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Decrease of Net Carbon Flux in Two Species of Mussels Caused by Extracts of Crude Oil

E.S. Gilfillan

University of Massachusetts, Marine Station; Gloucester, Massachusetts, USA

Abstract

Effects of crude oil and salinity stress on the metabolism of two common filter-feeding animals (*Mytilus edulis* and *Modiolus demissus*) have been investigated. Carbon budgets have been calculated for each species under a variety of combinations of oil content and salinity. Both reduced salinity and the presence of crude oil tend to decrease the net carbon flux for each species; stresses from each source interacted in their effects on experimental animals. Although similar responses to oil were shown by each species, *Mytilus edulis* appeared to be slightly more resistant to oil than *Modiolus demissus*.

Introduction

Studies made on effects of the Buzzard's Bay (USA) oil spill by Blumer *et al.* (1970 a,b) indicate an extensive immediate mortality, subsequent changes in population structure, and a long-term accumulation of hydrocarbons by plants and animals. North *et al.* (1964) report massive mortality in both animals and plants following an oil spill on the coast of Baja, California. These mortalities were followed by a protracted period of recovery lasting several years. On the other hand, results of post-spill studies in the Santa Barbara Channel (USA) suggest little adverse effects of oil pollution (Straughn, 1972). Likewise, Smith (1970) reports little long-term damage from oil alone as a result of the Torrey Canyon spill off Cornwall, England. The studies cited have emphasized gross changes in community structure as related to the oil chemistry in the ecosystem.

Little is known about the metabolic effects of crude oil on individual organisms. In this laboratory, results of preliminary experiments on the physiological effects of sea-water extracts of crude oil on *Mytilus edulis* indicated that water-soluble fractions of 7 different crude oils were not acutely toxic to this species (Gilfillan, unpublished data). One important result of these initial experiments was the discovery that small amounts of crude oil can decrease the amount of energy available for maintenance, growth, and reproduction. This paper reports on the combined effects of crude oil and salinity stress on the carbon budgets of two common filter-feeding molluscs, *Mytilus edulis* and *Modiolus demissus*.

Material and Methods

The crude oil used in this work was a blend of Mid-Continent Sweet Crudes, obtained from the Mobil Oil Corporation. Sea-water extracts were prepared by placing 900 ml glass-fiber-filtered seawater in a 1000 ml disposable beaker, along with 90 ml crude oil. Beakers were closed with an air-tight paper cap, and shaken at about 1 stroke/sec for 96 h.

Following the 96 h extraction period, the seawater was drained off through a hole made by a clean, hot nail. The extract has a powerful "oily" smell; it was either used immediately or held in a glass container for later use.

Fluorescence

Excitation and emission spectra have been obtained for a number of sea-water extracts of crude oil, including Mobil crude oil, using a Baird Atomic Model SF-1 Fluorispec spectrofluorimeter. Excitation and emission spectra resemble one another for all crude oils tested. Excitation maxima range from about 290 nm to about 320 nm. Emission maxima range from about 380 nm to about 420 nm. Routine fluorescence analysis of crude-oil extracts, using a Turner Model 110 fluorometer with a Corning 5840 excitation filter and a Corning 4015 emission filter, showed that extraction of fluorescent compounds is virtually complete after 96 h. There are many non-fluorescent compounds which could affect marine animals; the apparent saturation of seawater with fluorescent compounds may not hold true for non-fluorescent compounds. Boylan and Tripp (1971), however, were able to obtain reproducible extracts of both crude oil and kerosene using a method similar to ours.

A standard sea-water extract of Mobil Crude Oil contains about 12 ppm of hydrocarbons lighter than C_{20}. In order to obtain a higher oil concentration than could be obtained by extraction, a 0.0001% emulsion was prepared by emulsifying 0.1 ml crude oil in 1000 ml glass-fiber-filtered seawater. This emulsion was stable for several days.

Respiratory rates for *Mytilus edulis* and *Modiolus demissus* were determined polarigraphically in a water-jacketed plexiglass chamber. The output of a Yellow Springs semi-micro oxygen electrode mounted in the chamber's stopper was recorded on a Gilson Medical Electronics Oxygraph. Recordings were made for a period of 15 to 30 min for each mussel. The electrode was calibrated against Winkler titrations.

Filtration was determined using C^{14}-labeled unialgal cultures of *Dunaliella tertiolecta* as food. In the *D. tertiolecta* cultures used as food, the chlorophyll/carbon ratio is 0.00931 (Parsons *et al.*, 1961). Sufficient *D. tertiolecta* culture was added to 1 l of filtered seawater to yield a food concentration similar to that found in summer in local coastal waters, i.e. 215 mg C/m^3. Filtration rates were obtained by determining levels of radioactivity before and after a 3 h feeding period, according to the relation: $F = V\ (lnC_o\ lnC_t)/t$, where F = volume filtered per unit time, V = volume of experimental vessel, C_o = initial food concentration, C_t = final food concentration, and t = length of time mussels were allowed to feed.

After the feeding period, the experimental mussels were fed in a solution of non-radioactive algae for 12 h to flush radioactive algae from their guts. The soft parts of the mussels were freeze-dried, and weighed. Radioactivity retained in the soft parts was then determined using liquid-scintillation techniques. Counting efficiencies, determined using the channels-ratio method, were about 20 to 25% for the soft parts and greater than 70% for the filters. Pugh (1973) has suggested that the channels-ratio method of determining counting efficiency is subject to error at low counting efficiencies. However, because the soft parts of all the experimental individuals weighed close to 100 mg, errors introduced by color quenching and self absorbtion should not affect the relative changes shown by the results. Assimilation ratios were calculated by dividing the amount of radioactivity present in the soft parts by the amount of radioactivity removed from the water during the feeding period. This estimate is not corrected for respiration of radiocarbon; it is thus a minimum estimate of assimilation. Carbon budgets have been calculated for 100 mg specimens in terms of µgC/h. The quantity of carbon consumed in 1 h is calculated as the volume filtered/h in liters X 215; it is assumed that all carbon is removed from the water filtered. This value, when multiplied by the assimilation ratio, yields carbon assimilated. Carbon respired is calculated from the relation: Carbon respired/100 mg = 0.43 ml O_2 consumed/100 mg. This relation presumes an RQ (respiratory quotient) of 0.8; the algebraic sum of carbon assimilated and carbon respired is the net carbon flux.

Carbon budgets were determined for both *Mytilus edulis* and *Modiolus demissus* under essentially summer conditions (15°C). Experimental animals were taken from local populations; mussels in the size range used (25 to 30 mm) were approximately 1 year old. Three salinities: 31, 21, 11 °/oo were used. Five oil concentrations were used at each salinity 0, 1, 10 and 50% oil extract, and 0.0001% oil in water emulsion. All concentrations are expressed in terms of volume/volume units. Ten mussels of each species were acclimated to each treatment for 48 h. Five were used in feeding experiments; five were used to determine respiration.

Results

Although low concentrations of oil (1% extract) increase gross carbon consumption at 31°/ooS in *Mytilus edulis* (Fig. 1), this increase is nullified by an increase in carbon respired. At higher oil concentrations, both carbon consumption and assimilation decrease, so the net carbon flux is reduced by 50% with 10% oil, and is nearly 0 with 50% oil. In the oil-seawater emulsion, the net carbon flux is negative. At a reduced salinity (21°/oo; Fig. 2), essentially the same pattern of results emerges as at 31°/oo, but the net carbon flux is much more severely affected by the presence of oil. It is positive only at 0, 1, and 10% oil.

At a low salinity (11 °/oo; Fig. 3) the effects of crude oil on the carbon budget are largely masked by those of salinity stress. There is virtually no assimilation at any oil concentration used. The net carbon flux is equivalent to carbon respired and is negative.

Figs. 4, 5 and 6 show carbon budgets calculated for *Modiolus demissus*. In each of these experiments, assimilation ratios were much lower than those obtained for *Mytilus edulis*. These results suggest that *Dunaliella tertiolecta* is not acceptable food for *Modiolus demissus*. This inacceptability may result either from the former's small size (ca. 10 µ), or from some behavioral mechanism which causes *M. demissus* to reject it. Since *D. tertiolecta* used as food were taken from log-phase cultures, there is no reason to suppose that the cells might be unpalatable because of their stage of growth.

At 31 °/ooS (Fig. 4), regardless of any problems encountered with suitability of the food, a small amount of oil (1%) causes a drastic reduction in net carbon flux, both through decreasing assimilation and through increasing respiration.

At 21 °/ooS (Fig. 5), effects of salinity stress on the mussels' metabolism are such that the net carbon flux is always negative. Even so, 1% oil extract decreases net carbon-intake still further by increasing respiration. The assimilation ratio is so low that the increased gross carbon intake at 1% oil is offset by increased respiration.

Fig. 6 shows the carbon budget for *Modiolus demissus* at 11 °/ooS. As with *Mytilus edulis*, the effect of salinity stress on the mussels' metabolism is very great. Even so, the effects of oil are obvious. The presence of any oil extract is sufficient to completely stop feeding and assimilation.

Discussion

Because of the relative ease with which feeding, assimilation and respiratory rates can be obtained, filter-feeding animals offer excellent opportunities for determination of energy and carbon budgets. The primary object of this research has been to examine the combined effects of crude oil and salinity stress on feeding, respiration and assimilation in two common marine filter feeders, *Mytilus edulis* and *Modiolus demissus*.

The effects of oil alone are best seen in Figs. 1 and 4, which show carbon budgets calculated for both species under normal summer conditions. It is clear from these results that the presence of even small quantities of oil reduces both feeding and assimilation while, at the same time, increasing respiration. These trends are evident for both species, but are most apparent in the results for *Mytilus edulis* (Fig. 1), possibly because *Modiolus demissus* did not readily accept *Dunaliella tertiolecta* as food. The net result of these effects is that the net carbon-flux for both species is reduced by the presence of oil.

The effects of salinity stress on responses to oil begin to become apparent in the carbon budgets calculated for mussels held at 21 °/ooS; a moderately stressing salinity for both species (Figs. 2 and 5). Effects of salinity stress alone are apparent in the results for the control individuals. Feeding, respiration and assimilation are all reduced in the controls held at 21 °/ooS as compared with the controls held at 31 °/ooS. As a result, the net carbon-flux for both species is reduced by salinity stress at 21 °/ooS.

The pattern of response to oil at 21 °/ooS is somewhat different from that seen at 31 °/ooS. Both species show the characteristic elevation of respiratory rate. Feeding rates are higher than in controls at low oil concentrations (1 and 10% for *Mytilus edulis*; 1, 10 and 50% for *Modiolus demissus*). At 31 °/ooS, both species show greatly reduced feeding in the emulsion. The effect of oil on assimilation at 21 °/ooS is such that increased carbon consumption is nullified by reduced assimilation rates, so that the net result of the presence of oil is a reduction in net carbon balance for both species. Because of the unsuitability of *Dunaliella tertiolecta* as food for *Modiolus demissus*, once again, these effects are better seen in the results for *Mytilus edulis* (Fig. 2).

In carbon budgets calculated for mussels held at 11 °/ooS (a severely stressing salinity for both species), the effects of salinity stress dominate; control specimens of both species show greatly reduced feeding and somewhat reduced respiration. Only *Modiolus demissus* shows any assimilation whatsoever at 11 °/ooS. *Mytilus edulis* shows no apparent effects of oil at 11 °/ooS. *Modiolus demissus* shows the only clear-cut effects of oil at 11 °/ooS; the presence of any amount of oil completely blocks both feeding and assimilation.

The results of this study indicate that the effects of crude oil on these animals act to reduce the amount of carbon available for growth and re-

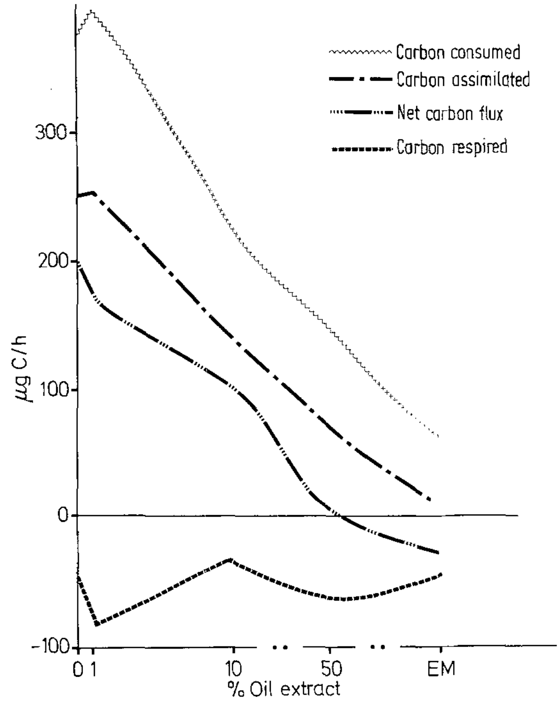

Fig. 1. *Mytilus edulis*. Effects of crude oil extract on carbon budgets calculated for 100 mg mussels held at 31°/ooS under summer conditions (15°C, 215 μgC/l). Note changes in scale on X-axis. EM, expressed as % oil extract, refers to volume of standard seawater extract used to make up experimental medium: EM = 0.0001% v/v oil in water emulsion

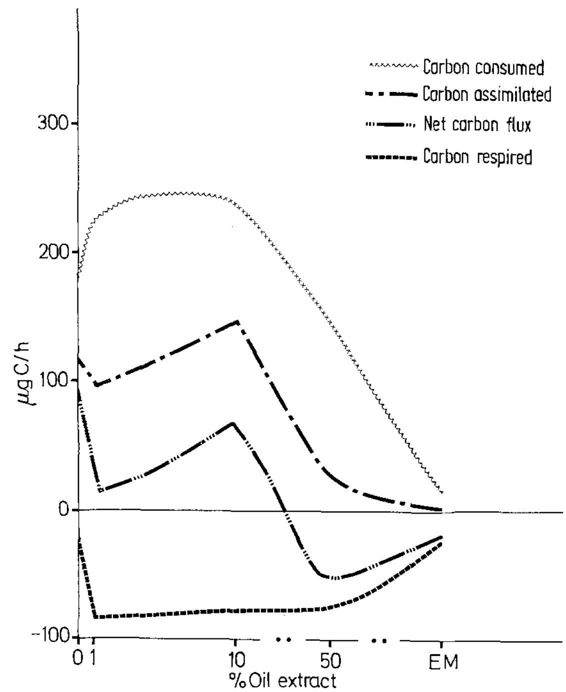

Fig. 2. *Mytilus edulis*. Effects of crude-oil extract on carbon budgets calculated for 100 mg mussels held at 21 °/ooS under summer conditions (15° C, 215 ugC/l). Note changes in scale on X-axis

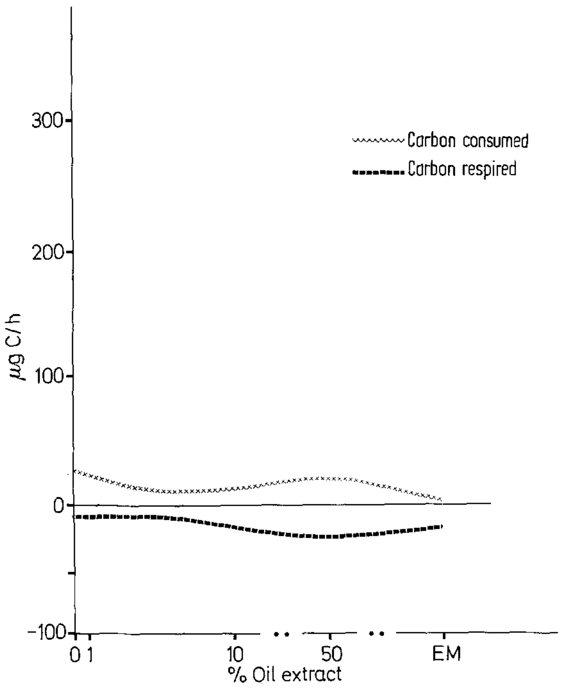

Fig. 3. *Mytilus edulis*. Effects of crude-oil extract on carbon budgets calculated for 100 mg mussels held at 11 °/ooS under summer conditions (15° C, 215 ugC/l). Note changes in scale on X-axis

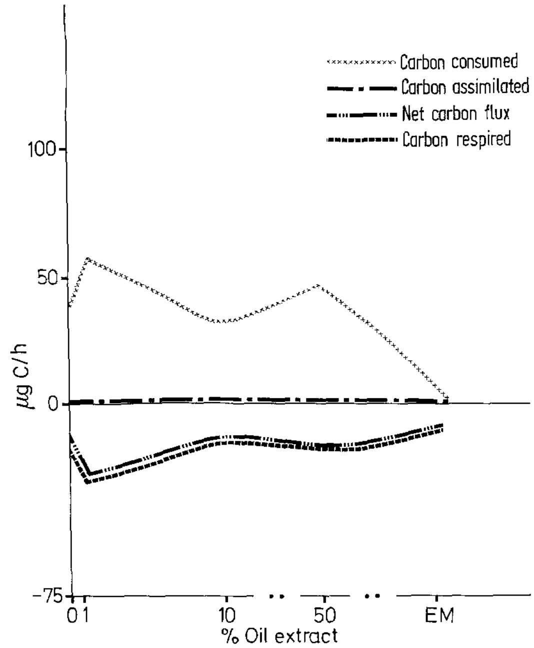

Fig. 5. *Modiolus demissus*. Effects of crude-oil extract on carbon budgets calculated for 100 mg mussels held at 21 °/ooS under summer conditions (15°C, 215 ugC/l). Note changes in scale on X-axis

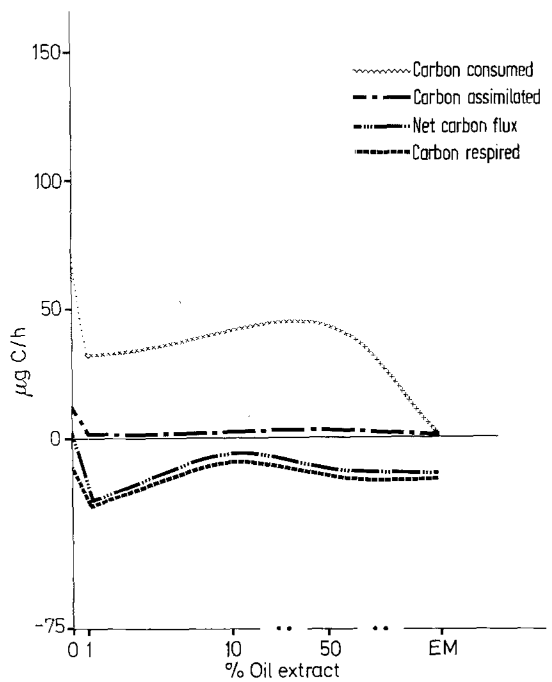

Fig. 4. *Modiolus demissus*. Effects of crude-oil extract on carbon budgets calculated for 100 mg mussels held at 31 °/ooS under summer conditions (15°C, 215 ugC/l). Note changes in scale on X-axis

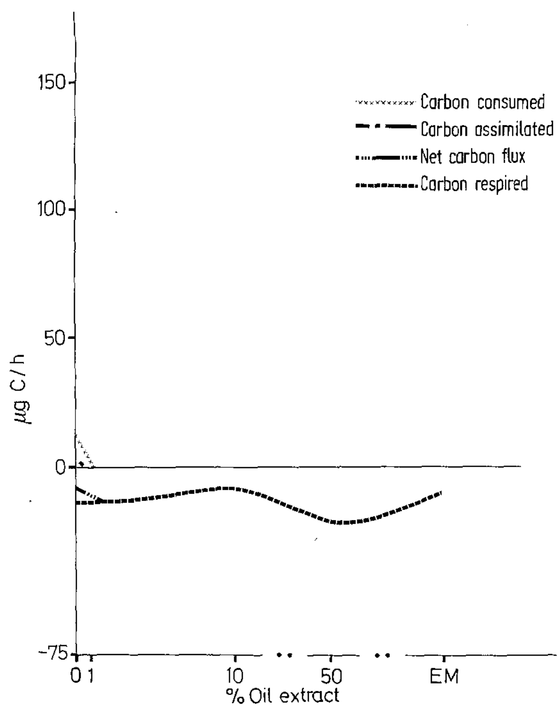

Fig. 6. *Modiolus demissus*. Effects of crude-oil extract on carbon budgets calculated for 100 mg mussels held at 11 °/ooS under summer conditions (15°C, 215 ugC/l). Note changes in scale on X-axis

production. One of the most interesting aspects of this effect is that even as little as 1 ppm causes a significant decrease in net carbon-flux. The degree of reduction in net carbon-flux does not vary directly with crude-oil concentration. In severe instances, the amount of carbon available falls below maintenance requirements. The results also clearly indicate that the actions of other stresses such as salinity stress serve to enhance the effects of the oil.

Widdows and Bayne (1971) reported that, during periods of acclimation to changed temperatures, *Mytilus edulis*' calorific balance is adversely affected. The results reported above for both *Mytilus edulis* and *Modiolus demissus* show a similar response to both salinity stress and the effects of crude oil. It appears likely that the decrease in net carbon-flux observed to result from salinity stress and the presence of oil may well be a manifestation of these animals' general response to stress. Because environmental stresses tend to interact in their effects, the addition of yet another (oil) may have drastic effects. Widdows and Bayne (1971) have reported that in the spring, when *Mytilus edulis* is acclimating to summer temperatures as well as maturing gonads, the mussels' energy balance becomes negative, and they are forced to mobilize energy reserves. Blumer (1971) reported that mussels (*M. edulis*), which survived the West Falmouth oil spill, did not breed the following year. In the light of the results of the present study, it is probable that these animals, which may well have begun the spring with reduced energy reserves as a result of the presence of oil, did not have sufficient energy reserves to develop gametes.

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E.S. Gilfillan
Bigelow Laboratory for Ocean Sciences
McKown Point
West Boothbay Harbor, Maine 04575
USA

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