The ability to feed in hypoxia follows a seasonally dependent pattern in shore crab *Carcinus maenas*

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**Abstract**

The ability of the adult shore crab *Carcinus maenas*, native to the Bay of Arcachon (SW France), to feed in hypoxia was determined at various seasons. Crabs previously kept at field temperature were fed after a 5-day fasting period at 15°C. Their blood oxygenation and pH regulation strategy and also their gill anatomy were analysed. From May to October, *C. maenas* feed at levels of O₂ partial pressure (pO₂) in the water, pwO₂ = 2 kPa (1 mg l⁻¹), without switching to their anaerobic metabolism. In March–April, before the main moulting period, the same food intake at pwO₂ = 4 kPa induced a systematic blood lactate increase associated with some mortality. An analysis performed at pwO₂ = 4 kPa at that time showed that in intermoult crabs the development of a coating of foreign material over the gill cuticle interfered with O₂-supply, preventing the small arterial pO₂ increases (from 0.7 to 1 kPa) which occurred at other seasons. This led to a cellular hypoxia despite a systematic postprandial blood-pH alkalinisation which favoured O₂-loading at gill level and increased arterial O₂ concentration. In March–April, alkalinisation appeared at pwO₂ values ≥ 6 kPa and from May to at least July at pwO₂ ≥ 2 kPa. Results are discussed in terms of season-related physiological performance, as hypoxic events mainly occur during the hot season. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Oxygen; Gill; Biological rhythm; Respiration; Blood O₂ transport; Feeding; Metabolism; Crustaceans; Lactate

1. **Introduction**

For two centuries, there has been a clear dynamic increase in the population of the European shore crab *Carcinus maenas* due to human activities. The crab is native to Europe and was first reported in the western Atlantic in 1817 (Cohen et al., 1995); it has

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now invaded coastal communities in the North Pacific as well as in Australia and South Africa (Fulton and Grant, 1900; Hutchings et al., 1989; Le Roux et al., 1990; Cohen et al., 1995). Due to its potential for extensive ecosystem alteration, it is today considered in a large body of literature as a ‘pest’ species. In response to this concern, it is fundamental that we improve our knowledge of its potential to colonise new ecosystems and gain a better understanding of its ability to successfully face hypoxic events, as this is a clear advantage during inter-species competition.

The main aim of the present work was to study the ability of *Carcinus maenas* to feed in hypoxic waters and to gain more insights into the physiological adaptations that allow or limit this ability. After feeding, the shore crab’s $O_2$ consumption increases by a factor $\approx 2$ due to the specific dynamic action of food (SDA; Brody, 1945; Beamish, 1974; Jobling, 1981; Houlihan et al., 1990). This is a severe physiological challenge to solve in hypoxia. In a previous paper we reported that the SDA 24 h after feeding was systematically associated with blood acidosis in normoxia and with blood alkalosis in hypoxia (Legeay and Massabuau, 1999). We suggested that in hypoxia, the alkalosis could favour $O_2$ loading at the gill level via a pH-induced increase in hemocyanin affinity (Truchot, 1992). In contrast, in normoxia, the acidosis could favour $O_2$ unloading at the cellular level via a pH-induced decrease in hemocyanin affinity.

The present study was carried out at various seasons from 1995 to 1997 in adult intermoult *C. maenas* native to the Bay of Arcachon (South-western France). Importantly, experiments were performed in laboratory conditions using procedures which brought about a respiratory behaviour close to that found in the natural habitat (Massabuau and Forgue, 1996). We report here that the performance of adult intermoult *C. maenas* in hypoxia varies with the season in relation to the mouling cycle. In summer, *C. maenas* that had moulted in the last 2–4 months could feed in a water $p_{O_2}$ as low as 2–3 kPa (1–1.5 mg l$^{-1}$ at 15°C) with only a minor switch to anaerobic metabolism. In contrast, in winter, with the water temperature in the Bay at its lowest in March–April, intermoult *C. maenas* are least resistant to hypoxia. An analysis of gill morphometry, blood oxygenation status and associated blood pH changes shows how the development of a coating of foreign material on the gill cuticle, which reaches a maximum in adult specimens before moulting in March–April, modifies its strategy and capacity to adapt to hypoxia at various seasons. Results are discussed in terms of the ecological performance of *C. maenas* in relation to the time of year. Unspecialised readers could read with interest *Principles of Respiratory Physiology* (Dejours, 1981) to improve their understanding of the fundamental but subtle physiological changes that are presently described.

For reference, 1 kPa = 7.5 mmHg. In water equilibrated with air, the $O_2$ fraction is 21% and $p_{O_2} = 21$ kPa. Seawater with a 3-kPa $p_{O_2}$ is approximately 14–15% $O_2$ saturated.

2. Materials and method

Experiments were performed at the Marine Biological Station, Arcachon on a total of 588 adult male *Carcinus maenas* in the intermoult stage (stage C from Drach, 1939),
weighing 61±1 g (range 35–100 g) and with carapace colours ranging from pale green to deep red. According to Reid et al. (1997), who suggested that the change in coloration corresponds to a photo-denaturation of shell pigments, in adult *C. maenas* the red coloration was assigned to prolonged intermoult crabs (found abundantly in winter) and green shells to animals which had recently entered the intermoult stage (found as from early spring). Crabs were collected on a regular basis in the Bay of Arcachon at various periods between 1995 and 1997. They were maintained all year round in large tanks supplied with running seawater at Bay temperature (8–20°C depending on the season; salinity 30–32‰, pH 8.3–8.4; O₂ partial pressure 20–21 kPa) and fed twice weekly with mussels. To standardise analyses, all experiments were performed at 15±0.5°C (pH 7.76±0.05; water pCO₂ = 0.1 kPa) after an acclimatization period of 5 days. During this period they were not fed at all to synchronise and stimulate food intake. Three days before the beginning of the experiments, the animals were prepared for arterial blood sampling by having a hole drilled in the carapace above the heart; a thin layer of cuticle was left in place and a piece of rubber glued over it. All experiments were carried out under natural light conditions and the tanks were provided with areas of shade to induce resting behaviour. To minimise external disturbance, tanks were isolated from vibrations with antivibrating benches and the crabs could not see the experimenters. Hypoxic water was obtained by bubbling an N₂/air gas mixture via mass flow controllers (model FC-260, Tylan General) driven by a laboratory-constructed programmable control unit.

2.1. Experimental protocol

When the experiments started (t₀), the animals were either kept in normoxia or exposed to different levels of hypoxia (water pO₂ = 4, 3 or 2 kPa). At each water pO₂, groups of 12–14 crabs were studied in two conditions. Crabs were either fed (at t₀ + 24 h with one mussel weighing ≈ 2 g per animal) or left unfed (to serve as a control group). In the fed group, a first blood sampling was performed just prior to feeding (at t₀ + 23 h) and a second at t₀ + 48 h. In the unfed control group, samplings were performed in parallel at t₀ + 23 and + 48 h. In hypoxic conditions care was taken to prevent crabs from reaching the water surface and from breathing air by using immersed plastic nets. Note that in both our laboratory conditions and in the Marine Biological Station facilities, *C. maenas* rarely accepts food more than three to four times a week.

2.2. Blood sampling

Blood samples were collected by removing crabs from water and using capillary glass tubes equipped with a needle to puncture (i) the rubber membrane for arterial sampling, and (ii) the arthrodial membrane at the base of a walking leg for venous sampling. Arterial and venous samples were obtained within the first minute of emersion and capillaries were plugged as soon as the samplings were completed. This sampling technique was critically assessed in Forgue et al. (1992a) and Massabuau and Forgue (1996). After sampling, blood was stored on ice to prevent clotting and to slow down metabolic reactions. It was analysed, or prepared for delayed analysis, within 1–10 min.
2.3. Analyses of blood gases, hemocyanin, lactate, acid-base balance and blood a,v O₂-capacitance

Arterial and venous $p_{O_2}$ (kPa) were determined within 3 min on 100-μl samples with a E5046 Radiometer polarographic electrode thermostatted at 15°C. The electrode was calibrated with sea-water equilibrated with O₂-free N₂ and precision low $p_{O_2}$ standards (O₂ fraction, $fO_2 = 2.35$ or 4.44%) obtained via mass flow controllers (Tylan General, model FC-260). The gas phase composition of these standards was regularly controlled using a paramagnetic O₂ analyser (Servomex 1100A) calibrated with high grade gas mixture ($fO_2 = 3.99 ± 0.04$%). As shown in Massabuau and Forgue (1996, see Fig. 2), this calibration procedure improves analysis quality in the low range yet does not preclude reading of high blood $p_{O_2}$ values. Arterial and venous blood O₂ concentrations ($c_{O_2}$, μmol l⁻¹) were determined using a modified Tucker chamber (Tucker, 1967) thermostatted at 37°C and calibrated with various volumes of normoxic distilled water (10–50 μl). Potassium cyanide (6 g l⁻¹) was used for releasing bound O₂ from the haemocyanin (Bridges, 1983). The blood a,v O₂ capacitance ($β_{a,v,O_2}$, μmol l⁻¹ kPa⁻¹) was calculated for each individual from the simultaneous measurements of $p_{O_2}$, $p_{O_2,a}$, $c_{O_2}$ and $c_{O_2}$ as $Δc_{a,v,O_2}/Δp_{a,v,O_2}$. The blood haemocyanin concentration ([Hc], Boehringer kit no. 124834), assuming that Hc contains 0.173% of copper (Truchot, 1978).

The concentrations of blood lactate were measured in the venous samples to check when C. maenas resorted to anaerobic metabolism during hypoxic exposure. The blood concentration of lactate [lact] was determined spectrophotometrically using an enzymatic method (Boehringer kit no. 139084) on deproteinized samples (50 μl), taking the precautions described by Gade (1984).

Venous pH was measured on 75-μl samples with a capillary G299A electrode thermostatted at 15°C and total CO₂ concentration (μmol l⁻¹) in the venous blood on 25-μl samples with a modified Cameron chamber (Cameron, 1971). Bicarbonate plus carbonate concentrations (mmol l⁻¹) and $p_{CO_2}$ in venous blood, $p_{CO_2}$ (kPa), were calculated using a CO₂ solubility of 374 μmol l⁻¹ kPa⁻¹ and apparent carbonic acid dissociation constants, $K'_1$ and $K'_2$, $pK'_1 = 6.027$ and $pK'_2 = 9.29$ (Truchot, 1976).

2.4. Water–blood barrier distances at gill level

The study was performed on a total of 15 crabs, five red crabs sampled in March, five green crabs sampled in April, and five orange-green crabs sampled in August. Analyses were performed on the anterior gill 5, which is assumed to be specifically involved in gas exchange as it possesses only thin epithelial cells, and on the posterior gill 8, which possess both thin and thick epithelial cells, typical features of ion-transporting tissues (Compère et al., 1989; Taylor and Taylor, 1992). The dorsal ends of gills 5 and 8 (sampled from 5 to 10 mm below the apex) were cut from crabs anaesthetised by chilling on ice before dissection. Pieces containing about 20 lamellae were immersed in a fixative for electron microscopy (6% glutaraldehyde buffered with 0.4 M sodium cacodylate, pH 7.4, osmolarity 1100 mosM l⁻¹) for 12 h at 4°C and subsequently rinsed.
in cacodylate buffer (0.4 M, NaCl 4%). They were embedded separately in Araldite in such a way that the lamellae could be cross-sectioned. Serial sections were prepared with a Reichert automatic ultra-microtome. About 30 samples were obtained from each crab. Ultra-thin sections were done in randomly distributed areas of the Araldite block. For each crab, two cross-sections of lamellae per gill were randomly selected. The percentage of obstructed area was estimated by measuring on enlarged images of lamellae (semi-thin preparations) the perimeter of the cross-section and the length covered by the coating material. The maximum and minimum water–blood barrier distances were measured after visual inspection of the ultra-thin sections (×500) using a Leica TCS 4D microscope. As no statistical difference was observed between data determined on gills 5 and 8, all results were pooled together.

2.5. Statistics

All data are presented as individual values and/or as means±1 standard error (S.E.). Paired design t-tests were used to analyse changes in blood O₂-status and acid–base balance before and after feeding. Otherwise, differences between normoxic and hypoxic conditions as well as between seasonal morphological status were evaluated using the Mann–Whitney U-test or Student’s t-tests. P < 0.05 was taken as the fiducial limit of significance.

3. Results

3.1. Seasonal rhythm of tolerance to hypoxia

When unfed and resting intermoult crabs Carcinus maenas — previously kept at Bay temperature and adapted to 15°C for 5 days — were abruptly transferred from normoxia to hypoxia (water pO₂ = 4 kPa; O₂ concentration = 2 mg l⁻¹) during 48-h periods, no mortality or switch on of anaerobic metabolism was ever observed, whatever the season (as revealed by any significant change of blood lactate concentration). In contrast, when crabs were fed 24 h after the beginning of the hypoxic exposure our observations were quite different. When experiments were performed in the period March–April, feeding specifically induced a large [lact] rise, occasionally accompanied by a noticeable mortality at +48 h. Fig. 1 illustrates the seasonal evolution of blood lactate response to hypoxia observed in 1995. Experiments started in early March. At that time, feeding at water pO₂ = 4 kPa induced a considerable mortality rate (36%, n = 8/22) and a rise in blood lactate which reached 22.1±4.7 mmol l⁻¹ 24 h after feeding in the surviving animals. Interestingly, in April, May and June the same feeding test in hypoxia did not lead to any significant switch on of anaerobic metabolism despite some exceptions in a few individuals, as in May at 3 kPa when in three crabs [lact] increased to 6.8, 8.4 and 37.0 mmol l⁻¹. In May and June, it was only when food was given to unfed C. maenas kept at 2 kPa that their anaerobic metabolism was systematically increased. Fig. 2 presents the cumulated individual data for 1995–97 in parallel with the corresponding temperature change occurring in the area where they were caught and in the tanks in
Fig. 1. Change in anaerobic threshold in adult *Carcinus maenas* fed in hypoxia from March to June 1995. Intermoult crabs regularly caught in the Bay of Arcachon were starved for 5 days and then fed at time \( t = 24 \) h of a 48-h exposure period in hypoxia (water \( P_{O_2} = 4, 3 \) or 2 kPa). Switch on of anaerobic metabolism was estimated by measuring blood lactate concentration, \([\text{lact}]_b\), at the end of hypoxic exposure. At water \( P_{O_2} = 4 \) kPa, \([\text{lact}]_b\) was maximum in March but not significantly different from zero between April and June (\( n = 14 \) crabs per experiments). In May and June, there was a significant switch on of anaerobic metabolism only when crabs were fed at water \( P_{O_2} = 2 \) kPa. * Values significantly different from normoxic control (0.18±0.07; Forgue et al., 1992a).

which they were stocked. Remarkably, this confirms that it was always at the same season, March–April, when the Bay water temperature was at its lowest, that SDA at water \( P_{O_2} = 4 \) kPa induced an increase in \([\text{lact}]_b\) and eventually some mortality.

Among the various physiological characteristics which could display an annual rhythm able to influence the capacity to face deep hypoxic events in intermoult adult crabs, the blood \( O_2 \)-carrying capacity can obviously play a key role. In this respect, Massabuau and Forgue (1996) showed that a blood hemocyanin concentration, \([\text{Hc}]_b\), lower than 5 g l\(^{-1}\) limits the blood \( O_2 \)-transport capacity. We thus analysed the variation in \([\text{Hc}]_b\) values in intermoult *C. maenas* throughout the year. Fig. 3 shows that whatever the season, the mean \([\text{Hc}]_b\) remains within a narrow range (\( \approx 30 \) g l\(^{-1}\); \( n = 10–28 \) crabs per data point; \( \text{min} = 25.5\pm4.2\), \( \text{max} = 36.8\pm2.3 \) g l\(^{-1}\)). Consequently, \([\text{Hc}]_b\) cannot by itself explain the seasonal change in sensitivity to hypoxia. We then considered the fact that in March–April the population of adult male *C. maenas* was mostly represented by red crabs, which did not moult for 10–12 months (Reid et al., 1997). As the gills of adult crabs can be significantly covered with a coating of phycomycetes, foreign material (Martin, 1973, 1977) and/or ectoparasites (Humes, 1941; Gannon, 1990), we consequently focused our attention on the role that such a possible gas-exchange obstruction could play on the blood oxygenation status after feeding.
3.2. Effect of gill coating on blood $O_2$-transport in red and green intermoult crabs prior to and after feeding in hypoxia

To study this problem we compared intermoult crabs before and after moulting in March–April. Fig. 4 shows first that in red crabs, the gill cuticle was covered with a thick coating which, in the example shown, multiplied the water–blood barrier distance by a factor of three in comparison with the green specimen. We did not observe any
other noticeable gill parasitism in the present experiments. Table 1 presents a study of the mean data on the morphometry of these gills and, in addition, data measured on intermediate intermoult C. maenas (orange-green crabs) sampled a few months later, in summer. As the coating, when present, was inhomogeneous over the gill cuticle of each sample, we present mean values of minimum and maximum thickness. Remarkably, in
Table 1
Water–blood barrier thickness at gill level in red and green Carcinus maenas

<table>
<thead>
<tr>
<th>Obstructed surface</th>
<th>Thickness of coating material (μm)</th>
<th>Thickness of water–blood barrier (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March, red crabs</td>
<td>80</td>
<td>1.5±0.2/4.8±0.6</td>
</tr>
<tr>
<td>April, green crabs</td>
<td>14</td>
<td>0* *<em>/0.1±0.1</em> **</td>
</tr>
<tr>
<td>June, orange-green crabs</td>
<td>69</td>
<td>0.8±0.3*/2.6±0.6*</td>
</tr>
</tbody>
</table>

*Min/max: as the thickness of coating material was not uniform over the gill cuticles, minimal and maximal thickness values per experimental condition are given. n = 5 crabs per data point. * Significantly different from red crabs; ** green crabs significantly different from green-orange crabs.

The red crabs, the percentage of obstructed surface attained ≈ 80% and the mean maximum thickness of the water–blood barrier was 8.1±0.4 μm (n = five crabs). In contrast, on the gills of the green crabs, the surface did not present any obstructed area and the maximum distance from water to blood was only 4.8±0.6 μm (n = five crabs). In summer, the coating already presented an extension quite close to that in March (= 70%) but the thickness of the water–blood barrier was only in the range 3–6 μm. Taken altogether therefore, these results were the first indication of how this cyclic gill obstruction could participate in a seasonal rhythm of resistance to hypoxia.

In a further step towards analysing the influence of this obstruction on gas exchanges, we determined the blood oxygenation status during exposures to hypoxia (water $p_{O_2} = 4$ kPa) in red and green intermoult crabs. To further assess the comparison, analyses were performed before and after feeding, i.e. at routine and stimulated metabolism (Fig. 5). Note first that in pre-prandial conditions, there was no difference between the arterial $p_{O_2}$ and $O_2$ concentrations in these animals. Both exhibited the low blood $p_{O_2}$ values and associated ‘normal’ $O_2$-contents already well described in various resting water-breathers (Forgue et al., 1992b). The results demonstrated that at water $p_{O_2} = 4$ kPa, the gill obstruction observed in red intermoult crabs (Fig. 4) did not limit their $O_2$-exchange rates in these resting and unfed conditions. However, the picture was quite different when the oxidative metabolism was enhanced after feeding, as two striking changes were evident. On the one hand, in green crabs with new and clean gill cuticle, the arterial $p_{O_2}$ increased significantly from 0.73±0.03 to 0.94±0.05 kPa (+ 30%), while the arterial $O_2$ concentration decreased from 154±23 to 107±11 μmol l$^{-1}$. On the other hand, in red crabs with obstructed gills, which were shown to rely usually on anaerobic metabolism (Figs. 1 and 2), the arterial $p_{O_2}$ did not increase and there was a significant rise in the arterial $O_2$ concentration. Note in addition that the venous $O_2$-concentration did not decrease (Fig. 5, bottom), which shows furthermore that these animals did not rely on their venous $O_2$-reserve.

These changes in blood $O_2$ concentrations, with or without variations in blood $p_{O_2}$, consequently suggested the existence of potential modulation of the blood $O_2$-affinity. Based on the $p_{O_2}$, $p_v$, and $c_{v,O_2}$ data (Fig. 5) we then studied the corresponding adjustments of blood $a_v$ $O_2$-capacitance ($\beta_{a_v,O_2}$) at the same water $p_{O_2}$ of 4 kPa. As $c_{v,O_2}$ and $p_v,O_2$ remained in the same low range (see below), $\beta_{a_v,O_2}$ was taken as an index of the in vivo blood $O_2$-affinity. Fig. 6A confirms first that at rest, before feeding,
Fig. 5. Change in blood oxygenation status in red (left column, \( n = 14 \)) and green (right column, \( n = 28 \)) intermoult *Carcinus maenas* 24 h after feeding in hypoxia (water \( p_{O_2} = 4 \) kPa). Crabs were starved for 5 days and then fed at time \( t = 24 \) h of a 48-h exposure period in hypoxia. Red crabs were studied in March and green crabs in April. \( p_{O_2} \), partial pressure of oxygen in the arterial blood; \( c_{O_2} \), total oxygen concentration in the arterial blood; \( c_{O_2} \), total oxygen concentration in the venous blood. In the green intermoult *C. maenas*, the arterial \( p_{O_2} \) increased significantly but this was not the case in the red intermoult *C. maenas*. In the green intermoult condition, this was associated with a tendency for the arterial \( c_{O_2} \) to decrease and with an absence of change in venous \( c_{O_2} \). In the red crabs, on the other hand, there was an increase in arterial \( c_{O_2} \). * Value significantly different from preprandial data.

the blood \( O_2 \)-status was independent of the gill obstruction status as there was no difference between red and green intermoult crabs (\( \beta_{O_2} = 382 \pm 46 \) and \( 459 \pm 95 \) \( \mu \text{mol l}^{-1} \text{kPa}^{-1} \), respectively). Fig. 6B illustrates the different changes occurring after a meal. In the animals with clean gills, the blood a,v \( O_2 \)-capacitance decreased significantly to \( 213 \pm 35 \) \( \mu \text{mol l}^{-1} \text{kPa}^{-1} \) which could be considered as a favourable adaptation to the \( O_2 \)-unloading at tissue level. It shows, as confirmed by a non-significant change in [lact] from \( 0.4 \pm 0.2 \) to \( 0.1 \pm 0.1 \) mmol \( \text{l}^{-1} \), that despite a doubling of their \( O_2 \)-needs and very small but significant changes in blood \( p_{O_2} \) in absolute values (before feeding, \( 0.73 \pm 0.03 \) kPa; after feeding, \( 0.94 \pm 0.05 \) kPa), these animals did not encounter any problem in \( O_2 \)-loading at gill level despite the low water \( p_{O_2} \). In contrast, in the red intermoult specimens in which the arterial \( p_{O_2} \) did not increase (pre-feeding value, \( 0.74 \pm 0.04 \) kPa; post-feeding value, \( 0.70 \pm 0.04 \) kPa), the tendency was to increase the blood a,v \( O_2 \)-capacitance (from \( 382 \pm 46 \) to \( 504 \pm 59 \) \( \mu \text{mol l}^{-1} \text{kPa}^{-1} \)) and to facilitate
Fig. 6. Relationships between mean values of blood $c_{\text{O}_2}$ and $p_{\text{O}_2}$ in hypoxia (water $p_{\text{O}_2} = 4 \text{ kPa}$, same experiment as in Fig. 5) before and after feeding in red and green intermoult *Carcinus maenas*. (A) Preprandial conditions; there was no significant difference in blood oxygenation status whatever the physiological condition of the crab. (B) Postprandial conditions; the blood a-v O$_2$ capacitance significantly decreased in the green intermoult and did not significantly change in the red intermoult animals (see text). For comparison, the preprandial blood a-v O$_2$ lines, as shown in panel A, are also plotted (dashed lines). Open triangles, arterial and venous blood in green intermoult crabs ($n = 28$); closed circles, arterial and venous blood in red intermoult crabs ($n = 14$).

The O$_2$-loading strategy at the gill level. Thus, the above results (i) describe the mechanisms underlying the astonishing ability of intermoult *C. maenas* to feed in hypoxia, (ii) confirm that *C. maenas* can cope with very small changes in arterial $p_{\text{O}_2}$ values to satisfy the increased O$_2$-consumption associated with SDA, and (iii) show how and to what extent the gill coating observed in red intermoult crabs restricts the O$_2$-supply mechanism after feeding: it prohibits any increase in arterial $p_{\text{O}_2}$ and leads to cellular hypoxia (see Figs. 1 and 2) despite a significant increase in the arterial O$_2$-concentration.

### 3.3. Blood pH regulation

In *C. maenas*, Legeay and Massabuau (1999) previously showed that blood pH adjustments associated with feeding vary as a function of the water $p_{\text{O}_2}$. Consequently, to analyse the role these could play in controlling the blood O$_2$-affinity changes described above, we focused our attention on any possible modification in the regulation of acid–base balance in relation to season, gill status and water oxygenation level. To do this, we performed a series of experiments during which crabs studied from April to July were fed at water $p_{\text{O}_2} = 21$, 4, 3 or 2 kPa. Fig. 7 shows first that when crabs were fed in normoxia, the acid–base balance regulation was systematically characterised by a blood pH acidosis, whatever the season (plotted in Fig. 7B and C as $\text{pH}_{\text{fed}} - \text{pH}_{\text{unfed}} < 0$). As
previously suggested by Legeay and Massabuau (1999) and illustrated above, this was in agreement with the hypothesis that O$_2$-unloading at cellular level is favoured in normoxic water, i.e. when there is no O$_2$-limitation from the environment. It was thus expected that in hypoxic waters, one would systematically observe the reverse, i.e. a blood pH alkalosis to favour O$_2$-loading at gill level. Interestingly however, the water p${\text{O}}_2$ threshold at which the blood pH adjustment was reversed to favour O$_2$-unloading at tissue level instead of O$_2$-loading at gill level varied with the season. For example, when
crabs were fed at an inspired $pO_2$ of 4 kPa the blood pH was systematically alkalinised in March–April and systematically acidified from May to July. In fact Fig. 7B and C show that the water $pO_2$ at which the blood pH started to be systematically alkalinised (to eventually favour $O_2$-loading) was $\geq$ 6 kPa in March–April and $\leq$ 3 kPa from May to April.

4. Discussion

The present work reports on the ability of intermoult adult *Carcinus maenas* living in the Bay of Arcachon, a site with low pollution levels and low concentrations of suspended matter (Bouchet et al., 1997), to feed in hypoxia and also on the seasonally related evolution of this ability. Present results show that it is in winter, before the major moulting period in the Bay and when the water temperature is at its lowest, that *C. maenas* is most sensitive to hypoxia. Remarkably, in spring and summer, intermoult crabs were able to feed in a water $pO_2$ of as low as 2–3 kPa with a systematic, but non-lethal, switch to anaerobic metabolism. An analysis of water–blood barrier thickness at gill level, blood oxygenation status and strategy of blood pH regulation showed that in March–April, a 5–10 $\mu$m coating of foreign material covered most of the gill cuticle of red intermoult crabs and prohibited the rise in arterial $pO_2$ that is observed for example in green intermoult animals at water $pO_2$ = 4 kPa upon feeding. In March, $O_2$-loading at gill level started to be favoured by a postprandial blood pH alkalinisation for water $pO_2$ values well above 6 kPa which suggested that it is at this water $pO_2$ that the cellular $O_2$-supply required by the SDA started to be influenced by the ambient hypoxia. In contrast, in spring and summer this postprandial alkalinisation appeared exclusively below a water $pO_2$ of 2–3 kPa. This illustrates both the remarkable potential of *C. maenas* to face hypoxic events, for at least medium-term periods during the hot season, and the effect that fouled gills can have on such performance.

In *C. maenas* the existence of variations in response to water oxygenation occurring during the intermoult period was first reported by Reid and Aldrich (1989). These authors associated the colour of intermoult *C. maenas*, from red to green, with differences in emersion responses to declining water $pO_2$. They showed that red *C. maenas* start to escape a water body in which $pO_2$ is declining at $\approx$ 6 kPa whereas green crabs only start to escape at $\approx$ 3.5 kPa. This fits well with the water $pO_2$ thresholds we report concerning the switch from acidosis to alkalosis and its relationship with the seasons and the moulting cycle. They also showed that the mortality of red crabs sealed in closed vials was greater than that of green specimens. Present results offer a rational basis for these observations. They strongly suggest that the development of a coating on the gill cuticle of these red intermoult animals impaired $O_2$ diffusion until an ultimate status is reached at the end of the intermoult period. To our knowledge, Martin (1973) was the first to report the presence of a thick coating on the gills of intermoult *C. maenas* and to describe the presence of a tight network of phycomycetes (Martin, 1977). He suggested that this network could act as a filter against the particles present in the water flowing through the gill chamber and that it would be rejected, with the old
cuticle, only at ecdysis. Interestingly, Martin (1973, 1974) also reported the existence in *C. maenas* and in *Cancer irroratus* captured in Terrance Bay, Canada, of iron accumulation in a particulate state which was probably trapped in this network and attained 20–40 \( \mu \text{m} \) in some places. In the present situation the maximum thickness we observed was much lower, at only \( \approx 10 \ \mu \text{m} \) (Fig. 4).

In normoxia, the effect of a gill obstruction on gas exchange was studied by Gannon and Wheatly (1992, 1995) who analysed the physiology of blue crabs *Callinectes sapidus* infested by the ectocommensal gill barnacle *Octolasmis muelleri*. This parasite filter feeds on particulate matter in the host ventilatory stream and it was expected to have a potentially harmful effect as it is most frequent at the optimal — i.e. most ventilated — respiratory sites, that is the bases of gills 3, 4, 5 and 6 (Gannon, 1990). Remarkably however, although the crabs were studied at rest and at exercise, no major disturbance was found in the crab’s hemolymph (Gannon and Wheatly, 1995). This is again entirely consistent with the present data, as in normoxia we observed no seasonal limitation of the gas exchange system during the increase of \( \text{O}_2 \)-consumption induced by the SDA.

In adult *C. maenas*, the thickening of the coating material on the gill cuticle reaches a maximum before moulting in March–April in the Bay of Arcachon and it was only at that time that we observed any impairment of the animal’s ability to face environmental hypoxia during periods of increased metabolism. As previously suggested by Reid et al. (1997) this confirms, from a respiratory standpoint, that intermoult crustacea prior to and after moulting are in completely different conditions to successfully face hypoxic events. The single reference to the moult stage (Drach, 1939) is incomplete in numerous ecophysiological and behavioural studies as the gill status for example can vary greatly according to both internal physiological rhythm and water quality. The present work helps to quantify these differences and give new insights into the role that can be played by a gill coating which must be highly variable from site to site as it is closely related to the local water physico-chemistry. In this context, it is important to note that in the Bay of Arcachon, the mean annual suspended matter concentration (MES) varies between 7 and 10 mg l\(^{-1}\), depending on the location and the tides (Robert et al., 1987), which is in the low range of most reported values. In the open sea the suspended matter concentration varies from 0.005 to 1 mg l\(^{-1}\) but in coastal waters concentrations of more than 100 mg l\(^{-1}\) can occur, while in estuaries and tidal channels values of up to \(10^2–10^3\) mg l\(^{-1}\) have been reported (Eisma, 1993). As crustaceans’ gills which are covered by a phycomycete can be easily clogged by foreign particles (Martin, 1977), the limits we determined here — in settled crabs exhibiting a minimal ventilatory activity in unstimulated laboratory conditions — is probably an extreme performance for the species. It is thus expected that they would be much more sensitive to hypoxia in estuaries and bays with higher concentrations of suspended matter although the turbidity of estuarine waters has surprisingly been considered by some authors as an unlikely cause of limiting species expansion (Wolff, 1972; McLusky, 1981). We propose on the contrary that in areas where the rainy season is synchronous with the *C. maenas* late intermoult period it is very likely to be a strong selection factor on the population. Interestingly, from March to April in the Bay of Arcachon, the suspended matter concentration can reach up to 110 mg l\(^{-1}\) in places and it is at this season that massive
mortality levels can be observed along the sea shore of some channels (Labourg and Auby, personal communication). Moreover, we suggest also that this difference in gill coating could at least partially explain some of the variations found in the literature on this crab species. For example, Taylor (1976) reported that *C. maenas* was unable to maintain its $O_2$-consumption constant below a critical pressure of 8–10 kPa, whereas Forgue et al. (1992a) determined an arterial $pO_2$ at the anaerobic threshold of 1 kPa in crabs exposed to water $pO_2 = 2.1–2.8$ kPa.

In water-breathers with partially obstructed gills, hyperventilation is the only possible means of compensating for the increased resistance to $O_2$ diffusion from water to blood. In the present experimental conditions, the thickness of the blood–water barrier increased progressively. This consequently shows that, at constant $O_2$-uptake, and in parallel to the increase of gill thickness, there should be a progressive increase in the ventilated gill surface and for in the water $pO_2$ profiles in the branchial cavity. Such a ventilatory compensation has already been recorded in normoxic blue crabs infested with gill parasites (Gannon and Wheatly, 1992). They have a systematically higher ventilatory frequency when compared with control animals.

Importantly, this study has also demonstrated the role of the pH-induced change in blood $O_2$-affinity which continuously counterbalances the changes in $O_2$-diffusion capacity at gill level and more generally, the fundamental role of acid–base balance regulation in *C. maenas* (see Truchot, 1992). When this crab is at rest and in settled conditions, this is especially important as the animal can satisfy its $O_2$-needs with quite low blood $pO_2$ values which are located in the upper-middle and lower sigmoid parts of the $O_2$-dissociation curves (Massabuau and Forgue, 1996). Alongside all considerations of *C. maenas*’ potential to adapt to environmental hypoxia, this change of strategy in acid–base balance regulation is a new and original means of testing the efficiency of the overall chain of $O_2$-transfer from gill to tissue in water-breathers. Before any appearance of lactate in the blood, the switch from postprandial acidification to postprandial alkalinisation is a preliminary alarm showing that the intracellular medium is becoming hypoxic. In addition, it is worthwhile noticing that in summer, when animals were able to feed at water $pO_2 \approx 2–3$ kPa, there was a systematic increase in the blood lactate concentration. As Truchot (1980) showed, an increase in $[\text{lact}]$ increases the $O_2$ affinity of crab hemocyanin. When the arterial $pO_2$ ranges lie within the values reported here, this property would obviously help in loading $O_2$ at gill level. In summer, this mechanism could play a key role in enabling feeding to occur in waters with extremely low levels of oxygenation. Interestingly, the failure of the $O_2$-delivery system which occurred in March–April was not associated with a decrease in arterial $O_2$-concentration but only with an absence of any increase in arterial $pO_2$. Together with the small changes on blood pH reported above, this further reinforces the key role of minor adjustments to blood parameters in the normal physiological repertoire of crustacean life (Forgue et al., 1992a, Clemens et al., 1998, Legeay and Massabuau, 2000).

In conclusion, the intrinsic capacity of adult *Carcinus maenas* to face at least medium-term periods of deep hypoxia is remarkably high, even during periods of increased metabolism as induced by SDA. However, this capacity varies throughout the year in relation to the seasons, to the intermoult cycle and ultimately, to gill cleanliness. Interestingly, it is during the hot season, i.e. when most hypoxic events occur in the
field, that this capacity is greatest. This appropriate timing is obviously of great importance in explaining the ecological success of *C. maenas* and in illustrating its ability to invade new biotopes.

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**References**


