ELSEVIER

Journal of Experimental Marine Biology and Ecology
279 (2002) 61–72

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Effects of a changing abiotic environment on the energy metabolism in the estuarine mysid shrimp *Neomysis integer* (Crustacea: Mysidacea)

Tim Verslycke*, Colin R. Janssen

Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, J. Plateaustraat 22, B-9000 Ghent, Belgium

Received 31 July 2002; received in revised form 2 August 2002; accepted 9 August 2002

Abstract

Adaptations to life in an estuary include a wide salinity tolerance, an extremely efficient osmoregulatory and respiratory physiology. These adaptive mechanisms are energy-consuming and relatively little data is available on the combined effects of abiotic stress factors on the energy metabolism of mysid shrimp. A new methodology (cellular energy allocation, CEA) to assess the energy budget was adopted for the estuarine crustacean *Neomysis integer* (Crustacea: Mysidacea). The biochemical composition of *N. integer* was determined: protein (7.39 ± 1.81% wet weight), lipid (3.99 ± 1.05% ww) and sugar (0.42 ± 0.18% ww). To assess the effect of natural variability on the energy metabolic processes in *N. integer*, a fractional factorial test design was set up with different naturally (Westerscheldt estuary, The Netherlands) occurring combinations of temperature, salinity and dissolved oxygen. The different abiotic factors had no significant effect on the energy metabolism of *N. integer* within the tested range. Temperature explained the decrease in lipid, protein and total energy reserves. Temperature, in general, had the most adverse effect on the CEA. Salinity was the most important factor explaining the effects on sugar reserves, with higher salinities causing an increased sugar demand. By modeling the influence of these abiotic stresses on the energy metabolism (CEA) of *N. integer*, it will be possible to use the CEA as an ecologically relevant biomarker of exposure to pollutants in estuaries.

Keywords: Abiotic stress; Biomarker; Cellular energy allocation; Energy metabolism; *Neomysis integer*

* Corresponding author. Tel.: +32-9-264-37-07; fax: +32-9-264-37-66.
E-mail address: tim.verslycke@rug.ac.be (T. Verslycke).

0022-0981/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0022-0981(02)00339-8

1. Introduction

Few environments expose their inhabitants to such a variety of abiotic variables and of such large magnitude, as does an estuary. The mysid *Neomysis integer* (Crustacea: Mysidacea) dominates the hyperbenthic fauna of the low-salinity regions of western European estuaries (Mees et al., 1995; Mees and Jones, 1997). In these upper estuarine regions, this species is exposed to large tidal and seasonal fluctuations in temperature, salinity and dissolved oxygen (Moffat and Jones, 1992). For example, *N. integer* maintains a relatively permanent position in the East Looe River Estuary (Cornwall, England) and the Westerscheldt estuary (The Netherlands), despite being exposed to large daily fluctuations in its abiotic environment (Mees et al., 1994; Roast et al., 1998). Adaptations to life in such an environment include a wide salinity tolerance (Mauchline, 1971), an extremely efficient osmoregulatory and respiratory physiology (hyper–hypo-osmoregulator) (McLusky and Heard, 1971; Roast et al., 1999). These adaptive mechanisms are energy-consuming and relatively little data is available on the combined effects of abiotic stress factors on the energy metabolism of mysid shrimp. Changes in the energy metabolism, in general, will ultimately influence future life characteristics such as growth and reproduction.

In this context, a new methodology (cellular energy allocation, CEA) to assess the energy budget was adopted for the estuarine crustacean *N. integer* (De Coen and Janssen, 1997). Available energy reserves (total sugar, lipid and protein contents) and energy consumption (electron transport activity) were quantified biochemically and integrated into a general stress indicator. To assess the natural variability of the energy metabolic processes in *N. integer*, a fractional factorial test design was set up with different naturally (Westerscheldt estuary) occurring combinations of temperature, salinity and dissolved oxygen. The influence of these natural variables on the energy metabolism in *N. integer* is described.

2. Material and methods

2.1. Animal collection and maintenance

Initial *N. integer* populations were collected from the shore by hand net in the Galgenweel (a brackish water with a salinity of 3–5 ‰ near the river Scheldt, Antwerp, Belgium). After a 24-h acclimation period to the maintenance temperature, the organisms were transferred to 200-l glass aquaria. Culture medium was artificial seawater (Instant Ocean® Aquarium Systems, France), diluted with aerated deionised tap water to a final salinity of 5 ‰. A 14-h light:10-h dark photoperiod was used during culturing and water temperature was maintained at 20 °C. Cultures were fed daily with 24–48-h-old *Artemia nauplii* ad libidum to prevent adult mysids from cannibalising their young. Hatching of the *Artemia* cysts was performed in 1-l conical vessels under vigorous aeration and continuous illumination at 25 °C.

2.2. Exposure to different abiotic variables

The range of the abiotic variables was chosen from field data for the Westerscheldt estuary. The Westerscheldt estuary is the lower part of the river Scheldt. It is the last

remaining true estuary of the Delta area and is characterised by a marked salinity gradient. The estuarine zone of the tidal zone extends from the North Sea (Vlissingen) to Antwerp, 80-km inland. The abiotic environment is described in Heip (1989).

All exposures were done in a temperature-controlled chamber (Liebher®, Laborimpex, Brussels, Belgium). Temperature was set at 5 or 20 °C (± 1 °C) and dissolved oxygen was maintained at 110% or 70% saturation by aerating with respectively atmospheric air or reduced-oxygen air (14% O_2, Air Liquide, Belgium). The required salinity (5‰ or 25‰) was obtained by diluting artificial seawater (Instant Ocean®) with carbon-filtered deionised tap water. Final salinity was confirmed with a portable refractometer (Digit 032, CETI, Belgium). Dissolved oxygen and temperature were measured at least two times a day (Oxi 191, WTW, Germany) and were within 5% of the desired value.

Test organisms of about equal size (average weight of all animals used: 11.9 ± 4.6 mg) were collected from the cultures and randomly distributed in 1-l glass beakers (10 organisms/beaker). Test animals were allowed a 24-h acclimation period, after which 20 organisms were collected (animals day 0), shock-frozen in liquid nitrogen and kept at -80 °C until analysis. The remaining organisms were exposed to a selected temperature–salinity–dissolved oxygen combination and were collected in the same way after 120 h (animals day 4). We decided not to feed the animals during the exposure period to avoid variance due to individual differences in feeding.

2.3. Factorial design

The influence of the different abiotic parameters on the energy metabolism of *N. integer* was evaluated in a factorial design (Statsoft, 1994). In such a design, factors are tested at two levels. This implies that only linear relationships can be detected because only two levels are tested per factor and curvature cannot be detected from that portion alone. The most intuitive approach to study the influence of multiple factors is to vary the factors of interest in a full factorial design to try all possible combinations of the settings. In this way, the number of necessary runs in the experiment increases geometrically. In this study, temperature, salinity and dissolved oxygen were varied according to a 2^3 full factorial design (eight designs were tested) and, to avoid systematic errors, the order of the runs was randomised (Table 1). Temperature, salinity and dissolved oxygen were varied within a naturally occurring range, representative of the physiological limits of *N. integer*.

2.4. CEA measurement

The CEA was measured according to De Coen and Janssen (1997) with major modifications. The different energy reserve fractions E_a (lipids, protein, sugar) were determined spectrophotometrically and transformed into energetic equivalents using their respective energy of combustion (17,500 mJ/mg glycogen, 24,000 mJ/mg protein, 39,500 mJ/mg lipid) (Gnaiger, 1983). The energy consumed (E_c) was estimated by measuring the electron transport activity according to Owens and King (1975). The quantity of oxygen consumed per mysid, as derived from the ETS data, was transformed into energetic

Table 1
Full factorial design to assess the effects of the abiotic variables temperature (T), salinity (S) and dissolved oxygen(DO) on the energy metabolism of the mysid *N. integer*

Design no.	Water parameters			Energy allocation					
	T (°C)	S (‰)	DO (%)	ΔSugar reserve[a] (mJ/mg ww)	ΔProtein reserve[a] (mJ/mg ww)	ΔLipid reserve[a] (mJ/mg ww)	ΔE_a (mJ/mg ww)	4d-E_c (mJ/mg ww)	CEA
1	5	5	110	20.8 ± 27.2	− 149.8 ± 651.4	− 695.4 ± 667.3[b]	− 824 ± 933[b]	6973 ± 1629[b]	− 0.118 ± 0.137
2	20	25	110	3.4 ± 13.0	448.1 ± 886.3	163.2 ± 743.8	614 ± 1157	5633 ± 1595	0.109 ± 0.208
3	20	5	70	13.5 ± 10.8[b]	1067.5 ± 437.4[b]	570.5 ± 422.7[b]	1651 ± 608[b]	4498 ± 2190	0.367 ± 0.224
4	5	25	70	24.9 ± 13.0[b]	183.5 ± 563.3	269.2 ± 269.1	478 ± 624	6393 ± 1735	0.075 ± 0.100
5	5	5	70	− 4.0 ± 14.0	37.3 ± 245.9	− 19.8 ± 222.0	13 ± 332	4724 ± 753	0.003 ± 0.070
6	20	25	70	17.7 ± 6.0[b]	68.6 ± 286.1	− 11.7 ± 270.1	75 ± 393	3359 ± 643[b]	0.022 ± 0.117
7	20	5	110	1.0 ± 19.3	− 39.8 ± 133.5	− 145.4 ± 373.7	− 184 ± 397	5091 ± 1443	− 0.036 ± 0.079
8	5	25	110	40.9 ± 29.6[b]	− 588.2 ± 134.5[b]	− 141.3 ± 455.8	− 689 ± 476[b]	4769 ± 1090	− 0.144 ± 0.105

[a] Energy reserve day 0 − energy reserve day 4.
[b] Significant change in energy reserve, energy consumption or CEA between days 0 and 4 ($\alpha = 0.05$).

equivalents using the oxyenthalpic equivalents for an average lipid, protein and sugar mixture (484 kJ/mol O_2) (Gnaiger, 1983).

The E_a, E_c and CEA value were calculated as follows:

ΔE_a (difference in 'available energy,' E_a) $= E_{a,\ day\ 0} - E_{a,\ day\ 4}$
E_c (average 'energy consumption,' E_c) $= 1/2*(E_{c,\ day\ 0} + E_{c,\ day\ 4})$
CEA (cellular energy allocation) $= \Delta E_a / E_c$

2.5. Statistical analysis

All data were checked for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's test, respectively, with an $\alpha = 0.05$. The factorial design was analysed using the experimental design module in the software package Statistica™. The effect of the treatment was tested for significance using a one-way analysis of variance (Tukey's test, Statistica™).

3. Results

The biochemical composition of *N. integer* was as follows: protein (7.39 ± 1.81% wet weight), lipid (3.99 ± 1.05% ww) and sugar (0.42 ± 0.18% ww). The changes in energy reserves during the 4-day exposure period are summarized in Table 1. Sugar reserves were mainly used as an energy source and higher salinities were correlated with higher sugar consumption. The protein fraction was quantitatively the most important energy fraction and exhibited large variation (1714.6 ± 467.5 mJ/mg ww). Similar large variations were found in the lipid content (1553.8 ± 493.6 mJ/mg ww). The energy consumption as measured by the electron transport activity was relatively unaffected after the 4-day exposure, except for a significant reduction in design 1 (5 °C-5 ‰ –110% DO) and design 6 (20 °C-25 ‰ –70% DO).

To determine individual effects of the tested abiotic factors, an analysis of variance was performed on the complete data set (Table 2). The different abiotic factors had no significant effect on the energy metabolism of *N. integer* within the tested range. The

Table 2
The influence of temperature (*T*), salinity (*S*) and dissolved oxygen (DO) on the energy metabolism of *N. integer*

Energetic fraction	Abiotic stress factor		
ΔSugar	*S* ($p = 0.24$, ↓)	*T* ($p = 0.31$, ↑)	DO ($p = 0.75$, ↓)
ΔProtein	*T* ($p = 0.15$, ↓)	DO ($p = 0.21$, ↑)	*S* ($p = 0.52$, ↑)
ΔLipid	DO ($p = 0.15$, ↑)	*T* ($p = 0.27$, ↓)	*S* ($p = 0.56$, ↓)
ΔE_a	DO ($p = 0.15$, ↑)	*T* ($p = 0.16$, ↓)	*S* ($p = 0.93$, ↑)
4d-E_c	DO ($p = 0.90$, ↑)	*T* ($p = 0.95$, ↑)	*S* ($p = 0.96$, ↑)
CEA	DO ($p = 0.15$, ↑)	*T* ($p = 0.16$, ↓)	*S* ($p = 0.70$, ↑)

The abiotic factors are ranked according to their importance in explaining the variance of the tested variable (full factorial design, ANOVA, Statistica™). The level of significance and the nature of the effect are shown (↑ positive; ↓ negative).

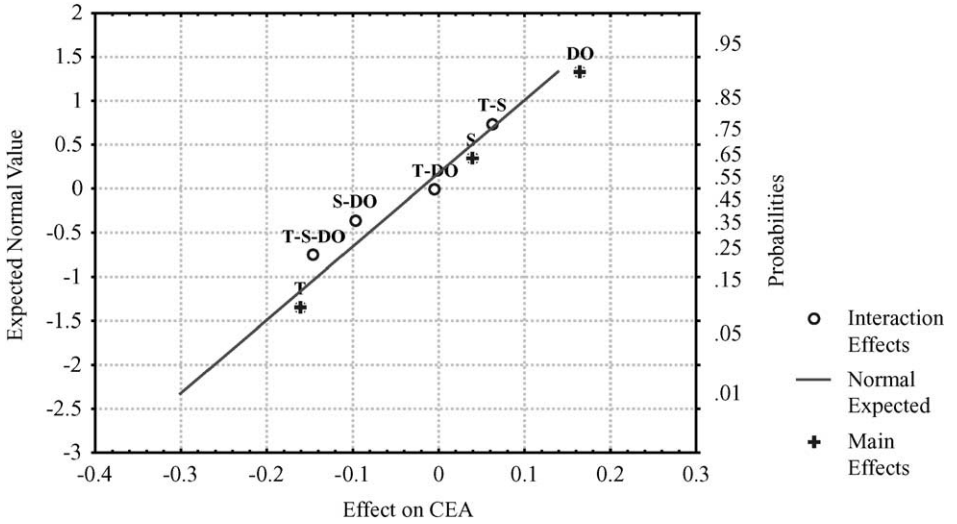

Fig. 1. Normal probability plot of the individual and interaction effects of the selected abiotic factors (*T*: temperature, *S*: salinity, DO: dissolved oxygen) on the energy metabolism of *N. integer*. True effects are seen as outliers on the graph (Statistica™).

small variance in energy consumption could not be explained by a single abiotic factor. Temperature explained the decrease in lipid and protein content, change in total energy reserves (ΔE_a) and CEA. Again, salinity was the most important factor explaining the effects on sugar reserves. Interaction effects of the different abiotic factors were also tested (Fig. 1). Although interaction effects were apparent (deviation from 0-effect on CEA in the abscissa), no significant effect could be found on the energy budget by the interaction of the three tested abiotic factors; temperature, in general, had the most adverse effect on the CEA.

4. Discussion

4.1. Biochemical composition of N. integer

Knowledge of the biochemical composition of a species is essential to the understanding of its metabolism. The different biochemical fractions of *N. integer* were determined simultaneously. Although lower than reported by Raymont et al. (1964), the protein content (7.39 ± 1.81% ww) of *N. integer* was found to be high and quantitatively the most important fraction. The low levels of sugar (0.42 ± 0.18% ww) and lipid (3.99 ± 1.05% ww) confirm earlier findings (Bhat and Wagh, 1992; Raymont et al., 1964). Differences in energy content reported in our study in comparison to other studies might be caused by the artificial feeding of *N. integer* with lipid-enriched *Artemia* (*Artemia* are enriched with lipid-containing SuperSelco-INVE, Aquaculture NV, Belgium), or as a result of the colorimetric methods used. Mysids are known to be omnivores and base their metabolism on food, which is more or less immediately available, and food

storage may be less significant (Fockedey and Mees, 1991; Mauchline, 1971; Tattersall and Tattersall, 1951). The artificial feeding of these animals in the laboratory may thus explain the different biochemical composition compared to that of field-collected mysids. Since the main objective of this study was to investigate changes in the energy metabolism during a relatively short period under different environmental conditions, feeding was stopped during the exposures. In this way, the metabolic use of energy reserves were quantified without having interference of effects on feeding behaviour and energy intake (energy was only lost during the experiment). Differences in initial energy reserves were of less importance for the described biomarker, since energy fractions were always measured relatively to the initial condition (day 0).

4.2. Effects on energy reserves

The effects of different combinations of temperature, salinity and dissolved oxygen on the energy status of *N. integer* were tested to gain insight into the natural variability of the energy metabolism under field-based conditions.

As the animals were not fed during the exposure period, no significant increase of any of the biochemical fractions was expected. The significant increase in some biochemical fractions is attributed to cannibalism (personal observation) and was taken into account when interpreting the results (only negative effects were seen as true effects).

With exception of design 5 (5 °C-5‰–70% DO), all tested designs resulted in a reduction of the sugar content (Table 1). Sugar depletion could be correlated, although not significantly ($p = 0.24$), with changes in salinity (Table 2). *N. integer* is a hyper- and hypoosmoregulator with its isoosmotic point situated around 18‰, and a high tolerance for salinities between 1‰ and 40‰ (McLusky and Heard, 1971; Roast et al., 2001). Thus, at salinities of 5‰ and 25‰, *N. integer* actively maintains its hemolymph respectively hyper- or hypo-osmotic to the external environment. The actual osmoregulatory mechanisms of mysids have not been demonstrated, and may be different from those of decapods (Tattersall and Tattersall, 1951). It is, however, obvious that when placed in a medium of higher or lower osmotic pressure, energy-consuming processes are required to maintain the internal osmotic pressure constant. Glucose delivers fast energy in the form of ATP via the process of glycolysis and oxidative phosphorylation and is the major circulating carbohydrate in crustaceans (Morris, 1999). Although lipid and protein utilisation is variable in crustaceans, generally, carbohydrate is used before lipid and protein as the preferred fuel for metabolic processes (Garret and Grisham, 1995; Morris, 1999). The mysids in this study were acclimated to a salinity of 5‰ and transferred into a test medium of either the same (5‰) or higher salinity (25‰). Transfer to a higher salinity requires the organism to shift its osmoregulation from hypo- to hyperosmotic, which may explain the significantly higher use of sugar reserves (in designs 4-6-8) as a fast energy source to fuel these processes.

The effects of the abiotic factors on the lipid reserves were not clear. Higher temperatures, higher salinities and lower dissolved oxygen concentrations resulted in increased lipid use. Linford (1965) found that *N. integer* starved for 96 h showed little decrease in lipid content. Contrary to this, Morris (1971) found significant changes in the lipid content (25% lipid loss after 7 days) of *N. integer* in a series of experiments to assess the effects of temperature and

salinity. Morris also suggested that the lipid fraction of *N. integer* contains a very conservative (in its fatty acid composition) element, which plays an important role in the metabolism of the animal. Our findings support the view of Morris that relatively large variations in lipid reserves are found in *N. integer*. Organisms were not sexed and consequently male and female organisms were randomly used for each experiment. The eggs of mysids contain high amounts of lipids and the lipid metabolism of ovigerous females can be assumed to be different from males (Linford, 1965). The large variations in lipid metabolism observed in our study might be partially caused by a sex-specific lipid metabolism.

The biochemical composition of the estuarine mysid *N. integer* contrasts sharply with the typical mammal in its low carbohydrate reserves and fat depots. The large amount of protein observed in our study might suggest that *N. integer* actively deaminates its body proteins, especially should food shortage occur. In this event, the mysid, a relatively small aquatic animal should experience no great difficulty in ridding its body of ammonia arising from deamination (Raymont et al., 1968). The potential use of protein as a metabolic reserve has also been suggested by Bhat and Wagh (1992) for marine zooplankton. The decline in protein reserves in our study after a 4-day exposure supports the hypothesis that *N. integer* can actively use protein as an energy source under starvation or stress conditions. Longer exposures will be performed to confirm these results. In these experiments, the effect of ecdysis on the protein metabolism and the general energy metabolism will also be assessed. The exoskeleton of *N. integer* contains large amounts of protein and ecdysis occurs in a temperature and age dependent way. In juvenile *N. integer*, the intermoult period varies from 2–3 up to 10 days at 9 and 16 °C, respectively (Astthorson and Ralph, 1984; Mauchline, 1985). Although no exoskeletons were observed in the exposure beakers (the exoskeleton is, however, rapidly consumed by other animals), large variations (as in design 3) could be attributed to ecdysis.

4.3. Effects on energy consumption

The oxygen consumption of *N. integer* is dependent on several abiotic (e.g. temperature, salinity) and biotic (age, weight, gender, reproductive status) parameters (Burggren and Roberts, 1991; Kinne, 1970, 1971; Newell and Branch, 1980; Roast et al., 1999; Schmidt-Nielsen, 1997). Each factor may have an independent effect, or all factors may interact and influence the respiratory metabolism, making it a difficult parameter to interpret. The method described in this study to measure respiration was adopted from Owens and King (1975). The method for measuring the activity of the electron transport system provides an estimate of the potential whole organism respiration rate and has been validated in our laboratory for *N. integer* against real-time respiration measurements with a respirometer (RC650 Strathkelvin Instruments). Both measurements were highly correlated ($R^2 = 0.94$; $p < 0.01$, data not shown).

From the analysis of variance on the average energy consumption (4d-E_c), it can be concluded that none of the three tested abiotic parameters had a significant effect on the measured ETS activity (Table 2). Biotic variables such as age, weight and gender probably have a larger influence on respiration rates. Furthermore, the ETS assay measures the maximum ETS activity under saturated substrate (NADH, NADPH) conditions and

observed changes in activity must therefore be realised through changes in the amount of enzymes produced by the organism. Consequently, ETS activity responds much slower to changes in the environment than the respiration rate (Båmstedt, 1980; Mayzaud, 1986; Skjoldal et al., 1984). Båmstedt (2000) has recently published an alternative to the Owens and King method based on natural levels of substrate in an attempt to achieve a better correlation between the ETS assay and ambient respiration rates. This method looks promising but needs further validation.

Taking into account the inherent properties of the ETS assay, the energy consumption reported in our study is an overestimation of the ambient respiration. This is clear when considering the energetic content calculations for the organisms. The average calculated energetic content ($E_{a,\ day\ 0}$) on day 0 was 3405 mJ/mg ww, the average energy consumption (4d-E_c) for the 4-day period derived from the ETS assay was 5180 mJ/mg ww. From these calculations *N. integer* would use up all of its energy resources in less than 3 days provided there is no energy intake. As mentioned before, the ambient respiration is a lot lower than calculated by the ETS assay, and mysids consumed considerably less energy during the exposure period (max. 1651 mJ/mg ww; ΔE_a, Table 1). Although energy consumption calculated by ETS activity is thus an overestimation of actual respiration and can also be derived more correctly from the decline in energy reserves, there are advantages associated with ETS measurements. Especially with reference to exposures with toxic compounds, the ETS assay can mechanistically explain alterations in the energy consumption through specific interaction of the toxicant with the electron transport system (Oberdörster et al., 1998; Spicer and Weber, 1991).

4.4. Interaction effects on the cellular energy allocation (CEA)

Although apparent interaction between temperature–salinity–dissolved oxygen and salinity–dissolved oxygen can be derived from the normal-probability plot shown in Fig. 1, none of these effects was significant. The energy metabolic processes of *N. integer* seem to be relatively unaffected by changes in the abiotic environment.

Temperature had the most effect (negative) on the CEA (Table 2). The relatively higher sensitivity towards changes in temperature might be explained by the fact that this parameter is also the least variable over a small time period in the environment. Where salinity and dissolved oxygen show high fluctuations linked to tidal influences in an estuary, temperature changes more slowly with a seasonal variability. This conclusion is supported by Roast et al. (2000) examining the effect of fluctuating abiotic factors (temperature, salinity) on the egestion rate of *N. integer*. In this study, temperature was the most important factor causing increased egestion rates in *N. integer*. These correspond with a lower CEA in our study, since there was no ingestion in our study. Weisse and Rudstam (1989) also found temperature to be the most important abiotic factor explaining variance in excretion and respiration rates in *N. integer*. We are currently performing an inter-laboratory validation of our CEA method with the Scope for Growth approach based assay of Roast et al. (Plymouth Marine Laboratory, UK), to further mechanistically explain our common results and examine the future use of CEA as a biomarker for exposure.

The CEA methodology described in this study not only provides an integrated quantification of the organism's energy budget but also helps to elucidate different modes

of action upon exposure to a varying abiotic environment and could potentially explain the different modes of action of toxicants. Furthermore the CEA methodology has been used with success with other organisms, e.g. the crustacean *Daphnia magna* (De Coen and Janssen, 1997) and catfish *Clarias gariepinus* where Nguyen (1997) demonstrated that the CEA criterion was predictive of long-term effects on growth. This illustrates the potential use of the cellular energy allocation as a methodology to assess the energy budget of organisms using simple colorimetric measurements. Due to the observed relationships with long-term population effects, as described by De Coen and Janssen (1997) for *D. magna*, this biomarker might also be useful in field monitoring programmes.

5. Conclusion

In addition to its potential as a biomarker of exposure to environmental toxicants, the CEA methodology can be very useful as an applied physiological research tool to study the energy metabolism of an organism, as shown in this study. In contrast to the Scope for Growth assay (Warren and Davies, 1967), the CEA methodology offers the possibility to assess (separately) both energy conversion and energy allocation. Although no significant effects of the tested abiotic variables were apparent during the 4-day exposure period, preliminary conclusions on the involved mechanisms and potential effects of natural variability in *N. integer*'s natural environment are possible. Generally, these results were in accordance with previous studies on the energy metabolism of *N. integer*. Studies like this allow a more integrative approach when studying field effects in laboratory experiments. In this respect, the used factorial design approach has a great potential when a large number of parameters need to be tested.

Future research will focus on the validation of the CEA methodology with other described methodologies and the use of the CEA as biomarker of exposure in laboratory and field. The use of the CEA assay is, in this context, also being tested in situ on resident mysid populations in the Westerscheldt estuary.

Acknowledgements

This research was supported by the Flemish Institute for the Promotion of Scientific and Technological Research in Industry (IWT) and the Ghent University Research Fund. The authors wish to especially recognize the work of Pieter De Smet. **[SS]**

References

Astthorson, O.S., Ralph, R., 1984. Growth and moulting of *Neomysis integer* (Crustacea: Mysidacea). Mar. Biol. 79, 56–61.

Båmstedt, U., 1980. ETS activity as an estimator of respiratory rate of zooplankton populations. The significance of variations in environmental factors. J. Exp. Mar. Biol. Ecol. 42, 267–283.

Båmstedt, U., 2000. A new method to estimate respiration of biological material based on the reduction of tetrazolium violet. J. Exp. Mar. Biol. Ecol. 251, 239–263.

Bhat, K.L., Wagh, A.B., 1992. Biochemical composition of zooplankton of Bombay High (oil platform) area in the Arabian Sea. Indian J. Mar. Sci. 21, 220–223.

Burggren, W., Roberts, J., 1991. Respiration and metabolism. In: Prosser, L. (Ed.), Environmental and Metabolic Animal Physiology. Wiley-Liss, New York, pp. 353–436.

De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing: IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. J. Aquat. Ecosyst. Stress Recovery 6, 43–55.

Fockedey, N., Mees, J., 1991. Feeding of the hyperbenthic mysid *Neomysis integer* in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. J. Mar. Syst. 22, 207–228.

Garret, R.H., Grisham, C.M., 1995. Biochemistry Saunders College Publications, Forth Worth.

Gnaiger, E., 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger, E., Forstner, H. (Eds.), Polarographic Oxygen Sensors, Aquatic and Physiologica Applications. Springer Verlag, Berlin, pp. 337–345.

Heip, C.H.R., 1989. The ecology of the estuaries of Rhine, Meuse and Scheldt in the Netherlands. Sci. Mar. 53, 457–463.

Kinne, O., 1970. Temperature-invertebrates. In: Kinne, O. (Ed.), Mar. Ecol., vol. 1. Wiley-Interscience, New York, pp. 407–514.

Kinne, O., 1971. Salinity-invertebrates. In: Kinne, O. (Ed.), Mar. Ecol., vol. 1. (Part 2). Wiley-Interscience, New York, pp. 821–995.

Linford, E., 1965. Biochemical studies on marine zooplankton: II. Variations in the lipid content of some Mysidacea. J. Cons. - Cons. Int. Explor. Mer 28, 354–363.

Mauchline, J., 1971. The biology of *Neomysis integer* (Crustacea, Mysidacea). J. Mar. Biol. Assoc. U.K. 51, 347–354.

Mauchline, J., 1985. Growth in mysids and euphausiids. In: Wenner, A.M. (Ed.), Factors in Adult Growth. A.A. Balkema, Rotterdam, The Netherlands, pp. 337–354.

Mayzaud, P., 1986. Enzymatic measurements of metabolic processes concerned with respiration and ammonium excretion. In: Corner, E.D.S., O'Hara, S.C.M. (Eds.), The Biological Chemistry of Marine Copepods. Oxford Univ. Press, Oxford, UK, pp. 226–259.

McLusky, D.S., Heard, V.E.J., 1971. Some effects of salinity on the mysids *Praunus flexuosus*. J. Mar. Biol. Assoc. U.K. 51, 709–715.

Mees, J., Jones, M.B., 1997. The hyperbenthos. Oceanogr. Mar. Biol. Annu. Rev. 35, 221–255.

Mees, J., Abdulkerim, Z., Hamerlynck, O., 1994. Life history, growth and production of *Neomysis integer* in the Westerschelde estuary (SW Netherlands). Mar. Ecol., Prog. Ser. 109, 43–57.

Mees, J., Fockedey, N., Hamerlynck, O., 1995. Comparative study of the hyperbenthos of three European estuaries. Hydrobiologia 311, 153–174.

Moffat, A.M., Jones, M.B., 1992. Bionomics of *Mesopodopsis slabberi* and *Neomysis integer* (Crustacea: Mysidacea) in the Tamar Estuary (S.W. England). In: Köhn, J., et al. (Eds.), Taxonomy, Biology and Ecology of (Baltic) Mysids (Mysidacea: Crustacea). Rostock Univ. Press, Rostock, pp. 110–120.

Morris, J., 1971. Seasonal and environmental effects on the lipid composition of *Neomysis integer*. J. Mar. Biol. Assoc. U.K. 21, 21–31.

Morris, S., 1999. Integration of physiological responses of crustaceans to environmental challenge. S. Afr. J. Zool. 33, 87–106.

Newell, R.C., Branch, G.M., 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. Adv. Mar. Biol. 17, 329–396.

Nguyen, T.H.L., 1997. Potential and limitations of early life stage toxicity tests with fish. PhD thesis. Ghent University, Belgium, p. 241.

Oberdörster, E., Rittschof, D., McClellan-Green, P., 1998. Induction of cytochrome *P*450 3A and heat shock protein by tributyltin in blue crab, *Callinectes sapidus*. Aquat. Toxicol. 41, 83–100.

Owens, T.G., King, F.D., 1975. The measurement of respiratory electron transport activity in marine zooplankton. Mar. Biol. 30, 27–36.

Raymont, J.E.G., Austin, J., Linford, E., 1964. Biochemical studies on marine zooplankton: I. The biochemical composition of *Neomysis integer*. J. Cons. - Cons. Int. Explor. Mer 28, 354–363.

Raymont, J.E.G., Austin, J., Linford, E., 1968. Biochemical studies on marine zooplankton: V. The composition of the major biochemical fractions in *Neomysis integer*. J. Mar. Biol. Assoc. U.K. 48, 735–760.

Roast, S.D., Widdows, J., Jones, M.B., 1998. The position maintenance behaviour of *Neomysis integer*

(Peracarida: Mysidacea) in response to current velocity, substratum and salinity. J. Exp. Mar. Biol. Ecol. 220, 25–45.

Roast, S.D., Widdows, J., Jones, M.B., 1999. Respiratory responses of the estuarine mysid *Neomysis integer* (Pericarida: Mysidacea) in relation to a variable environment. Mar. Biol. 133, 643–649.

Roast, S.D., Widdows, J., Jones, M.B., 2000. Egestion rates of the estuarine mysid *Neomysis integer* (Peracarida: Mysidacea) in relation to a variable environment. J. Exp. Mar. Biol. Ecol. 245, 69–81.

Roast, S.D., Widdows, J., Jones, M.B., 2001. Effects of salinity and chemical speciation on cadmium accumulation and toxicity to two mysid species. Environ. Toxicol. Chem. 20, 1078–1084.

Schmidt-Nielsen, K., 1997. Animal Physiology (Adaptation and Environment), 5th ed. Cambridge Univ. Press, Cambridge, England.

Skjoldal, H.R., Båmstedt, U., Klinken, J., Lain, A., 1984. Changes with time after capture in the metabolic activity of the carnicorous copepod *Euchaeta norvegica* Boeck. J. Exp. Mar. Biol. Ecol. 83, 195–210.

Spicer, J.I., Weber, R.E., 1991. Respiratory impairment in crustaceans and molluscs due to exposure to heavy metals. Comp. Biochem. Physiol. 100C, 339–342.

Statsoft, 1994. Experimental Design. In: Statistica, vol. III. Tulsa, OK, USA, pp. 3613–3682.

Tattersall, W.M., Tattersall, O.S., 1951. The British Mysidacea. Ray Society, London, p. 460.

Warren, C.E., Davies, G.E., 1967. Laboratory studies on the feeding, bioenergetics and growth in fish. In: Gerking, S.D. (Ed.), The Biological Basis of Freshwater Fish Production. Blackwell, Oxford, pp. 175–214.

Weisse, T., Rudstam, L.G., 1989. Excretion and respiration rates of *Neomysis integer* (Mysidacea): effects of temperature, sex and starvation. Hydrobiologia 178, 253–258.