Ecophysiology and stress response of marine and brackish water *Gammarus* species (Crustacea, Amphipoda) to changes in salinity and exposure to cadmium and diesel-oil

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ABSTRACT: There is a current debate whether organisms living in estuaries should be more sensitive to pollution than their marine relatives, but few comparative studies have been made. Amphipods of the genus *Gammarus* are generally euryhaline and 5 species are common to the brackish Baltic Sea (7 % S) and the North Sea (30 % S). Laboratory measurements on the metabolism of *G. duebeni* and *G. oceanicus* from the 2 areas showed that respiration and oxygen:nitrogen ratios of *G. duebeni* were generally less affected by salinity changes and/or additions of diesel oil and cadmium than *G. oceanicus*. We interpret this as a higher tolerance of *G. duebeni* to pollutants and salinity changes, probably coupled to this species being physiologically broad-niched and having evolved in, and being adapted to, physically more variable environments. The Baltic populations of *G. duebeni* and *G. oceanicus* were generally more sensitive to salinity changes and treatments with diesel-oil than their North Sea conspecifics. The higher sensitivity to pollutants of the Baltic populations may be due to a number of factors such as changes in the characteristics of toxic substances (metals) with salinity, the higher relative ionic concentration of a given amount of poisonous substance in the low-saline Baltic Sea than in the North Sea, and direct interactions of toxicants with membrane permeability and osmo-regulatory mechanisms, which are already under strain at low salinities.

INTRODUCTION

The widely distributed amphipod genus *Gammarus* inhabits marine, brackish- and fresh-water habitats. To cope with a range of environmental conditions in their particular aquatic habitats, species diversity and niche variability are high (e.g. Kolding 1985), yielding a promising material for experimental investigations on adaptation and underlying physiological mechanisms. Considerable experimental and theoretical work has been done on the physiological characteristics of *Gammarus* spp. (Kinne 1952, 1959, 1964, 1971, Bulnheim 1972, 1979, Dorgelo 1973, Sutcliffe 1984) as well as on life cycle characteristics, population dynamics and genetics of the genus (Fenchel & Kolding 1978, Kolding & Fenchel 1979, Bulnheim & Scholl 1981, Kolding 1981, 1986, Kolding & Fenchel 1981). In the Baltic Sea 5 species of *Gammarus* are found that also inhabit the North Sea. These are *G. oceanicus* Segerstråle, *G. locusta* (L.), *G. salinus* Spooner, *G. zaddachi* Sexton and *G. duebeni* Liljeborg.

Although many studies have been performed on the effects of a variety of toxicants on marine organisms, there are relatively few on estuarine animals (McLusky et al. 1986), and estimation of the effects of a certain pollutant on estuarine ecosystems have often been derived from experiments with marine organisms that have been subjected to combinations of lowered salinity and toxicants, and generally not from comparative studies between estuarine organisms and their marine conspecifics in their respective environments.

Thus, the general sensitivity of the brackish Baltic Sea and the marine North Sea has been much debated (Gray 1981, Landner et al. 1982). some regarding the Baltic Sea as more sensitive and some considering the North Sea as more vulnerable to pollution. Those regarding the Baltic as more sensitive base their opinion on the concept that organisms living near to the
limit of their tolerance range (e.g., salinity) should be more susceptible to any additional stress (e.g., McLusky et al. 1986). This theory has been experimentally validated for a number of different species and for toxicants such as cadmium (Theede 1980, 1984), heavy metals (Jones 1975) and diesel oil (Tedengren & Kautsky 1987). The contrary view was put forward by Gray (1974, 1981), Jernelov & Rosenberg (1976) and Rosenberg (1976), proposing that since estuarine species have a wider tolerance to salinity, temperature and oxygen levels, this tolerance will pre-adapt the organisms to tolerate pollution stress. The field evidence for this would be that these species surviving in polluted marine areas are often the same as those found in estuaries. Sensitivity to pollution may thus be related to niche width of the organism (Levins 1968, Fisher 1977).

Using Gammarus duebeni and G. oceanicus as model organisms we compare the physiological responses of these species from marine and brackish environments to acute salinity changes and exposure to cadmium and diesel oil. Since G. duebeni is more tolerant to fluctuating salinities (Bulnheim 1972, 1979) and has a broader salinity range (Kolding 1985) than other Gammarus spp., we wanted to test if this broad-niched species would also be more resistant to pollutants. We also wanted to investigate whether there are any differences in sensitivity between Baltic and North Sea populations of G. oceanicus towards acute exposure to diesel oil and cadmium, representing 2 types of toxic substances, and whether the sensitivity of a homosmotic species to pollution acts synergistically with a salinity decrease, as in the poikilosmotic Mytilus edulis (Tedengren & Kautsky 1987). The work described here is concerned with changes in physiological parameters including oxygen uptake, nutrient release, and O:N and O:P ratios.

**MATERIALS AND METHODS**

Experiments were performed at the Askö Laboratory, Sweden, in the northern Baltic proper (58° 49' N, 19° 39' E) in June 1986 and at the Tjarno Marine Biological Laboratory, Sweden, on the North Sea (58° 53' N, 11° 09' E) in July and October 1986.

Gammarus oceanicus were obtained from the Fucus belt at a depth of 1 to 3 m at both localities. Ambient salinities and temperatures at collection sites were 6.5 % S, 8°C and 24.0 % S, 14°C for the Baltic and North Seas respectively. G. duebeni were, in both areas, collected from rock-pools of varying salinities (0 to 30 % S). Specimens were kept for 24 h in aerated aquaria of 6.5 and 24.0 l (Frank & Robertson 1979). Regarding normal concentrations of hydrocarbons there is little informa-
tion, but during an oil spill situation they will certainly reach concentrations well above those used here.

During the experiments, oxygen consumption was measured every hour with a polarographic oxygen electrode (Yellow Springs Instruments 59) modified with a stirring device, thus giving 30 replicate measurements for each treatment. NH$_4$-N and PO$_4$-P excretion were determined according to Carlberg (1972) and the amphipods were dried at 70°C and weighed. O:N and O:P ratios were calculated in atomic equivalents. The ratio of oxygen consumed to nitrogen excreted (O:N), is a measure of the relative balance between carbohydrate and protein substrates in the metabolism and has been used to describe the physiological status and stress level of marine invertebrates (e.g. Bayne & Sculillard 1977, Widdows 1978, Widdows et al. 1981, 1984, Bayne et al. 1985, Tedengren & Kautsky 1986, 1987).

The physiological response of the amphipods to salinity changes and treatments with diesel oil and cadmium is expressed as a Perturbation Index (PI) (cf. Lindblad et al. 1986, in press). Each measured value for respiration rate and excretion of NH$_4$-N and PO$_4$-P in the treated specimens is divided by the corresponding value of the control specimens.

To obtain an overall measure of disturbance an Absolute Disturbance Index (ADI) was calculated according to Lindblad et al. (in press) and André et al. (in press). ADI is the absolute distance between the metabolic activities of an undisturbed system and a disturbed point in a multidimensional space where each dimension represents the PI of a measured parameter (X). The ADI value is proportional to the disturbance, and larger values indicate a more disturbed situation. The Absolute Disturbance Index is expressed as:

$$\text{ADI} = \sqrt{\frac{1}{n} \sum (\text{PI}(X) - 1)^2}$$

**RESULTS**

Table 1a, b, c gives the respiration and excretion rates and the ratios between oxygen consumption and excretion of nutrients for Baltic and North Sea populations of *Gammarus duebeni* and *G. oceanicus* exposed to acute salinity changes, diesel-oil and cadmium treatments. The perturbation indexes (PI) for these data are given in Fig. 1.

Any change from ambient salinity increased metabolic activity as indicated by increased respiration and excretion of nutrients (Table 1a; Fig. 1). There were no significant differences between the effects of raising or lowering of salinity, although raised salinities seemed to have only minor effects on respiration of North Sea amphipods. O:N ratios were lowered with altered salinities, more obviously if salinity was lowered. O:P ratios were generally unaffected by alterations in salinity, but were of different magnitude in different experiments (Table 1a). The metabolism of *Gammarus oceanicus* was in both areas more affected by salinity changes than that of *G. duebeni*. The *G. duebeni* population from the North Sea was less affected in all PIs as well as ADI compared both to North Sea *G. oceanicus* and to Baltic Sea *G. duebeni* (Figs. 1 and 2).

Exposure to diesel oil generally resulted in decreased oxygen consumption and a rise in nutrient excretion, leading to lowered O:N and O:P ratios (Table 1b; Fig. 1). The response was higher for *Gammarus oceanicus* in both areas and was aggravated by simultaneous changes in salinity, both if salinity was lowered and if raised. The PIs (Fig. 1) suggest that this effect is more pronounced if salinity is lowered, and also that it is of a higher magnitude in the Baltic Sea, but these differences could not be shown to be statistically significant. The *Gammarus duebeni* population at Tjärnö, when exposed to diesel oil, was more resistant in all PIs as well as ADI ($p < 0.05$) (Figs. 1 and 2).

A salinity-dependent decrease in respiration following exposure to cadmium was observed in all populations (Table 1c; Fig. 1). Excretion of nutrients increased, leading to lower O:N and O:P ratios. The response to cadmium was enhanced at low salinities and reduced if salinity was raised, showing that the toxic effect of cadmium is aggravated by low-salinity conditions. *Gammarus duebeni* was more resistant to cadmium exposure than *G. oceanicus*, especially in the North Sea populations (Figs. 1 and 2).

**DISCUSSION**

**Salinity effects**

In our study we observed the lowest respiration rates at ambient salinities. When salinity was lowered respiration increased strongly, while raised salinities increased respiration only to a minor extent, especially in North Sea amphipods. In accordance with this Kinne (1952) found that oxygen consumption of *Gammarus duebeni* increased in suboptimal salinities (5 % S – fresh water), but contrary to our data he found that respiration decreased in supraoptimal salinities (22 to 30 % S). Suomalainen (1958) measured constant rates of respiration between 1 and 20 % S in 4 *Gammarus* species and McLusky (1969) could not assess significant differences in respiration of the amphipod *Corophium volutator* between 1 and 30 % S. Dorgelo (1973) obtained variable results with 3 *Gammarus* species, but the dominating effect of salinity was a
Table 1. *Gammarus* spp. Respiration, excretion and atomic O:N and O:P ratios (with SE in parentheses) compared between Baltic Sea and North Sea populations, exposed to (a) acute changes in salinity, (b) treatment with diesel-oil and (c) treatment with cadmium. Mean values per g dry weight. Duration of adaptation to experimental conditions 24 h, exposure and measurements of metabolism 6 h

<table>
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<tr>
<th>Species</th>
<th>Location</th>
<th>Salinity (% S)</th>
<th>Respiration (mg O₂ g⁻¹ h⁻¹)</th>
<th>Excretion NH₄-N (µg g⁻¹ h⁻¹)</th>
<th>PO₄-P (µg g⁻¹ h⁻¹)</th>
<th>O:N ratio (atomic)</th>
<th>O:P ratio (atomic)</th>
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* Ambient salinities

decrease in oxygen consumption with increasing salinity (0 to 36 % S).

Increased locomotor activity during the initial period of salinity stress may raise respiration. This period will, however, last only about 1 to 5 h after which a new level of routine O₂ demand is reached and maintained for 30 to 40 h (Bulnheim 1972). Since our specimens had been acclimated to experimental salinities over 24 h prior to start of the experiment they would have been in the phase of routine O₂ demand.

When euryhaline crustaceans adapt to environmental salinity variations, significant changes in ammonia excretion are observed. Decreasing salinity generally raises ammonia excretion and vice versa...
Fig. 1. *Gammarus* spp. Perturbation indexes (PI) for respiration and O:N ratios compared between Baltic Sea and North Sea populations exposed to acute changes in salinity and treatments with diesel oil and cadmium. Mean value of control (non-treated specimens at ambient salinity) is set to 1 indicated by the line. SE of means are shown.
Fig. 2. *Gammarus* spp. Absolute disturbance index (ADI) calculated from perturbation indexes for all measured physiological parameters. Disturbance of control (non-treated specimens at ambient salinity) is zero. Legend as Fig. 1 (Spaargaren 1982, 1985, Regnault 1984). In our experiments, any deviation from ambient salinity caused increased NH$_4$ excretion, indicating higher metabolic activity and increased osmoregulatory work. As indicated by the perturbation index for O:N ratios (Fig. 1), this effect was more pronounced when salinity was reduced than when it was raised. This is probably due to NH$_4$-N being used as counter-ion in the mechanisms of active uptake of Na$^+$ and K$^+$, and by partly replacing losses of these ions through urine in diluted media (Spaargaren 1982, Gilles & Pequeux 1983).

*Gammarus* species are capable of hyperosmotic regulation at low salinities (*G. duebeni* up to 23 %, Kinne 1971), but become osmoconformers at higher salinities. *G. duebeni* exhibits especially strong osmoregulatory abilities (Bulnheim 1972, 1979), as also indicated in our study by the PIs of *G. duebeni*, for both respiration and O:N ratios, being closer to control values than those of *G. oceanicus* (Fig. 1). In these homoiosmotic amphipods respiration and O:N ratios were highest at ambient salinities, indicating that energy expenditures for osmoregulatory work will increase with any change in salinity. However, the reduction of this index was generally more pronounced if salinity was lowered. In the poikilosmotic mussel *Mytilus edulis*, Tedengren & Kautsky (1986) found that O:N ratios decreased if salinities were lowered, but always increased with raised salinities. This was assumed to indicate a generally less favourable energy balance only at lower salinities, resulting in the lower growth rates and smaller maximum sizes observed in the lower growth rates and smaller maximum sizes observed in the Baltic Sea (Kautsky 1981, 1982).

**Diesel-oil treatment**

The effects of oil on various physiological functions of marine invertebrates are assumed to be concentration-dependent (Edwards 1978, Laughlin & Neff 1981). At low concentration, hydrocarbons exert effects predominantly through their action as narcotics (Crisp et al. 1967), which would affect oxygen consumption indirectly by decreasing locomotor activity. Narcosis would be expected to be a major effect mainly at low concentrations, or early in the course of exposure, when oil concentrations in the tissue are still relatively low. Ponat (1975) showed that oxygen consumption was reduced to 40 % in *Gammarus salinus* at 15 °C and 15 °C owing to the narcotic influence of crude oil.

Higher oil concentrations have been shown to increase respiration rates in isolated mitochondria from amphipods (Percy 1977). This was explained in terms of enhancement of some aspect of electron transport through effects on mitochondrial membranes. Larval lobsters exposed to sublethal concentrations of crude oil (0.25 mg l$^{-1}$ for 4 d) exhibited significant reductions in respiration rates and O:N ratios (Cappuzzo & Lancaster 1981). The reduction in O:N ratios was explained...
by an increased dependence on protein catabolism for immediate energy needs as a result of the oil exposure.

Lindén (1976) observed behavioural disturbances when Gammarus oceanicus was exposed to oil (5 to 20 mg l\(^{-1}\)). The first symptoms were a continuous and hectic swimming, probably an avoidance reaction; later the amphipods stopped swimming and began to crawl on the bottom. This might be explained by the above-mentioned narcotic effect, or by surfaces and fine structures, such as receptor cells and external glandular tissues, becoming clogged and their efficiency reduced. Carr & Lindén (1984) also observed decreased O:N ratios for G. salinus exposed to oil as well as oil/dispersant.

In the present study, we could not detect any signs of impaired swimming or other effects of a physical or mechanical nature. The amphipods exhibited decreased respiration rates and generally showed reduced O:N ratios when exposed to an acute oil-spill. We interpret the decreased O:N ratios in our experiments as indicators of increased protein catabolism. The effects were more pronounced in combinations with changed salinity (p < 0.05), especially if the salinity was lowered. In the poikilosmotic Mytilus edulis acute oil exposure also resulted in reduced O:N ratios, but it was found that lowered salinity and diesel oil acted synergistically to a higher degree in Baltic (p < 0.001) than in North Sea mussels (p < 0.1) (Tedengren & Kautsky 1987). In the present study of homoiosmotic gammarids no synergistic effects, but only additive effects, could be detected in the experiments with diesel oil and changed salinities. Nevertheless, we assume that diesel oil may have direct effects on the osmoregulatory activity of Gammarus spp. As indicated by lower ADIs (Fig. 2), G. duebeni was less sensitive to oil exposure than G. oceanicus (p < 0.001).

**Cadmium treatment**

Cadmium exposure also reduced respiration and increased excretion of NH\(_4\)-N and PO\(_4\)-P, leading to lowered O:N and O:P ratios. These effects were found to be strongly salinity-dependent with the PI:s more deviant from control, and the highest ADIs obtained, at the lowest salinities (p < 0.001). The salinity-dependent toxicity of cadmium and several other heavy metals is well known (Theede et al. 1979, Fischer 1986, review in McLusky et al. 1986) and has been assumed to be due mainly to the formation of less toxic complexes with chloride-, hydroxy- and carbonate-ions in more saline regimes (Olson & Harrel 1973, Jones 1975, Jackim et al. 1977, Phillips 1977, Bryant et al. 1984, 1985). George et al. (1977), Bouquegneau & Gilles (1979) and Phillips (1980) also suggested direct salinity effects on metal uptake processes involving competition between cadmium and calcium/magnesium as cations at uptake sites, which would thus affect osmoregulation. This would lead to relatively lower toxicity at high salinities both through reduction of free Cd ions and through lower rates of bioaccumulation. Sublethal effects of cadmium in crustaceans have been shown to interfere with osmoregulation, oxygen consumption, gill structure, moult regeneration and larval development (Phillips 1980), and cadmium was found to cause osmotic elevation in crab serum and to reduce the rate of oxygen consumption in Carcinus maenas and C. irroratus (Thurberg et al. 1973).

The absolute disturbance index (ADI) showed that Gammarus duebeni is less sensitive to additions of cadmium than G. oceanicus, especially in the North Sea population (p < 0.001) (Fig. 2). However, a comparison between the G. oceanicus populations from the Baltic and North Seas provided no significant differences in response to cadmium treatment. We suggest that 1 mg l\(^{-1}\) cadmium may have been too toxic to reveal any differences in sublethal physiological response, as such effects may be observed at as low as 1 and 7 \(\mu\)g l\(^{-1}\) in fresh and marine waters, respectively (Taylor 1981).

**Comparison of stress responses between Baltic and North Sea populations**

In both areas, the stress sensitivity expressed as the perturbation index (PI) of respiration and O:N ratio (Fig. 1), as well as the absolute disturbance index (ADI) (Fig. 2), were significantly (p < 0.001) higher in Gammarus oceanicus than in G. duebeni following exposure to acute salinity changes and/or treatments with diesel-oil or cadmium.

We believe that the higher tolerance of Gammarus duebeni is coupled to this species having evolved in, and being adapted to, physically more variable environments. G. duebeni is more euryhaline, and has the lowest oxygen demand of gammarids studied (Bulnheim 1979). This reduces metabolic energy expenditures and is considered of adaptive significance to the harsh conditions of the upper shore environment. As shown by Bulnheim (1972), G. duebeni also exhibits the highest developed capabilities for compensatory adjustments to rapid salinity changes, which leads to less extensive alterations in respiratory rates and to faster adaptation following osmotic stress than in other gammarids.

Fisher (1977) suggested that organisms which have evolved in and adapted to physically variable environments would, because of their adaptations, be better able to tolerate any toxic compound (and possibly...
every perturbation) than would morphologically similar organisms adapted to stable environments. This ability involves the evolution of a tolerance of the effects produced by other toxic substances, which may confer upon a cell resistance to exotic chemicals producing similar effects. Essentially, these are also the lines along which Gray (1974), Jerne-Jelov & Rosenberg (1976) and Rosenberg (1976) argue. Our data suggest this to be true for related broad- and narrow-niched species, but it does not seem to be valid when the sensitivity of the same species in 2 areas of different background stress is compared.

Exposure to diesel oil in combination with changes in salinity exerts more pronounced effects in the Baltic population of Gammarus oceanius than in the North Sea population. G. duebeni in the North Sea also exhibits a higher degree of resistance to both cadmium and diesel-oil exposure than in the Baltic Sea (p < 0.01), whereas the ability to withstand fluctuations in salinity is about the same in both areas.

Previous studies on the euryhaline and poikilosmotic blue mussel Mytilus edulis by Tedengren & Kautsky (1987) also showed that physiological stress, as indicated by decreasing O:N ratios, is aggravated by lowering of salinity and that Baltic mussels were more sensitive than North Sea mussels to additional stress (diesel-oil) superimposed on the already existing salinity stress.

The generally more pronounced effect of cadmium or diesel-oil on the Baltic populations can be explained by differences in salinity, through a number of mechanisms. Our data indicate that poisonous substances may interact with membrane permeability and osmoregulatory mechanisms, which are in any case generally more activated in low-saline areas. Furthermore, organisms from low salinity areas will generally be relatively more exposed to substances in the water as they will generally process more water due to osmoregulation and will thus pass a relatively larger amount of a specific substance through their bodies, which then can accumulate and/or exert effects. This is enhanced by the lower Ca^++ concentration in the Baltic Sea, which will increase the permeability of cell membranes (Schlepier 1971), and increase toxicity. Furthermore, the relative ionic concentration of a given amount of poisonous substance in the low salinity Baltic Sea will be higher than in the North Sea. Finally, the characteristics of the poisonous substance may change with salinity, and generally lead to increased toxicity with lowered salinities. This is definitely valid for cadmium and most other heavy metals, while it is of minor importance for petroleum hydrocarbons.

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LITERATURE CITED


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