ELSEVIER

Aquaculture 191 (2000) 209–245

Aquaculture

www.elsevier.nl/locate/aqua-online

Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans

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Received 1 March 2000; accepted 9 May 2000

Abstract

Exposure to water-borne pollutants, environmental stressors and pathological agents of a crustacean capable of osmoregulation usually results in a decrease of its Na^+ and Cl^- regulation and/or of its osmoregulatory capacity (OC: difference between the osmotic pressures of the hemolymph and of the external medium, at a given salinity). The partial or complete loss of osmoregulatory and ionoregulatory capacity is generally linked to disruptions of the osmotic and ionic regulations. Different causes of these variations include alterations in the structure and ultrastructure of the branchial and excretory organs and changes in Na^+,K^+-ATPase activity, ionic fluxes and surface permeability.

OC appears therefore as a nonspecific bioindicator. Hyper-OC and/or hypo-OC can be measured according to the type of osmoregulation of the species, respectively, in low-salinity media and in seawater or high-salinity media. Provided several precautions are taken (control of salinity and temperature, size, nutritional status, developmental stage, molt stage, use of controls), measurement of OC variations is proposed as a convenient and reliable way to monitor the physiological condition and the effect of stressors in crustaceans.

Keywords: Crustaceans; Osmoregulation; Osmoregulatory capacity; Pollutants; Stressors

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0044-8486/00/$ - see front matter
PII: S0044-8486(00)00429-4

1. Introduction

Rapid methods for the evaluation and understanding of stressors (pollutants, physical disruptions of the environment, pathological agents) on marine organisms are increasingly needed so that effective management decisions can be made and further environmental degradation averted (Malins and Ostrander, 1991; Holdway et al., 1995). Responses to stressors have traditionally been observed at the population, community or ecosystem levels of organization. Effects, however, are also manifested at the organismal level by impairing molecular, cellular and physiological functions (with reviews in Vernberg and Vernberg, 1974; Gilles and Péqueux, 1983; Hebel et al., 1997). Different histological, physiological, biochemical and molecular measures of toxicant-induced stress — referred to as biomarkers — have therefore been suggested and used as sensitive predictors of effects of stressors at the level of response of whole animals (with reviews in Haya and Waiwood, 1983; Giesy et al., 1988; Giesy and Graney, 1989; Mayer et al., 1992; Livingstone, 1993; Cajaraville et al., 1995; Holdway et al., 1995; Walker, 1995; Wedderburn et al., 1998). Osmoregulation maintaining osmotic homeostasis has already been used as a biomarker in fish species (Clarke and Blackburn, 1977; Eddy, 1981; Wendemeyar and McLay, 1981; Wendelaar Bonga and Lock, 1992) and can be considered as another possible marker for crustaceans (Charmantier et al., 1989; Lin et al., 1991, 1992; Young-Lai et al., 1991; Mayer et al., 1992; Bambang et al., 1995a,b; Lignot et al., 1997, 1998a). Osmoregulation, which is one of the most important regulatory functions an aquatic animal has to perform, has been extensively studied in many crustaceans (with reviews in Mantel and Farmer, 1983; Péqueux, 1995). Many crustacean species that effectively osmoregulate, either as hyper-isoregulators or as hyper–hypo-regulators, occur in marine, brackish and freshwater conditions (with reviews in Mantel and Farmer, 1983). Monitoring the physiological condition of crustaceans by using osmoregulation could therefore have a potential use as a biomarker in natural waters, such as inland freshwater lakes and rivers as well as in coastal areas, estuaries, lagoons, i.e., in the most exposed parts of the aquatic environment. The use of osmoregulation might also be considered in aquaculture in the early detection of adverse rearing conditions from different origins, including water quality.

The aim of this manuscript is to review the effects of various stressors on osmoregulation, and to evaluate the potential use of the ability to osmoregulate for monitoring the physiological condition of wild and cultured individuals and species of aquatic crustaceans submitted to different stresses. Temperature, although an important external factor and potential stress, is not taken into account in this review since the interrelations between temperature and osmoregulation have already been reviewed (Charmantier, 1975, 1987; Burton, 1986).

Since Na^+ and Cl^- ions are known to make up 90% or more of the osmotic pressure of hemolymph in most crustaceans (Prossner, 1973; Castille and Lawrence, 1981a,b; Mantel and Farmer, 1983), the changes in ionoregulation following stress will also be reviewed. In aquatic crustaceans and particularly in decapods, the organs of the branchial chambers are the primary site of osmotic and ionic regulation (as well as respiration and excretion) (with reviews in Mantel and Farmer, 1983; Taylor and Taylor, 1992; Péqueux, 1995). The large area for adsorption, the large volume of water passing

over the ion-transporting cells (or ionocytes) and the relatively small biomass compared to their surface area are indications that tissues of the branchial cavities can potentially suffer from structural and functional alterations after exposure to stress. Comparable alterations can also affect the excretory organs, in particular the antennal glands of decapods involved in volume regulation, and also in the regulation of solutes, divalent ions and nutrients (Mantel and Farmer, 1983; Wheatly and Gannon, 1995). The effect of water-borne toxicants and stressors on the structure of crustacean ion-transporting organs will therefore be reviewed. Modifications of the activity of Na^+,K^+-ATPase, one of the most important enzymes involved in active ion-transport in crustaceans (Towle, 1990; Lucu, 1993), and changes in ionic fluxes and surface permeability will also be considered.

To understand the impact of pollutants on aquatic organisms, the notion of stress that has been extensively reviewed (Bayne, 1975, 1985; Ivanovici and Wiebe, 1981; Broom, 1991) has to be defined. From all those available, Bayne's (1975) definition, derived from mollusc studies, is the best suited to the scope of this review: he defined stress as "a measurable alteration of a physiological (or behavioural, biochemical, or cytological) steady-state that is induced by an environmental change and that renders the individual (or the population, or the community) more vulnerable to further environmental change." An important aspect in this definition is that the modification (i.e., the alteration from a steady-state, or control, value) in the chosen physiological parameter must be "measurable". In addition, the "environmental" qualification of the change will be broadened in this review to changes affecting natural environments and artificial culture conditions.

2. Effect of stress on osmoregulation

2.1. Definition of the osmoregulatory capacity

Osmoregulatory capacity (OC) is defined, for a given osmoregulating species, as the difference between the osmotic pressures of the hemolymph and of the external medium, at a given salinity (Charmantier et al., 1989). Hyper-OC and/or hypo-OC can be measured according to the type of osmoregulation of the species, respectively, in low-salinity media, and in seawater or high-salinity media (Robertson, 1960).

The measurement of hemolymph osmolality that permits OC evaluation has been successful in numerous crustaceans from small isopod and gammarid species (Charmantier, 1975; Einarson, 1993) to peneid shrimps (Williams, 1960; Pannikar, 1968; Castille and Lawrence, 1981a,b; Dall, 1981; Ferraris et al., 1987; Lin et al., 1991; Bambang et al., 1995a,b; Lignot et al., 1997, 1998a,b, 1999b), homarid lobsters (Dall, 1970; Thuet et al., 1988; Young-Lai et al., 1991) and crabs (Warburg et al., 1987; Diamond et al., 1989). Measurement of hemolymph osmolality can also be evaluated in embryos and early developmental stages (Charmantier et al., 1988; Morritt and Spicer, 1995; reviewed in Charmantier, 1998). The technique implies several precautions. Experimental salinities and temperatures must be chosen in such a range that mortality is kept to a minimum and OC values are kept at maximum, in order to better reflect the changes induced by stress. When the evaluation of OC requires a change in salinity, animals

Table 1
Effects of different stress factors on OC, osmoregulation and ionic regulation of different species of crustaceans. When available, size, weight, sex, developmental stage, dose or value of exposure to stress, and experimental salinity(ies), are indicated. OC: osmoregulatory capacity (difference between osmotic pressures of hemolymph and external medium, at indicated salinity); OR: osmoregulation (osmotic pressure of hemolymph at the indicated salinity); Na^+R, Cl^-R: sodium, chloride regulation (ionic concentration in hemolymph at the indicated salinity); CTL: cephalothorax length; CTW cephalothorax width; TL: total length; FW: freshwater; SW: seawater; DSW: diluted seawater; ~ : about; ↘ : significant decrease of; ↗ : significant increase of ($p < 0.05$)

Pollutant or stress	Species, stage, size and weight	Dose	Effect on osmoregulation and/ or ionic regulation	References
Oil				
Fuel oil	*Penaeus aztecus* (5 g)	20% solution of water soluble fraction	no effect on OR at 10‰ and 30‰	1
Crude oil	*Homarus americanus* (~ 500 g)	40–500 μg l^{-1} (total oil concentration)	↘ OR by 2% in SW but no effect on Na^+R and Cl^-R	2
Pesticides–PCBs				
Alachlor	*Rhithropanopeus harrisii*	25, 50 mg l^{-1}	no effect on OR during adaptation from 20‰ to 1‰	3
Aroclor 1254 (PCB)	*P. aztecus* (adult, 11.5–13.7 cm TL)	3 μg l^{-1}	no significant effect on OR ↘ Na^+R by 16% at 10‰ ↘ Cl^-R by 19% at 10‰	4
	Palaemonetes pugio (adult and juvenile)	14–60 μg l^{-1}	no effect on OR from 32‰ to 2‰	1
	Pa. pugio (adult and juvenile) (juvenile)	7, 21.5 μg l^{-1}: 1.8 μg l^{-1}:	no effect on Cl^-R from 35‰ to 10‰ ↘ Cl^-R by 10% at 7‰	5
DDT, Mirex	*Callinectes sapidus* (35–50 mm CTW)	0.53 and 3.2 μg/week (DDT) and 0.03, 0.06, 0.14 μg/week (Mirex) in food	no effect on OR, Na^+R, Cl^-R from 0.7‰ to 48‰	6
DDT	*Carcinus maenas* (40 g)	0.2 μg l^{-1}	↘ Na^+R by 18% at 1.7‰	7
Endosulfan	*Oziotelphusa senex senex* (32 ± 1 g)	6.2, 18.6 mg l^{-1}	↘ Na^+R by 7% and 13% in FW ↘ Cl^-R by 10% and 16% in FW	8
Fenitrothion	*P. japonicus* (early adult at stages C and D_0, 43 mm CTL)	0.25, 0.5, 1 μg l^{-1}	↘ OR by 58% in SW (37‰)	9
	P. japonicus (juvenile at stage C, 13 ± 1 g and 20 ± 1 g)	0.5, 0.75, 1 μg l^{-1}	time- and dose-dependent ↘ OC, up to 62% at 37‰ and up to 18% at 19‰	10

	P. stylirostris (juvenile at stage C, 13.5 ± 1.4 g)	6, 8, 10, 12 μg l^{-1}	time- and dose-dependent ↘ OC, up to 22% at 36‰	11
	P. vannamei (juvenile at stage C, 14.2 ± 2.0 g)	5, 10, 15, 20 μg l^{-1}, 25 μg l^{-1}	no effect from 5 to 20 μg l^{-1} in SW (36‰) ↘ OC by 21% at 25 μg l^{-1} in SW (36‰)	11
Lindane	*Gammarus duebeni* (60–90 mg)	3.2–10 μg l^{-1}	↘ Na^+R by 7–12% at 0.7‰	12
Methoxychlor	*Cancer magister* (adult, 80–100 mm CTW)	0.01 mg l^{-1}	no effect on OR from 6‰ to 31‰ no effect on Na^+R	13
	Hemigrapsus nudus (15–30 mm CTW)	0.01 mg l^{-1}	no effect on OR from 9‰ to 34‰	13
Metals				
Aluminium	*Astacus astacus* (adult at stage C_4)	0.25, 0.5, 1 mg l^{-1}	↘ Na^+R by 10–15% in FW (at pH 5.0)	14, 15
	A. astacus (7–10.5 cm TL)	180 μg l^{-1}	↘ Cl^-R by 35% in FW (at pH 6.3)	16
	A. astacus (23–40 g)	25 μmol l^{-1}	↘ OR by 38% in FW (at pH 4) ↘ Na^+R by 44% in FW (at pH 4) ↘ Cl^-R by 17% in FW (at pH 4)	17
	Pacifastacus leniusculus (adult at stage C_4)	0.25, 0.5, 1 mg l^{-1}	↘ Na^+R by 10% in FW (at pH 5.0)	14
Arsenite	*C. maenas* (55–72 mm CL)	0.01, 0.1, 1 mg l^{-1}	no effect on OR during adaptation from 30‰ to 5‰	18
Cadmium	*Jaera albifrons*	10, 20 mg l^{-1}: **10 mg l^{-1}**	no effect on OR at 3.4‰ and 34‰ ↗ OR by ~ 40% at 17‰	19
	Idotea baltica	10 mg l^{-1}	no effect on OR at 27‰	19
	I. emarginata	10 mg l^{-1}	no effect on OR at 27‰	19
	I. neglecta	10 mg l^{-1}	↘ OR at 27‰	19
	P. japonicus (juvenile at stages C and D_0, 13 ± 1 g)	2, 4 mg l^{-1}	time- and dose-dependent ↘ OC, up to 53% at 38‰ and time- and dose-dependent ↘ OC, up to 47% at 16‰	20
	Palaemon elegans (juvenile at stage C, 200–300 mg)	1–7.5 mg l^{-1}	↗ OR by 13% in SW (7.5 mg l^{-1}), no effect on OR in 60% SW, dose-dependent ↘ OR up to 33% in 20% SW	21
	H. americanus (adult)	3–6 μg l^{-1}	no effect on OR at 25 ± 1‰	22
	Ca. sapidus (juvenile, 15–20 mm CTL)	50, 100 μg l^{-1}	dose-dependent ↘ OR up to 25% at 2.5‰	23
	C. maenas (18–30 g)	0.5–8 mg l^{-1}	dose-dependent ↗ OR by 20–50% according to salinity	24

(continued on next page)

Table 1 (*continued*)

Pollutant or stress	Species, stage, size and weight	Dose	Effect on osmoregulation and/or ionic regulation	References
	C. maenas (25–50 g)	1 mg l^{-1}	no effect on OR and Na^+R from 13.6‰ to 24‰	25
	Can. irroratus	25 μg l^{-1}	↘ OR	26
	Chasmagnathus granulata	1.25, 5 mg l^{-1}	↘ Na^+R in 2.5‰ but unaffected in 30‰ no effect on Cl^-R in 2.5‰ and 30‰	27
	Uca rapax	1, 5, 25 mg l^{-1}	Na^+- and Cl^-R: unaffected at 25% and 125% SW	28
Chromium	*Praunus flexuosus*	16 mg l^{-1}	↘ OR by 21, 16 and 12% at 4.5‰, 9‰ and 27‰, respectively. no effect on OR at 18‰	29
Copper	*P. japonicus* (juvenile at stages C and D_0, 13 ± 1 g)	0.5–1.5 mg l^{-1}	time- and dose-dependent ↘ OC, up to 100% at 37‰ and up to 33% at 17‰	30
	Pal. elegans (juvenile at stage C, 200–300 mg)	1–7.5 mg l^{-1}	dose-dependent ↗ OR up to 38% in SW dose-dependent ↘ OR up to 19% in 60% SW and up to 53% in 20% SW	21
	A. leptodactylus and *Orconectes limosus* (42–56 mm)	1 mg l^{-1} (max.)	↘ Na^+R by 30% in FW ↘ Cl^-R by 30% in FW	31
	C. maenas (18–30 g)	2.5–40 mg l^{-1}	dose-dependent ↘ OC, up to 100% at 15‰	24
	C. maenas (adult)	0.25–1 mg l^{-1}	dose-dependent ↘ OR, Na^+R and Cl^-R up to 30%, 12% and 16%, respectively, at 13.6‰	32
	C. maenas (male, 40–70 g)	1, 2 mg l^{-1}	no major effect on Na^+R and Cl^-R at 33‰	33
	C. maenas (male, 40–70 g)	0.5 mg l^{-1}	no major effect on OR at 33.5‰ but ↘ OR, Na^+R and Cl^-R by ~ 14% at 17.5‰	34
	C. maenas (adult, intermolt male)	10 mg l^{-1}	↘ Na^+R, up to 40% at 10‰	35
	C. maenas (55–72 mm CL)	0.1, 0.5, 1 mg l^{-1}	dose-dependent ↘ OR up to 42% (0.5 mg l^{-1}) after transfer from SW (30‰) to DSW (5‰)	18
	C. maenas (adult)	0.75 mg l^{-1}	↘ Na^+R and Cl^-R by 21% and 11% at 10‰ ↗ Cl^-R by 30% at 30‰	36
	Can. irroratus (28–50 g)	0.3–5 mg l^{-1}	dose-dependent ↘ OC, up to 90% at 15‰	24

Lead	*Cherax destructor* (adult at stage C, 75–100 mm)	0.5 mg l^{-1}	no effect on OR, Na^+R and Cl^-R in FW	37
Mercury	*J. albifrons*	1 mg l^{-1}	↗ OR by 5% at 33‰	19
		0.1 mg l^{-1}	↗ OR by 14% at 1.7‰	
	Pal. elegans (juvenile at stage C, 200–300 mg)	20–150 μg l^{-1}	↗ OR up to 7% in SW (0.15 mg l^{-1}) dose-dependent ↘ OR up to 10% in 60% SW and up to 23% in 20% SW	21
	O. propinquus	1 μg/Hg g^{-1}		38
		mercury in water	↘ Na^+R by 21%	
		mercury in food	↘ Na^+R by 17%	
	H. americanus (adult)	3–6 μg l^{-1}	no effect on OR at 25 ± 1‰	22
	C. maenas (adult)	0.5–10 mg l^{-1}	dose-dependent ↘ OR, Na^+R and Cl^-R, up to 20%, 25% and 35%, respectively, at 13.6‰. Effects vary during the year	39
	Petrolisthes armatus	50 μg l^{-1}	no effect on Cl^-R from 7‰ to 35‰	1, 40
	Eriocheir sinensis	10 μg l^{-1}	↘ Na^+R by 40% in FW ↘ Cl^-R by 30% in FW	41
Methylmercury	*G. duebeni* (60–90 mg)	56–320 μg l^{-1}	↘ Na^+R by ~ 8% at 0.7‰	12
Nickel	*Pra. flexuosus*	64 mg l^{-1}	↘ OR by ~ 17% at 4.5‰ and 9‰ no effect on OR at 18‰ and 27‰	29
Zinc	*Pra. flexuosus*	16 mg l^{-1}	↘ OR by 11%, 20% and 9% at 4.5‰, 9‰ and 27‰, respectively no effect on OR at 18‰	29
	G. duebeni	0.5 mg l^{-1}	↘ OR by 12% at 10‰ ↘ Na^+R by 13% at 10‰	42
	J. albifrons	20 mg l^{-1}	↘ OR by 20% at 3.4‰	19
	I. baltica	20 mg l^{-1}	↘ OR by 15% at 34‰	19
	I. neglecta	10 mg l^{-1}	no effect on OR at 27‰ and 34‰	19
	Pal. elegans (juvenile at stage C, 200–300 mg)	1–15 mg l^{-1}	↗ OR up to 9% in SW (15 mg l^{-1}) dose-dependent ↘ OR up to 9% in 60% SW and up to 17% in 20% SW	21
Tributyltin oxide (TBTO)	*P. japonicus* (juvenile at stage C)	100, 200 μg l^{-1}	time- and dose-dependent ↘ OC, up to 79% at 38.5‰ and up to 86% at 19‰	43

(continued on next page)

Table 1 (*continued*)

Pollutant or stress	Species, stage, size and weight	Dose	Effect on osmoregulation and/ or ionic regulation	References
Phenols				
Phenol	*Mesidotea entomon*	50, 80, 120, 180, 270, 400 mg l^{-1}	dose-dependent ↘ Na^+R, up to 25% at 6‰	44
4-Chlorophenol	*M. entomon*	45 mg l^{-1}	↗ Na^+R by 20% at 6‰	44
		100 mg l^{-1}	↘ Na^+R by 10% at 6‰	
Potassium	*H. americanus* (20–55 mm CTL)	0.8 g l^{-1}	no effect on iso-OR from 31 to 48‰	45
Ammonia	*P. japonicus* (juvenile at stages C and D_0, 8.5–11.5 g)	16, 48 mg l^{-1}	dose-dependent ↘ OC, up to 100% at 36‰	46, 47
		32, 48, 64 mg l^{-1}	dose-dependent ↘ OC, up to 40% at 15‰	
	H. americanus (st. IV, stage C):;	32, 64, 96 mg l^{-1}	dose-dependent ↘ OC, up to 47% at 17‰	48
	(adult, 65–75 mm CTL, st. C)	100, 150, 200 mg l^{-1}	dose-dependent ↘ OC, up to 45% at 17‰	
Nitrite	*P. japonicus* (juvenile at stage C, 11.4 ± 1.4 g)	0.01, 0.07, 0.37, 0.72, 1.43 mM	dose-dependent ↗ OC, up to 47% at 30‰	49
	Macrobrachium rosenbergii (23.3 ± 3.5 g)	2, 10 mg l^{-1}	dose-dependent ↘ OR, up to 5.5% at pH 7.3 and up to 4.1% at pH 7.7	50
	A. astacus (18–45 g)	0.8 mM	↘ Na^+R by 5% in FW ↘ Cl^-R by 23% in FW	51
	A. astacus (30–60 g)	0.5, 1 mM	dose-dependent ↘ Na^+R up to 17% in FW dose-dependent ↘ Cl^-R up to 38% in FW	52
	Pac. leniusculus (33 ± 1 g)	1 mM	no significant effect on OR in FW ↘ Na^+R by 12% in FW ↘ Cl^-R by 25% in FW	53
Low pH	*Artemia franciscana* (nauplii)	pH 5.7–8.3	↗ Na^+R by 79% (pH 5.8) and by 32% (pH 6.0) at 35‰ No significant variation from pH 6.5 to 8.3 pH-dependent ↘ Na^+R from pH 7.0 to 5.7, up to 39% at 7‰ pH-dependent ↘ Cl^-R from pH 7.0 to 5.7, up to 64% at 7‰	54
	Ar. franciscana (adult)	pH 4.5–8.3	↗ Na^+R and Cl^-R by ~ 160% (pH 4.5) at 35‰ No significant variation from pH 5.0 to 8.3 pH-dependent ↘ Na^+R from pH 7.0 to 5.25, up to 42% at 7‰	54

			pH-dependent ↘ Cl^-R from pH 7.0 to 5.25, up to 48% at 7‰	
	Branchinecta paludosa	pH 3.0	↘ Na^+R by 61% in FW ↘ Cl^-R by 40% in FW	55
	Daphnia middendorfiana	pH 3.0	↘ Na^+R by 50% in FW ↘ Cl^-R by 16% in FW	55
	Diaptomus arcticus	pH 3.0	↘ Na^+R by 64% in FW ↘ Cl^-R by 44% in FW	55
	Lepidurus arcticus	pH 3.5	↘ Na^+R by 63% in FW ↘ Cl^-R by 76% in FW	55
	P. monodon (juvenile)	pH 5.6	↘ OC by 15% at 30‰ ↘ OC by 7% at 15‰	56
	A. astacus (adult at stage C_4)	pH 4.0	↘ Na^+R by 19% in FW ↘ Cl^-R by 20% in FW	14, 15
	A. astacus (23–40 g)	pH 4.0	↘ OR by 25% in FW ↘ Na^+R by 60% in FW ↘ Cl^-R by 20% in FW	17
	Ch. destructor (adult)	pH 4.5	no effect on OR but ↘ Na^+R by 30% in FW	57
	O. propinquus (4 ± 1 g)	pH 4.0	↘ Na^+R by 21% in FW ↘ Cl^-R by 17% in FW	58
	O. rusticus (15–19 g)	pH 4.0	↘ Na^+R by 15% in FW ↘ Cl^-R by 14% in FW	58
	Procambarus clarkii	pH 5.2	↘ Cl^-R by 11% in FW no effect on Na^+R	59
	Pr. clarkii (40–60 g)	pH 3.8	↘ Na^+R by ~ 20% in FW no effect on Cl^-R	60, 61
Gamma radiation	*Cyathura polita* (juvenile)	44,000 r	↘ OC by 43% at 40‰	62
	Cy. polita (adult)	44,000 r	↘ OC by 16% at 40‰	
Hypoxia	*P. vannamei* (juvenile at stages C and D_0, 10 g)	3 mg O_2 l^{-1}	↘ OC by 20% at 15‰ and 36‰	63
	Pal. adspersus (adult)	O_2: 10–15 mm Hg	↘ Cl^-R by 27% at 15‰	64
	Crangon crangon (4–5 cm TL)	O_2: 10–30 mm Hg	no effect on OR at 10‰ no effect on Na^+R at 10‰ ↘ Cl^-R by 34% at 10‰	65

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Table 1 (*continued*)

Pollutant or stress	Species, stage, size and weight	Dose	Effect on osmoregulation and/or ionic regulation	References
	C. maenas (adult)	0 mm Hg	no effect on OR in SW	66
	C. maenas (50–150 g)	20 Torr	no effect on Na^+R at 13.5‰ ↘ Cl^-R by 10% at 13.5‰	67
Starvation	*Apus* (*L. productus*)		↘ OR	68
	Branchipus sp.		↘ OR	68
	Corophium volutator	72–192 h	↘ OR by 19% at 10‰	69
	D. magna		↘ OR by 30% in FW (?)	70
	P. stylirostris (juvenile at stage C, 9.7 ± 1.3 g)	24 h	↘ OR by 6% in SW	71
Fungal infection	*P. japonicus* (juvenile at stages C and D_0, 13.4 ± 2.8 g)	Injection of *F. oxysporum* in juveniles	↘ hypo-OC by 53% in SW 14 days after infection ↘ hyper-OC by 26% in DSW (20‰) 16 days after infection	72
	A. leptodactylus and *Au. pallipes* (adult at stage C)	36 h of exposure to *Fusarium oxysporum* (conidia)	↘ OR by 14%, 23% and 11% after, respectively, 1, 2 and 3 weeks in FW after infection.	73
Turbidity	*P. japonicus* (juvenile at stages C and D_0, 9.7 ± 1.3 g)	35 ± 15 NTU	↘ hypo-OC by 69% at 42‰ ↘ hyper-OC by 35% at 15‰	74

References: (1) Anderson et al., 1974; (2) Payne et al., 1983; (3) Diamond et al., 1989; (4) Nimmo and Bahner, 1974; (5) Roesijadi et al., 1976; (6) Leffler, 1975; (7) Jowett et al., 1981; (8) Rajeswari et al., 1988; (9) Langy, 1991; (10) Lignot et al., 1997; (11) Lignot et al., 1998b; (12) Inman and Lockwood, 1977; (13) Caldwell, 1974; (14) Appelberg, 1985; (15) Appelberg, 1987; (16) Fjeld et al., 1988; (17) Jensen and Malte, 1990; (18) Bamber and Depledge, 1997; (19) Jones, 1975; (20) Bambang et al., 1995a; (21) Chen and Slinn, 1980; (22) Thurberg et al., 1977; (23) Guerin and Stickle, 1995; (24) Thurberg et al., 1973; (25) Bjerregaard, 1991; (26) Dawson; (in) Thurberg et al., 1977; (27) Vitale et al., 1998; (28) Rojas and Zanders, 1998; (29) McLusky and Hagerman, 1987; (30) Bambang et al., 1995b; (31) Chaisemartin, 1973; (32) Bjerregaard and Vislie, 1986; (33) Boitel and Truchot, 1989; (34) Boitel and Truchot, 1990; (35) Hansen et al., 1992; (36) Weeks et al., 1993; (37) Ahern and Morris, 1998; (38) Wright and Welbourn, 1993; (39) Bjerregaard and Vislie, 1985; (40) Roesijadi et al., 1974; (41) Bouquegneau and Hibaude, cited in Gilles and Péqueux, 1983; (42) Johnson and Jones, 1990; (43) Lignot et al., 1998a; (44) Oksama and Kristoffersson, 1980; (45) Charmantier et al., 1985; (46) Lin et al., 1991; (47) Lin et al., 1993; (48) Young-Lai et al., 1991; (49) Chen and Cheng, 1996; (50) Chen and Lee, 1997; (51) Jensen, 1990; (52) Jeberg and Jensen, 1994; (53) Harris and Coley, 1991; (54) McMahon and Doyle, 1997; (55) Havas and Hutchinson, 1983; (56) Allan and Maguire, 1992; (57) Ellis and Morris, 1995; (58) Wood and Rogano, 1986; (59) Zanotto and Wheatly, 1993; (60) Morgan and McMahon, 1982; (61) McMahon and Stuart, 1989; (62) Kelley and Burbanck, 1972; (63) Charmantier et al., 1994; (64) Hagerman and Uglow, 1981; (65) Hagerman and Uglow, 1982; (66) Spaargaren, 1977; (67) Johnson and Uglow, 1987; (68) Krogh, 1939; (69) McLusky, 1970; (70) Fritsche, 1916; (71) Lignot et al., 1999b; (72) Souheil et al., 1999; (73) Maestracci and Vey, 1989; (74) Lin et al., 1992.

must be kept in the new medium until full osmotic equilibration (Charmantier-Daures et al., 1988). Since the OC value also changes with size, nutritional status, developmental stage and molt stage, only starved animals at the same size and developmental stage, and at intermolt stage C must be used (Lignot et al., 1999b). Controls, not subjected to the studied stress but maintained under the same experimental conditions, must also be used for comparisons of OC values.

2.2. Effect of stress on ionic regulation and OC

The effects of different stress factors on OC, osmoregulation and ionic regulation of crustaceans are summarized in Table 1. They have been reported mostly in decapods, for a lesser part in isopods and amphipods and very seldom in other groups. Stress was mainly applied on animals in hyper-regulating situations, i.e., on marine or brackish water species in dilute media or on freshwater species in their medium. In the vast majority of experimental cases, application of a stress alters osmoregulation and/or ionic regulation and reduces the OC. The reduction is mostly observed for crustaceans living in (or acclimated to) seawater and diluted media, i.e., for hyper–hypo- and hyper–iso-regulators. In isosmotic crustaceans (osmoconformers), alterations in OC and/or ionic regulation are not usually significant since OC is close to zero.

In freshwater and diluted media, significant decreases of hyper-OC range from a few percent to usually 20–50% and up to 90–100% in some cases (Thurberg et al., 1973). Hyper-OC decreases are particularly apparent on exposure to ammonia, low pH, TBTO, fungal infections of the gills, some pesticides and most of the studied metals, particularly aluminium, cadmium and copper (see Table 1). In some cases, exposures to oil, pesticides, PCBs and metals do not affect osmoregulation (Anderson et al., 1974; Caldwell, 1974; Leffler, 1975; Diamond et al., 1989; Bjerregaard, 1991; Bamber and Depledge, 1997; Ahern and Morris, 1998). These observations might result from the application of too low doses, over too short exposure times, from specific differences of sensitivity to pollutants and/or from the absence of interference of these pollutants with osmoregulatory processes. In some rare occurrences, exposure to pollutants increases OC or sodium regulation, as in the case of cadmium and mercury in *J. albifrons* (Jones, 1975) and 4-chlorophenol in *M. entomon* (Oksama and Kristoffersson, 1980). In the latter species, however, a higher dose of 4-chlorophenol lowers sodium regulation. Although little is known about the threshold of pollutant detection, an activation of ionic uptake following pollutant exposure could be initiated in some species as the first reaction to a change of the ambient medium, in order to maintain the internal hemolymph homeostasis. This compensating mechanism might also explain the lack of effect or even some of the observed OC increases.

In seawater, stress exposure generally induces a decrease in hypo-OC and/or Na^+ and Cl^- regulation. This is particularly apparent on exposure to ammonia, low pH and most of the metals studied, particularly aluminium, cadmium and copper. Hypo-OC can decrease up to 100%, rendering the animals isosmotic with the medium, as in *P. japonicus* exposed to ammonia (Lin et al., 1991) or to copper (Bambang et al., 1995b). As in low-salinity media, osmoregulation in seawater may not be affected by pollutants in crustaceans that are either weak hyporegulators (Thurberg et al., 1977) or isosmotic

Table 2
Effects of different stress and pollutants on the morphology, histological structure and ultrastructure of branchial chambers and excretory organs. When several concentrations or several times of exposure to pollutants were used, the maximum deleterious effect on tissues was described. H: hour; CL: carapace length; TL: total length; FW: freshwater; SW: seawater; S‰: salinity

Pollutant	Species, size and weight	Dose, time and medium of exposure	Effect on morphology (M), histological structure (H) and ultrastructure (U)	References
Branchial organs				
Oil and dispersants				
Crude oil	*Charybdis lucifera*	0.1–0.8 ml oil l^{-1}; 1 day; S = 35.5‰	H: branchial cell necrosis, rupture and fusion of lamellae, accumulation of hemocytes.	1
	Scylla serrata	1–4 ml oil l^{-1}; 1 day; S = 35.5‰	H: branchial epithelial damage but no lamellar disruption or fusion.	1
Oil emulsions and Dispersants	*Pal. serratus*	200–5000 mg l^{-1}	H: swelling of gill lamellae, cell vacuolization, nuclear pycnosis, rupture of pillar cells.	2
Pesticides, fungicides				
Busan 85	*Pa. pugio* (22–28 mm TL)	5–60 μg l^{-1}; 14 days; S = 15‰	M, H: swelling of gill lamellae, blackened tip of lamellae, accumulation of hemocytes. U: cytolysis of branchial epithelial cells, swollen mitochondria with sparse cristae, dilated rough endoplasmic reticulum without attached ribosomes.	3
Fenitrothion	*P. japonicus* (13–20 g)	0.5, 0.75, 1 μg l^{-1}; 4 days; SW	H: gill and epipodite necrosis, accumulation of particles between the branchial lamellae, breakage of the	4

			cuticular lining, hemocytic congestion (thrombosis).	
	Ma. kistnensis (50 ± 10 mm TL, 0.6 ± 0.1 g)	3.1 μg l^{-1}, 14 days; 0.097 μg l^{-1}, 30 days	H: necrosis of gill tissue, hemocytic congestion and separation of the cuticle from the epithelium.	5
Gusathion A	*P. monodon*	75, 150 μg l^{-1}, 4 days	H: hyperplasia of the gill epithelium, degeneration and necrosis of gill tissue.	6
Metals				
Aluminium	*A. astacus* (7–10.5 cm TL)	80 μg l^{-1} pH 6.7; 10 days; FW	M, H: brown colored gill filaments, shrunken with an irregular surface. U: branchial epithelial cells severely degraded, electron dense layer on surface of the epithelium.	7
Cadmium	*Ja. nordmanni*	10, 20 mg l^{-1}; 8–24 h; S = 10% SW	H: numerous hemocytes in gill haemolymph spaces. U: gill basal membrane infoldings fragmented, distended microvilli, dilated endoplasmic reticulum, swollen mitochondria.	8
	P. duorarum (43–100 mm TL)	5 mg l^{-1} 4 days; 1 mg l^{-1} 21 days; S = 20‰	M, H: partly blackened gills, accumulation of hemocytes, necrosis of epithelium, dissolution of exo- and endocuticular layers.	9
	P. duorarum	763 μg l^{-1} 15 days; S = 20‰	M, H: partly blackened gills, disorganization of the epithelial structure, necrosis.	10

(continued on next page)

Table 2 (*continued*)

Pollutant	Species, size and weight	Dose, time and medium of exposure	Effect on morphology (M), histological structure (H) and ultrastructure (U)	References
			U: gill cell vacuolization, cellular membrane degeneration, nuclear pycnosis.	
	P. japonicus (12 ± 1 g)	2000, 4000 μg l^{-1} 4 days; SW	M, H: blackened and necrosed gills and epipodites, thickening of the epithelial layer, swollen epipodites, separation of the cuticle from the epithelium. U: increased number of nephrocytes in the gills, autolysis and loss of cytoplasmic organelles, microvilli and basal lamina, swollen mithochondriae, vacuolization and nuclear pycnosis.	11
	Paratya tasmaniensis	30, 50 μg l^{-1} 4 days; FW	U: branchial mitochondria degeneration, dilatation of intercellular spaces and of the rough endoplasmic reticular system.	12
	Pal. serratus	5, 25, 50 mg l^{-1}; 44 h; S = 30‰	U: mitochondria in the gills are the most affected organelles (disruption of the bounding membranes, loss of cristae, swelling), impairment of the pinocytotic process and of the membrane system involved in osmoregulation, fewer infoldings of the apical and basal plasma membrane.	13

	Pa. vulgaris	75 μg l^{-1}; 10 days; S = 20‰	H: melanized and necrotic gill lamellae distended by numerous hemocytes.	9
	Cr. crangon	5, 25, 50 mg l^{-1}; 20 h; S = 30‰	U: mitochondria in the gills are the most affected organelles, swollen endoplasmic reticulum, fewer invaginations of the apical plasma membrane and more cytoplasmic vesicles than in controls.	14
	Pr. clarkii (20.5–30.4 g)	1 mg l^{-1}; 4 days; FW	H: extensive disorganization of the gill filaments, the medial septum cannot be observed.	15
Chromium	*Pa. pugio* (22–28 mm TL)	0.5, 1, 2, 4 mg l^{-1}; 30 days; S = 10‰	H: relatively few degenerative changes in the gills, lamellar irregularity. U: decrease in epithelial cytoplasmic density in the gill filaments, swollen mitochondria with fragmented cristae, diminution of plasmalemmal infoldings.	16
Copper	*Ja. nordmanni*	1, 10 mg l^{-1}; 8–24 h; S = 10% SW; 8–96 h; S = 50% SW	U: numerous hemocytes, irregularly outlined microvilli, large intracellular vacuoles, disrupted infolded basal membrane, swollen mitochondria with a reduction in cristae, dilated and fragmented endoplasmic reticulum.	8
	P. japonicus (12–15 g)	500, 1000 μg l^{-1}; 4 days; SW	M, H: dose-dependent blackening of gills and	17

(continued on next page)

Table 2 (*continued*)

Pollutant	Species, size and weight	Dose, time and medium of exposure	Effect on morphology (M), histological structure (H) and ultrastructure (U)	References
			epipodites, necrosis, swollen epipodites. U: increased number of gill nephrocytes, swollen epithelial cells, vacuolization, degraded microvilli and basal infoldings and disorganized cytoplasmic structures.	
	Ma. kistnensis	50, 100 μg l^{-1}; 6–8 days; FW	M, H: no blackened gills, distension of gill lamellae, vacuolization and necrosis of gill tissue.	18
	Caridina sp.	50, 100 μg l^{-1}; 6–8 days; FW	H: partly blackened gills, distension of gill lamellae, vacuolization and necrosis of gill tissue.	18
	C. maenas (50–80 g)	0.5, 2 mg l^{-1}; 5–6 days; S = 30–33‰	U: gill necrosis, cellular hyperplasia, vacuolization, thickening of the gill epithelium and reduction of haemolymph spaces.	19
	C. maenas (15–20 g; 40–45 mm CL)	50 μg l^{-1}; 10 days; S = 10, 35‰	U: decrease in the number of plasma membrane infoldings and associated mitochondria in the gill epithelium, vacuolization, change in ribosomal distribution, disruption of the microtubular network.	20
Lead	*Pr. clarkii* (21.5–28.4 g)	200 mg l^{-1}; 4 days; FW	M, H: gill filaments grossly blackened, pronounced	21

			disorganization, medial septum not visible.	
	Pr. clarkii (20.5–30.4 g)	100 mg l^{-1}; 4 days; FW	M, H: gross blackening of almost all gill filaments observed, irregular profiles of the filaments, structural disorganization.	14
	Pr. clarkii	100 mg l^{-1}; 4 days; FW	H: branchial epithelial layer separated from the cuticle, reduction of cellular organites, nuclear degeneration, numerous vesicles containing electron dense material.	22
Mercury	*Ja. nordmanni*	0.1, 1 mg l^{-1}; 8–24 h; S = 10% SW	H: numerous hemocytes, cellular break down and large sub-cuticular spaces. U: irregularly outlined microvilli, large intracellular vacuoles, disrupted infolded basal membrane, swollen mitochondria with a reduction in cristae, dilated and fragmented endoplasmic reticulum.	8
	Cr. crangon (4.5–6.6 cm TL)	17 μg l^{-1}; 14 days; S = 20‰	H: accumulation of mercury on the gills surface and within the lamellar epithelial cells, the septum and the hemocytes. U: mercury deposits in lysosome-like bodies and mainly in nephrocytes.	23
Zinc	*Ma. hendersodyanum* (1.1 g)	3 mg l^{-1} 15 days; 6–15 mg l^{-1} 4 days; FW	H: gill lamellae disrupted, accumulation of hemocytes, necrosis of epithelium, nuclear pycnosis.	24

(continued on next page)

Table 2 (*continued*)

Pollutant	Species, size and weight	Dose, time and medium of exposure	Effect on morphology (M), histological structure (H) and ultrastructure (U)	References
TBTO	*P. japonicus* (9–12 g)	200, 400 μg l^{-1}; 4 days; SW	H: multiple gill and epipodite necrosis, hemocytic congestion, severe nephrocyte hyperplasia.	25
Fungal infection	*A. leptodactylus, Au. pallipes* (adults)	36 h of exposure to *Fusarium oxysporum* (conidia), up to 3 weeks; FW	H: black spots on the gills with melanotic deposit in contact with the hyphae, hemocytic reaction with encapsulation processes. U: cellular alteration of the gill epithelium, vacuolization, dilatation and alteration of mitochondria, microvilli acquiring a loose structure.	26
Antennal glands				
Fungicides				
Busan 85 Aquareat (DNM 30)	*Pa. pugio* (22–28 mm TL)	5–60 μg l^{-1}; 14 days; SW 40–120 μg l^{-1}; 3–35 days; S = 10‰	U: labyrinth: channel virtually occluded, microvilli distended and ruptured, dilated mitochondria, numerous vesicles; coelomosac: deterioration of podocyte organization, nuclear pycnosis.	27

Metals				
Chromium	*Pa. pugio* (22–28 mm TL)	0.5, 1, 2, 4 mg l^{-1}; 30 days; S = 10‰	U: labyrinth: cytoplasmic vacuolization, swollen mitochondria with vesiculization of cristae, exfoliation of microvilli, decrease in number and extent of the basal membrane infoldings, exfoliation of cells; coelomosac: epithelium generally normal.	16
Copper	*O. rusticus* (12–15 g)	0.5 mg l^{-1}; 2 days; FW	H: labyrinth: cells vacuolized then completely necrosed; nephridial canal: less affected.	28
Mercury	*Cr. crangon* (4.5–6.6 cm TL)	17 μg l^{-1}; 14 days; S = 20‰	H: coelomosac: blackened cells. U: labyrinth: mercury deposits within lysosomes and along the basal lamina; coelomosac: large black inclusions in vacuoles and cytoplasm of podocytes and along the basal membrane.	23
	Pac. leniusculus	2.5 mg l^{-1}; 2 days; FW	H: labyrinth, coelomosac: nuclear pycnosis, cellular disruption.	29

References: (1) Chandy and Kolwalkar, 1984; (2) Papineau and Cheze, 1984; (3) Pawar and Katdare, 1982; (4) Lignot et al., 1997; (5) Doughtie and Rao, 1983a; (6) Baticados and Tendencia, 1991; (7) Fjeld et al., 1988; (8) Bubel, 1976; (9) Nimmo et al., 1977; (10) Couch, 1977; (11) Soegianto et al., 1999a; (12) Lake and Thorp, 1974; (13) Papathanassiou and King, 1983; (14) Papathanassiou, 1985; (15) Torreblanca et al., 1989; (16) Doughtie and Rao, 1984; (17) Soegianto et al., 1999b; (18) Ghate and Mulherkar, 1979; (19) Nonnotte et al., 1993; (20) Lawson et al., 1995; (21) Torreblanca et al., 1987; (22) Rubio et al., 1991; (23) Andersen and Baatrup, 1988; (24) Patil and Kaliwal, 1989; (25) Lignot et al., 1998a; (26) Maestracci and Vey, 1989; (27) Doughtie and Rao, 1983b; (28) Hubschman, 1965; (29) Ellis and Fuller, 1979.

with the external seawater (Roesijadi et al., 1974; Jones, 1975; Thurberg et al., 1977; Boitel and Truchot, 1989, 1990; Bjerregaard, 1991). Osmoregulation can even be increased by pollutants (Jones, 1975; Chen and Slinn, 1980; McMahon and Doyle, 1997).

Most authors do not indicate whether or not they used individuals at selected molt stages (stage C usually). Water permeability, water content and the concentration of inorganic ions in the hemolymph are known to change during the molt cycle (Robertson, 1960; Bursey and Lane, 1971; Lockwood, 1977; Exbrayat and Bourguet, 1982; Mantel and Farmer, 1983; Wheatly and Ignaszewski, 1990), resulting in variations of the hemolymph osmotic pressure and therefore of OC (Mantel and Farmer, 1983; Ferraris et al., 1987; Charmantier et al., 1994). If molt stage selection is not carried out prior to measuring OC or ion concentrations, results can be variable enough to either decrease the difference between control and exposed groups, or even abolish any significant difference. This might account for several "no effect" results in Table 1.

2.3. Effect of toxicant-induced stress on the structure and ultrastructure of the branchial chambers and excretory organs

Ion-transporting cells or ionocytes, located in the gills and epipodites, in extra-branchial epithelia (e.g., pleurae, branchiostegites) and in antennal glands are characterized by the presence of apical microvilli and basolateral membrane infoldings associated with numerous mitochondria (Berridge and Oschman, 1972; Cioffi, 1984; Taylor and Taylor, 1992; Bouaricha et al., 1994; Haond et al., 1998). The ionocytes show extensive structural changes within the same species according to external salinity (Compère et al., 1989; Shires et al., 1994; Freire and McNamara, 1995; Haond et al., 1998).

Ion-transporting branchial organs are severely affected by toxicant-induced stress (Table 2). Gills and epipodites of crustaceans exposed to pollutants and fungal infections generally blacken and are the site of necrosis and hemocytic congestions. At the ultrastructural level, damages to branchial organs have been reported in numerous studies carried out on crustaceans exposed mainly to metals (Table 2). Common features of cellular alterations are fewer and swollen mitochondria, nuclear pycnosis, intracellular vacuolization, decrease or fragmentation of the basolateral membrane infoldings and occurrence of pseudomyelinic structures. In the antennal glands of decapods, the effects of pollutants generally result in alterations in the structure and ultrastructure of the epithelial cells of the coelomosac and labyrinth, with necrosis, nuclear pycnosis, swollen mitochondria and vacuolization.

The blackening and concomitant disorganization of the ion-transporting epithelial cells (tissue necrosis, thrombosis, vacuolization, disruption of the basolateral infoldings) are usually time- and dose-dependent and appear within a few days of exposure. The progressive destruction of the transporting cells after exposure to stressors can be related to the ambient salinity (Lawson et al., 1995) and can partially explain the decrease of OC. It is generally accepted that the influx of Na^+ ions across the ionocytes involves a two-step process implicating a Na^+,K^+-ATPase pump and a Na^+/H^+ antiporter (Siebers et al., 1985, 1987; Péqueux et al., 1988; Lucu, 1990; Towle, 1990; Pierrot et al.,

1995; Péqueux, 1995), respectively located along the basolateral infoldings and the apical microvilli (Towle and Kays, 1986; Péqueux et al., 1988; Towle, 1990; Taylor and Taylor, 1992; Lignot et al., 1999a). The observed alterations of these plasma membranes may therefore decrease the surface area available for ion-exchange, disrupt the function of the ion-transferring sites, particularly the sodium pumps, and thus impair ionic exchanges and osmoregulation mechanisms. The decrease in the number of mitochondria and the disruption of their internal membranes (swelling) where ATP is synthesized, can also result in the reduction of available energy necessary for the ion-exchanging pumps. Vacuolization may also indicate a failure to regulate water content. The rate of entry of water into the cell and then, into the hemolymph, can furthermore be decreased by the subcuticular spaces and vacuoles creating a longer pathway for water movement (Bubel, 1976; Lawson et al., 1995).

2.4. Stress effects on the activity of the Na^+,K^+-ATPases in the gills

The effects of stressors on the gill Na^+,K^+-ATPase activity are summarized in Table 3. Na^+,K^+-ATPase activity generally decreases after exposure to stress despite varying effects according to the pollutants. A discrepancy between in vivo and in vitro response of Na^+,K^+-ATPase activity to stress also exists. In *C. maenas* exposed to DDT, a surprising opposite response in vivo and in vitro has been observed (Jowett and Rhead, 1978; Jowett et al., 1981). In *Ch. destructor* exposed to lead and in *Cha. granulata* exposed to cadmium, gill Na^+,K^+-ATPase also appeared more sensitive to the pollutant in vitro than in vivo (Ahern and Morris, 1998; Rodriguez-Moreno et al., 1998). This could result from the binding of the pollutant to intracellular proteins or organelles (Andersen and Baatrup, 1988; Rodriguez-Moreno et al., 1998).

Since Na^+,K^+-ATPase is directly involved in active ion transport, its functional disruption can be related to the ionic and osmotic alterations observed in Table 1. This applies to *C. maenas* exposed to DDT (Jowett et al., 1981), to *A. leptodactylus*, *O. limosus* and *C. maenas* exposed to copper concentrations (Chaisemartin, 1973; Hansen et al., 1992) and to *Ch. destructor* exposed to chronic sublethal lead concentrations (Ahern and Morris, 1998). The observed decrease of the Na^+,K^+-ATPase activity might be due to the inhibition of the phosphorylated enzyme intermediate necessary for ion translocation (Ahern and Morris, 1998) and/or to the disruption of the basolateral infolding membranes within the ionocytes, as mentioned above. A decrease in Na^+,K^+-ATPase activity has also been observed in *Can. magister* after sublethal acute exposure to methoxychlor (Caldwell, 1974) and in *Ch. destructor* after long-term exposure to lead (Ahern and Morris, 1998), despite the absence of any effect on ionic regulation. These alterations have been explained either by the fact that the degree of inhibition of the enzyme may be insufficient to significantly affect osmotic and ionic regulation, or by increased ion uptake and reabsorption from the urine in the freshwater species in order to regulate the ionic homeostasis.

In a few cases, the Na^+,K^+-ATPase activity increases in stress-exposed animals (Jowett and Rhead, 1978; Lin et al., 1992, 1993). A short-term increase in Na^+,K^+-ATPase activity can result from hormonal stimulation by biogenic amines like dopamine

Table 3
Effects of different stress and pollutants on the activity of the gill enzyme Na^+,K^+-ATPase. Abbreviations as in Tables 1 and 2; in vitro: pollutant added to the incubation medium of gill homogenates or membranes

Pollutant	Species (length and weight)	Dose, time and medium of exposure	Effect on Na^+,K^+-ATPase activity	References
Oil and dispersants				
Dispersants	*Pal. serratus*	2000–15,000 mg l^{-1}; 24 h; SW	↘ 17–30%	1
Emulsions	*Pal. serratus*	200–1000 mg l^{-1}; 24 h; SW	↘ 22–57%	1
Oil	*Pal. serratus*	5000 mg l^{-1}; 24–96 h; SW	↘ 46–49%	1
Pesticides				
DDT	*C. maenas*	0.03–3500 μg l^{-1} (in vitro)	↗ 217–140%	2
	C. maenas (~ 40 g)	0.2 μg l^{-1}; 25 days; 15% SW	↘ 43.5%	3
	Can. irroratus (170–250 g)	0.5–4 mg l^{-1} (in vitro)	↘ 30–38%	4
		0.1 mg kg^{-1} weight (injection)	↘ 4 h: 26% 30 h: 41% 50 h: 42%	
Endosulfan 35 EC	*Barytelphusa guerini* (male, 25.0 ± 0.5 mm)	6 μg l^{-1}; 4 days; FW	↘ 15% and 22% after 72 and 96 h of exposure	5
Methoxychlor	*Can. magister* (80–100 mm CTW)	0.01 mg l^{-1}; 14 days; S = 30–33‰	↘ 50%	6

Metals							
Cadmium	*Pr. clarkii* (~ 30.5 g)	1 mg l^{-1}; 4 days; FW	no effect				7
	H. americanus	6 μg l^{-1}; 30 days; S = 24–26‰	no effect				8
		control: 4 μg Cd g^{-1} dry gills contaminated: 70 μg Cd g^{-1} dry gills	no effect				9
	Can. irroratus	1, 10 mg l^{-1} (in vitro)	1 mg l^{-1}: no effect 10 mg l^{-1}: ↘ 37%				10
	Sc. serrata (~ 25 g, 4.5–5.5 cm TL)	0.3–24 mg l^{-1}; 4–30 days; S = 30‰	0.3 mg l^{-1}	0.6 mg l^{-1}	1.5 mg l^{-1}	24 mg l^{-1}	11
		4 days				↘ 62%	
		10 days	↗ 11%	↘ 47%	↘ 58%		
		20 days	↘ 26%	↘ 63%	↘ 71%		
		30 days	↘ 26%	↘ 30%	↘ 45%		
	Cha. granulata (28.0 ± 0.7 mm CTW)	0.1, 0.25 mg l^{-1}: 18 days; S = 30‰ (in vivo)	no significant effect				12
		0–100 mg l^{-1}: 40 min (in vitro)	dose-dependent ↘ up to 97% with 100 mg l^{-1} Cd^{2+}				
Copper	*A. leptodactylus* and *O. limosus*	0.03–3 mg l^{-1} (in vitro)	0.03 mg l^{-1}: no effect 1 mg l^{-1}: ↘ 60% 3 mg l^{-1}: ↘ 87%				13
	C. maenas (adult)	10 mg l^{-1}	↘ 50%				14
Lead	*Ch. destructor* (adult at stage C, 75–100 mm)	0.5 mg l^{-1}; 21 days; FW (in vivo)	↘ 40%				15
		120, 240 mg l^{-1}; 21 days; FW (in vitro):	↘ 50% and 80%, respectively				

(continued on next page)

Table 3 (*continued*)

Pollutant	Species (length and weight)	Dose, time and medium of exposure	Effect on Na^+,K^+-ATPase activity	References
	Pr. clarkii (~ 30.5 g)	100 mg l^{-1}; 4 days; FW	no effect	7
	Can. irroratus	1, 10 mg l^{-1} (in vitro)	no effect	10
Mercury	*Pr. clarkii* (~ 30.5 g)	0.25 mg l^{-1}; 4 days; FW	no effect	7
Zinc	*H. americanus* (476 ± 47 g)	25 mg l^{-1}; 4 days; SW	↘ 67%	16
Mixture of cadmium, copper and zinc	*Nephrops norvegicus* (male: 3.63 ± 0.07 cm, female: 3.62 ± 0.10 cm)	Cd: 1, 5, 25 μg l^{-1}; 18 days; SW Cu: 1, 5, 25 μg l^{-1}; 18 days; SW Zn: 8, 40, 200 μg l^{-1}; 18 days; SW	1, 1, 8 μg l^{-1}: no effect 5, 5, 40 μg l^{-1}: ↘ 43% 25, 25, 200 μg l^{-1}: ↘ 30% no effect on female	17
TBTO	*P. japonicus* (juvenile at stage C, 9–12 g)	100, 200 μg l^{-1}; 4 days; SW	no effect	18
Ammonia	*P. japonicus* (juvenile at stages C and D_0, 8.5–11.5 g)	48 mg N l^{-1}; 2 days; S = 36‰	↗ 20.5%	19
Turbidity	*P. japonicus* (juvenile at stages C and D_0, 9.7 ± 1.3 g)	35 ± 15 NTU; 3 weeks; S = 15‰ and 42‰ 65 ± 15 NTU; 3 weeks; S = 15‰ and 42‰	S = 15‰: ↗ 33.5% S = 42‰: ↗ 16.5% S = 42‰: ↗ 35%	20

References: (1) Papineau and Le Gal, 1983; (2) Jowett and Rhead, 1978; (3) Jowett et al., 1981; (4) Neufeld and Pritchard, 1979; (5) Reddy et al., 1992; (6) Caldwell, 1974; (7) Torreblanca et al., 1989; (8) Tucker, 1979; (9) Haya et al., 1980; (10) Tucker and Matte, 1980; (11) Dhavale et al., 1988; (12) Rodriguez-Moreno et al., 1998; (13) Chaisemartin, 1973; (14) Hansen et al., 1992; (15) Ahern and Morris, 1998; (16) Haya et al., 1983; (17) Canli and Stagg, 1996; (18) Lignot et al., 1998a; (19) Lin et al., 1993; (20) Lin et al., 1992.

known to stimulate Na^+,K^+-ATPase activity in *C. maenas* (Sommer and Mantel, 1988), while long-term activation can be due to a higher concentration of gill Na^+,K^+-ATPase (Wheatly and Gannon, 1995). This increased activity could also result from a compensating mechanism for the impairment of ionic regulation, e.g., from an increased surface permeability (Lin et al., 1993).

2.5. Stress effects on water and ionic fluxes and surface permeability

The effects of pollutants and stress on ionic fluxes and surface permeability are presented in Table 4. In general, the permeability and net ion outflux increase whereas the water outflux and net ion influx decrease (Chaisemartin, 1973; Inman and Lockwood, 1977; Spaargaren, 1990; Harris and Coley, 1991; Wright and Welbourn, 1991; Rasmussen et al., 1995). These exchanges thus result in a decrease in hemolymph ionic concentrations and OC (Chaisemartin, 1973; Inman and Lockwood, 1977; Harris and Coley, 1991). In stress-exposed crustaceans, hemolymph circulation and movements of water around the branchial ion-transporting organs are also decreased through reduced ventilation and cardiac activity (Depledge, 1984; Nonnotte et al., 1993), therefore lowering gill perfusion and ionic influx. It has also recently been demonstrated that the cadmium inhibitions of Na^+,K^+-ATPase and active ion influxes in *C. maenas* are directly interdependent (Postel et al., 1998). Structural lesions of the ion-transporting epithelia also likely contribute to the reduction in ion influx and to the increase in permeability. The decrease in water outflux might be related to the appearance of vacuoles and of subcuticular spaces in ionocytes. Permeability and active transport are also closely interrelated (Spaargaren, 1990). An increase in permeability also usually results in a higher passive ion outflux that would therefore require a compensating increase in Na^+,K^+-ATPase activity, as observed in *P. japonicus* exposed to low ammonia concentrations (Lin et al., 1993). The reduction of Na^+ influx can thus be correlated to the functional disruption of Na^+,K^+-ATPase also linked to the impairment of the ion-transporting epithelia.

3. Conclusion

It appears from the available literature that exposure to stress most often results in a disruption of ionic regulation (mostly of Na^+ and Cl^- regulation) and therefore of osmotic regulation in osmoregulating crustaceans. From the data gathered in Table 1, the ability to ionoregulate or to osmoregulate following exposure to stress was affected in 79% of the species, with a decrease of this ability in 90% of them. In cultured species, osmoregulation was disrupted in 93% of peneid shrimp and in 100% of crayfish exposed to various stresses. Several thorough studies have shown that the effect of stress upon osmotic and ionic metabolism was time- and dose-dependent. When data are available, they most often demonstrate that the changes in iono- or osmoregulation are induced by sublethal doses of stressors.

Table 4
Effects of different stress and pollutants on water and ionic fluxes and surface permeability. Fluxes were measured in total organisms except for Chaisemartin (1973, perfused gills). Abbreviations as in Tables 1 and 2

Pollutant or stress	Species, size and weight	Dose, time, medium of exposure	Effect on water and ionic fluxes and surface permeability	References
Pesticides				
Lindane	*G. duebeni* (50–80 mg)	1.8 μg l^{-1}; 2 days; S = SW and 2% SW	After transfer in 2% SW: Na^+ influx: ↘ 28% Na^+ outflux: ↘ 17%	1
Aroclor 1254 (PCBs)	*Pa. pugio*	31–51 μg l^{-1}; 4 days; S = 2, 17 and 32‰	Cl^- outflux: no effect at S = 2‰, 17‰ and 32‰ Water outflux: no effect at S = 2, 32‰ but ↘ 66% at S = 17‰	2
Metals				
Cadmium	*C. maenas* (31–41 mm CTW)	4, 8 mg l^{-1}; 5 days; S = 14‰	Water outflux: ↘ 68% (4 mg l^{-1}) and 71% (8 mg l^{-1})	3
Copper	*A. leptodactylus*, *O. limosus*	0.3 mg l^{-1} in incubation medium of perfused gills	Na^+ influx: ↘ Na^+ outflux: ↗	4
Lead	*C. maenas* (31–41 mm CTW)	1 mg l^{-1}; 1 h; S = 15‰	Water outflux: ↘ 62%	3
	Ch. destructor (75–100 mm)	0.5 mg l^{-1}; 21 days; FW	Na^+ influx: ↘ 50% Na^+ outflux: no effect	5

Methylmercury	*G. duebeni* (50–80 mg)	100 μg l^{-1}; 2 days; S = SW and 2% SW	After transfer in 2% SW: Na^+ influx: ↘ 20% Na^+ outflux: ↘ 14%	1
	As. aquaticus	8–1000 μg l^{-1}; 2 h; FW	Na^+ influx: ↘ 56–79% Ca^{2+} influx: ↘ 67–72%	6
Mercury	*As. aquaticus*	8–1000 μg l^{-1}; 2 h; FW	Na^+ influx: ↘ 50–56% Ca^{2+} influx: ↘ 43–78%	6
	C. maenas (31–41 mm CTW)	1 mg l^{-1}; 1 h; S = 15‰	Water outflux: ↘ 71%	3
Zinc	*Cr. crangon* (36–47 mm TL)	500 μg l^{-1}; 1, 3 days; S = 15‰	Water outflux: ↘ ~82% (1 day) and ~76% (3 days)	3
Nitrite	*Pac. leniusculus* (33.0 ± 1.1 g)	46 mg l^{-1}	Cl^- influx: ↘ 41% Cl^- outflux: ↘ 20%	7
Ammonia	*A. pallipes* (10 g)	0.2–1 mg l^{-1}; FW	Na^+ influx: ↘ 20–80%	8
	C. maenas (15–60 g)	3.6–36 mg l^{-1}; 2–10 min; S = 24‰	ionic surface permeability: ↗ net salt outflux: ↗	9
Low pH	*A. pallipes* (10 g)	pH 4.0	Na^+ influx: ↘ 70–80%	8

References: (1) Inman and Lockwood, 1977; (2) Roesijadi et al., 1976;(3) Rasmussen et al., 1995; (4) Chaisemartin, 1973; (5) Ahern and Morris, 1998; (6) Wright and Welbourn, 1991; (7) Harris and Coley, 1991; (8) Shaw, 1960; (9) Spaargaren, 1990.

The disruption of osmoregulation due to stressors originates from physiological and cellular alterations. The activity of Na^+,K^+-ATPase was — most often negatively — affected in 74% of species exposed to stressors. Water and ion fluxes were also altered in all reported cases. Stressors also induced different types of damage to the transporting epithelia located in the branchial chambers and/or the excretory organs, with different levels of cellular alterations affecting the ionocytes of these organs.

It therefore appears that an integrated series of events occurs in most crustaceans following exposure to a supra-liminal, but sublethal, level of stressor. According to the physico-chemical nature of the stressor, it can induce deleterious effects on the cellular structure of ion-transporting epithelia and/or variations in the activity of the enzyme Na^+,K^+-ATPase. These events lead to alterations in water and ionic fluxes and surface permeability, finally resulting in disruption of ionic regulation and thus of osmotic regulation.

As underlined above, the effect of stressors on osmoregulation is usually detectable at sublethal levels, often before any mortality is noticeable among exposed animals. The variations in osmotic and ionic regulation, and thus in OC and/or Na^+ or Cl^- regulation, can therefore be considered as an early warning of sublethal stress in crustaceans.

We thus confirm that one of these parameters, in particular OC, can be used as a reliable biomarker to monitor the physiological condition and the effect of stressors in osmoregulating crustaceans (Charmantier et al., 1989; Young-Lai et al., 1991; Lin et al., 1992, 1993; Bambang et al., 1995a,b; Charmantier et al., 1994; Lignot et al., 1997, 1998a,b). From experimental studies conducted under laboratory conditions, the use of OC as a stress biomarker has been successfully applied in peneid shrimps under pilot medium-scale culturing conditions (Lignot et al., 1999b) and has begun to be used in aquaculture farms (Cochard et al., 1997).

Future research might concentrate in several directions. Further stressors should be tested on the osmoregulatory ability of diverse species of crustaceans, leading to an improved knowledge and understanding of the effect of different stress on the physiology of ionic and osmotic regulation. The cellular and molecular effects of low levels of stressors should be studied through electron and confocal microscopy, immunocytochemistry, etc...., and through molecular approaches, to detect their effect on the specific structures of ionocytes (apical microvilli, basolateral infoldings, mitochondria), and the location, activity and regulation of Na^+,K^+-ATPase and of other enzymes involved in osmoregulation such as carbonic anhydrase and V-ATPases. The determination of OC variations can also be used to assay endocrine and neuroendocrine factors susceptible to control osmotic and ionic regulation (McNamara et al., 1991; Freire and McNamara, 1992; Charmantier-Daures et al., 1994). Another direction of research should be to continue studies in cultured species, mostly in peneid shrimp, and also in crayfish and *Macrobrachium* species. Their objective would concentrate on the practical use of OC as a biomarker. Some types of stress, in particular pathological stress, have been seldom studied as yet (Maestracci and Vey, 1989; Souheil et al., 1999). The effects on osmoregulation of viral, bacterial and fungal infections affecting cultured species should be studied, with the aim of also using OC as an early warning of these fast-spreading diseases.

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