# SUBLETHAL EFFECTS OF OIL ON MOLLUSC SPECIES FROM THE BALTIC SEA

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**Abstract.** Sublethal effects of Iranian crude oil were studied using molluscs from the brackish Baltic Sea (salinity: 6 to 7‰ S). In blue mussels (*Mytilus edulis*) low concentrations caused a reduction in byssal attachment. The sensitivity of the mussels appeared to increase with increasing size. The burrowing behavior of the bivalve *Macoma baltica* was found to be affected at oil concentrations of 70 $\mu g\ l^{-1}$ as measured by a spectrophotometer. Also small individuals appeared less sensitive to the oil than large ones. The effect of oil on the crawling rate of the snail *Theodoxus fluviatilis* was studied. Low concentrations apparently increased the rate of crawling initially (0 to 45 min) while at the end of exposure time (45 to 120 min) the activity decreased. At higher concentrations the crawling rate was very low throughout the experimental period and stress symptoms like complete immobilization occurred.

## 1. Introduction

The effects of acute oil exposure to marine organisms are relatively well documented both from laboratory studies and from field situations (reviewed by Anderson, 1975). Relatively little is known, however, about sublethal effects of oil on individual organisms or at the community level. As chronic oil pollution is potentially more damaging to ecosystems than isolated larger spills, the problem of low level oil pollution warrants special concern (see e.g. Evans and Rice, 1974).

The brackish-water ecosystem of the Baltic Sea is, due to some typical characteristics, considered to be extremely sensitive to discharges of oil and other persistent toxic substances. Thus, obvious features of the Baltic Sea are low annual mean temperature, shortage of oxygen in bottom water and extremely long renewal time of the water (see e.g. Jansson, 1972). Studies on the biological effects of oil pollution in the Baltic area are scarce, however. Some investigations into the effects of oil on Baltic littoral model ecosystems have been made by Notini and Hagström (1976). These studies showed that structural changes in the *Fucus* communities due to oil pollution could be rapidly and easily detected in ecosystems enclosed in basins on the shore. Studies have also been carried out on the effects of oil on the metabolism of Baltic bladder-wrack (*Fucus vesiculosus*) (Ganning and Billing, 1974). These investigations indicated a considerable reduction of community metabolism in the presence of low concentrations of oil in the water.

Furthermore, a few studies on the effects of low concentrations of oil on certain Baltic sea organisms have been carried out indicating behavioral and reproductive disturbances, acute and chronic toxic effects and physiological effects (Lindén, 1975, 1976a, b, c).

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As current and projected demands for energy are prompting accelerated development of offshore petroleum reserves in the Baltic area, and also expanded oil tanker traffic, intensified studies of the effects of low-level oil pollution in the area seem urgently needed. The present paper deals with effects of low concentrations of crude oil on some behavioral patterns of three Baltic Sea molluscs. Sublethal effects of the oil are indexed by measurement of byssus formation among blue mussels *Mytilus edulis* L., burrowing behavior in the clam *Macoma baltica* L., and crawling rate in the intertidal snail *Theodoxus fluviatilis* L.

## 2. Experimental

### 2.1 TEST ANIMALS

The animals were collected in the littoral zone in the archipelago off Tvärminne Zoological station, at the entrance of the Gulf of Finland. The salinity of the sea water in this area is normally 5 to $7^{o}/_{oo}$ S. The experiments were performed during spring and summer. The blue mussels (*Mytilus edulis*) and the snails (*Theodoxus fluviatilis*) were collected from plants of bladder-wrack (*Fucus vesiculosus*) from moderately exposed rocky shores. The bivalve (*Macoma baltica*) was sifted from soft bottom sediments, collected in a sheltered bay. The snails and blue mussels were acclimatized to laboratory conditions 1 to 2 weeks before each experiment, kept in basins containing bladder-wrack and with continuously renewed seawater. The temperature was kept 2 to 3 °C over the normal temperature of the ambient water. The bivalve *Macoma baltica* was collected 12 to 24 h before each experiment.

### 2.2 TEST MEDIA

Crude oil samples were obtained from the Neste Oil Company, Helsinki. The original oil source was the Agha-Jari (Iran) field located off-shore near the head of the Persian Gulf. The Agha-Jari crude has the following major characteristics according to Neste Oy: specific gravity at 15 °C :0.849; S: 1.43% by weight; bottom sediments and water: 0.1% by volume; NaCl: 19.1 mg $l^{-1}$; asphaltenes: 1.9% by weight; V: 74 ppm, Ni: 22 ppm.

The test concentrations were prepared by mixing 10 ml oil with 1 l sea water. The mixture was vigorously shaken for 30 min, after which it was left to weather in an open glass jar. Twenty-four hours later the water under the oil film at the surface was tapped off. This water constituted a stock solution, and the different test solutions were serial dilutions of the stock solution.

The amounts of oil in the water used in each experiment were measured in $CCl_4$ extracts by an IR spectrophotometer according to a method described by Carlberg and Skarstedt (1972). The final concentrations used in the experiments ranged between 6300 and 70 $\mu$g $l^{-1}$. The concentrations used in each experiment are given in Figures 1, 2 and 3.

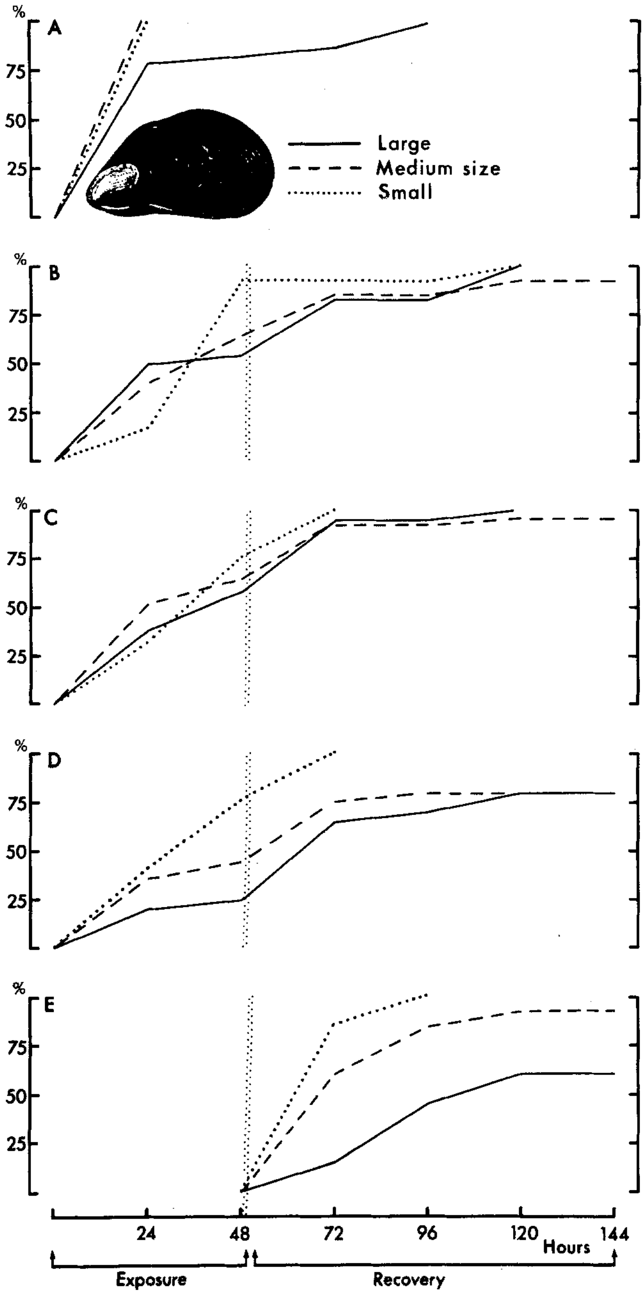


Fig. 1. Percentage of blue mussels (*Mytilus edulis*) byssally attached each 24 h, exposed to the following concentrations of petroleum hydrocarbons in the water: (A) control, (B) 130 μg $l^{-1}$, (C) 190 μg $l^{-1}$, (D) 380 μg $l^{-1}$, (E) 6300 μg $l^{-1}$.

## 3. Experimental Performance

### 3.1 BYSSUS FORMATION OF BLUE MUSSELS

Mussels of three length-classes (small: 5 to 15 mm, medium size: 15 to 25 mm and large: 25 to 35 mm) were placed on petri-dishes (10 cm dia.) which were immersed in the test solutions. Twenty to 25 animals of each length-class were exposed to each concentration. The exposures were made in several glass jars containing 1.5 l of test media.

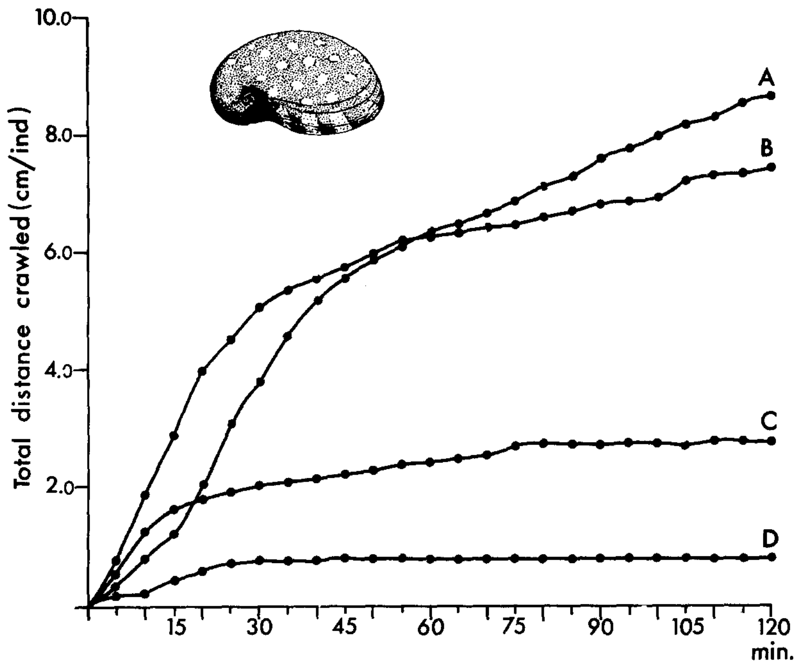


Fig. 2. Average distance crawled (cm/ind) by 20 snails (*Theodoxus fluviatilis*) exposed to the following concentrations of petroleum hydrocarbons in the water: (A) control, (B) 140 $\mu g\, l^{-1}$, (C) 1350 $\mu g\, l^{-1}$, (D) 6750 $\mu g\, l^{-1}$.

At most, 8 mussels were exposed in each jar, and no more than 3 individuals were placed on each dish. Twenty-four to 48 h after the start of the exposure the number of animals that had adhered to the dish was noted. After the 48 h exposure period, the petri-dishes were transferred for a recovery period to a continuous flow of sea water. The number of adhered individuals was observed each 24 h. The temperature was maintained at $10 \pm 2°C$ during the experiment.

Oxygen concentration of the test water was measured at regular time intervals during the experiment. Normally the amount of $O_2$ varied between 85 and 90% saturation at the end of the exposure time. In one jar (D, see Figure 3), however, the $O_2$ concentration was found to be as low as 67% saturation after 48 h of exposure.

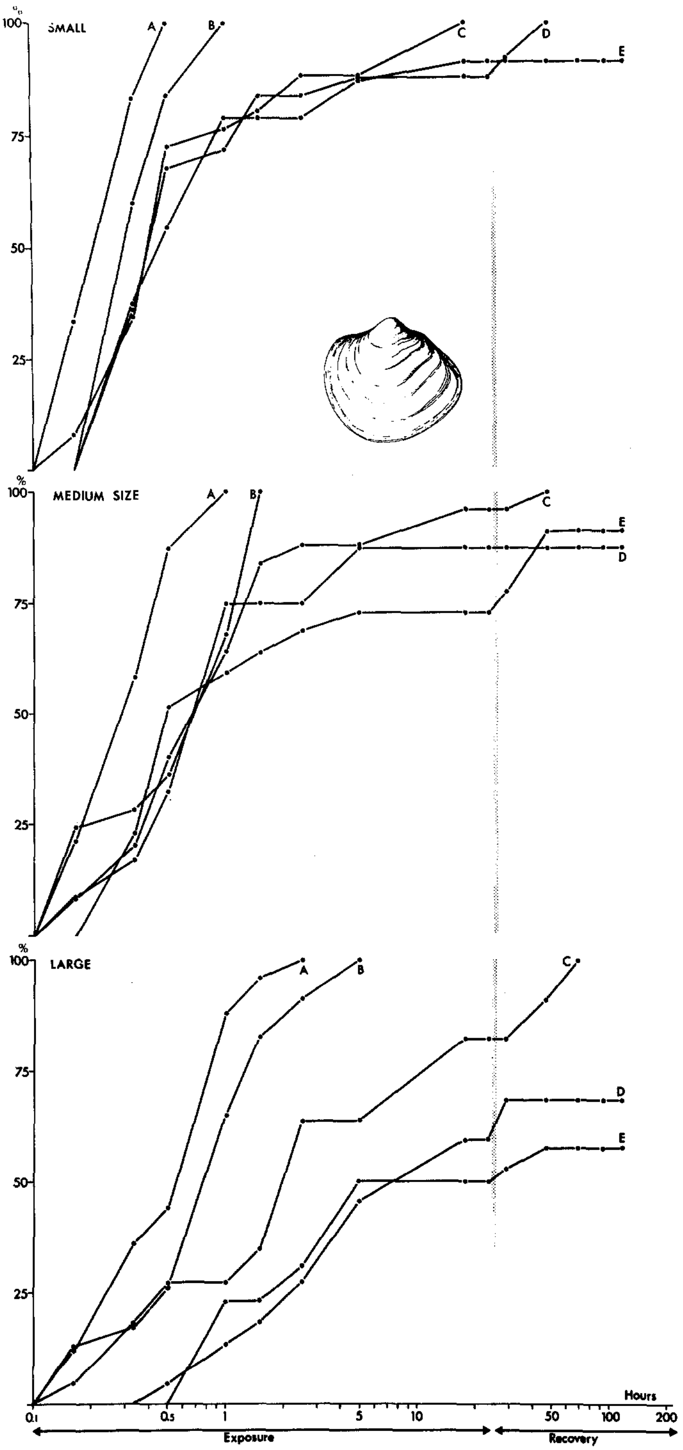


Fig. 3. Percentage of buried clams (*Macoma baltica*) exposed to the following concentrations of oil hydrocarbons in the water: (A) control, (B) 70 $\mu g\ l^{-1}$, (C) 660 $\mu g\ l^{-1}$, (D) 1650 $\mu g\ l^{-1}$, (E) 3300 $\mu g\ l^{-1}$.

3.2 BURROWING BEHAVIOR OF THE BIVALVE *MACOMA BALTICA*

Twenty-two to 26 clams of each of three length-classes (small: 4.5 to 6.5 mm, medium size: 9 to 11 mm and large: 15 to 18 mm) were exposed to 4 concentrations of test media. The clams were spread out over the surface of a 5 cm thick sand layer in several 2 l glass jars. Not more than one size group were put together in one jar. The number of clams that had buried completely was counted at several time intervals (see Figure 3). After a 24 h exposure, the glass jars were immersed in basins with uncontaminated, continuously renewed sea water, and the burrowing behavior was observed each 24 h during 4 days. The temperature was maintained at $10 \pm 2\,°C$ during the experiment.

3.3 CRAWLING IN THE SNAIL *THEODOXUS FLUVIATILIS*

Twenty snails were exposed to each of the experimental concentrations of test media in glass jars containing 1.5 l of test media. They were placed in the center of petri-dishes and the total distance crawled by each snail was measured every 5 min during a 2 h exposure to the test media. A temperature of $16 \pm 2\,°C$ was maintained during the experiment.

## 4. Results

4.1 BYSSUS FORMATION OF BLUE MUSSELS

Figure 1 shows the percentage of mussels byssally attached in the different concentrations of test medium during the course of the experiment. The majority of the mussels of all size groups was byssally attached to the substrate after a 48 h exposure in the two lowest concentrations of test medium. In the highest concentration, however, no mussels at all had attached at that time, but at the end of the recovery period the majority of the mussels in all size groups was byssally attached. No clearcut difference in sensitivity between the different size groups was observed during the course of the experiment. There seems to be a slight tendency towards a higher byssal activity during the first 24 h of exposure compared to the second. The byssal activity increased again during the recovery period.

4.2 BURROWING BEHAVIOR OF THE BIVALVE *MACOMA BALTICA*

The rates of burrowing of the bivalve are shown in Figure 2. On the whole, the control animals showed the highest burrowing rate, and all individuals of all sizes had buried completely within 1 h and 20 min. In the higher concentrations and among animals of large and medium size, between 10 and 50% of the mussels had not buried completely during the 24 h exposure to test media.

4.3 CRAWLING IN THE SNAIL *THEODOXUS FLUVIATILIS*

The results of the experiment are shown in Figure 3. Crawling rates for the control group of snails were found to be relatively high during the first 45 min, after which it decreased somewhat. The crawling rate for the snails exposed to the lowest out of the medium concentrations of test media was in the beginning higher than for the control snails. At the end of the experiment, however, the crawling rate had decreased and was lower than the rate for the control snails. The snails in the highest concentration showed some crawling during the first 30 min of the exposure time. This activity ceased, however, and almost no crawling at all could be observed during the rest of the experiment.

## 5. Discussion

The effects of oil on the byssus thread production of blue mussels have been observed under more marine conditions than in the Baltic Sea (Smith, 1968; Swedmark *et al.*, 1973). Comparison between the results, however, is difficult, as these authors used other methods, especially to establish the amount of oil in the test water. Reduction or absence of the byssus production may be due to decreased pedal activity by shell closure or by direct interference with the synthesis or combination of byssus components (Roberts, 1975a). From the observations in this experiment it seems that initially the ceased byssal activity is caused by shell closure. Near the end of the exposure time, however, the shells were slightly open, indicating either a depression of the activity of the thread-producing pedal gland or direct interference with the synthesis or combination of byssus components. Eisler (1973) using crude oil from Iran observed an ability of 10% of the Red Sea mussels *Mytilus variabilis* to adhere to the substrate after immersion in 10 ml $l^{-1}$ oil for 168 h. The result of this experiment indicates that there may be a difference in sensitivity between small and large mussels. The small specimens appear generally to be less affected by the oil over the experimental period and their recovery from higher concentrations seems to be better. The results are somewhat contradictory, as increased sensitivity in older individuals of a species compared with younger ones is rarely the case in aquatic toxicology (see e.g. Marchetti, 1965; Wilson, 1972). However, Stirling (1975) observed the same relationship in the bivalve *Tellina tenuis* exposed to copper and phenol. Dicks (1973) doing experiments with the limpet *Patella vulgata* found no obvious difference in the effect of Kuwait crude oil on large and small specimens. However, when using pesticides and PCB's Roberts (1975a) found that the sensitivity of blue mussels decreased with increasing size.

The observation that the small individuals possibly exhibit fewer apparent reactions than the larger specimens to the oil recur in the experiments with *Macoma baltica* reported in this paper. The burrowing behavior of the large specimens appears to be more affected compared with that of the small ones. Thus, almost 50% of the large clams had not burrowed at the end of the recovery period in the highest concentration of oil, while less than 10% was the corresponding figure for the small clams. Burrowing behavior in bivalve species under the influence of other toxicants has been reported in studies under more marine conditions. Thus Roberts (1975b) found impaired burrowing behavior in the cockle *Cerastoderma edule* exposed to the chlorinated hydrocarbon Endosulfan. Swedmark *et al.* (1971) reported abnormal burrowing in

*Cardium edule, Astarte sulcata* and *A. montagui* as a reaction to sublethal concentrations of oil spill dispersants.

The crawling rate of the snail *Theodoxus* is initially increased in the presence of the lower concentrations of crude oil. Before the end of the exposure period, however, the crawling rate decreases. In the highest concentration, immobilization occurred almost immediately and the snails stopped crawling completely and showed no tendency to retract into the shell in response to mechanical stimuli. Hargrave and Newcombe (1973) doing experiments with the snail *Littorina littorea* observed an increase in the crawling rate of snails treated with Bunker C oil at 18 °C, but no significant effect at 4 °C. The authors observed, however, no following decrease similar to that observed in the present study, although the observation period was shorter. The concentrations of oil ranged from 750 to 800 $\mu g\,l^{-1}$ measured by spectrophotometer. Eisler *et al.* (1973) performed experiments with Red Sea snails (*Drupa granulata* and *Nerita forskali*) exposed to Iranian crude oil from the same oil field as the oil used in this study. The authors found distress syndromes at sublethal concentration of oil (*Nerita*: 3 $ml\,l^{-1}$, *Drupa*: 30 $ml\,l^{-1}$). They were characterized by 'extreme sluggishness in closure of operculum following tactile stimulation, inability to complete closure of operculum, failure to adhere to sides or bottoms of jar and excessive mucous secretion'. Compared to the effects observed in the present study, at least some of these syndromes appear to be applicable to the observations of *Theodoxus* in the highest concentration. In lower concentrations, however, the stress symptoms observed in *Theodoxus* appeared to be less accentuated.

The results reported on these pages have demonstrated some effects of crude oil on Baltic Sea molluscs, appearing at truly sublethal concentrations. The amount of petroleum hydrocarbons in areas in the Baltic Sea not subjected to immediate oil pollution is below 0.5 $\mu g\,l^{-1}$ (Rudling, 1976). Although exact figures are lacking for Baltic Sea waters subjected to chronic oil pollution or substantial oil spills, the concentration of oil under such conditions is likely to be much higher and would certainly amount to the lower values tested here. Investigations 3 months after a spill of Bunker C oil in Chedbucto Bay, Nova Scotia, showed the presence of 10 to 90 $\mu g\,l^{-1}$ at stations up to 20 mi from the spill (Levy, 1971). Concentrations under acute spills in eastern Canada were up to 800 $\mu g\,l^{-1}$ (Gordon *et al.*, 1973). In areas with moderate but constant pollution in the north-west Atlantic region concentrations from 2 to 70 $\mu g\,l^{-1}$ are reported (Michalik and Gordon, 1971; Gordon and Prouse, 1973; Gordon *et al.*, 1974).

As the experiments were performed under laboratory conditions, the ecological consequences of the observed effects, however, are still unproven. Effects observed in laboratory tests are thus not necessarily effects on the littoral ecosystem, although they may permit prediction of actual effects in a field situation, provided that the ecology of the affected organisms is known. Under natural conditions, however, all behavioral disturbances will most probably in the long run be unfavorable to the organisms. Blue mussels incapable of byssal thread production will be unable to remain in their natural habitat, as the threads are used as mooring lines among both the post-larvae and the adults (Young, 1962; Van Winkle, 1970). They will consequently be washed away and their chances of reattachment at a suitable place are probably small. Digging clams like *Macoma baltica* unable to burrow normally may have their food finding affected as they are deposit feeders. Furthermore, the importance of burrowing behavior in the predator-prey relationship is obvious. The decrease in crawling rate, and the high number of detached snails may well affect their survival in a number of ways, e.g. interference in the food finding or delayed defence reactions like slow or incomplete retraction into the shell.

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