SCOPE FOR GROWTH IN MUSSELS EXPOSED IN WESTERN AND EASTERN SCHELDT

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Summary

The application of the Scope For Growth (SFG) in mussels Mytilus edulis as a stress-parameter was tested. Groups of mussels were exposed to cadmium or PCBs in an experimentel set-up; others were placed at stations in more or less polluted coastal areas in the Netherlands. The different components of the SFG of the mussels at the stressed sites were not always significantly different from those at reference sites. The SFG measurement was especially usefull for the detection of local pollution. In all cases the consumption of energy (and clearance rate) was the main component of the SFG that was changed.

1 - Introduction

Objectives

Effects of pollutants on marine and estuarine ecosystems are an actual topic in water quality management in the Netherlands. Much attention is given to improvement of the quality of the Western Scheldt and the North Sea. To estimate the effects of pollutants in time and space, and to evaluate measures, national and international monitoring programmes, such as the "Mussel Watch", have been performed. Considerable data bases exist on concentrations of contaminants in water, sediment and certain organisms. However, on the in situ effects of pollution on populations and ecosystems less is known. Field surveys in most cases follow a retrospective approach and show weak relations between dose and response. To develop indicative parameters on different levels of integration a project has been set up by the Tidal Waters Division. As part of the project a study is performed in cooperation with the Delta Institute for Hydrobiological Research to test the usefulness of food budget (Scope for Growth, furtheron SFG) estimations of the blue mussel for biological effect monitoring purposes. In 1987 and 1988 the method was set up according to Widdows & Bayne, 1971, Bayne et al., 1985 and Widdows and Johnson, 1988. The method was tested under several circumstances (Smaal & Korporaal, 1988) and applied in the Western and Eastern Scheldt. Results given in this report consider testing of the measuring practice and of technical problems of in situ exposure.

The aim of the present study is to investigate whether it is possible to apply the SFG as a stress-parameter in the coastal waters of the Netherlands. The Western Scheldt and Eastern Scheldt are chosen as a test location. The Western Scheldt is selected for several reasons; a pollution gradient can be found and this estuary includes a variety of environments that will enable us to distinguish between effects of pollutants and abiotic variation. The Eastern Scheldt is a more homogeneous, and relatively unpolluted sea-arm, that can be interpreted as a control, however, with some polluted harbours.

The SFG is a determination of the surplus of energy, expressed in Joule, that is available for growth. By measuring the amount of energy uptake(consump-

tion) and expenditure(respiration) the SFG can be calculated by substracting the latter, the outcome will give the amount of energy that can be used for growth. Growth can be expressed in somatic growth, increase of dry weight or shell length and in development of gametes, developmental stage of the gonads, during the reproductive cycle.

When the SFG is applied as a stress-parameter test it gives a general indication whether or not mussels could survive a certain environment and subsequently the ecosystem could be affected by the disappearance of one or more species. The SFG does not give information concerning the molecular or cellular mechanism by which the toxicity is generated.

Effects of pollutants on the SFG could be divided into three groups;
- substances affecting the filtration rate, either by exerting a narcotic effect on the ciliary activity of gill tissue, so, filtration rate decreases or eventually ceases (Redpath and Davenport 1988, Widdows and Donkin 1989), or by an acute effect on a specific tissue, e.g. nerve tissue, resulting in valve closure; in this case filtration rate should be zero at all times.
- substances affecting the oxygen consumption, for several toxicants, e.g. cadmium(Jacobs 1956) and organo-tin compounds, it has been proven that these uncouple the oxidative phosphorylation, so the normal interaction between carbohydrate metabolism and oxygen consumption is disrupted.
- substances affecting the digestion and/or absorption of nutrients in the gut compartment. This results in a lower absorption efficiency and a lower intake of energy. The relation between pollution and absorption efficiency has not been subject for intensive investigation, the relation between food quality and absorption efficiency in bivalves have been extensively studied (Bayne and Newell, 1983).

History of scope for growth as biological monitoring tool

After the development of the technique for the measurement of the SFG of fish, the method was adapted for the measurement of the energy budget for mussels. At first the method was applied under laboratory conditions(Widdows and Bayne 1971, Bayne 1975, and Widdows 1978). Later, the SFG was applied in field situations on translocated mussels, special attention was given to changes induced by abiotic factors(Bayne and Widdows 1978,Bayne et al. 1979, and Widdows et al. 1981). Gilfillan used SFG to investigate effects of oil pollution(Gilfillan 1975, and Gilfillan et al. 1979). Nelson(1985) used the SFG to investigate effects of polluted sludge(contaminated with PCB, Cu and Cr).

Several investigators were able to find a correlation between the gradient of pollution and coinciding changes in the SFG of mussels translocated to more or less enclosed waters(Widdows et al. 1982: Sullom Voe(GB), Widdows et al. 1980: Narragansett bay(USA), Severeid 1983: San Fransico bay(USA), Nelson 1985: Black Rock harbor(USA). In the Netherlands Rijkswaterstaat and TNO-Den Helder performed research into the applicability of SFG in Dutch coastal waters.

The SFG technique was also used during an international workshop held in Oslo -GEEP-(Bayne et al. 1988). In this study a large number of different stress-parameter tests was performed on both a transect through a pollution gradient in a fjord and an artificial exposure system was set up in which animals were exposed to copper. From all these tests the SFG best reflected the gradient of pollution and the different Cu concentration used in the exposure systems (Widdows and Johnson 1988).

Recently, Widdows found a good correlation between the QSAR parameter of small PAH's and their effect on the clearance rate(Widdows and Donkin 1989). In the study of Widdows it was demonstrated that particularly LMW hydrocarbons showed a linear relation with the concentration resulting in 50 % inhibition of the filtration rate.

Th use of SFG in mussels

- mussels have a global distribution and play a vital role in the coastal and intertidal communities.
- mussels accumulate pollutants in their soft tissue by virtue of their filter-feeding lifestyle, also for this reason mussels give a kind of integrated information of the level of contaminants in their environment. Due to this, mussels enable us to detect low concentrations of pollutants.
- mussels are very stress resistant animals, in the sense that they can be handled without causing any detectable stress after a sufficient recovery period.
- mussels can be used in both field and laboratory set up, so reference experiments can be performed under standardized conditions.
- relatively much literature is available on the mussel describing its physiology and various aspects of their toxicology, mainly under laboratory conditions.

However, besides the effects of toxic agents on the SFG of mussels, other factors could influence the outcome of a SFG measurement. These factors can be divided into two groups; intrinsic biotic factors and abiotic factors, i.e.

body size, reproduction and temperature, salinity, oxygen concentration, food concentration, and aerial exposure, respectively. As a detailed description on the effects of these factors on the SFG is provided by Widdows(1985), a concise representation will be given, together with their practical implication for the method used in this study.

Body size - Some components of the SFG increase in relation to body size, according to an allometric relation. In general, all mussels used for exposure at the field sites were selected on their shell length which in turn is related to body weight. By only using mussels with a shell size from 40 to 50 mm, a rather homogenous and uniform group was used for exposure and testing.

Reproduction - The reproductive cycle causes an increase of clearance rate and oxygen consumption during the period of spawning. As a consequence the stage of the gonads should be taken into account when interpreting SFG data in the spawning period.

Temperature - SFG is dependent on temperature, mussels are capable to adapt to temperatures ranging from 5 to 20 °C. As metabolic rate in the mussel is temperature dependent, only oxygen consumption is influenced by this parameter. When the ambient temperature increases from 5 to 20 °C, respiration will increase from about 5 to 8 J/g/h. Overall, the influence of temperature does not have to generate important considerations while reference and test site are always compared at ambient temperatures.

Salinity - Salinity has no marked effect on the measurement of the SFG ranging from 30 to 20 %. , but at salinity values below 20 %. the energy budget decreases due to a reduction of energy uptake. As all SFG measurements are performed at a salinity of about 30 %., at the site of the field station, this should cause no problems, as mussels adapt their osmotic value to higher salinity very rapidly, in about 2 to 4 hours. Adaptation of the internal osmolarity to reduced salinities requires an acclimatization period between 12 to 24 hours. This factor again does not play a role in the comparison of reference and test sites.

Oxygen concentration - As long as the oxygen concentration is kept above 40 % saturation no effect of this parameter can be discerned. So, only during the measurement of the oxygen consumption the concentration of oxygen should have to be watched carefully and the duration of the anaerobic incubation would be as short as possible, still giving an accurate determination of the oxygen decline.

Food concentration - The concentration of food particles should be maintained at a sufficient level in order to obtain a positive outcome of the SFG. SFG is

proportional to the food concentration, when food concentrations are extremely high, filtration activity and thus consumption can be found to be reduced. When the measurement of SFG is performed in this study, food concentration expressed as number of algal cells per mL is kept constant within the range of 25,000 to 30,000 cells per mL for sequential measurements and throughout the year.

Aerial exposure - During aerial exposure the SFG can not be measured whilst the animals are not submerged in seawater. But anoxia does exert an effect on the SFG during the initial phase of recovery from aerial exposure. During this initial phase lasting up to 2 hours the metabolic rate to the mussel is enhanced as reflected in an increased oxygen consumption. In this initial period the anaerobic endproducts, formed during exposure, are metabolized, thereafter the metabolism is restored at its normal rate. This implies that no SFG determination should start before at least a 2 hours recovery period is provided. As defecation in the procedure lasts about 6 hours this does not interfere with the recovery of an aerial exposure.

2 - Materials and Methods

Animals

All mussels used for active bio-effect monitoring were collected from cultured mussel plots in the Eastern Scheldt. Specimens with a shell length ranging from 40 to 50 mm were cleaned and selected, and divided at random in groups of 80 individuals. During this procedure the animals were kept in running seawater at the field station of the Tidal Waters Division. After the measurements the mussels were dried for 48 h. at 70 °C, to establish dry weight(DW).

Field Exposure

Each group of mussels was put in wire baskets and six baskets were placed in a steal frame. These frames were attached to buoys at different locations in both Eastern and Western Scheldt. At different incubation times a group of animals was retrieved from an exposure site and brought to the field station. where the mussels were kept in running seawater. Within two days SFG measurements were performed.

Field exposure sites

Sophia-harbour	field station(reference location), Eastern Scheldt
Jacoba-harbour	field station(future reference location),Eastern Scheldt
Hammen	Eastern Scheldt, location of cultured mussel plots
Colijnsplaat	yachting-harbour, Eastern Scheldt
Vlissingen	Western Scheldt, west part
Walsoorden	Western Scheldt, east part

The geographic location of the different sites is given in figure 1.

Experimental Exposure

At the field station, Sophia-harbour, mussels were exposed to 25 ppb Cd or 0.5 ppb PCB in a tank with a volume of 1200 L. More details of this set up are given in the annual report of Stresspar 1987 (Den Besten and Veldhuizen, 1988). Twice, after 10 and 11 weeks of exposure, SFG was measured in comparison with a control group kept under identical conditions.

Scope for Growth measurement

A detailed description of the SFG technique is given by Widdows(1985). Therefore,this par. will be restricted to a description of the actual practical performance.

The SFG set up consists of 16 cells, volume 700 mL, equipped with peristaltic pumps and magnetic stirrers. Different parts of the SFG were measured in these cells; clearance rate, food absorption, respiration rate and ammonia excretion. Each part is described below, calculations are added in a supplement.

Clearance rate

By means of peristaltic pumps filtered sea water, to which Phaeodactylum tricornutum was added(25,000-30,000 cells/mL), was passed through the measuring cells with a flow of 7 to 12 L/h.

First, mussels were placed in the cells for 5 to 6 hours for acclimatization to the diet. After this period, all cells were opened and rinsed with filtered sea water in order to remove faeces. Mussels were placed in the clean cells and incubated overnight(16 h), during which the outflow was sampled regularly. The algae concentration in the in- and outflow were determined by means of a Coulter counter, each sample was measured three times, the mean of this triplicate was used to calculate the filtration and clearance rate.

By measuring the difference in number of algal cells(particles/l), by means of a coulter counter, in the outflow of empty cells compared to those containing one mussel, multiplied by flow rate (l/h), the filtration rate (particles-/h) is established. The filtration rate divided by the outflow concentration results in the clearance rate (l/h). Division by outflow is chosen in stead of inflow because it is assumed that the internal concentration is equal to the outflow concentration, because water in the cell is stirred and there is a high flow rate.

Absorption efficiency

The amount of particulate organic matter of the diet was determined by filtration of 1 L seawater, spiked with algae, through a preashed Whatman GFC filter (1 um mesh size). Thereafter, the filter was dried at 70°C and weighed before and after ashing at 450°C. Dry weight at 70°C relates to the total amount of suspended matter, while the decrease of dry weight after ashing is the amount of particulate organic matter(POM).

After overnight incubation, feeding with algae was stopped and the cells were circulated with filtered sea water, during this the mussels could continue to produce faeces for 1 hour. After opening the cells and removing the mussels, faeces was collected quantitatively with a pipet. Thereafter, faeces was processed similar to diet, described above, and suspended matter and POM from faeces was measured. By means of the Conover ratio(Conover, 1966), absorption

efficiency was calculated.

Because of the sensitivity of the Conover ratio for small differences in weight, resulting in a fluctuating absorption efficiency, an alternative calculation of absorption efficiency is performed by using the difference of total ingestion and defecation.

Respiration and Ammonia excretion

After collecting faeces the mussels were put back in the cells and filtered sea water was circulated through the cells for 1 hour. After sampling the outflow for the oxygen concentration, both in- and outlet of the cells were closed. Depending on the ambient temperature, the mussels were left in the sealed cells for 45 to 120 minutes. A second sample was collected from each cell to measure the oxygen decrease during the incubation. By means of a Winkler titration the oxygen concentration was determined. The oxygen consumption was calculated by subtracting the oxygen concentration from both samples and correcting this for the duration of the incubation.

For Ammonia excretion, separate samples were collected at the same time as the samples for oxygen measurement.

After completing this cycle, the mussels were removed from the cells and dissected, dry weight was determined by drying the soft tissue at 70°C for 48 hours.

The recalculation of the different parameters into energetics is given in a supplement.

Methodological Discussion

At first a methodological problem with respect to the absorption efficiency has to be solved. Two methods were used: the Conover ratio (Conover, 1966) and the Balance Calculation.

Calculating the absorption efficiency with the Conover ratio resulted in fluctuating values, sometimes the efficiency was found to be negative.

The calculation of the Conover ratio is extremely sensitive for small variations in the estimation of percentage ashfree dryweight, this is best illustrated by an example. Fictive results are estimated as :

organic content faeces - 60 % $\pm$ 2.5 , so ranging from 57.5 to 62.5 %

organic content algae - 80 % $\pm$ 2.5 , so ranging from 77.5 to 82.5 %

Using the above extremes for adw faeces and algae, the Conover ratio ranges

from 0.51 to 0.71. When the standard deviation is larger, for example 5 % then the ratio will diverge from 0.38 to 0.78. This shows the sensitivity of the Conover ratio by mere calculus, resulting in a ratio differing a factor two.

Using the Balance Calculation method, the passage time through the digestive track has to be subtracted from the total incubation time, following :

$$U = \frac{(I-N)}{I} ,$$

in which; I = total amount of ingested organic matter
(clearance rate * POM-food * duration)
N = total amount of organic matter in faeces
U = absorption efficiency

In earlier studies it was concluded that the gut passage took about six hours (Traas, 1985). A second assumption had to be made, i.e. faeces was collected quantitatively.

When the Balance Calculation was performed on basis of the inorganic fraction, a difference between the amount of inorganic substance ingested and defeacated became clear (calculated ingestion of inorganics was much higher than defeacation). This discrepancy might be explained, by assuming that after digestion a considerable part of the anorganic fraction became soluble and by the gentle stirring in the cells this was "washed" out by the outflow. One might assume that the inorganic part of the algae is indeed altered after digestion through a mussel and the silica skeletons of especially an algae as Phaeodactylum could be dissolved in the passing sea water.

Although, no conclusive evidence can be presented for either method, the balance calculation method has been used for processing the results.

The different absorption efficiency and its implication for the calculated SFG obtained with the Conover ratio and the budget calculation are given in table 6 and 7.

Ammonia excretion was found to be marginal, it mounted up to mJ while the SFG could expressed as J. So, in calculating the results the excretion of ammonia has been omitted.

3 - Results

Annual cycle Sophia-harbour

In order to get a general impression of the existence of an annual cycle of the SFG and its components, all data derived from mussels exposed in Sophia-harbour will be examined as a group. All measured parameters are presented in table 1, the results for the SFG are given after the SFG for individual animals was calculated.

The SFG decreased from November until March (Fig. 2). Thereafter, a rapid increase is shown in four weeks time, followed by a plateau. In September, the SFG showed a small decrease.

The clearance rate showed a pronounced annual cycle, similar to that of the SFG, (Fig. 3), it remained low from November up to March. Thereafter, a sharp increase in clearance rate was observed until the end of April, this was followed by a gradual decrease in clearance rate.

The absorption efficiency (U) varied between 0.85 and 0.98 (Fig. 3). From November until the beginning of April this parameter was almost constant at 0.85, whereafter, its value increased to 0.98. In September a small decrese was registered

The amount of energy absorbed is depicted in figure 4, the shape of this curve is comparable to that of the SFG. This is not surprising, as the amount of energy absorbed is the largest term in the calculation of the SFG.

The amount of energy used by respiration was about 10 % of the absorbed energy (Fig. 4). From November until March oxygen consumption was circa 4 J/h.g DW, in April this increased to more than 9 J/h.g. During the spring and summer (from April to July) oxygen consumption was more or less constant at about 5 J/h.g.

Field exposure Eastern Scheldt

The location Jacoba-harbour and Hammen can be interpreted as reference points. Results of the SFG measurements are presented in table 2 and figure 5.

In March '88, no significant difference could be detected between Sophia-harbour and Hammen in the SFG components or the SFG itself. Standard deviations of the parameters measured with mussels from Hammen were larger compared to those at Sophia-harbour.

The location of the new field station; Jacoba-harbour , measured in July '88 is with respect to the SFG or its components comparable with the results from mussels exposed at Sophia-harbour (Fig. 5). No significant differences

could be demonstrated (table 2); so the site Jacoba-harbour could also serve as a good reference point in the future.

At location Colijnsplaat, mussels were exposed in a marina. Therefore, it was expected that the water quality at this site would stress the animals. Pollution could originate from antifouling paint, petrol derived contaminants and household waste.

After 8 and 17 weeks of exposure, the SFG at Colijnsplaat was significantly reduced compared to the control at Sophia harbour (table 3, Fig. 6). After 17 weeks the difference with the reference point has become smaller, this could point at some adaptation of the mussels during sustained exposure.

Energy consumption and related absorbed energy were significantly lowered ($p \leq 0.01$) at 8 weeks of exposure. This decrease is mainly caused by a reduction of the filtration rate, the absorption efficiency was also somewhat lower although not significant ($p \leq 0.2$).

After 17 weeks of exposure, the consumption and its correlated quantity of absorbed energy are lowered significantly ($p \leq 0.05$). In this case the absorption efficiency was not influenced, while Oxygen consumption was increased significant ($p \leq 0.02$) compared to Sophia-harbour.

Field exposure Western Scheldt

In the Western Scheldt mussels were exposed at the sites Vlissingen (buoy; W12) and Walsoorden (buoy; A48), Sophia-harbour was used as reference. After an exposure of 5 weeks, November '87, no difference could be measured nor in the SFG neither in one of its components (table 4, Fig. 7). At Vlissingen, March '88, the frame containing the baskets with mussels was lost, so no animals could be retrieved from this site. At the same time, after 21 weeks of exposure, mussels exposed at Walsoorden showed a significant reduction of their SFG ($p \leq 0.01$), this was mainly caused by a significant lowering of the consumption ($p \leq 0.01$) and therefore also in absorbed amount of energy. Six weeks later, after 27 weeks of exposure, the SFG was relatively more reduced ($p \leq 0.001$). Besides a lowered consumption ($p \leq 0.01$) and absorption, also respiration was significantly reduced.

Experimental exposures

Mussels exposed in experimental tanks at the field station, Sophia-harbour, were measured during two separate incubations, after 10 and 11 weeks of exposure (table 5 A and B, Fig. 8).

In April (table 5A), a significant decrease ($p \leq 0.001$) of the SFG was observed in mussels that had been exposed to cadmium. The consumption was reduced and the respiration was somewhat higher. After exposure to PCB there was no effect on the SFG or most of its components, respiration was significantly increased($p \leq 0.02$).

The control mussels used for the second measurement, showed a lowered SFG compared to mussels exposed in field conditions at Sophia-harbour. So, it can be concluded that the experimental exposure at the field station had a disturbing effect on the SFG. At this occasion no effect of the pollutants was detectable.

4-Discussion

From the different measurements of SFG of mussels exposed in Sophia harbor-(reference site) an annual cycle can be drawn. Some reservations for a detailed interpretation should be made due to the fact that only a limited number of measurements are performed in the winter period. In spring (March) the SFG is low, thereafter the amount of energy available for growth increased rapidly until the end of April. Following this increase the SFG was rather constant throughout the growing season of mussels. From July to September, SFG showed a tendency to decrease, this reduction in growth potential seems to continue throughout the winter until the next spring. Especially the winter period and early spring should be more carefully examined with a more frequent sampling.

A strong depression of the SFG in March, in comparison with the summer (-growing) period, can be related with the occurrence of spawning that normally takes place during this time of the year (Hummel et al., 1988). With respect to the energy budget, mussels recover very quick, within a month.

The amount of energy absorbed closely resembles the course of the SFG, this component forms the largest contribution to this parameter. There is no clear evidence of an annual cycle in the absorption efficiency. The highest efficiency is found in the summer period(April to July), this correlates with both the highest amount of particulate organic matter and the growing season of mussels

In April, respiration showed a marked increase, during the rest of the summer oxygen consumption was more or less constant. From July until March the respiration decreased. Increased oxygen consumption correlated with spawning, possibly the maturation of gametes contributed to a higher metabolic rate. Another explanation could be that following the spawning residues of the reproductive tissue and/or gametes are resorbed and metabolized,which results in an enhanced metabolism and coupled higher oxygen consumption.

Smaal and Korporaal(1988) performed a comparable study into the SFG at different sites, when the unpolluted sites are combined into a annual cycle it is clear that SFG is lowest during the reproductive period, in April about 4 J/h.g and even negative in May, increased during the summer to 16.6 and 35.1 J/h.g in august and september respectively. An intermediate SFG was registered in February, about 9 J/g.h. In the summer period the consumption was found to be high, while in the reproductive season the respiration was enhanced to the same extent as in the present study. A complicating factor in this comparison is the fact that all control samples were from different sites, so one may not exclude

the possibility that an annual cycle can be disturbed by the variability of abiotic factors.

With respect to the different components of the SFG measurement both locations Hammen and Jacoba harbor are not significantly different from the reference site Sophia harbor.

After 8 weeks of exposure in the yachting harbor Colijnsplaat, mussels showed a significant change in their consumption and therefore in SFG. Prolonged exposure upto 17 weeks, led to an enhanced respiration in these animals and to a lowered SFG. At this location it was expected that the SFG would be lowered as this site could be polluted by the harbor activity. But, as no concentration of contaminants is determined neither from this group nor another subgroup of mussels, a correlation can only be based on this expectation. The possibility that this lowered SFG could be caused by abiotic or biotic environmental factors is less likely, while Colijnsplaat is situated close to Sophia harbor and has an open connection with the Eastern Scheldt. As there is no evidence for contamination of the surrounding waters, the SFG measurement is useful for the detection of pollution at smaller sites.

The results at the sites Vlissingen and Walsoorden showed no differences for SFG, when compared to the reference site, after 5 weeks of exposure. After 23 weeks, SFG in Walsoorden was significantly reduced by a reduced uptake of energy. Respiration was found to be lowered at 27 weeks of exposure. These last two observations can not be compared with Vlissingen, at this location the frame containing the mussels was washed away. Possibly, SFG in Walsoorden is reduced by a lower salinity, about 15 %. . Widdows(1985) reported that the SFG was affected by salinity lower than 20 %. , so this could be an explanation. Another explanation is the chronical pollution by the outflow of the river Scheldt. Data about the differences between pollution in the east and west part of the Western Scheldt are presented in table 9.

In order to check if the differences in energy consumed are also reflected by a reduced clearance rate, the results of this parameter is given in table 8. From this result it is clear that all the significant differences at the various sites in the SFG or its components, especially consumed energy, correlate with a significant effect on the clearance rate.

Measurements of mussels exposed in an artificial exposure system at the fieldstation(Stresspar) showed no uniform response of SFG on Cd or PCB. At first, Cd had a clear effect on the growth potential by a reduced energy uptake. In the PCB exposure mussels showed an enhanced respiration, this can be

understood by the fact that PCBs are metabolized. This metabolism can eventually lead to an activation of the basic metabolism and result in a higher respiration.

In the second experiment no effect of toxicants on the SFG were found. This can be explained by a malfunction in the exposure system, while control mussels showed a lower SFG than mussels exposed at the reference site.

Due to the fact that only a few measurements per location or exposure were realized, it is difficult to draw general conclusion at this stage. However it is clear that when the SFG is significantly affected, in all cases the consumption of energy is the main component of the SFG that has changed.

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Tables

Table 1: SFG and its components measured in mussels exposed at Sophia harbor. The provisional year cycle is derived from these data. Values are the mean and SD of 4 animals

Table 2: SFG and its components of mussels exposed at Sophia harbor, Hammen en Jacoba harbour. Values are the mean of 4 animals and SD

Table 3: SFG and its components measure in animals exposed at Sophia harbour and Colijnsplaat, a yachting harbour in the Eastern Scheldt. Values are the mean and SD of 4 mussels

Table 4: SFG and its components measured in mussels exposed in the Western Scheldt, at Vlissingen and Walsoorden, and Sophia harbour in the Eastern Scheldt. Values are the mean and SD of 4 specimens

Table 5: SFG and its components of mussels exposed at the field station to 25 ppb cadmium and 0.5 ppb PCB, compared with either mussels exposed at Sophia harbour or in the control tank. Values are the mean and SD of 4 mussels

Table 6: A comparison of absorption efficiencies calculated according to Conover and "balance calculation". Data of all exposure sites are presented. Values are the mean and SD of 4 mussels

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Table 9 A: Different gradioents measured at two location in the Western Scheldt (Stronkhorst 1988)

Table 1: SFG and its components measured in mussels exposed at Sophia harbor. The provisional year cycle is derived from these data. Values are the mean and SD of 4 animals

DATE	absorp. coeff.	consumed energy (J/h.g)	absorbed energy (J/h.g)	oxygen consumption (J/h.g)	SFG (J/h.g)
04/11/87	.88 ± .01	70.4 ± 27.3	61.7 ± 24.1	4.39 ± .67	57.4 ± 23.5
13/03/88	.85 ± .02	16.4 ± 3.1	14.1 ± 2.9	3.34 ± 1.17	10.7 ± 4.1
14/04/88	.87 ± .05	50.5 ± 8.6	43.6 ± 7.3	9.72 ± 1.08	33.8 ± 6.6
28/04/88	.98 ± .02	84.4 ± 7.1	79.4 ± 17.4	5.41 ± .77	79.0 ± 7.5
05/07/88	.98 ± .01	101.1 ± 28.7	99.2 ± 29.0	6.37 ± 1.17	105.6 ± 15.9
08/09/88	.91 ± .12	75.2 ± 32.4	71.6 ± 34.4	5.74 ± .60	65.9 ± 34.8

Table 2: SFG and its components of mussels exposed at Sophia harbor, Hammen en Jacoba harbour. Values are the mean of 4 animals and SD

date/ location	absorp. coeff.	consumed energy (J/h.g)	absorbed energy (J/h.g)	oxygen consumption (J/h.g)	SFG (J/h.g)
Sophia-harbour*					
13/03/88	.85 ± .02	16.4 ± 3.1	14.1 ± 3.0	3.34 ± 1.17	10.7 ± 4.1
Hammen					
13/03/88	.78 ± .08	17.3 ± 11.4	14.0 ± 10.5	4.23 ± 1.22	11.4 ± 13.1
Sophia-harbour*					
05/07/88	.98 ± .01	101.1 ± 28.7	99.2 ± 29.0	6.37 ± 1.17	92.8 ± 28.6
Jacoba-harbour**					
05/07/88	.99 ± .01	95.0 ± 5.2	93.4 ± 4.8	6.92 ± .53	86.5 ± 4.4

*: reference location, **: location new field station

Table 3: SFG and its components measure in animals exposed at Sophia harbour and Colijnsplaat, a yachting harbour in the Eastern Scheldt. Values are the mean and SD of 4 mussels

date/ location	absorp. coeff.	consumed energy (J/h.g)	absorbed energy (J/h.g)	oxygen consumption (J/h.g)	SFG (J/h.g)
Sophia-harbour*					
05/07/88	.98 ± .01	101.1 ± 28.7	99.2 ± 29.0	6.37 ± 1.17	92.8 ± 28.6
Colijnsplaat					
05/07/88	.88 ± .12	21.4 ± 15.9	19.9 ± 15.2	6.44 ± 2.54	13.5 ± 13.4
p:	≤0.20	≤0.01	≤0.01		≤0.001
Sophia-harbour*					
08/09/88	.91 ± .12	75.2 ± 32.4	71.6 ± 34.4	5.74 ± 0.60	65.9 ± 34.8
Colijnsplaat					
08/09/88	.93 ± .04	36.9 ± 16.2	34.8 ± 15.9	7.33 ± 1.25	27.4 ± 16.1
p:		≤0.05	≤0.05	≤0.02	≤0.05

*: reference location

Table 4: SFG and its components measured in mussels exposed in the Western Scheldt, at Vlissingen and Walsoorden, and Sophia harbour in the Eastern Scheldt. Values are the mean and SD of 4 specimens

date/ location	absorp. coeff.	consumed energy (J/h.g)	absorbed energy (J/h.g)	oxygen consumption (J/h.g)	SFG (J/h.g)
Sophia-harbour*					
04/11/87	.88 ± .01	70.4 ± 27.3	61.7 ± 24.1	4.39 ± 0.67	57.4 ± 23.5
Vlissingen					
04/11/87	.87 ± .05	83.5 ± 28.0	72.5 ± 25.8	4.37 ± 0.27	68.1 ± 25.6
Walsoorden					
04/11/87	.85 ± .06	65.5 ± 22.5	54.6 ± 14.9	3.14 ± 2.25	51.4 ± 16.8
Sophia-harbour*					
13/03/88	.85 ± .02	16.4 ± 3.1	14.1 ± 2.9	3.34 ± 1.17	10.7 ± 4.1
Walsoorden					
13/03/88	.81 ± .27	4.7 ± 4.1	3.8 ± 3.7	2.95 ± 0.64	0.8 ± 4.2
p:		≤0.01	≤0.01		≤0.01
Sophia-harbour*					
14/04/88	.87 ± .05	50.5 ± 8.6	43.6 ± 7.3	9.72 ± 1,08	33.9 ± 6.6
Walsoorden					
14/04/88	.70 ± .28	3.3 ± 2,3	1.7 ± 2.5	6.09 ± 1.85	1.2 ± 4.3
p:	≤0.40	≤0.01	≤0.001	≤0.02	≤0.001

*: reference location

Table 5: SFG and its components of mussels exposed at the field station to 25 ppb cadmium and 0.5 ppb PCB, compared with either mussels exposed at Sophia harbour or in the control tank. Values are the mean and SD of 4 mussels

date/ incubation	absorp. coeff.	consumed energy (J/h.g)	absorbed energy (J/h.g)	oxygen consumption (J/h.g)	SFG (J/h.g)
A/ 11 weeks of exposure;					
Sophia-harbour*					
28/04/88	.98 ± .02	84.4 ± 7.1	79.4 ± 17.4	5.41 ± 0.77	79.0 ± 7.5
Cd-exposure					
28/04/88	.98 ± .02	28.7 ± 17.8	28.3 ± 18.0	7.39 ± 1.47	20.9 ± 18.8
p:		≤0.01	≤0.01	≤0.10	≤0.001
PCB-exposure					
28/04/88	.99 ± .01	84.9 ± 6.8	78.0 ± 18.4	8.54 ± 1.51	69.4 ± 18.5
p:				≤0.02	
B/ 10 weeks of exposure;					
Control-tank					
10/08/88	.99 ± .01	48.8 ± 18.9	48.5 ± 18.9	5.03 ± 2.33	44.6 ± 20.7
Cd-exposure					
10/08/88	.99 ± .01	56.8 ± 27.9	56.2 ± 27.8	5.74 ± 1.63	50.5 ± 26.9
PCB-exposure					
04/08/88	.97 ± .03	53.4 ± 16.7	51.8 ± 16.8	5.19 ± 2.16	46.6 ± 17.2

*: reference location

Table 6: A comparison of absorption efficiencies calculated according to Conover and "balance calculation". Data of all exposure sites are presented. Values are the mean and SD of 4 mussels

Location	date	Conover	balance
Western Scheldt	(reference location)		
Sophia-harbour	04/11/87	.73 ± .11	.88 ± .01
	17/03/88	.67 ± .06	.85 ± .02
	14/04/88	.85 ± .03	.87 ± .05
	05/07/88	.22 ± .20	.98 ± .01
	08/09/88	.30 ± .07	.91 ± .12
Western Scheldt			
Jacoba-harbour	05/07/88	.50 ± .10	.99 ± .01
Hammen	17/03/88	.67 ± .06	.78 ± .08
Colijnsplaat	05/07/88	.40 ± .14	.88 ± .12
	08/09/88	.70 ± .15	.93 ± .04
Eastern Scheldt			
Vlissingen	04/11/87	.57 ± .15	.87 ± .05
Walsoorden	04/11/87	.71 ± .08	.85 ± .06
	17/03/88	.64 ± .13	.81 ± .27
	14/04/88	.71 ± .08	.70 ± .28

Table 7: A comparison of the eventual results of the SFG calculation with the Conover ratio or "balance calculation" at the different locations tested. Values are the mean and SD of 4 measurements

Location	date	SFG Conover	SFG balance
Western Scheldt	(reference location)		
Sophia-harbour	04/11/87	45.6 ± 22.5	57.4 ± 23.5
	17/03/88	7.8 ± 4.0	10.7 ± 4.1
	14/04/88	33.2 ± 7.6	33.9 ± 6.6
	05/07/88	13.3 ± 21.6	105.6 ± 15.9
	08/09/88	16.9 ± 10.1	65.9 ± 34.8
Western Scheldt			
Jacoba-harbour	05/07/88	40.6 ± 10.3	86.5 ± 4.4
Hammen	17/03/88	8.0 ± 8.0	11.4 ± 13.1
Colijnsplaat	05/07/88	3.4 ± 6.2	13.5 ± 13.4
	08/09/88	18.4 ± 11.6	27.4 ± 16.1
Eastern Scheldt			
Vlissingen	04/11/87	43.3 ± 23.2	68.1 ± 25.6
Walsoorden	04/11/87	42.3 ± 12.7	51.4 ± 16.8
	17/03/88	-5.5 ± 8.6	0.8 ± 4.2
	14/04/88	-4.1 ± 1.5	1.2 ± 4.3

Table 8: Clearance rates (L/h.g) of mussels exposed at different sites. Values are the mean and SD of 4 animals

date	reference location (Sophia-harbour)	Test location; Eastern Scheldt Hammen	Jacoba-harbour	Colijnsplaat
17/03/88	0.9 ± 0.2	0.9 ± 0.6	----	----
05/07/88	4.2 ± 1.2	----	4.0 ± 0.2	0.9 ± 0.7*
07/09/88	2.7 ± 1.1	----	----	1.3 ± 0.6*

		Test location; Western Scheldt Walsoorden(east)	Vlissingen(west)
04/11/87	1.4 ± 0.5	1.3 ± 0.4	1.7 ± 0.6
17/03/88	0.9 ± 0.2	0.3 ± 0.2*	----
14/04/88	4.1 ± 0.7	0.3 ± 0.2*	----

*; indicates a significant difference, compared to reference location.
----; not determined.

Table 9 A: Different gradioents measured at two location in the Western Scheldt (Stronkhorst 1988)

	Salinity ‰	POC/Part.mat. %	PCB ng/g part.mat.	PAH	Cd* ug/L
Hansweert (east)	18	4.9	77	3181	0.07
Vlissingen (west)	24	3.5	31	1737	0.02

*; soluble fraction

Table 9 B: Concentrations of different contaminants in the soft tissue parts of mussels at three different sites in the Western Scheldt (Stronkhorst 1988)

	Cd ug/gdw	PCB ug/g lipid	PAH ug/g lipid
Hansweert (east)	2.3	2.1	n.a
Terneuzen (middle)	1.3	2.3	5.2
Vlissingen (west)	0.7	1.3	n.a

n.a; not available

Figures

Figure 1. A map of the Scheldt estuary area, The different sites used for exposure are indicated by an open circle, 1; Sophia harbour (reference site), 2; Jacoba harbour(future reference site) 3; Hammen, 4; Colijnsplaat, 5; Vlissingen, and 6; Walsoorden.

Figure 2. Annual cycle of the SFG of mussels exposed at Sophia-harbour, fieldstation, serves as reference site. Each point represents the mean of 4 animals, vertical bars indicate SD.

Figure 3. Annual cycle of the clearance rate(top) and absorption efficiency(bottom) of mussels exposed at Sophia-harbour, each point represents the mean of 4 mussels, vertical bars indicate SD.

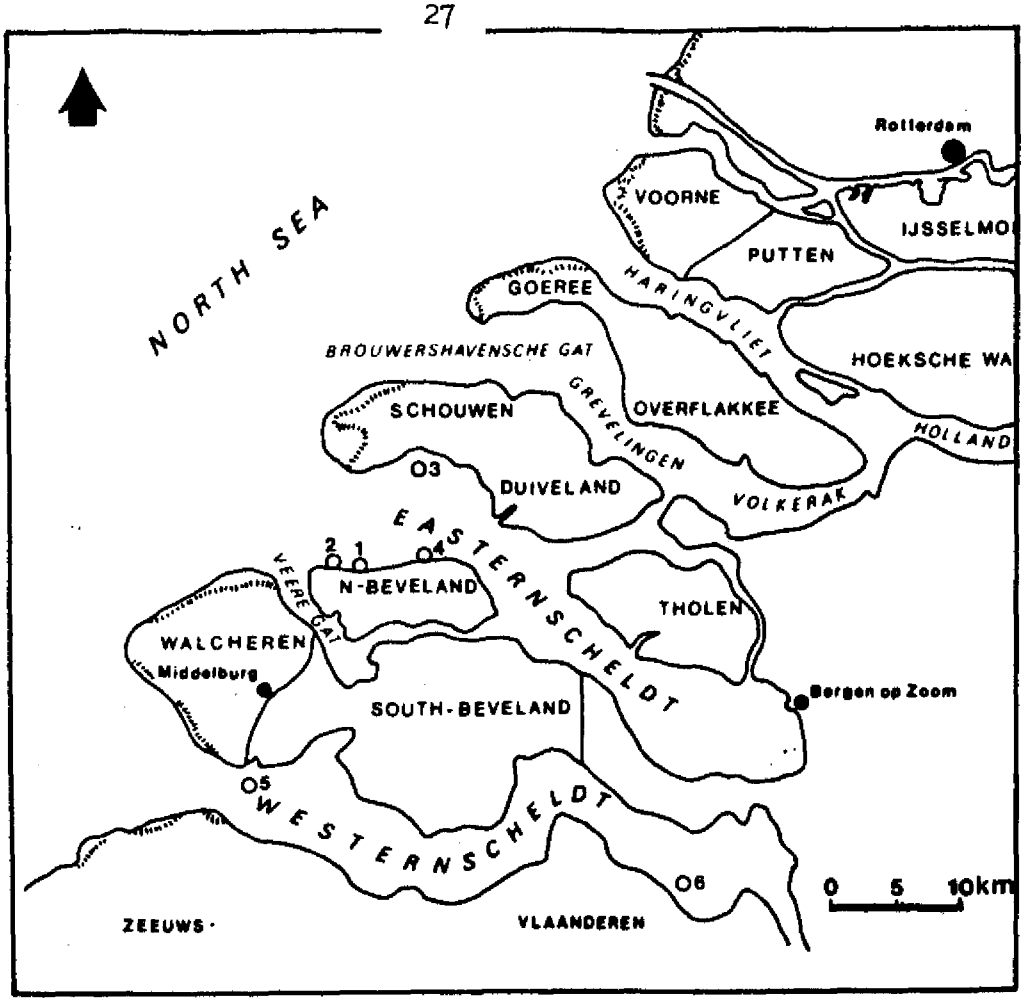
Figure 4. Annual cycle of the amount of energy absorbed(top) and oxygen consumption(bottom) of mussels exposed at Sophia-harbour, each point represents the mean of 4 animals, vertical bars indicate SD.

Figure 5. The different components of the SFG and the SFG determined in mussels exposed at Sophia-harbour and Hammen, and Sophia-harbour and Jacoba-harbour(future fieldstation site), the top and bottom respectively. Each bar represents the mean of 4 mussels.

Figure 6. The SFG and its components measured in mussels exposed at Sophia-harbour and Colijnsplaat(yachtingharbour) at two different time intervals. Each bar represents the mean of 4 animals.

Figure 7. The SFG and its components measured in mussels exposed at Sophia-harbor and at the locations Vlissingen and Walsoorden in the Western Scheldt at three different exposure times. The cage exposed at Vlissingen was wash away after the first retrieval. Each bar represents the mean of 4 animals.

Figure 8. The SFG and its constituents determined with mussel exposed in an artificial exposure at the fieldstation. Mussels were exposed to 25 ppb cadmium and 0.5 ppb PCB for 10 and 11 weeks in two different experiments, top and bottom respectively. Each bar represents the mean of 4 mussels.



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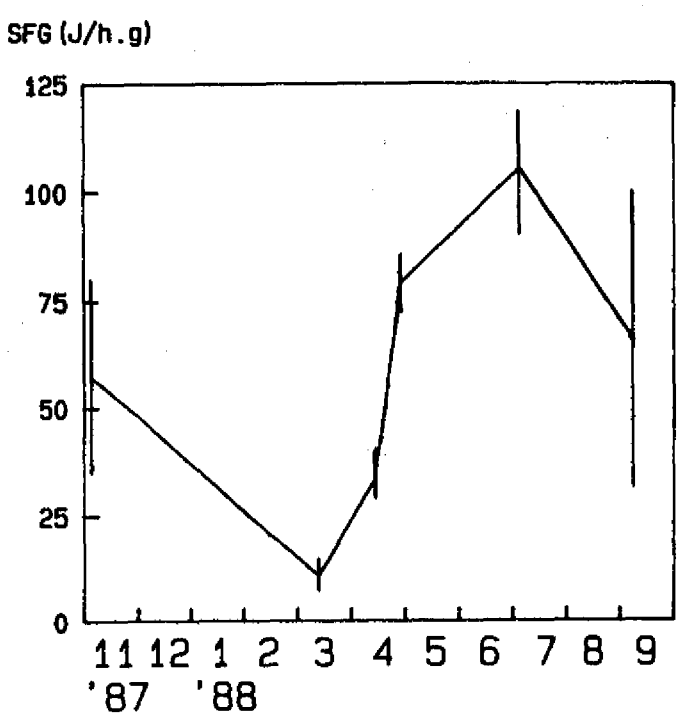

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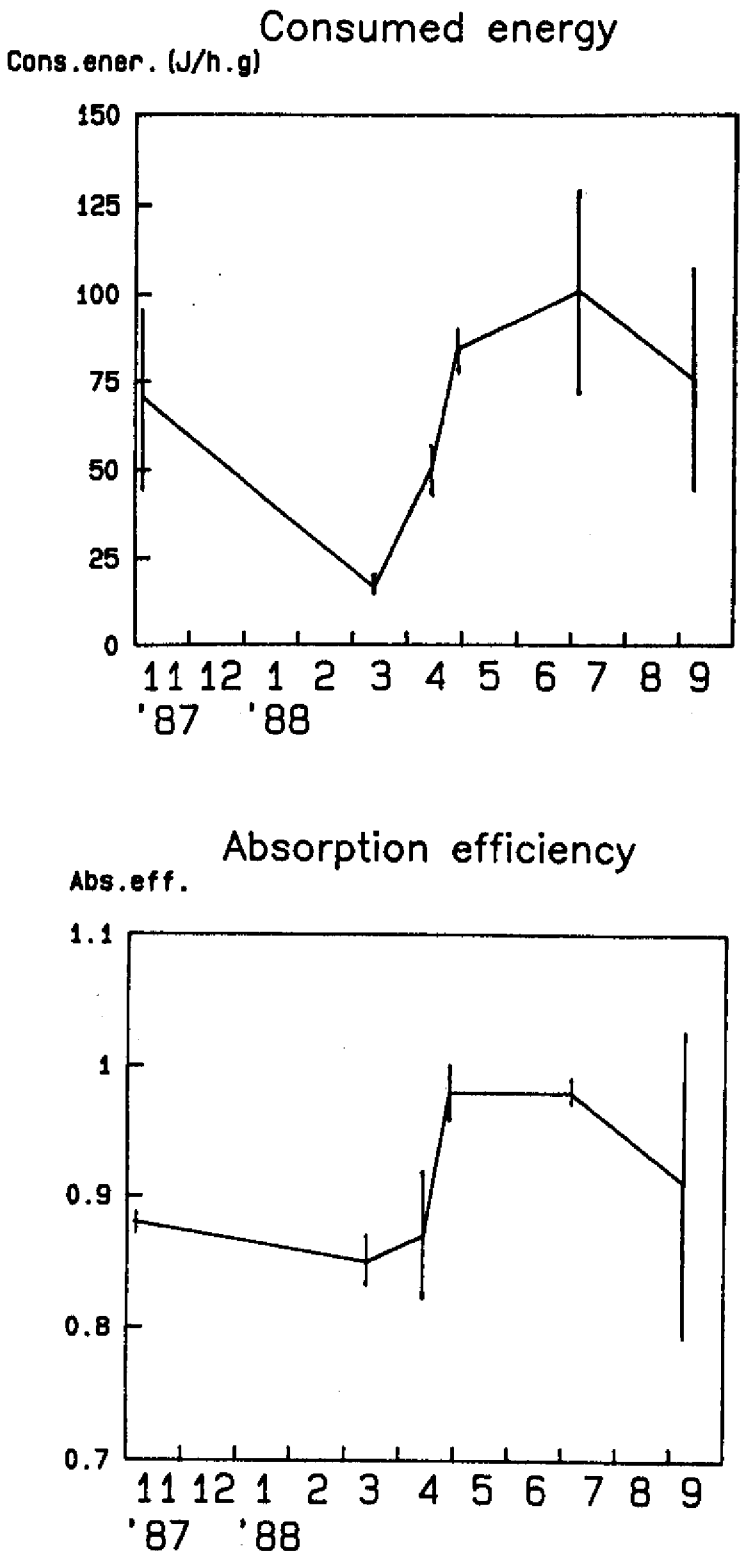

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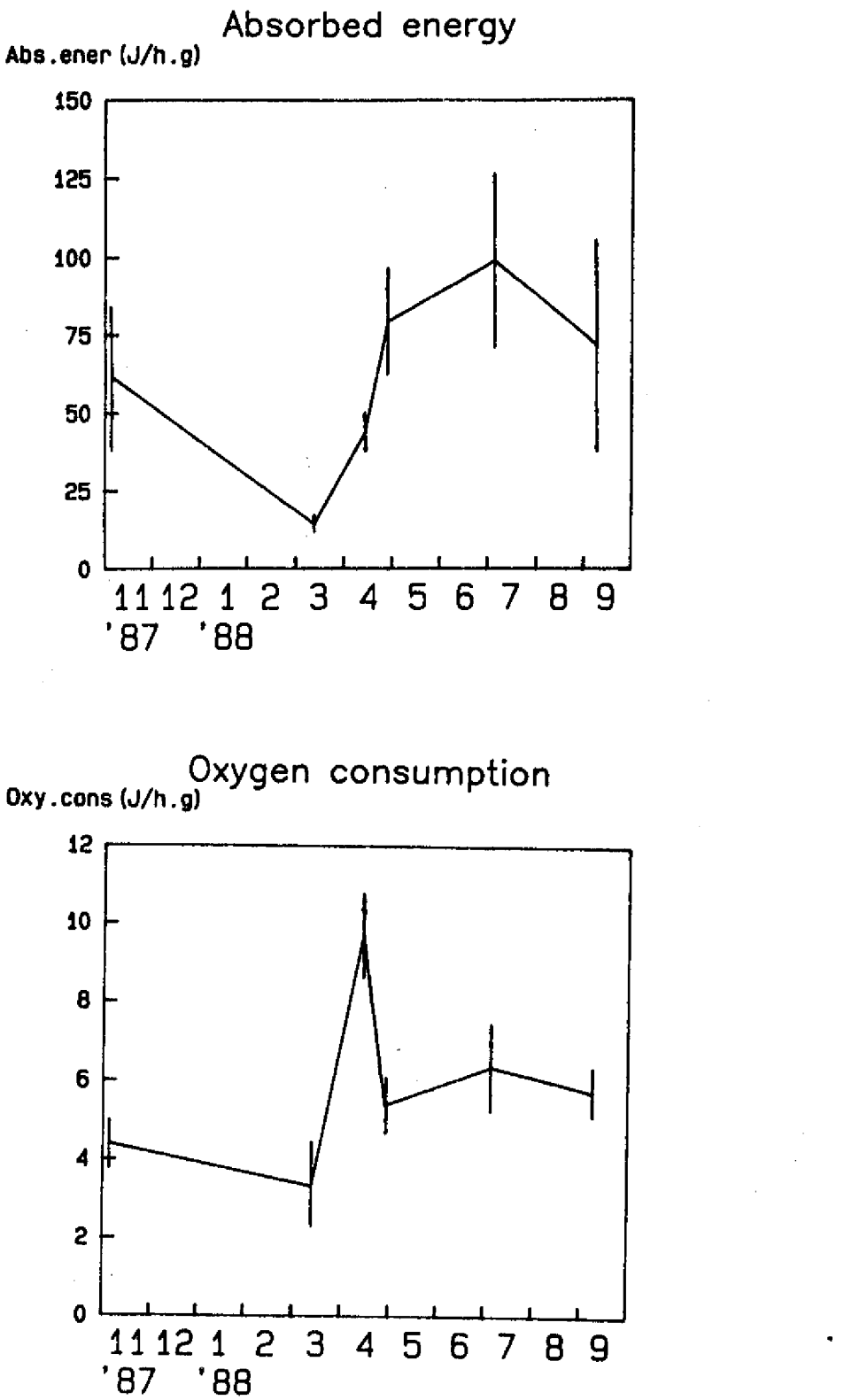

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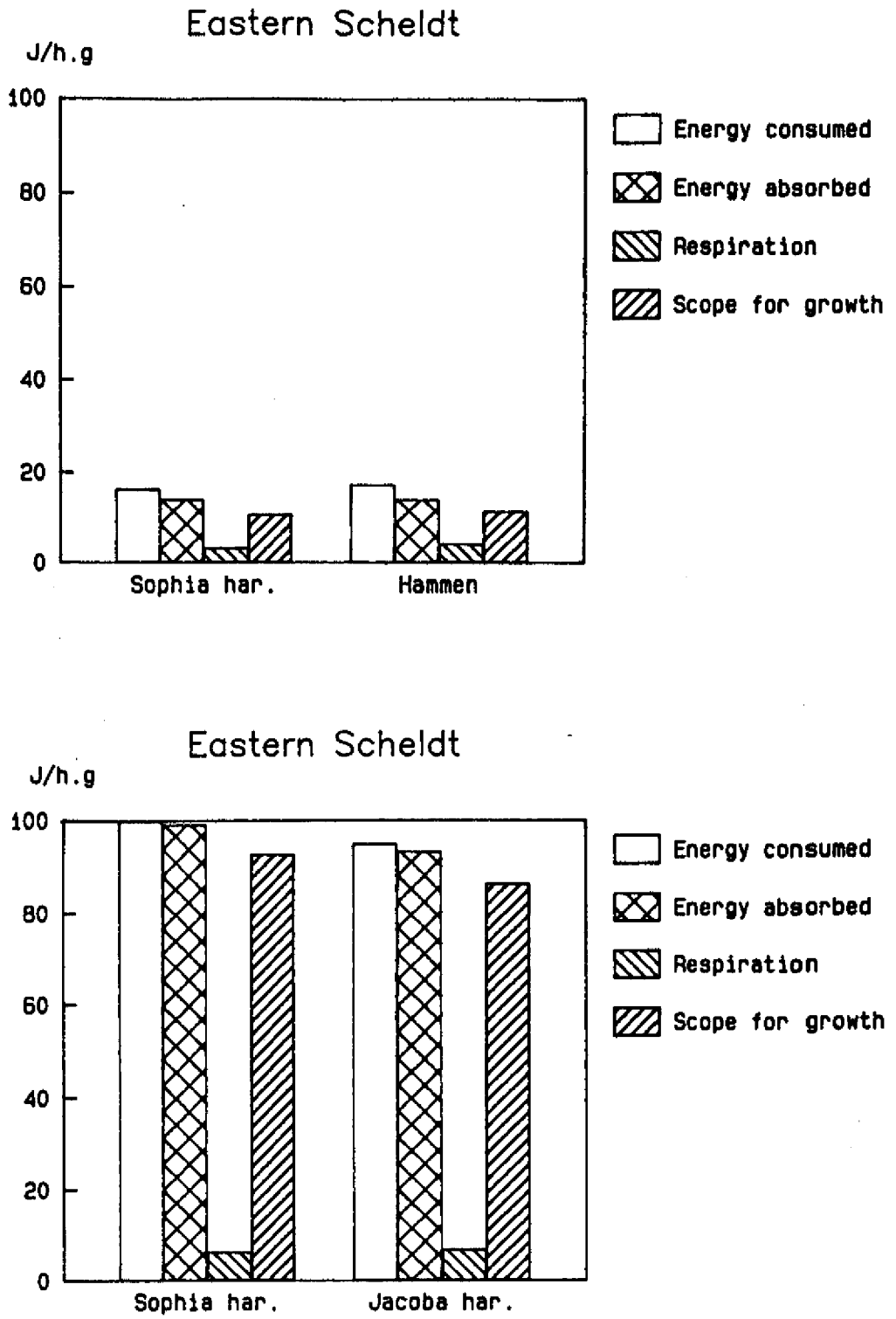

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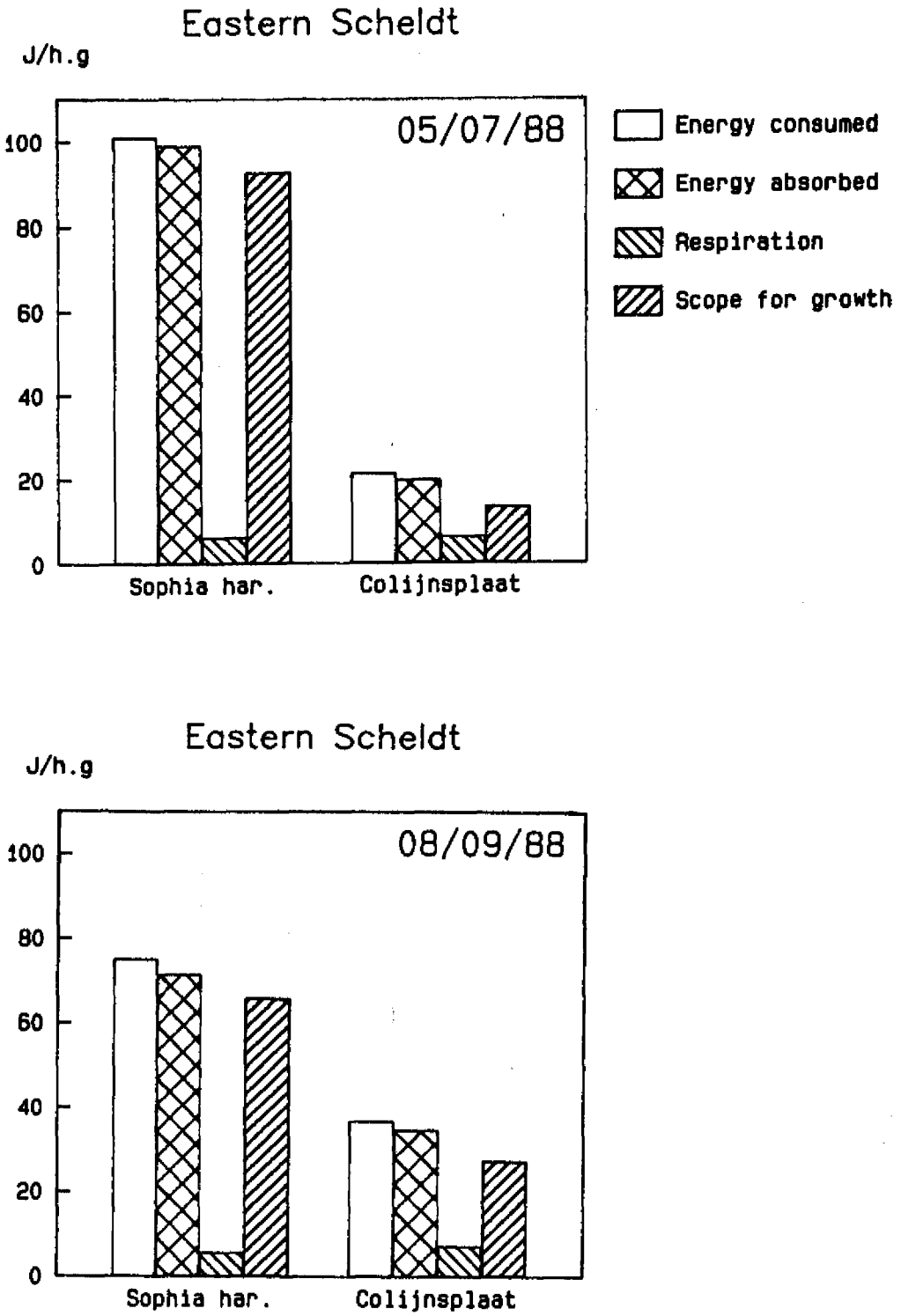

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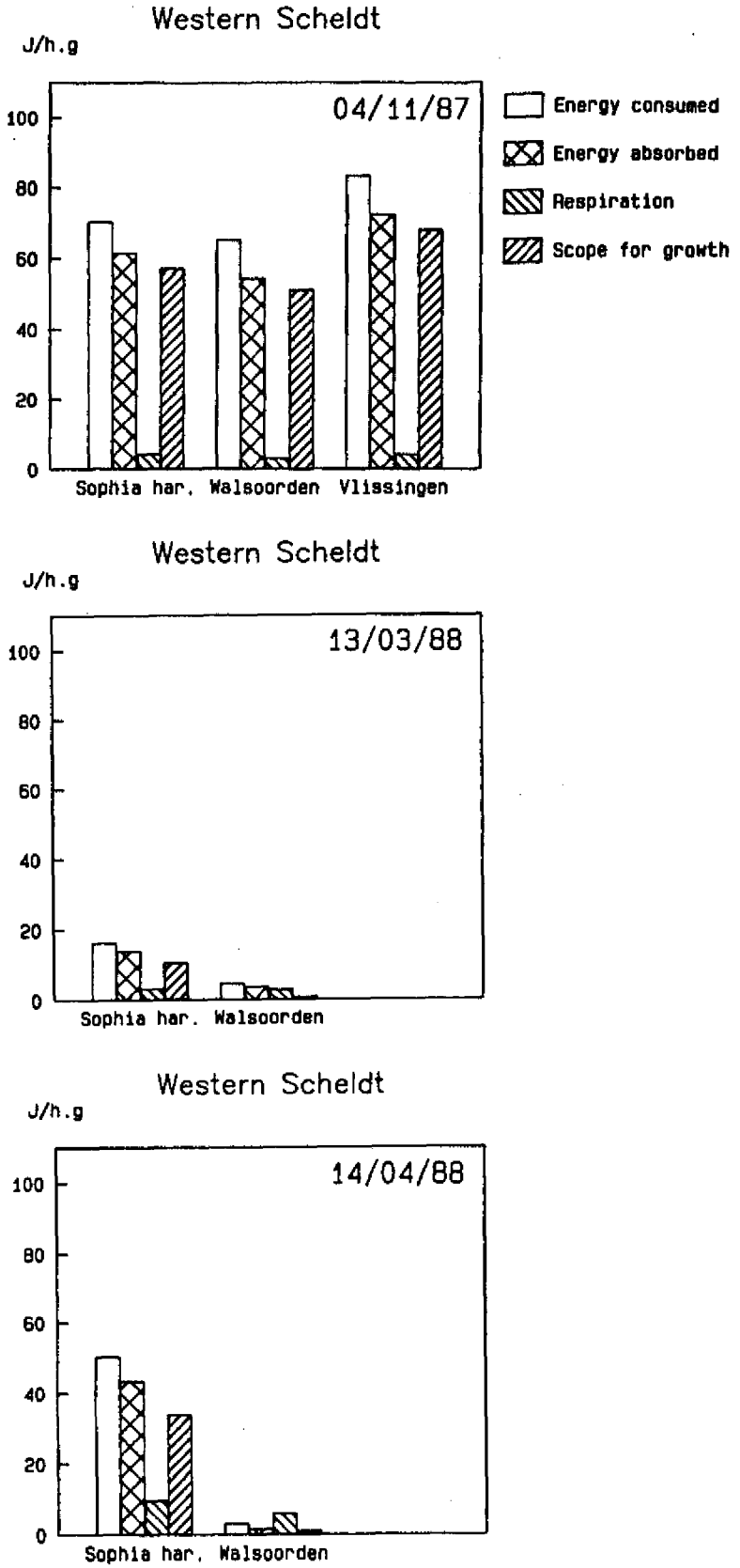

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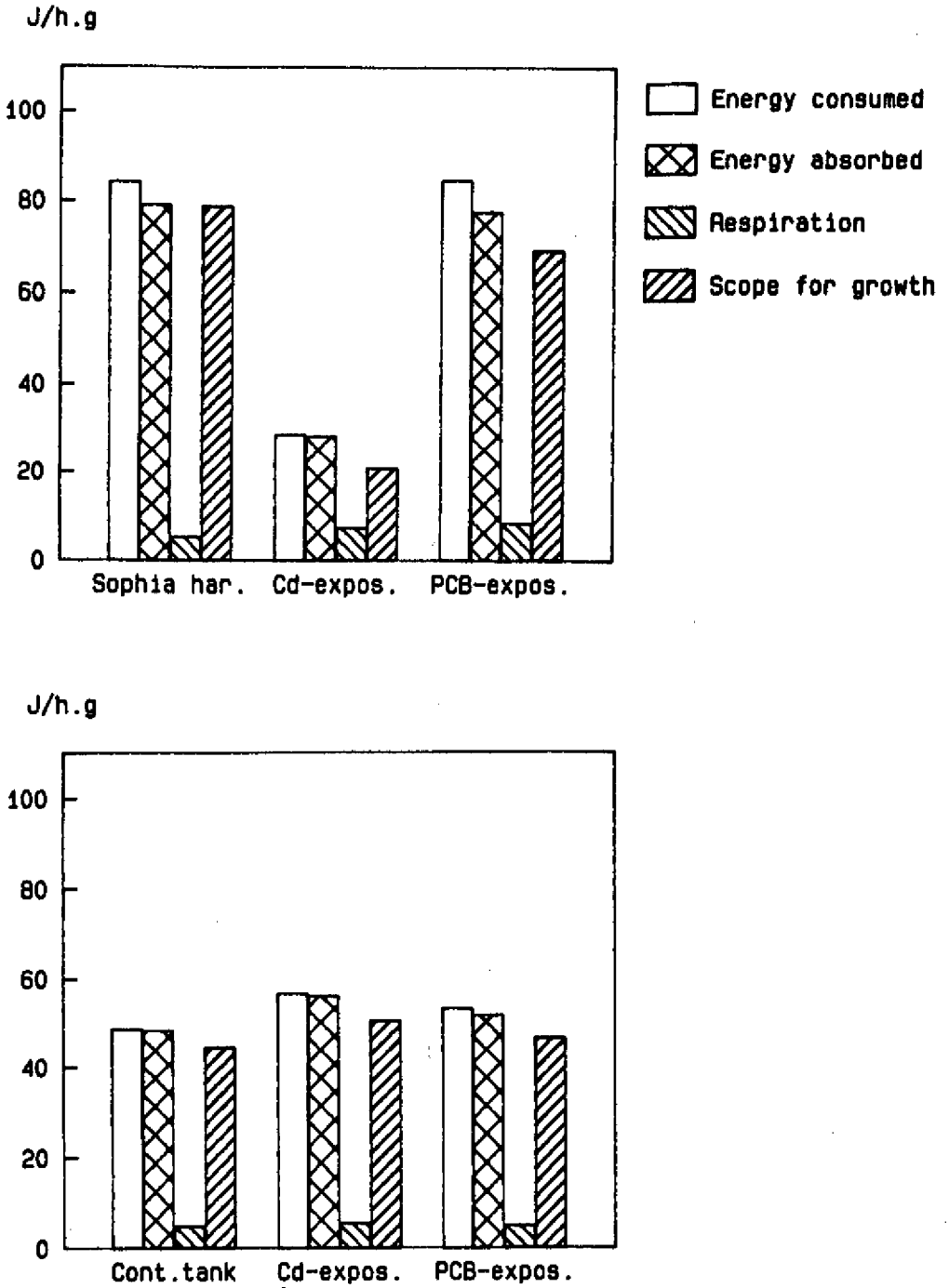

Figure 8. The SFG and its constituents determined with mussel exposed in an artificial exposure at the fieldstation. Mussels were exposed to 25 ppb cadmium and 0.5 ppb PCB for 10 and 11 weeks in two different experiments, top and bottom respectively. Each bar represents the mean of 4 mussels.

Supplement

Formulas used for the calculation of the different components of the SFG.

Clearance rate

Filtration; $\frac{\text{Inflow - outflow}}{\text{outflow}}$ **(all expressed in number of algae/mL)**

Clearance rate (L/h.mussel); filtration x flow rate of the pump

For a comparison between individuals, the clearance rate has to be corrected on dry weight basis, using the following formula;

$$Y = a * X^b$$

where Y = the parameter to be calculated

X = dry weight

a and b = coefficiences

For the clearance rate b has the value 0.56.

Clearance rate (L/h.gdw); clearance rate(L/h.mussel)/ gdw^0.56

Consumed energy

The amount of energy consumed by a mussel can be calculated by multipying the clearnce rate with the energetic content of the diet.

Energy in diet(mg/L); ashfree dry weight per litre(POM/L)

As the algae contain 23.5 J/mg the amount of energy consumed can be calculated;

clearance rate(L/h.g) x POM/L(mg/L) x 23.5(J/L) = (J/h.g)

Absorbed energy

The amount of energy absorbed by the mussel is the amount of energy consumed multiplied by the absorption efficiency, the Conover ratio.

Conover ratio; $\frac{F - E}{(1 - E) F}$

where F = ashfree dry weight food / dry weight food

E = ashfree dry weight faeces / dry weight faeces

The amount of energy absorbed;

consumed energy x Conover ratio = absorbed energy(J/h.g)

Respiration

The amount of oxygen consumed by a mussel is determined by measuring the difference in oxygen concentration in an incubation cell at the start and

after a preset incubation time.

The following equation was used;

Oxst - Oxend x ((Vol.cell - Vol.mus.) x (60/inc.time) = oxygen consumed(mg/h)

Where Oxst - Oxygen concentration at the start(mg/L)

Oxend - Oxygen concentration after the incubation(mg/L)

Vol.cell - Volume of the cell(L)

Vol.mus. - Volume of the mussel(L)

inc.time - incubation time(minutes)

Oxygen consumption per gram dry weight;

$$\frac{\text{Oxygen consumed}}{\text{dry weight}^{0.7}} = \text{Oxgen consumption (mg/h.g)}$$

This value can be converted into Joules by a multiplication of 14.77 J/mg oxygen(Widdows, Feith and Worrall 1979).

Scope for growth

The scope for growth can be caculated by taking the outcomes of some caculations above together in the following calculation;

SFG = Absorbed energy - Respiration

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