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ACID-BASE HOMEOSTASIS IN AQUATIC ANIMALS EXPOSED TO NATURAL AND PERTURBED ENVIRONMENTS

by

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SUMMARY

Keeping an appropriate acid-base state in the various body compartments of animals is of prime importance for many basic living processes. What is preserved is not a constant pH value but rather a constant relationship between pH and body temperature, which tends to stabilize the protein electrical charge and, more generally, conformation and function of macromolecules. Acid-base homeostasis requires a balance between metabolic production and controlled excretion of two classes of acids or bases : the volatile carbonic acid whose elimination depends on respiratory regulations ; and fixed acids and bases, usually excreted in association with ion exchanges. In aquatic animals, these functions are heavily challenged by large natural changes of respiratory gases, oxygen and carbon dioxide, as well as of total salinity or of particular ions in the environment. The effects of each of these factors in isolation have been well studied in laboratory conditions, but integrated responses to the changes of many factors as it occurs in the natural setting are less well known. Variations of ambient or internal CO_2 are not a strong stimulus to breathing in aquatic crustaceans and fishes, and respiratory compensations are thus of little importance in acid-base homeostasis. On the contrary, aquatic organisms are usually able to quickly get rid of large fixed acid or alkaline loads by coupling their excretion with gill ionic exchanges. Such excretory processes also serve to compensate acid-base disturbances induced by changes of the respiratory qualities of the water. The well-known impact of various pollutants (heavy metals, ammonia, acid waters...) on gill structure and ionoregulatory mechanisms can also considerably disturb acid-base balance in aquatic animals. Such disturbances may serve as very sensitive tests of sublethal toxicity.

Keywords : acid-base regulation, aquatic animals, gill ion exchange, intertidal rockpools, pollutants.

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INTRODUCTION

The maintenance of an appropriate hydrogen ion activity is probably one of the most basic requirements of living systems. Much recent work has revealed that a large range of animal cells are able to efficiently compensate cytosolic acid-base deviations arising from endogenous metabolic byproducts or from extracellular disturbances. The well-known constancy of blood pH in man and homeothermic vertebrates probably represents a first line of defense that facilitates cellular acid-base homeostasis. More recently, it has become clear that such a precise extracellular acid-base regulation is also working in lower vertebrates and invertebrates. Rather than extensively reviewing the abundant literature on this topic (see TRUCHOT, 1987), the present short account will focus on aquatic animals and try to answer a few simple questions. Do water-breathing animals possess mechanisms to maintain acid-base homeostasis ? Are these mechanisms similar to or different from those at work in air breathers ? How are these mechanisms used in natural aquatic environments ?

But before examining these particular questions in some detail, it is important first to raise a more general problem which has recently been much clarified thanks to studies on lower animals.

WHAT IS THE REGULATED ACID-BASE VARIABLE AND WHY ?

Acid-base homeostasis is commonly thought to mean maintenance of a constant blood pH. But this concept is valid only as long as body temperature remains steady. In poikilotherms, many observations have shown that blood pH in fact decreases as body temperature increases (RAHN *et al.*, 1975 ; REEVES, 1977). This pH change depends both on the thermal properties of body fluid buffers and on physiological adjustments that maintain a new steady value after a temperature change (*e.g.* TRUCHOT, 1978). The slope of the relationship between blood pH and temperature has raised some debate, but it is generally agreed that it is similar *in vivo* and in an *in vitro closed* system, its value amounting to 0.015 to 0.020 pH units/°C (reviewed by TRUCHOT, 1987). This is just the value required to keep a constant difference between the physiological pH and the neutral pH of pure water, which also decreases at increasing temperature. This means that blood pH is regulated in such a way that the *relative alkalinity* (or the ratio $[OH^-]/[H^+]$, see RAHN and HOWELL, 1978) is kept constant (Fig. 1). Another striking consequence of the observed pH/temperature slope is that the degree of dissociation of the most important protein buffer group at physiological pH, the imidazole group of histidine, is also kept nearly constant (REEVES, 1972). This so-called *alpha-imidazole regulation* results in the maintenance of a relatively unchanged electrical charge on proteins, which presumably stabilizes their structure and functional properties in the face of temperature changes (WHITE and SOMERO, 1982 ; SOMERO, 1986). Beyond the maintenance of a constant blood pH, or of a constant blood pH/temperature relationship, acid-base regulation may thus have the meaning of an *homeostasis of*

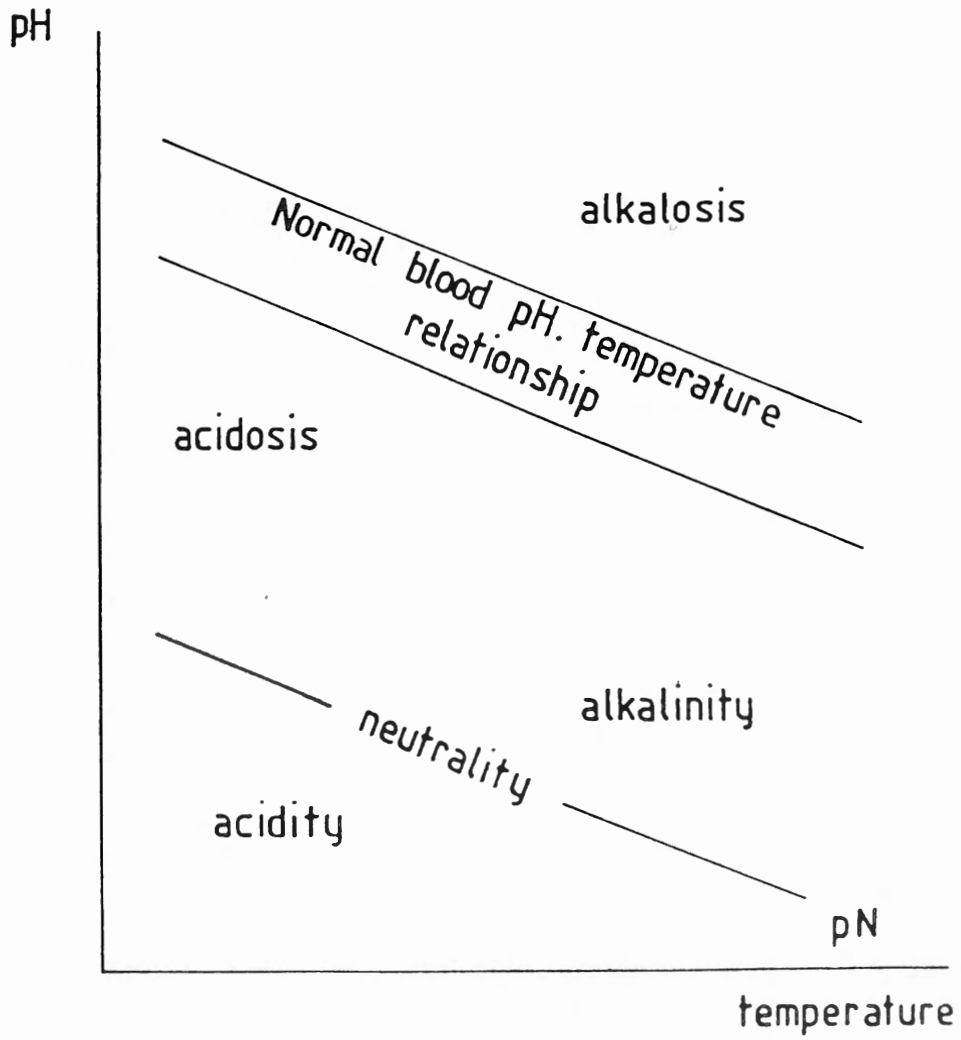

Fig. 1. — The normal blood pH *vs* body temperature relationship observed in poikilothermic animals. The regulated blood pH value decreases as temperature increases in such a way that the difference between biological pH and the neutral pH of pure water (pN) remains constant through the whole temperature range. In the same way as acidity and alkalinity of aqueous solutions are defined by deviations from the pN/temperature line, acidosis and alkalosis designate departures not from an unique pH value but from the normal blood pH/temperature relationship.

protein function. A similar relationship between intracellular pH and temperature has been found (*e.g.* RODEAU, 1984), showing that the concept also applies to intracellular fluids.

SOME ASPECTS OF ACID-BASE HOMEOSTASIS IN AQUATIC ANIMALS

Acid-base regulatory mechanisms

Body fluid acid-base balance is permanently challenged by acidic and alkaline substances which are produced by cell metabolism and must thus be eliminated. There are in fact two broad categories of such products : first, the volatile acid CO_2 whose excretion is controlled by respiratory gas exchanges, and second, non volatile or so-called fixed acidic or alkaline equivalents, whose elimination must comply with electroneutrality constraints and is by consequence necessarily coupled with ion exchanges. The primary purpose of gas and ion exchanges being not acid-base homeostasis, it should be pointed out that these processes can be involved as *disturbing factors* as well as *regulatory mechanisms* of acid-base balance. For example, reduced CO_2 elimination relative to its production leads to an increase of body fluid Pco_2 (hypercapnia) and by consequence to a pH decrease of respiratory origin (respiratory acidosis). Conversely, a restricted excretion of metabolically-produced acidic equivalents or an excessive elimination of alkaline equivalents (both taking place mainly via the urinary route in higher terrestrial vertebrates) could lead to a decrease of body fluid pH of metabolic origin (metabolic acidosis). In mammals, respiratory acid-base disturbances are known to be compensated at least partly by modulation of renal excretion of acidic or alkaline equivalents (metabolic compensation). In addition, ventilatory adjustments of blood Pco_2 in response to pH changes can contribute to the regulation of metabolic acid-base disturbances (respiratory compensation).

In many fishes and crustaceans, acid-base disturbances of respiratory origin are efficiently compensated with a progressive recovery of blood pH resulting from an increased bicarbonate concentration. This response has been much studied by exposing the animals either to ambient hyperoxia, which entails a reduced ventilatory activity and an endogenously-generated elevation of internal Pco_2, or to ambient hypercapnia which leads to CO_2 loading from the external medium. As shown in Fig. 2, the increase in plasma [HCO_3^-] compensating for the hyperoxia-induced respiratory acidosis is obtained in trout by a sustained net outflux of fixed acid to the ambient water via the gill route. At return to normoxia, a transient alkalosis disappears rapidly thanks to a branchial base efflux (or acid influx). These responses affecting fixed acid or base excretion are very similar to those observed in terrestrial vertebrates, but the contribution of urinary excretion is here always minor (Fig. 2). As for ionic regulation, the role of the gills in acid-base control is prominent. Similar acid-base compensations have been observed in crustaceans (*e.g.* TRUCHOT, 1979).

Branchial excretion of acid-base equivalents is in fact coupled to ion exchanges. It has long been postulated that freshwater animals take up Na^+ and Cl^- ions from the ambient water in exchange for excretion of H^+ (or NH_4^+) and HCO_3^-, respectively, in order to maintain ion homeostasis. Coupled Na^+ and H^+ movements probably do not take place by a direct Na^+/H^+ antiport but rather by

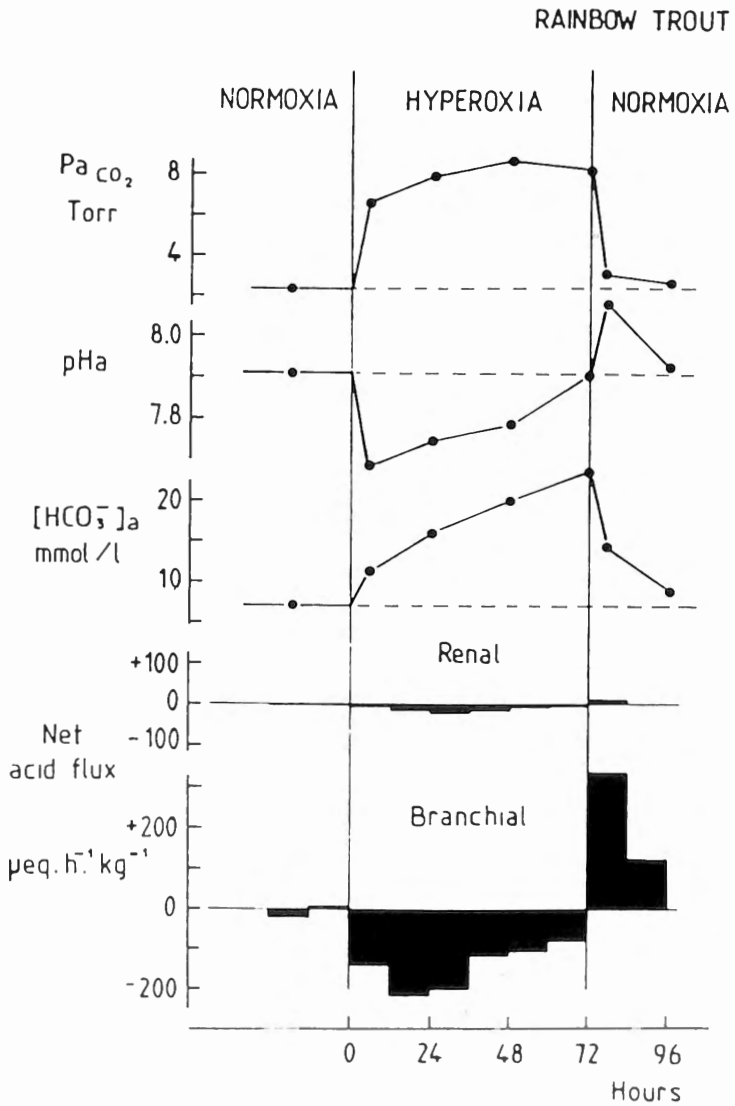

Fig. 2. — Simultaneous changes of arterial blood acid-base status (pH, Pco_2 and plasma HCO_3^-) and of net transfer of acidic equivalents via renal and branchial routes in the rainbow trout *Oncorhynchus mykiss* during exposure to hyperoxic water followed by recovery in normoxic water. Net acid flux is considered negative when fixed acid is lost, as measured by titration of ambient water. During hyperoxia, a transient respiratory acidosis brought about by an increase of blood Pco_2 is progressively compensated thanks to an increase of HCO_3^- concentration. This increase results from a mainly branchial acid loss, which is readily reversed to an influx of acid (or base efflux) upon return to normoxic water whereas [HCO_3^-] rapidly decreases. Drawn from values published by HOBE *et al.* (1984), WHEATLY *et al.* (1984) and WOOD *et al.* (1984).

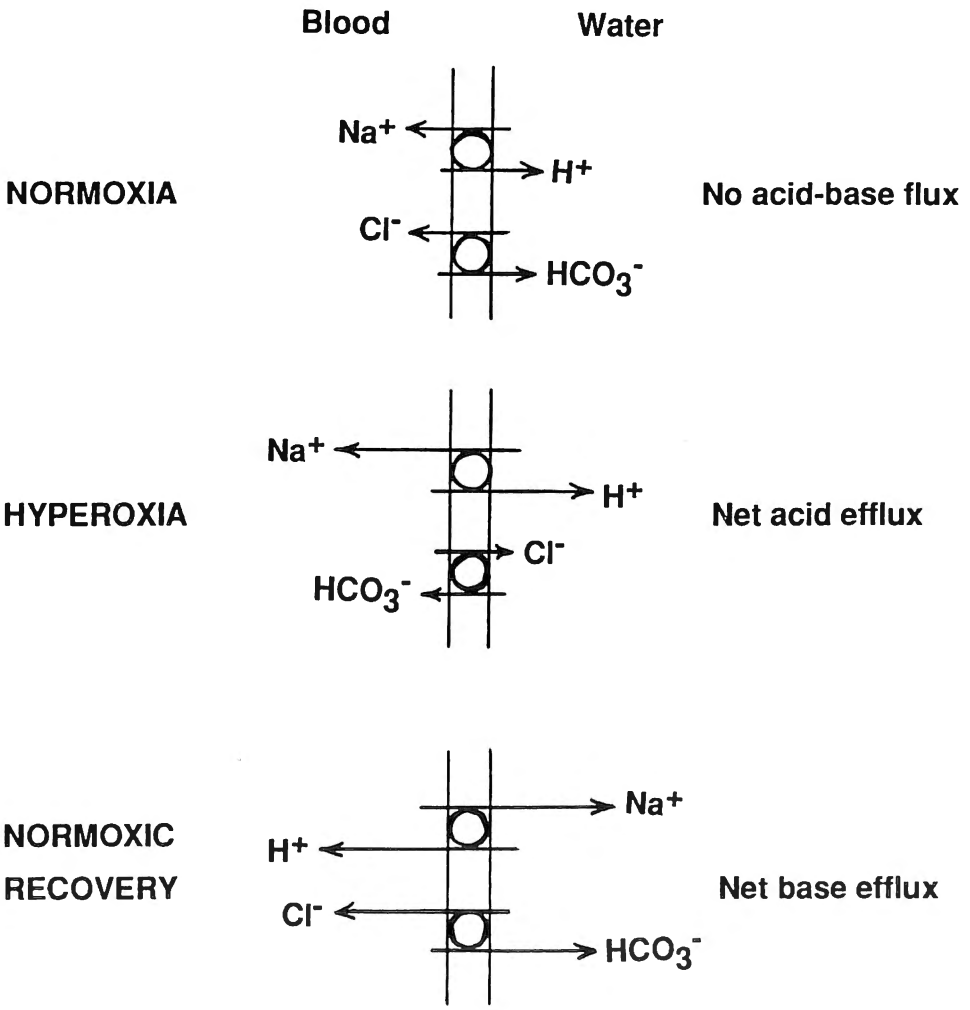

Fig. 3. — General principles of adjustments of the transfers of acid-base equivalents coupled to gill Na^+ and Cl^- exchanges in the rainbow trout *Oncorhynchus mykiss*, in response to respiratory acid-base disturbances (Derived from flux values published by WOOD *et al.*, 1984). Sodium and chloride are stoichiometrically exchanged against acid and base equivalents labelled H^+ and HCO_3^-, respectively. In normoxic steady acid-base state, NaCl uptake against equal effluxes of H^+ and HCO_3^- result in no measurable net acid-base flux. Hyperoxia induces a respiratory acidosis (see Fig. 2), accompanied by an increased Na^+ net influx and a reversed Cl^- net flux. Both increased H^+ excretion and bicarbonate uptake result in a net acid efflux increasing plasma bicarbonate and compensating the respiratory acidosis. During normoxic recovery, a transient alkalosis (see Fig. 2) is also compensated by a net base efflux resulting from H^+ uptake and HCO_3^- excretion, respectively coupled to increased Cl^- influx and Na^+ outflux.

a proton pump electrically coupled to an apical Na^+ channel (AVELLA and BORNANCIN, 1989). But, whatever the exact mechanisms, it has been shown that gill ion exchanges can be modulated to ensure acid-base compensations (WOOD, 1991). Indeed, measurable directional changes of Na^+ and Cl^- fluxes consistently explain associated acid-base movements in trout (Fig. 3), and a 1/1 linear relationship between the net acid-base flux and the difference of Na^+ and Cl^- net fluxes indicate that the Na^+/H^+ and Cl^-/HCO_3^- exchanges are the only mechanisms involved (WOOD *et al.*, 1984).

Whether respiratory modulation of blood Pco_2 can compensate for metabolic disturbances in aquatic animals remains little documented and controversial. Regulation of ventilatory activity in water-breathers is mainly oriented to meet oxygen demand, presumably because oxygen is poorly available in water due mainly to its low solubility (DEJOURS, 1981). The poor responsiveness of ventilatory control to increased Pco_2 and/or decreased pH is clearly illustrated by the sustained depression of gill water flow rate under hyperoxia, despite a prevailing and often marked respiratory acidosis. Some data nevertheless suggest that ventilation may be responding to acid-base disturbances in certain circumstances, and particularly when ion exchange limitations presumably impede metabolic compensations. For example, crayfish exposed to very poorly mineralized water typically exhibited a metabolic acidosis which was, however, moderated by a marked decrease of Pco_2 apparently resulting from a higher than normal ventilatory activity (BURTIN *et al.*, 1986).

Acid-base balance in naturally variable aquatic environments

Many aquatic environments may undergo wide spatial and temporal changes of many physical and chemical factors among which ion and gas composition are potentially disturbing for acid-base balance. We will focus here mainly on respiratory gases, O_2 and CO_2, and pH, the variations of which are mainly caused on a diel basis by biological processes, respiration and photosynthesis. These variations are particularly marked in small water bodies such as rockpools left at low tide on the seashore (TRUCHOT and DUHAMEL-JOUVE, 1980). Typically, these biota become hypoxic at night while water Pco_2 increases moderately and pH decreases. Conversely, during the day, photosynthesis is active and water Po_2 usually reaches high levels, accompanied by a large reduction of Pco_2 and a huge increase of pH. Additionally, in temperate regions, diel changes of temperature are well marked and may also strongly affect acid-base balance.

Obviously, situations encountered in nature are often very different from those explored in single-factor laboratory experiments, in that some variables can act synergistically and some others antagonistically on acid-base balance. For example, during the day, ambient hyperoxia will induce gill hypoventilation and respiratory acidosis, while concomitant reduction of water Pco_2 and increase of pH could be expected to favor internal alkalosis. Organismal acid-base disturbances resulting from such antagonistic influences can hardly be predicted and must be directly studied either *in situ* or in simulated environments, which has been rarely done. As

an exemple, we will comment some data we have obtained on shore crabs *Carcinus maenas* put in an outdoor tank populated with algae, a device which has proved to qualitatively and quantitatively reproduce quite well natural variations of many factors as observed in rockpools (TRUCHOT, 1986). The most interesting outcome of these data was that blood pH variations were much more moderate that could be predicted from changes of ambient water oxygenation alone, essentially because they were damped by the influence of concomitant Pco_2 variations. In addition, changes of blood bicarbonate concentration were so moderate that they could not be taken as evidence that metabolic compensations of respiratory acid-base disturbances took place in these conditions. Rather interestingly, when measured pH values were plotted as a function of water temperature, they gave a slope much alike that observed at variable temperature but constant gas conditions in laboratory experiments. A rather surprising conclusion can be drawn from these data. Even if the shore crab possesses elaborate mechanisms to maintain acid-base homeostasis in the face of environmental changes, these mechanisms are apparently not used when the animal is exposed to natural rockpool conditions. In fact, the concerted changes of ambient factors in this environment seems appropriate enough to lead passively to the optimal acid-balance.

Effects of pollutants on acid-base balance in aquatic organisms

Acid-base homeostasis in aquatic animals is strongly dependent on a proper functioning of the gill which is the main site of respiratory and ionic exchanges, two processes involved in acid-base compensations. Being actively irrigated by the respiratory water flow, the large surface and the thin epithelial lining of the gill is also the most prominent interface between the aquatic organism and the environment. As a consequence, it has been shown to be very sensitive to toxicant action (MALLATT, 1985). In fact, the gill epithelium of many fishes and crustaceans is apparently the first target of many pollutants as demonstrated by important and relatively unspecific cytological damages, even upon exposure to sublethal levels. Among accompanying physiological perturbations, restriction of gas exchange is caused mainly by thickening of the water-blood barrier or by mucus accumulation. Although less studied, acid-base disturbances are probably always present and may even constitute the first and most sensitive symptom of sublethal contamination. As shown by our observations on the shore crab, *Carcinus maenas,* exposed to sublethal copper levels, there is first a progressively developing metabolic acidosis (Fig. 4), which does not result from anaerobic lactic acid generation but is probably ascribable to some yet undefined perturbation of gill ion exchanges (BOITEL and TRUCHOT, 1989). Indeed, even if no changes of hemolymph ion concentrations are apparent in full strength sea water where the crab is isosmotic and almost isoionic, copper exposure causes a typical loss of hemolymph ions in dilute sea water (BOITEL and TRUCHOT, 1990). Concomitant changes of hemolymph Pco_2 may or not ensure partial compensation of the primary metabolic acidosis. Interestingly, these acid-base disturbances appear reversible after weeks of sublethal exposure to copper in the shore crab (Fig. 4). Disappearance of the metabolic acidosis coincides with

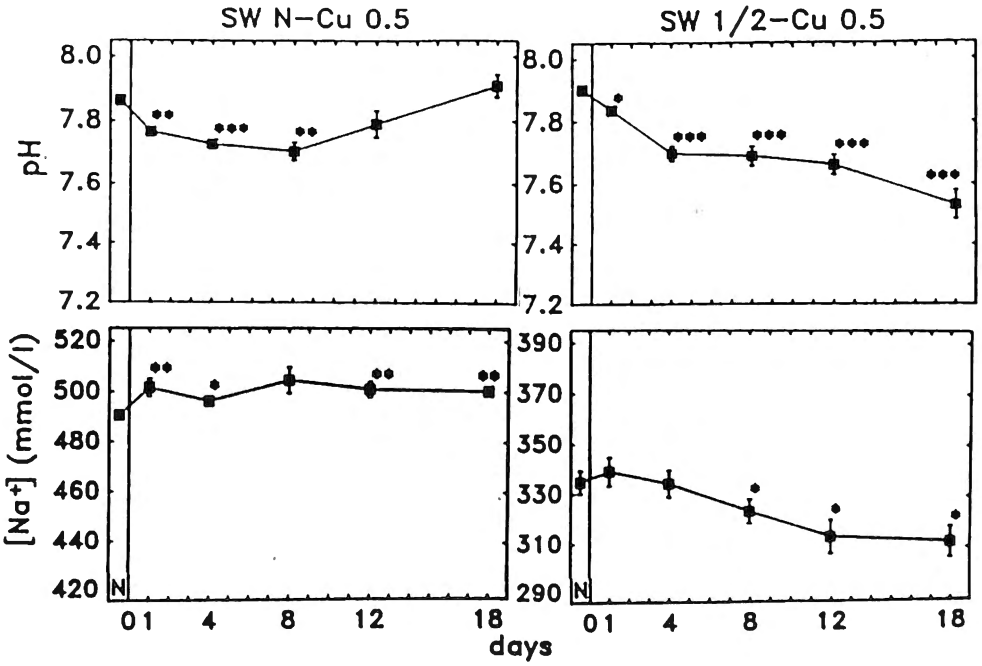

Fig. 4. — Changes of hemolymph pH and Na^+ concentration induced by sublethal exposure to waterborne copper (0.5 mg/l) in the shore crab *Carcinus maenas* acclimated to full strength (SW N) and dilute (SW 1/2) seawater. A well marked acidosis progressively recovers after 18 days in full strength but not in dilute sea water. Copper exposure induces no important ionic disturbance in full strength sea water but the animals progressively lose sodium in a dilute medium when exposed to the toxicant. N : values measured the day before copper exposure. (Redrawn in a modified form from BOITEL and TRUCHOT, 1990).

anatomical repair of the gill epithelium and recovery of normal oxygen levels in hemolymph (NONNOTTE *et al.*, 1993). These and other data indicate that acid-base disturbances could provide very sensitive tests of toxicant sublethal exposure. Inasmuch as the toxicity of many pollutants depends on water chemistry, studies of acid-base effects could prove appropriate to understand such variations.

CONCLUSION

Like higher terrestrial vertebrates, aquatic animals are endowed with efficient mechanisms to achieve extracellular acid-base homeostasis. Among these mechanisms, transfer of acid-base equivalents in coupling with ion exchanges at the gill level appears of prime importance while respiratory compensations are likely minor or absent. Being related to both respiratory and ion exchange functions, acid-base parameters are particularly useful to evaluate physiological, pathological

and adaptational responses of aquatic animals to any natural or anthropogenic changes in their environment.

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