Influence of temperature, salinity, and oxygen on the cadmium balance of mussels *Mytilus edulis*

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ABSTRACT: Juvenile mussels *Mytilus edulis* L. were cultivated in controlled flow-through aquaria to investigate the influence of environmental temperature, salinity, and dissolved oxygen on cadmium balance. (1) Inverse relations between Cd concentration of soft tissues and physical condition of mussels were observed in response to temperature. The relation between Cd body burden and shell weight (Cd/shell-wt index) is independent of temperature between 7 and 25°C. (2) Reducing salinity (mg kg⁻¹), while keeping dissolved Cd (µg l⁻¹) constant, increases the molar ratio of Cd and seawater salts. Accumulation of Cd in the soft tissues and of Ca in the shells of mussels is determined by Cd and Ca concentrations within the range of 15 to 30 ‰ salinity. Residues of Cd in mussels, therefore, are higher in samples from lower salinity. Below 15 ‰ the Cd/shell-wt index is disproportionately higher, corresponding to reduced shell growth in lower salinities. Slightly lower index values were observed in 35 ‰. (3) Dissolved oxygen concentrations between 2.5 and 6.5 ml l⁻¹ have no influence on the Cd balance of mussels. (4) Experimental results were reproducible and corresponded to field observations. (5) Modifications of growth and performance were in accordance with the literature. Lowest experimental temperature, salinity, and oxygen tested inhibited both growth of mussels and Cd uptake within a small laboratory gradient. Growth in the concerned environment is discussed as an essential aspect of research on metals and molluscs, and in relation to the mussel watch concept.

INTRODUCTION

Mussels (*Mytilus edulis* and related species) have been suggested for monitoring of coastal water quality on a global scale. Numerous 'mussel watch' (Goldberg 1975) projects have confirmed increases in contaminant levels in mussels from industrialized shores. Numerous laboratory studies have documented the molluscan capacity of bio-concentrating aquatic contaminants (reviews: Phillips 1980, Eisler 1981). Using mussels as biological indicators has entered national and international administrative programmes in marine pollution control (USA: Stephenson et al. 1979, Goldberg et al. 1983; North Atlantic: ICES 1982; North Sea: BMI 1986).

When the biological content indicator concept evolved from empirical evidence, molluscs may have been envisaged as a type of self-maintaining ion exchange device – a substitute which could help to overcome difficulties in seawater chemistry. A decade of research, however, has discovered considerable problems when evaluating mussel watch data. The effort spent in this matter (Bryan 1980, review) suggests that comparable difficulties would have been

found in other organisms if they had been investigated as intensively. Mussels respond to a large variety of external stimuli. Phillips (1976) developed sampling strategies to eliminate the influence of some environmental variables on metal concentrations in mussels. Sexual maturation has been identified as a source of biological variability (Cossa et al. 1979, Orren et al. 1980). Some new alternative suggestions are based on models in metal biochemistry (Zaroogian 1980, Simkiss & Taylor 1981).

When using organisms as indicators of contaminants, biological variability must be recognized. This can be a matter of choosing an appropriate independent variable. Recently the accumulation of cadmium has been interpreted as a result of organismic immobilisation, and shell weight has been chosen as a correlate of similar cumulative character. The influence of individual size, spawning, food supply, and tidal exposure could be eliminated from mussel watch programmes by introducing shell weight as an independent variable in relation to the amount of cadmium in soft tissues (Cd/shell-wt index, Fischer 1983; Table 1). The former study resulted from a methodological field survey. Apart from describing an experimental verifi-

cation of a new approach, the work reported here provides information on the influence of temperature, salinity, and dissolved oxygen on the cadmium balance of mussels. Methodological aspects relate to the scaling of laboratory studies on the accumulation of metals by molluscs, and to growth as an essential aspect in the mussel watch concept.

Table 1. *Mytilus edulis*. Cadmium balance of field-collected samples. Where the Cd concentration of soft tissues varies depending on physical condition, the Cd/shell-wt index is not affected (abstracted from Fischer 1983)

Mussels are	Cd in meat Meat weight	Meat weight Shell weight	Cd in meat Shell weight
Very young			
Very old			
Starved	Increased	Decreased	Not affected
Spawned			
Tidally exposed			

MATERIAL AND METHODS (GENERAL)

Laboratory cultivation

Juvenile blue mussels *Mytilus edulis* L. of approximately 7 mm shell length (experiment on influence of temperature: 3 to 4 mm) were collected from their natural habitat in Kieler Förde, Western Baltic Sea. They were kept in a series of flow-through aquaria and fed a chemostat algal culture. Experimental water and feed algae were introduced with a peristaltic pump. Filtration rates of juveniles as reported by Riisgård et al. (1980) were used to scale the experimental flow rates. Unless being controlled at intervals as experimental variables, standard environmental conditions were as follows: temperature, 15°C; salinity, 15‰; dissolved oxygen, air saturated, i.e. 6.5 ml l⁻¹; dissolved Cd, 1 µg l⁻¹ added to the natural concentration of approximately 0.1 µg l⁻¹. The experiments were terminated after 9 to 20 wk, according to the observed growth.

Water supply. Natural seawater (salinity 16 to 19‰) was pumped from a depth of 4 m in Kieler Förde with all-plastic pumps, passed through 20 µm mesh, and stored in polyethylene containers until use. Experimental water was prepared by adjusting salinity to 15‰ (Kieler Förde annual average for surface water) with distilled water. Cd was added as CdCl₂. Results of Cd water analysis were not significantly different from calculated Cd concentrations. A radiotracer experiment showed that virtually all (97 %) added Cd was in the 'dissolved' (<0.4 µm) phase.

Algal culture. The green coccal alga *Nannochloris*

sp. was used because of its resistance to large differences of salinity (cf. Witt et al. 1981). Algal culture water was prepared after Betz (1977) with analytical grade chemicals: 100 mg l⁻¹ NaNO₃, 20 mg l⁻¹ Na₂HPO₄, 80 mg l⁻¹ NaHCO₃, and 2 mg l⁻¹ FeCl₃·6H₂O. The use of EDTA was strictly avoided. Illumination was by fluorescent tube lights in a 20 h light/4 h dark cycle.

Dimensions. Ten juvenile mussels were each attached to perspex supports and suspended in aerated flow-through aquaria with a volume of 600 ml. The daily water exchange was approximately 600 (start) to 1200 ml plus 60 to 120 ml of algal suspension. The volume of the chemostat culture was adjusted to give a daily renewal of approximately 20 %.

Experimental routine. At weekly or biweekly intervals the length of individual mussels was measured by a calibrated ocular micrometer in a stereo microscope. Pooled live weights were determined. Temperature and dissolved oxygen were measured directly; salinity was measured or calculated from flow rates of peristaltic pumps. New algal batch cultures were inoculated every month and used in the chemostat after 14 d.

Analytical steps

Before sample preparation and analysis, juvenile mussels were kept for 1 d without food to allow elimination of gut contents. Mussels were killed by wet heat at 60°C, detached from their supports, and soft tissues were removed from the valves. Shells were dried overnight, weighed, and measured for length. Individual soft tissues were freeze-dried, oven-dried at 100°C for 2 h, and weighed. Digestion was carried out in quartz test tubes, with loose teflon lids, by a 1:1 mixture of concentrated nitric and perchloric acids for 2 h at 90°C. The samples were diluted with distilled water and analysed for Cd by flameless AAS. Subsamples of 20 mg of reference material TORT-1 (available from the National Research Council, Ottawa, Canada) were analysed for quality control.

Calculations

All data refer to the dry weight of soft tissues and shells. Outliers ($|\bar{x} - x_i| > 3 \text{ SD}$) were eliminated. Error terms in comparisons relate to the 95 % confidence intervals of means.

Individual growth increments were estimated basing on initial shell length and the relations

$$\text{shell wt} = a \text{ shell length}^{2.85} \quad (1)$$

$$\text{meat wt} = b \text{ shell wt} \quad (2)$$

The coefficients were determined in subsamples before starting the experiments. The exponent of 2.85 was determined empirically from a large number of individual measurements. Reverse calculations produced deviations not greater than $\pm 5\%$.

Constants of daily growth, k , were determined from measured final weight data and calculated initial weight (Eq. 1 & 2), using the formula

$$W = W_0 e^{kt} \quad (3)$$

Growth constants depend on individual size (review: Jörgensen 1976), a problem eliminated here by conducting experiments with juveniles of similar size.

A condition index (CI) of mussels was calculated in terms of % soft tissue weight of total dry weight:

$$CI = \frac{\text{soft tissue dry wt}}{\text{total dry wt}} \cdot 100 \quad (4)$$

CONTROLLED CULTIVATION EXPERIMENTS

Temperature

Experimental techniques

Temperature was preset at 5 °C intervals between 5 and 25 °C by placing the flow-through aquaria in controlled water baths. Thermometer readings were recorded every week. Constancy was within ± 0.2 °C.

Temperature influences growth of mussels, which results in changing food requirements. Equivalent nutrition was approximated by maintaining a high rate of water and food supply. Differences in growth were allowed for by adjusting flow rates to the pooled live weight of mussels in each aquarium. Seventy-five % of

experimental water was recirculated. A scheme of experimental arrangements is given in Fig. 1.

Results

Cadmium balance

Cd concentration of soft tissues. Chemical analyses of soft tissues showed that Cd concentration in soft parts was highest in mussels grown at 25 °C (Fig. 2a). Maximum dry tissue concentrations exceeded $80 \mu\text{g g}^{-1}$. Lowest tissue concentrations were approximately $35 \mu\text{g g}^{-1}$ in mussels grown at 7 and 11 °C.

Condition index. The reverse relation was observed in the physical condition of mussels. The CI was 20 % in mussels from 7 °C, but those grown in 25 °C exhibited only half that value.

Cd/shell-wt index. Relating Cd body burden with shell weight gave a mean of $8.2 \pm 1.1 \mu\text{g g}^{-1}$ over the range of experimental temperatures from 7 to 25 °C (Fig. 2b, shaded).

Fig. 1. Flow-through system for testing effects of temperature. W: water; F: feed algae; P: peristaltic pump; M: mixing cell; A: series of thermostated aquaria. Some flow-rates are given

Fig. 2. *Mytilus edulis*. Influence of temperature on Cd balance (15 ‰; air-saturated; $1.1 \mu\text{g Cd l}^{-1}$). (a) Higher Cd concentrations in soft tissues and lower condition are found at elevated temperature. (b) The Cd/shell-wt index remains unaffected. Cd accumulation is inhibited in the lowest experimental temperature. Bars: 95 % confidence intervals of means; shaded: common arithmetic mean

Inconsistency. The results observed in mussels from 5.6 °C was in remarkable contrast to this consistent pattern. Their Cd concentration, CI, and Cd/shell-wt index were considerably lower than the other data.

Growth and performance

The experiment was terminated after 8 wk. Within the range 7 to 25 °C the increase in pooled live weights (in terms of the constant of daily growth) was lowest at the lowest temperature (Table 2). Maximum growth was observed at 15.4 °C. At higher temperatures mean growth declined. Ten % mortality occurred at 25 °C though the majority of individuals continued to grow at this highest experimental temperature. Modifications of shell proportions were not observed except in the juveniles grown at 15 °C. Their valves appeared to be flatter, but variability was substantial and differences were not significant. Length-normalized shell weight was used as an indicator of thin (lightweight) shells when environmental variables promote the growth of mussels. The allometric equation $W = a L^{2.85}$ was applied, and the data were normalized to a shell length of 10 mm.

Discussion

Temperature is estimated to be a major environmental variable influencing cadmium in molluscs (reviews: Phillips 1980, Eisler 1981). Experimental studies in general have been short-termed. They provide no information on a final equilibrated state. There are no field observations concerning effects of temperature on Cd balance of mussels.

Apart from its descriptive character, this experiment was designed to provide a verification of the Cd/shell-wt index as a reliable indicator of environmental Cd. The conceptual frame was as follows. (1) A rise in temperature accelerates life processes in mussels including those of concern in the binding of Cd in soft tissues, and of calcium carbonate deposition in shells. (2) The higher the temperature the lower the net

growth efficiency (cf. Bayne et al. 1973), which reduces growth of soft tissues. (3) Below the optimum, deposition of calcareous shells appears to be directly governed by temperature (cf. Mann 1979). Consequently, mussels grown at an elevated temperature were expected to be of a lower physical condition, and to exhibit higher Cd concentrations in their soft tissues. If the relation between soft-tissue Cd burden and shell weight was a reliable index of environmental Cd, the Cd/shell-wt index should not be affected by temperature. This was verified between 7 and 25 °C (Fig. 2).

The additional observations on growth and performance are in accordance with the findings of others. A growth optimum between 15 and 20 °C has also been found by Coulthard (1929), Richards (1946), and Almada-Villela et al. (1982). Particle clearance follows a similar pattern (Theede 1963, Schulte 1975). The onset of mortality at 25 °C (see also Mann & Ryther 1977) can be attributed to a sharp decline in energy balance when temperature exceeds 20 °C (Bayne et al. 1973). With respect to shell dimensions the experimental results reported here confirm field observations of mussels producing light (thin) shells when their growth rate is high. This has often been mentioned in connection with nutrition (e.g. Havinga 1929, Boje 1965, Seed 1968), but it seems to be a general interrelation – and *vice versa*: slow growth and heavy shells coincide in case of suboptimal or supraoptimal temperature (this study), tidal emersion and wave action (Baird & Drinnan 1957, Harger 1970, 1971), or insufficient food supply (Fischer 1983).

With respect to the scope of this study these additional results are marginal. However, they provide information on the method applied and require consideration for this reason.

Salinity

Experimental techniques

This experiment was conducted with a constant ratio between dissolved Cd and natural seawater salts in a sequence of salinities (see Fig. 4). Cadmium (4.25

Table 2. *Mytilus edulis*. Influence of temperature on the growth constant k of pooled live weight, and of length-normalized shell weight. Increased growth at the optimum experimental temperature coincides with the formation of light shells (95 % confidence intervals of means)

	Temperature (°C)				
	7.1	10.9	15.4	20.4	25.2
k (d ⁻¹) of live weight	0.0060	0.0115	0.0308	0.0196	0.0095
Final shell-wt (mg) of 10 mm individuals	34 ± 3	34 ± 4	26 ± 3	31 ± 2	37 ± 4
n	15	15	15	15	15

Fig. 3. Water supply and flow-through system for testing effects of salinity. NSW: North Sea water; B: brine; S: hypersaline stock; D: deionized water; W: water; F: feed algae; P: peristaltic pump; A: aquaria. Some salinities and Cd concentrations are given

$\mu\text{g l}^{-1}$) was added to hypersaline water (37.5 ‰) which was then diluted with deionized water to give a number of experimental stocks. Further dilution with the feed algae suspension (15 ‰; $1.7 \mu\text{g l}^{-1}$ Cd) was by the multichannel peristaltic pump, which operated the flow-through arrangement (Fig. 3). Salinities of the experimental stock water were calculated from measured flow rates of water and algae, and adjusted to give desired final salinities between 7.5 and 35 ‰ in the flow-through aquaria. Final salinities were determined by density measurements, or calculated from measured flow rates. There were no significant differences between these methods. Variability was within $\pm 5\%$ of the mean data.

The use of tap water for dilution was strictly avoided to exclude possible sources of contamination. For the same reason commercial seawater salt was not used. The hypersaline sea water was prepared from offshore North Sea water (30 to 32 ‰) by adding a brine (50 to 65 ‰) which had been separated from thawing North Sea water ice (cf. Fig. 3).

Juvenile mussels were collected in the field (16 ‰ S sea water) and adapted at 5 ‰ intervals to experimental salinities over a period of 3 wk. Comparable growth in all salinities except the lowest showed that the juveniles were well adapted (cf. Böhle 1972).

Results

Cadmium balance

Cd concentration of soft tissues. Cd concentrations were not significantly different in mussels grown in salinities between 10 and 35 ‰ (Fig. 4a). An average

Fig. 4. *Mytilus edulis*. Influence of salinity on Cd balance; equimolar availability of Cd and seawater salts (15°C; air-saturated; $4 \mu\text{g Cd/35 g}$ seawater salts). (a) Cd concentration of soft tissues is independent of salinity. Condition increases in the lower salinities. (b) Cd/shell-wt index is independent of salinity between 15 and 30 ‰. Inhibition of Cd accumulation occurred in lowest experimental salinity. Bars: 95% confidence intervals of means; shaded: common arithmetic means

Cd concentration of $65 \pm 8 \mu\text{g g}^{-1}$ corresponds to a constant ratio of 114 ng Cd per g natural seawater salts in the culture water, according to the experimental design.

Condition index. Independence of salinity was also observed in the CI which was $14 \pm 1.5\%$ in mussels from 20 to 35 ‰. At lower salinities the CI increased and showed the highest experimental value, 23%, in mussels from the lowest salinity.

Cd/shell-wt index. This index appeared to increase from 9.5 to 12 when salinity was reduced from 35 to 15 ‰. The trend, however, was not statistically significant due to high variability in the 30 ‰ results. A mean Cd/shell-wt index of $11.3 \pm 1.2 \mu\text{g g}^{-1}$ was calculated for mussels from 15 to 30 ‰ (Fig. 4b, shaded). The Cd/shell-wt index of mussels from 35 ‰ was some 15% lower. In mussels from 10 ‰ the Cd/shell-wt index was significantly higher.

Inconsistency. A lower tissue concentration and a

Table 3. *Mytilus edulis*. Influence of salinity on daily growth constants k of meat and shell weight, and on length-normalized shell weight. Growth and performance are constant over a wide range of salinities. Growth rates are markedly reduced below 10 ‰ (95 % confidence intervals of means)

	Salinity (‰)						
	7.5	10	15	20	25	30	35
k (d ⁻¹) of meat weight	0.0235 ± .0028	0.0329 ± .0074	0.0367 ± .0053	0.0339 ± .0073	0.0317 ± .0075	0.0329 ± .0043	0.0290 ± .0056
k (d ⁻¹) of shell weight	0.0143 ± .0023	0.0274 ± .0047	0.0331 ± .0039	0.0321 ± .0104	0.0310 ± .0066	0.0347 ± .0047	0.0305 ± .0044
Weight (mg), 10 mm shells	33 ± 5	30 ± 3	32 ± 6	32 ± 2	29 ± 4	32 ± 4	29 ± 3
n	6	10	9	8	9	9	10

reversed trend in the Cd/shell-wt index were observed in mussels from the lowest experimental salinity (7.5 ‰).

Growth and performance

The flow-through experiment was conducted over a period of 9 wk. As reflected by the constants of daily growth, there were only minor differences of soft-tissue growth at salinities between 10 and 30 ‰ (Table 3). A slightly lower growth rate was noted at 35 ‰, and a marked reduction was observed in 7.5 ‰. Dilution of full-strength sea water (35 ‰) had no detectable effect on shell weight down to 15 ‰. A further dilution produced a decrease in final shell weight, which coincided with a smaller final shell length. Starting from an initial shell length of 7.6 ± 0.2 mm, juvenile mussels kept in 7.5 and 10 ‰ grew to a length of 10.6 ± 1 and 13.6 ± 0.6 mm. The final average in 15 to 35 ‰ was 15.1 ± 1.3 mm. Length-normalized shell weight (Table 3) indicated that shell thickness was rather uniform in all salinities.

Discussion

Considerable interaction of salinity on Cd accumulation by molluscs has frequently been reported, low salinity promoting experimental uptake rates (reviews: Phillips 1980, Eisler 1981). Higher soft tissue Cd concentrations in mussels from the Baltic Sea are well documented (Karbe et al. 1977, Phillips 1977, Theede et al. 1979). Comparative investigations of mussels from clean shorelines have shown North Sea samples (mean salinity 30 ‰) to have less than half the Cd/shell-wt index of mussels from the Western Baltic (mean salinity 15 ‰; Fischer 1983). Dissolved Cd, however, has been reported to be almost the same in both areas (Kremling et al. 1979, Schmidt 1980, Sperling 1985).

A higher percentage of free Cd²⁺ in low salinity (cf.

Mantoura et al. 1978) has been considered for increased uptake rates by organisms subjected to large laboratory gradients, and for higher acute toxicity of Cd (Sunda et al. 1978, Engel & Fowler 1979). Competitive interactions with calcium have been discussed (Westernhagen et al. 1974, Wright 1977, Lehnberg & Theede 1979). Phillips (1977) generalized, from field observations, that availability of Cd to mussels was greater in the Baltic. He suggested that only mussels from the same salinity should be compared.

In general, contaminant concentrations of aquatic environments are given in terms of weight per volume, ($\mu\text{g l}^{-1}$), without considering other dissolved components. Reducing salinity while keeping contaminant concentration constant therefore has the effect of increasing the contaminant amount in relation to natural sea-water salts. This alone could account for an effect of salinity on Cd accumulation in molluscs. To test this assumption, the experimental design maintained an equimolar relation of Cd and natural salts in a sequence of salinities. According to this specific laboratory situation the null-hypothesis was that no difference in Cd accumulation would be detected. With respect to Cd concentration of soft tissues, this was verified in the range of salinities between 10 and 35 ‰ (Fig. 4a).

When using shell weight as an independent variable in relation to Cd content of molluscs, influences of salinity on calcification must be considered. This study shows that the increment of shell weight is reduced when salinity falls below 15 ‰ (Table 3). Growth of soft tissues is less affected, and the CI increases. Consequently the relation between Cd in soft tissues and (inhibited) shell weight is higher in mussels from the lower end of the salinity range.

Correction factors for direct comparisons of mussel watch data from different salinities can be derived from Fig. 5. Measured Cd/shell-wt indices were divided by experimental Cd concentrations (cf. Fig. 4). Hence, recalculated Cd/shell-wt indices relate to

Fig. 5. *Mytilus edulis*. Influence of salinity on Cd/shell-wt index. Cd/shell-wt index of mussels is determined by the concentrations of Cd and Ca in the water (bars, dotted line; left scale) rather than by free ions (broken line calculated after Mantoura et al. 1978; right scale). Bars: 95 % confidence intervals of means, recalculated for $1 \mu\text{g l}^{-1}$ dissolved Cd; x: data from experiments with $1 \mu\text{g l}^{-1}$ dissolved Cd

$1 \mu\text{g l}^{-1}$ dissolved Cd in all salinities. Experiments actually conducted with this concentration produced similar results (Fig. 5, x). In salinities between 15 and 35 ‰ the Cd/shell-wt index is close to the ratio of dissolved Cd/Ca (dotted line). Obviously the concentrations of dissolved metals determine the balance of Cd and Ca in mussels when grown in a physiological steady-state with their environment. The free ions (Fig. 5, broken line; calculated according to Mantoura et al. 1978) may dominate acute toxicity of metals and influx rates (cf. Sunda et al. 1978, Engel & Fowler 1979) when organisms are subjected to high concentrations of contaminants in short-term tests.

Some tentative calculations may illustrate the capacity of mussels to concentrate Cd and Ca from the water. One l of full-strength sea water contains approximately 400 mg Ca, which is incorporated to produce 1 g CaCO_3 in the shells. A CI of 14 % (Fig. 4) means that a shell of 1 g covers a soft body of 170 mg dry weight, or roughly 1 g wet weight. One can conclude from these relations that mussels concentrate Ca from full-strength sea water by a factor of approximately 1000.

The concentration factor (CF) of Cd in soft tissues is roughly 2500, corresponding to a Cd/shell-wt index of $2.3 \pm 0.2 \mu\text{g g}^{-1}$ of mussels grown in 35 ‰ salinity and $1 \mu\text{g l}^{-1}$ dissolved Cd (Fig. 5). The CFs of Cd and Ca increase in lower salinity. It should be noted that the above estimates include reference to soft tissue weight, which is rather variable in field populations. Consequently, derived variables such as CI, CF, and Cd concentration of soft tissues fluctuate as well. Though being modified by reduced shell growth in lower salinity, the Cd/shell-wt index should be preferred for the biological monitoring of Cd in coastal waters. Salinity must be recorded to allow comparisons.

Compared to the impressive amount of information on the effects of temperature, relatively little is known about salinity (cf. Kinne 1971, review). Juvenile blue mussels produced smaller shells in salinities below 15 ‰, confirming the well-established reduction of size in suboptimal salinities (cf. Remane 1940). Disproportional growth of meat resulted in well-developed mantle tissues of small (10 mm) individuals from 7.5 ‰, similar to ripe adults just before spawning. It was interesting to note that shell thickness (in terms of length-normalized shell weight) obviously was not markedly influenced by dilution of seawater down to 10 ‰ (Table 3). This is in contrast to Schlieper & Kowalski (1957) who attributed different shell thickness of field-collected mussels to the influence of salinity. Newcombe & Kessler (1936) mentioned that difference in shell thickness (of soft shell clams *Mya arenaria*) 'seems not to be necessarily related to salinity. Heavier shell may be a natural concomitant of increased size in optimum salinities rather than a relation *per se* of heavier shell to salinity' (see also Eisma 1965, on *Cardium edule*). The experimental results reported here indicate that shell proportions of juvenile *Mytilus edulis* are relatively independent over a wide range of salinities. Between 10 and 35 ‰, shell thickness of field-collected mussels may be determined by other environmental variables, such as tidal exposure (Baird & Drinnan 1957), wave action (Harger 1970), or nutrition (Havinga 1929, Winter 1974). Further investigation is needed, the more so since recording shell weight of mussels has been adopted internationally for contaminant monitoring programmes (ICES 1985, BMI 1986).

Dissolved oxygen

Experimental techniques

A sequence of experimental dissolved oxygen (DO) levels was set up using an initial degassing step at different temperatures up to 70°C. On its way to the

Fig. 6. Flow-through system for testing effects of oxygen deficiency. W: water; F: feed algae; P: peristaltic pump; DU: series of thermoregulated (ΔT) degassing units; A: series of gas-tight aquaria

flow-through aquaria the water was cooled to the experimental temperature of 16°C. This physical process resulted in a graded series of oxygen deficiencies according to the degassing temperatures (Fig. 6). DO was determined at 7 to 14 d intervals by the Winkler method. Measured data varied by $\pm 0.5 \text{ ml l}^{-1}$. They were approximately 1.5 ml l^{-1} lower than the equilibrium in the degassing units, attributable to the oxygen demand of mussels, microorganism fouling of the walls, and organic debris.

Stoppered glass bottles with a magnetic stirrer were used as flow-through aquaria. All tubing was made from oxygen-tight material (Tygon® and stainless steel). One aquarium was aerated as a control. The experimental water was initially heat-sterilized and reaerated before being delivered to the thermal degassing devices.

Routine operations included determination of salinity ($15.2 \pm 0.2 \text{ ‰}$), temperature ($16.2 \pm 0.2^\circ\text{C}$), and pH (7.7 ± 0.1). These environmental variables could have been influenced by degassing of the water at different temperatures, but no such differences were recorded.

Results

Cadmium balance

Cd concentration of soft tissues. Chemical analysis of soft tissues showed that differences of Cd concentration were insignificant in mussels grown at DO levels between 2.5 and 6.5 ml l^{-1} (Fig. 7). The mean value was $63 \pm 10 \mu\text{g g}^{-1}$.

Condition index. The mean physical condition was $9.6 \pm 1.9 \%$ meat of total dry weight. In DO concentrations below 3 ml l^{-1} there was a tendency towards lower CI values, but this observation was not statistically significant due to substantial variability at the lowest DO level tested (1.5 ml l^{-1}).

Fig. 7. *Mytilus edulis*. Influence of dissolved oxygen on Cd balance (16°C ; 15 ‰ ; $1.1 \mu\text{g Cd l}^{-1}$). Cd concentration of soft tissues, condition index, and Cd/shell-wt index are independent of dissolved oxygen between 2.5 and 6.5 ml l^{-1} . Inhibition of Cd accumulation occurred in the lowest experimental oxygen level. Bars: 95 % confidence intervals of means; shaded: common arithmetic means

Cd/shell-wt index. There were no significant differences in the Cd/shell-wet indices of mussels grown at DO levels between 2.5 and 6.5 ml l^{-1} . The mean value was $7.0 \pm 1.4 \mu\text{g g}^{-1}$.

Inconsistency. In mussels from the lowest DO level tested the uptake of Cd was significantly reduced as indicated by lower Cd concentration of soft tissues and lower Cd/shell-wt index.

Growth and performance

The flow-through experiment was conducted over a period of 20 wk. It was terminated when the controls had grown by a factor of 4.5; growth was reduced at lower DO levels. Calculations of experimental increments showed that there had been virtually no growth of soft tissues at 1.5 ml l^{-1} DO (Table 4). Growth of shells, however, was by a factor of 1.5. This resulted in a lower CI of mussels from this lowest experimental DO level (Fig. 7). Besides that, growth of meat and

Table 4. *Mytilus edulis*. Influence of dissolved oxygen on growth in a 20 wk experiment. Growth gradually declines with lower oxygen. A steep decrease of growth occurred below 2 ml l⁻¹ DO (95 % confidence intervals of means)

	DO (ml l ⁻¹)				
	1.54	2.39	3.17	4.23	6.50
	± .36	± .43	± .49	± .54	± .47
Meat-Wt/Meat-Wt ₀ n = 10	1.06 ± .28	2.99 ± .56	3.71 ± .73	3.88 ± .61	4.65 ± 1.37
Shell-Wt/Shell-Wt ₀ n = 10	1.53 ± .28	2.74 ± .38	3.07 ± .51	3.36 ± .31	4.15 ± 1.39

shells occurred at about the same rate, with a gradual increase of growth at higher DO levels. The largest experimental increment was by a factor of approximately 4.5 in air-saturated seawater. This corresponds to a growth constant of 0.0107 ± 0.0022 , or a daily growth of 1.1 ± 0.2 % of actual weight.

Discussion

Oxygen deficiency is a regular seasonal phenomenon in semi-enclosed ecosystems such as the Limfjord (Jørgensen 1980) and the bays of SW Kieler Bucht (Krey et al. 1978, Gerlach 1984). In September 1981 extensive water masses in Kieler Bucht were devoid of oxygen and saturated with hydrogen sulfide (Ehrhardt & Wenk 1984). Severe mortality of benthic fauna was recorded (Weigelt 1985). Blue mussels have been shown to resist hydrogen sulfide for several weeks (Theede et al. 1969). Following the 1981 period of anoxia a considerably lower Cd/shell-wt index was observed in mussels from the affected area (Fischer 1983). This has been interpreted as a result of a lower availability to biota of Cd after the formation of cadmium-sulfur compounds (Fischer 1984, cf. Kremling 1983).

In most cases hydrogen sulfide is confined to bottom water. Mussels, living in the upper water layer, may be frequently subjected to lowered oxygen levels. This could affect their ability to reflect environmental levels of dissolved cadmium. The experiment has shown that Cd/shell-wt indices were independent of DO between 2.5 and 6.5 ml l⁻¹. Irrespective of DO in the tested range, lower Cd/shell-wt indices of mussels indicate lower concentrations of (biologically available) Cd in the water.

Compared to the controls, a lower growth rate of juvenile mussels was recorded at decreased oxygen concentrations. This is in accordance with experimental observations on fish (cf. Davis 1975, review) and clams (Neuhoff 1983), and with field data on mussels (Riisgård & Poulsen 1981). The growth rate of controls

in air-saturated water was only 1/3 the value observed in the experiments on temperature and salinity. It is not clear why. The tests were conducted in winter. Pretreatment of the experimental water with heat may have denatured seasonally low levels of dissolved organic matter. Such substances may contribute significantly to the nutrition of marine invertebrates including mussels (Siebers & Winkler 1984). In other experiments conducted in winter (unpubl.) mussels also grew more slowly, but retardation was less than reported here.

Comparative evaluation

Inconsistent results observed at the lowest experimental levels of temperature, salinity, and dissolved oxygen are not discussed here. They require consideration in a methodological context and will be dealt with in a subsequent section. Observations on Cd concentration and CI from different experiments cannot be compared because growth conditions were not the same. Comparisons must be restricted to the Cd/shell-wt index.

Consolidation of experimental data

The average Cd/shell-wt indices observed in the experiments on effects of temperature (Fig. 2b, shaded) and DO (Fig. 7, shaded) relate to Cd concentration of approximately 1.1 µg l⁻¹ in a salinity of 15 ‰. The mean Cd/shell-wt index of mussels grown in different salinities relates to 1.7 µg l⁻¹ Cd in 15 ‰. Since the molar ratio of dissolved Cd and seawater salts was constant, this value represents salinities between 15 and 30 ‰ (Fig. 4b, shaded). Growth experiments with a graded series of Cd concentrations have shown that Cd accumulation by mussels is proportional to environmental levels (unpubl. data). Anticipatively, the Cd/shell-wt indices observed in the experiments reported here are corrected for the same concentration of 1 µg l⁻¹ dissolved Cd, dividing mean values by the respective experimental Cd concentrations (Table 5).

Table 5. *Mytilus edulis*. Laboratory observations of Cd/shell-wt indices from 3 cultivation experiments. Correcting measured data for the same salinity (15 ‰) and for the same Cd concentration ($1 \mu\text{g l}^{-1}$) results in a common overall mean (arithmetic means of 95 % confidence intervals)

	Temperature 7–25 °C	Experimental variable Salinity 15–30 ‰	Oxygen 2.5–6.5 ml l ⁻¹
Dissolved Cd ($\mu\text{g l}^{-1}$) in 15 ‰ salinity	1.1	1.7	1.1
Cd/shell-wt index ($\mu\text{g g}^{-1}$)	8.2 ± 1.1	11.3 ± 1.2	7.0 ± 1.4
Cd/shell-wt index ($\mu\text{g g}^{-1}$) corr. for $1.0 \mu\text{g l}^{-1}$ Cd in 15 ‰ salinity	7.5 ± 1.0	6.5 ± 0.7	6.4 ± 1.4
Overall mean		6.8 ± 1.0	

Differences of these calculated data are not significant. Therefore a Cd/shell-wt index of $6.8 \pm 1.0 \mu\text{g g}^{-1}$ can be used as an overall estimate for mussels grown in a salinity of 15 ‰ with $1.0 \mu\text{g l}^{-1}$ Cd. This value represents 13 experimental regimes of temperature, salinity, and dissolved oxygen. The 95 % confidence interval corresponds to 9 to 10 individuals analysed in a sample.

Field correlates

Are the experimental results consistent with Cd/shell-wt indices of mussels grown in their natural habitat? Two sets of data can be used for comparative calculations (Table 6). (1) Mussels (Fischer 1983, and unpubl. data) and water (Sperling 1985) were

repeatedly sampled at the same time in the German Wadden Sea NE of the island of Sylt (salinity 30 ‰). (2) Mussels were sampled in the Western Baltic Sea in November 1980 (salinity 18 ‰). Mussel watch results can be related to water analyses of Kremling et al. (1979), assuming a relative constancy over the years according to measured data and a comparable hydrographic situation (cf. Fischer 1983). The sequence of calculations is: correcting Cd/shell-wt indices for a salinity of 15 ‰ (this study), and dividing them by data of dissolved Cd reported by others. This results in a hypothetical Cd/shell-wt index of field-collected mussels if salinity had been 15 ‰ and if dissolved Cd had been $1 \mu\text{g l}^{-1}$. These data are close to the overall mean of experimental results relating to the same salinity and Cd concentration.

Table 6. *Mytilus edulis*. Cd/shell-wt index of field samples in relation to dissolved Cd. Correcting field data for a salinity of 15 ‰ and a Cd concentration of $\mu\text{g l}^{-1}$ produces hypothetical values of field data which are close to experimental mean values (95 % confidence intervals of means)

	Aug 81	List, Wadden Sea 30 ‰ Feb 82	Aug 82	W Baltic 18 ‰ Nov 80	Experiment ^d 15 ‰
Cd/shell-wt index ($\mu\text{g g}^{-1}$) of field samples ^a	0.07 $\pm .005$ (n = 52)	0.10 $\pm .008$ (n = 49)	0.06 $\pm .005$ (n = 25)	0.24 $\pm .02$ (n = 64)	
Cd/shell-wt index ($\mu\text{g g}^{-1}$) corr. for 15 ‰	0.14	0.20	0.12	0.29	6.8 ± 1.0 (n = 10)
Dissolved Cd ($\mu\text{g l}^{-1}$)	0.028 ^b	0.056 ^b	0.015 ^b	0.071 ^c $\pm .016$ (n = 27)	1.0
Hypothetical Cd/shell-wt index ($\mu\text{g g}^{-1}$) calculated for $1 \mu\text{g l}^{-1}$ Cd in 15 ‰	5.0	3.6	8.0	4.1	6.8

^a Fischer 1983, and unpubl. data
^b Sperling 1985
^c Kremling et al. 1979
^d This study, cf. Table 5

GENERAL CONSIDERATIONS

Scaling of experimental studies

The high variability found in natural environments presents major difficulties in determining the influence of specific field variables on response variables. Therefore it was felt impossible to derive the influence of temperature, salinity, and dissolved oxygen on the Cd balance of mussels from comparative field investigations. Experimental physiologists and toxicologists are aware of the need to perform long-term studies with low contaminant levels if ecologically valid results are sought. Regarding the time scales involved in long-lived species such as mussels, however, studies on tissue residues have been considered to be most difficult (cf. Perkins 1979).

The 'normal' condition of molluscs in the field is growth in a physiological steady-state with respect to the environment. Conducting controlled cultivation experiments with a low Cd concentration was considered to be an adequate laboratory approach. When choosing a time scale in relation to growth, such experiments may be regarded as being long-termed if mussels had achieved a major portion of their weight in a laboratory environment. The use of juveniles is recommended for 3 reasons. (1) Juveniles have a higher growth rate than adults (review: Jörgensen 1976), which reduces duration of experiments. (2) Cultivating juveniles reduces the volumes of clean sea water, especially when conducting flow-through experiments, and of feed algae cultures (filtration rates: Winter 1978, review). (3) Initial uptake of contaminants by juveniles within a (small) laboratory gradient is faster than in larger specimens (cf. Schulz-Baldes 1974, Cunningham & Tripp 1975). Growth, however, is not an absolute scale in relation to physiological functions. High temperature, for example, accelerates metabolic turnover, but growth decreases in temperatures higher than the optimum (Table 2). In the case of salinity, mussels may need a prolonged period to achieve a new physiological steady state if changes exceeded their normal range of 10 to 30 % or more (cf. Kinne 1971, review). If mussels failed to grow, experiments were short-termed with respect to a time scale in relation to growth, and results might be less reliable. Organismic properties and needs must be considered when using biological indicators in contaminant monitoring and related research.

Growth and cadmium accumulation

In contrast to the consistency of results within the study (Table 5) and in relation to field correlates (Table

6), accumulation of Cd was significantly reduced in the lowest experimental levels of temperature (Fig. 2), salinity (Fig. 4), and dissolved oxygen (Fig. 7). Growth rates were also lower (Tables 2, 3 & 4). Obviously the life functions of Cd accumulation and growth are linked to each other. Both were seriously impaired when mussels were subjected to unfavourable conditions. There is a corresponding observation on oysters *Crassostrea virginica*: uptake of Cd was confined to temperatures higher than 6°C (Zarogian & Cheer 1976), or the growth season.

Accumulation of Cd is a normal biochemical function (cf. Nordberg & Kojima 1979, review). This implies a 'normal' metabolic state, which is indicated by growth of mussels. If life functions were retarded, e.g. by low levels of temperature, salinity, and dissolved oxygen, or by toxic levels of other metals (e.g. Zn; unpubl.), uptake of Cd from the environment was also reduced. The dependency of growth rates on age of mussels (review: Jörgensen 1976) may be responsible for low uptake rates of Cd from contaminated environments by large (old) individuals: a balanced state of mussels in relation to Cd has been observed neither in laboratory studies, even with low Cd levels (e.g. Jakim et al. 1977, Westernhagen et al. 1978), nor in field transplant experiments (de Kock 1983).

In this context adequate nutrition might be mentioned as a prerequisite of growth in animals. If food was inadequate in composition, the accumulation of Cd could be affected (as demonstrated with rats; Whanger & Oh 1979). The observation of higher uptake rates of Cd by mussels when given food (cf. Janssen & Scholz 1979, Köhler & Riisgård 1982, Borchart 1985) may be interpreted considering this aspect.

Importance of growth in the mussel watch concept

A low reactivity of mussels to environmental changes is necessary to dampen environmental oscillations, but it appears to be contradictory to the concept of using mussels as indicators of average environmental levels including their changes.

Biological indicators have been defined as 'time integrators' of variable contaminant levels (cf. Phillips 1980, review), a suitable view considering the cumulative storage of Cd (and of other contaminants with long biological half lives) in the soft tissues of molluscs, and of Ca (as carbonate) in the shells. Changes of environmental Cd concentration influence the amount of further additions to the Cd body burden. Theoretically, a new equilibrated Cd/shell-wt index could never be achieved. An approximation, however, is possible, depending on the quantity of Cd and Ca accumulated under changed conditions.

The consistency of results observed in this study suggests that a balanced state could have been attained. At present it is not possible, however, to substantiate a suggestion how long mussels should have grown in a new environment to provide reliable information on Cd contamination. A factor of 1.5 by shell length, or 3 to 4 by weight, in a normal range of environmental variables may be regarded as a tentative estimate. In any case weight increments should be as high as possible to increase significance of results.

For this principle reason, fast-growing juveniles are recommended when conducting laboratory studies on cumulative contaminants and molluscs, or performing field transplant experiments. The mussel watch concept implies growth in the environment of concern as an essential methodological aspect, both in research and field work.

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