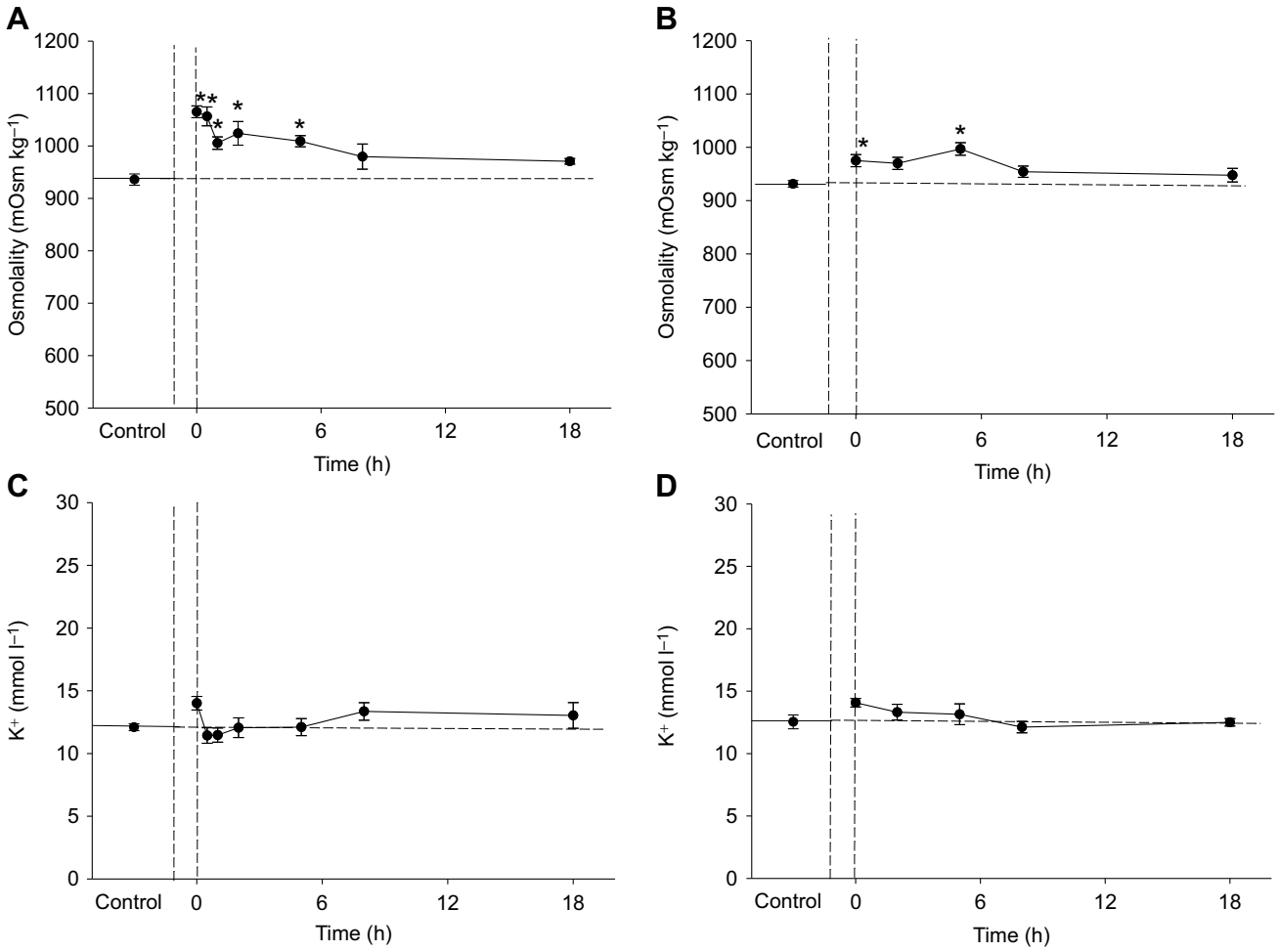

Fig. 7. Haemolymph osmolality and K^+ concentration of *C. maenas* in response to exercise or emersion (series 3). (A,B) Haemolymph osmolality and (C,D) [K^+] responses to 1 h of exercise in air (left, *N*=6–7) or 1 h of simple emersion in air (right, *N*=6–7), followed by 18 h recovery in seawater. Two-way ANOVA during recovery for osmolality: treatment *P*<0.001, time *P*<0.001, interaction *P*=n.s. For K^+: treatment *P*=n.s., time *P*=n.s., interaction *P*=n.s. Asterisks indicate significant difference from control. Other details as in Fig. 2.

may complicate interpretation, but is environmentally realistic. The experiments were performed in August–September, and these temperatures closely matched those that we recorded on cloudy days in shaded areas on the local shoreline [see also fig. 2 of Sokolova and Boulding (2004) and table 2 of Simonik and Henry (2014)]. Body temperatures were not measured, but the green crab is known to benefit from evaporative cooling (Wheatly and Taylor, 1979; Depledge, 1984), so core temperatures were probably below air temperature. Handling stress markedly affects key physiological parameters in this species (Jouve-Duhamel and Truchot, 1985; Wilkens et al., 1985), particularly by elevating $\dot{M}_{O_2}$ (Houlihan et al., 1984; Wilson et al., 2021). However, our animals (4 days fasted, ≥24 h in small darkened respirometers that minimized spontaneous movement) appear to have been extremely well rested and non-stressed under control conditions, with $\dot{M}_{O_2}$ around 530 µmol kg^{-1} h^{-1}. Other reports of resting $\dot{M}_{O_2}$ for comparably sized green crabs at 10–15°C range from about 850 µmol kg^{-1} h^{-1} (Taylor et al., 1977; Johnson and Uglow, 1985; Wilson et al., 2021) to 2400 µmol kg^{-1} h^{-1} (Hamilton and Houlihan, 1992) and 4200 µmol kg^{-1} h^{-1} (Simonik and Henry, 2014), with many records in the 1200–1600 µmol kg^{-1} h^{-1} range (e.g. Arudpragasam and Naylor, 1964; Taylor and Butler, 1978; Wilkens et al., 1984; Houlihan et al., 1984; Nancollas and McGaw, 2021). Control haemolymph lactate levels in our crabs (Fig. 5A,B) were lower than in most of these reports, and ammonia excretion rates (C.M.W., in preparation) were similarly low. Notably, by the end of the recovery period, our crabs had returned to these low $\dot{M}_{O_2}$ (Fig. 1), ammonia excretion and lactate levels (Fig. 5).

Our animals were acclimated, in large outdoor holding tanks, to continuous submersion for 3–6 weeks prior to experimentation. There are reports that exercise capacity in seawater declines when green crabs are held in captivity (Houlihan et al., 1984; Houlihan and Mathers, 1985). Furthermore, Nancollas and McGaw (2021) found that animals acclimated to a tidal emersion cycle are better able to sustain aerobic metabolism and have lower metabolic costs during air exposure than those acclimated to continuous submersion. Thus, it is possible that exercise capacity would have been greater and physiological disturbances less marked had our crabs been freshly collected from the intertidal zone.

Exercise performance

The velocity versus fatigue time data fitted a classic inverse relationship with maximum speeds of 2.0 BL s^{-1} sustainable for several minutes, and 0.25 BL s^{-1} sustainable for 30 to >60 min (Fig. 1A). Houlihan et al. (1984) reported a similar but up-shifted relationship for *C. maenas* exercising in seawater, with about

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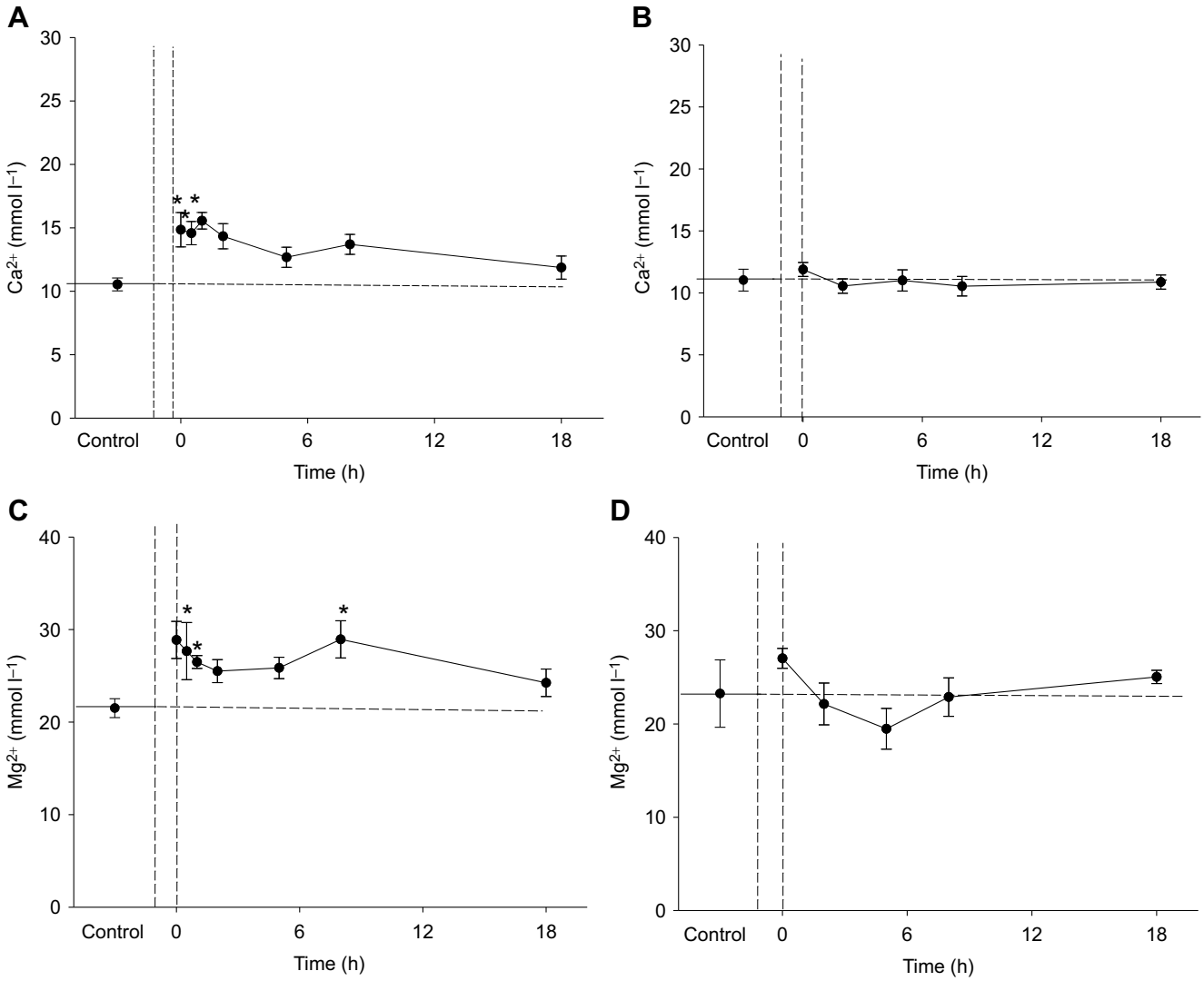

Fig. 8. Haemolymph Ca^{2+} and Mg^{2+} concentration of *C. maenas* in response to exercise or emersion (series 3). (A,B) Haemolymph [Ca^{2+}] and (C,D) [Mg^{2+}] responses to 1 h of exercise in air (left, *N*=6–7) or 1 h of simple emersion in air (right, *N*=6–7), followed by 18 h recovery in seawater. Asterisks indicate significant difference from control. Two-way ANOVA during recovery for Ca^{2+}: treatment *P*<0.001, time *P*=n.s., interaction *P*=n.s. For Mg^{2+}: treatment *P*<0.002, time *P*=n.s., interaction *P*=n.s. Other details as in Fig. 2.

3.0 BL s^{-1} sustainable for 2 min, and 0.4 BL s^{-1} sustainable for 10–50 min. The green crab's ability to exercise almost as well in air where it must also carry its own weight is remarkable, especially considering that *C. maenas* is a portunid, well-designed for swimming in water. Indeed, performance in air was similar to that of more terrestrial decapods such as the true land crab *Cardisoma carnifex* (Wood and Randall, 1981a) and the high-shore crab *Cyclograpsus lavauxi* (Waldron et al., 1986). However, some very small, completely terrestrial crabs exhibit severalfold greater burst and sustainable speeds (Full and Herreid, 1983; Full et al., 1985). Dehydration did not contribute to fatigue in *C. maenas*: crabs exhausted in a few minutes at high speed lost only about the same amount of mass as those quietly emersed in air for 60 min, and crabs continuing to exercise for 60 min at slow speed lost more than twice as much mass (Fig. 1B).

Aerobic and anaerobic metabolism

Simple emersion for 1 h elevated $\dot{M}_{O_2}$ about 2.7-fold (to 1450 µmol kg^{-1} h^{-1}, measured in air), yet induced an anaerobic component as shown by significant EPOC (Fig. 2B) and lactate elevation to about 4 mmol l^{-1} (Fig. 5B) when returned to seawater. This EPOC (2649 µmol kg^{-1}) would be sufficient to fuel control metabolism (530 µmol kg^{-1} h^{-1}) for 5 h. Undoubtedly, handling stress played a role, as $\dot{M}_{O_2}$ subsequently declined by 30% by 10–12 h in air. Yet, $\dot{M}_{O_2}$ in air remained almost 2-fold elevated, probably reflecting both the higher temperature and the cost of body support against gravity. Relative to previous data on emersed *C. maenas* (see Introduction), this represents the greatest fold-elevation, probably reflecting the lower resting $\dot{M}_{O_2}$ under our control conditions, as both the absolute $\dot{M}_{O_2}$ and lactate levels in air were not unusual.

While $\dot{M}_{O_2}$ during exercise in air was not measured, there was clearly a very large anaerobic component as demonstrated by the 3-fold greater EPOC, with $\dot{M}_{O_2}$ (Fig. 2A) and haemolymph lactate (Fig. 5A) both remaining elevated for at least 8 h. The EPOC (7636 µmol kg^{-1}) would support the control metabolic rate for >14 h, and the immediate post-exercise lactate concentrations (>25 mmol l^{-1}) appear to be the highest ever recorded in this species. In comparison, after 5–10 min of exercise in seawater at 0.4–0.8 BL s^{-1}, elevations in $\dot{M}_{O_2}$ (by 3- to 4-fold) and

lactate (by 2-fold) were corrected in less than 0.5 h (Houlihan et al., 1984; Hamilton and Houlihan, 1992). If haemolymph lactate concentrations at 0 h were representative of those in the entire body water compartment (~0.70 l kg^{-1}; C.M.W., unpublished data), then the measured EPOCs were sufficient to explain only about 15.5% (after exercise) and 36% (after emersion) of lactate removal by oxidation (3 O_2 consumed per lactate oxidized). More probably, most of this valuable fuel was not oxidized, but rather converted back to glucose and glycogen during recovery, as in fish (Richards et al., 2002) and as suggested for other crabs (Henry et al., 1994). If this occurred by reversal of the pyruvate kinase reaction for which the O_2 cost would be about 0.4 O_2 per lactate (Moyes et al., 1992), then the observed EPOCs would approximately cover the cost for the exercise group, and would be severalfold greater than needed for the emersed crabs. The post-treatment elevation in haemolymph glucose (Fig. 5C,D) may have reflected this flux reversal. Notably, in *C. maenas*, the haemolymph itself is the major site of both glucose and glycogen storage (Williams and Lutz, 1975).

Acid–base regulation

After 1 h of exercise in air, the crabs exhibited a profound haemolymph acidosis, of both respiratory (P_{CO_2} elevation) and metabolic origin (ΔH^+_m elevation, HCO_3^- decrease), whereas much smaller, qualitatively similar changes occurred after 1 h of quiet air exposure (Figs 3, 4 and 5). These samples were collected <1 min after re-submersion. Even greater elevations of P_{CO_2} might have been measured had the animals been sampled while still in air, though it is unlikely that haemolymph ΔH^+_m or HCO_3^- concentrations would have changed much in this short interval. Comparable P_{CO_2} elevations during emersion have been seen in many previous studies (Truchot, 1975a,b; Truchot, 1979; Depledge, 1984; Taylor and Butler, 1978; Wheatly and Taylor, 1979; Dejours and Truchot, 1988) and are the direct physico-chemical consequence of breathing air rather than water (Dejours, 1989). However, the P_{CO_2} elevation after exercise (to about 9 torr) was larger than reported in any of these previous studies, and likely reflected increased CO_2 generation from both elevated metabolic rate and titration of HCO_3^- stores by metabolic acid mobilization (Fig. 5). Upon return to water, rapid correction and sustained reversal of respiratory acidosis occurred such that P_{CO_2} remained significantly below pre-exercise levels throughout the 18 h recovery (Figs 3A and 4A). This was clearly a key contributor to the rapid restoration of haemolymph pH (Fig. 3C) in the face of long-lasting metabolic acidosis (Fig. 4A). A similar trend for sustained P_{CO_2} reduction, though not significant, occurred after emersion (Fig. 3D).

This sustained post-exercise respiratory compensation was not seen in *C. maenas* (Hamilton and Houlihan, 1992) or other species (McDonald et al., 1979; Booth et al., 1984; Milligan et al., 1989) exercised and recovered in seawater, or even in the high-shore crab *Cyclograpsus lavauxi* exercised in air and recovered in seawater (Waldron et al., 1986). However, it occurred in several terrestrial crabs exercised and recovered in air (Smatresk et al., 1979; Wood and Randall, 1981b; Smatresk and Cameron, 1981) and was associated with sustained increases in ventilation during the recovery period (Herreid et al., 1979, 1983; Wood and Randall, 1981b). Respiratory compensation for post-exercise metabolic acidosis is common in air breathers but rare in water breathers (Truchot, 1987; Henry et al., 2012). It is exceptional that this 'terrestrial' strategy was exhibited by *C. maenas* exercised in air but recovered in seawater. Indeed, it was this finding that prompted us to record ventilation and heart rate (Fig. 6) in series 4.

The elevations in both ΔH^+_m and lactate (Fig. 5A) were greater than those in any of the above-cited exercise studies in crabs breathing either water or air, indicating the severity of the disturbance in *C. maenas*. The high lactate concentrations would undoubtedly have been beneficial to O_2 transport by increasing haemolymph O_2 affinity, thereby off-setting the negative effects of low pH (Truchot, 1980; Booth et al., 1982; Morris et al., 1986). Notably, the metabolic acid load in the haemolymph remained substantially below the lactate load throughout recovery, similar to three exercise and recovery studies on entirely aquatic crabs (McDonald et al., 1979; Booth et al., 1984; Milligan et al., 1989), as well as to the high-shore crab exercised in air but recovered in seawater (Waldron et al., 1986), In contrast, in terrestrial crabs exercised and recovered in air, the loads were either identical initially (Wood and Randall, 1981b), or else ΔH^+_m was initially greater than Δlactate (Smatresk et al., 1979; Smatresk and Cameron, 1981). In both, ΔH^+_m later declined below Δlactate. In all these studies, the metabolic acidosis would classically be interpreted as a lactacidosis (Hill et al., 1924) due to 'lactic acid' production. However, the modern view (Robergs et al., 2004) is that during anaerobic metabolism, lactate (produced by glycolysis) and metabolic protons (produced in approximately equal amounts by non-mitochondrial ATP breakdown), are generated by different mechanisms. Furthermore, after exercise, lactate anions and metabolic acid can move across cell membranes and external epithelia at different rates and by different mechanisms, resulting in unequal concentrations in the extracellular fluid, a phenomenon seen in both fish (Wood and Perry, 1985; Wood and Wang, 1999) and crabs (McMahon, 1981; Fehsenfeld and Weihrauch, 2017).

Three aquatic exercise studies (McDonald et al., 1979; Booth et al., 1984; Milligan et al., 1989) recorded intense excretion of acidic equivalents to the external seawater as the major explanation for lower ΔH^+_m than Δlactate in the haemolymph during recovery. This clearly did not occur in *C. maenas* exercised in air and recovered in seawater (Fig. 2C). Instead, as discussed below, there was evidence of an alternative strategy, metabolic acid neutralization by carapace buffering, the mechanism used by terrestrial crabs exercised and recovered in air (Wood and Randall, 1981b; Waldron et al., 1986) and some aquatic decapods exposed to air (Defur et al., 1980; Taylor and Wheatly, 1981, 1989). Surprisingly, after emersion, *C. maenas* did excrete acidic equivalents to the external seawater at high rates (Fig. 2D) without any evidence of carapace buffering. These rates, though highly variable, were much greater than apparently required to correct the relatively small haemolymph metabolic acid load (Fig. 5B). This raises the prospect of production of intracellular metabolic acid of unknown origin and its clearance from the tissues, a possibility that has been raised in other studies (Smatresk et al., 1979; Smatresk and Cameron, 1981; Booth et al., 1984). To our knowledge, the only previous measurements of acidic equivalent excretion in *C. maenas* were made by Truchot (1979), who confirmed that the gills, rather than the urine, were the major site of flux. The author noted that these measurements were problematical as they failed to take ammonia excretion into account. They did, however, indicate that acidic equivalent excretion occurred during the first few hours of acute respiratory acidosis, when the animal was compensating by accumulating basic equivalents in the haemolymph. At least in part, this could be the explanation for the present results on *C. maenas* after emersion, where the crabs were restoring basic equivalents, thereby bringing ΔH^+_m below Δlactate in the haemolymph (Fig. 5B). Variability in these responses may relate to differences in feeding

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history (Wheatly and Henry, 1992) and/or timing in the moulting cycle (Cameron and Wood, 1985).

Ventilation and heart rate

The initial response to air exposure was a profound reduction in scaphognathite rate maintained throughout the emersion period (Fig. 6B) despite the higher temperature. In the face of the added burden of exercise, this reduction in ventilation was progressively attenuated (Fig. 6A). These reductions do not mean that less O_2 was delivered to the gills, as the O_2 concentration in air is 20–30 times greater than in seawater, and air is lighter and less viscous, making it easier to pump (Dejours, 1981, 1989). Indeed, there was a progressive tachycardia during exercise (Fig. 6C), and the initial bradycardia of air exposure alone was reversed as emersion continued (Fig. 6D), presumably to support increased $\dot{M}_{O_2}$ during both treatments. The emersion observations were similar to those of Simonik and Henry (2014), who employed a comparable temperature and air-exposure routine with *C. maenas*.

We performed these experiments primarily to assess whether cardiac and ventilatory changes could explain the sustained decreases in haemolymph P_{CO_2} (Fig. 3C,D) that were so important in correcting acidosis after exercise in air (Fig. 4A), and, to a lesser extent, after simple emersion (Fig. 4B). 'Hypocapnic alkalosis' associated with persistent hyperventilation has been observed in several water breathers, including *C. maenas*, during chronic hypoxia (Taylor, 1982; Truchot, 1987). We observed a clear hyperventilation after both treatments (Fig. 6A,B), with scaphognathite rates remaining approximately twice as high as control levels for at least 2 h in the exercised crabs. While obviously facilitating increased $\dot{M}_{O_2}$ (Fig. 2A,B), this certainly contributed to the depression of haemolymph P_{CO_2} below control levels. However, other factors (e.g. altered haemolymph and water flow patterns, decreased diffusive conductance of the gills) were probably important at later times because $\dot{M}_{O_2}$ remained elevated for at least 8 h (Fig. 2A) and P_{CO_2} remained depressed for 18 h (Fig. 3C). These 2-fold ventilatory increases were similar to or lower than the 2- to 4-fold increases measured in two other aquatic crabs (McMahon et al., 1979; Booth et al., 1982) as well as in *C. maenas* (Hamilton and Houlihan, 1992) after intense exercise in seawater. However, in all these aquatic studies, the restoration of resting rates occurred much more quickly. In contrast, in true land crabs exercised and recovered in air, post-exercise hyperventilation was of comparable magnitude but longer lasting (Herreid et al., 1979; Wood and Randall, 1981b), as in the present study with *C. maenas* exercised in air but recovered in seawater. Wood and Randall (1981a,b) suggested that this was driven by long-lasting depressions of haemolymph pH, rather than by blood gases, which were not greatly altered. A similar explanation could apply to the present observations on *C. maenas* (cf. Fig. 3E). In general, the heart rate elevations after both exercise in air (Fig. 6C) and simple emersion (Fig. 6D) paralleled elevations in scaphognathite rate (Fig. 6A,B), indicating ventilation–perfusion matching (Taylor, 1982). This has been seen in previous studies on both terrestrial (Wood and Randall, 1981b; Herreid et al., 1983) and aquatic crabs (McMahon et al., 1979; Booth et al., 1984).

Haemolymph osmolality and ions

Increases in osmolality after exercise (14%) and emersion (5–7%) were greater than could be explained by dehydration of the extracellular fluid volume (ECFV) (Table 1). If we assume that all of the losses of body mass (Fig. 1B) occurred solely from the ECFV (32.96 ml 100 g^{-1} in *C. maenas*; Harris and Andrews, 1982), this could account for elevations of only 9% (exercise) or 3% (emersion) in osmolality. These are likely over-estimates because some of the mass deficit occurred from loss of branchial chamber fluid (C.M.W., in preparation) and, undoubtedly, there would be some dehydration of the intracellular compartment accompanying ECFV dehydration. Table 1 illustrates that many measured haemolymph metabolites, particularly lactate, glucose, ammonia, urate-N and urea-N (plus probably other unmeasured ones) increased more than could be explained by dehydration, and more than the increases in osmolality, to which they obviously contributed. Interestingly, haemolymph K^+ did not increase after exercise (Fig. 7C). This is very different from many vertebrates, both air breathing (e.g. Medbø and Sejersted, 1990) and water breathing (e.g. Wang et al., 1994), where K^+ floods out of depolarized muscle into the ECFV after high-intensity exercise.

Table 1. Haemolymph osmolality and ion concentration changes in response to 1 h of treadmill exercise in air or simple emersion in air

	Exercise	Emersion
Lactate	×29.87*	×8.44*
Total ammonia	×3.50*	×2.31*
Glucose	×3.44*	×2.20*
Urate-N	×2.53*	×1.84*
Urea-N	×2.13*	×2.27*
Ca^{2+}	×1.41*	×1.08
Mg^{2+}	×1.34*	×1.16
K^+	×1.16	×1.12
Glutamate	×1.15	×1.06
Osmolality	×1.14*	×1.05*
Glutamine	×1.10	×1.17
Dehydration‡	×1.09	×1.03
HCO_3^-	×0.56*	×1.06

Data are the fold-change, relative to the mean pre-treatment control values, and are from the present paper and C.M.W. (in preparation). *Significant change (P<0.05). ‡Increase expected from evaporative water loss from the extracellular fluid volume alone, based on the mass loss data of series 1 (Fig. 1B).

Particularly notable were significant post-exercise increases (34–41%) in haemolymph, [Ca^{2+}] (Fig. 8A) and [Mg^{2+}] (Fig. 8C), far exceeding elevations attributable to the general increase in osmolality (Table 1). Very likely, this represents mobilization of bicarbonate buffer from the carapace, where these ions are stored as calcium and magnesium carbonates (Travis, 1963; Greenaway, 1985; Compère et al., 1993). Solubilization of these precipitates will yield two moles of basic equivalents (i.e. 2 HCO_3^- from each CO_3^{2-}) for every mole of Ca^{2+} or Mg^{2+}. Therefore, the recorded 4.3 and 7.4 mmol l^{-1} elevations of [Ca^{2+}] and [Mg^{2+}], respectively, would provide 23.4 mmol l^{-1} of basic equivalents. This is more than needed to account for the 14–16 mmol l^{-1} discrepancy between ΔH^+_m and Δlactate in the first 2 h post-exercise (Fig. 5A). Carapace buffering has been seen in some terrestrial crabs exercised in air (Wood and Randall, 1981b; Waldron et al., 1986) and in some aquatic decapods exposed to air (Defur et al., 1980; Taylor and Wheatly, 1981; Morris et al., 1986; Taylor and Whiteley, 1989). It explains why there was no post-exercise excretion of acidic equivalents (Fig. 2C). In contrast, after simple emersion, haemolymph [Ca^{2+}] or [Mg^{2+}] was not elevated (Fig. 8B,D) perhaps because the time was too short or the acidosis too small, and there was substantial excretion of acidic equivalents. Similarly, mobilizations of Ca^{2+} and Mg^{2+} ions into the haemolymph were negligible in aquatic crabs exercised and recovered in seawater but there was substantial excretion of acidic equivalents (Booth et al., 1984). In general, prior studies (Defur et al., 1980;

Henry et al., 1981; Cameron, 1985; Burnett, 1988) have indicated that carapace buffering predominates only when an aqueous medium is unavailable for acidic equivalent excretion.

The current results in amphibious *C. maenas* do not fit cleanly into this paradigm. Indeed, their acid–base regulatory strategy (carapace buffering) during recovery in seawater following exercise in air was similar to that of true terrestrial crabs recovering in air. However, their strategy (acidic equivalent excretion) following simple emersion in air was similar to that of aquatic crabs exercised and recovered in seawater, rather than that of aquatic crabs emersed in air and recovered in seawater. It is also notable that haemolymph [Mg^{2+}] elevation was greater than [Ca^{2+}] elevation on an absolute basis (Fig. 8A,C). To our knowledge, this has not been reported previously. $MgCO_3$ is much less abundant than $CaCO_3$ in the carapace (Greenaway, 1985; Compère et al., 1993). However, the presence of $MgCO_3$ increases the solubility of carbonates in skeletal material (Chave et al., 1962). In *C. maenas*, both Ca^{2+} and Mg^{2+} have been shown to elevate the O_2 affinity of the haemolymph, and Mg^{2+} additionally helps improve O_2 delivery by increasing the Bohr factor (Truchot, 1975b), yet at the same time depresses aerobic demand by an anaesthetic-like effect (Frederich et al., 2000). Together with elevated lactate (Fig. 5A) (Truchot, 1980), these two cations help to protect O_2 transport during post-exercise recovery.

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Conceptualization: C.M.W.; Methodology: C.M.W., B.H.K.P.; Formal analysis: C.M.W., B.H.K.P.; Investigation: C.M.W., B.H.K.P.; Resources: C.M.W.; Data curation: C.M.W.; Writing - original draft: C.M.W.; Writing - review & editing: B.H.K.P.; Supervision: C.M.W.; Project administration: C.M.W.; Funding acquisition: C.M.W.

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