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Evaluation of Metabolic Responses of *Artemia salina* to Oil and Oil Dispersant as a Potential Indicator of Toxicant Stress

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Oil represents an obvious hazard for the coastal environment and studies on its impact on marine organisms are necessary.Solvent based oil dispersants constitute one of the most important means for removing oil from shores.Although recently new dispersants have been developed(usually by replacement of the highly toxic aromatic hydrocarbons of their solvent part by aliphatic hydrocarbons),which are much less toxic than the first ones,dispersants still remain toxic substances.

Insight into the impact of a pollutant on marine organisms may be gained from four types of biological and chemical investigations: a) short-term acute toxicity tests,b)organism-environment transfer studies,c)physiological studies and d)field studies.In the case of oil pollution,field studies have limited value for the estimation of the impact of oil on natural communities since results are often confused with the effects of "detergents"used in cleanup processes. Both types of laboratory studies(acute toxicity and sublethal toxicity tests)provide valuable information for the toxicity of oils and oil dispersants.Since in the case of oil pollution treatment, oils and detergents are acting in combination,a realistic approach of laboratory studies must also include the combined action of these substances on marine organisms.Although acute toxicity studies which measure the mortality due to a toxicant are very useful for the determination of the range of animal tolerance,other effects causing physiological alterations may be detrimental to a population's survival.

This paper concerns research on the effects of an oil,an oil dispersant and of the mixture of oil and dispersant on a physiological process;the respiration of the brine shrimp Artemia salina.

MATERIAL AND METHODS

Adults of Artemia hatched from commercially available cysts(New Technology Artemia salina Revolution)were used as test animals.The hatching of the cysts and rearing of Artemia was performed in constant temperature rooms at 22+0,5°C in synthetic sea water (Synthetica). The three toxic solutions tested were a crude oil(tunesian crude oil zarzaitine type),an oil dispersant(Finasol-OSR$_2$)and their mixture. The type of oil/water mixture tested was oil-water dispersion(OWD).

OWDs have a hydrocarbon composition very closely resmbling that of the parent oil(Anderson et al.,1974).OWDs and Finasol solutions were prepared by diluting a known amount of stock solution in one liter of synthetic sea water.(Synthetica type).After the addition of the toxicant the stock solutions and the test conc.were shaken at aproximately 1000 cycles/min(30 min.for the oil and 15 for Finasol).Detailed characteristics of the tested OWDs and resulting conc.of hydrocarbons could not be obtained.The mixture of oil and dispersant contained equal parts of oil and Finasol.A wide range of toxicants was used (see Table 1).

For oil and Finasol three series of tests were performed.In the first the test conc.were prepared from a stock solution diluted immediately before the experiment(0h test).In the second the stock solution was prepared 48h before the experiment(48h test) and in the third 96h(96h test).For the oil/dispersant mixture only a 0h solution was tested.This was done because in a previous study (Verriopoulos and Moraitou-Apostolopoulou,1982)we noticed that the toxicity of oil and especially of Finasol was reduced with time ("age" of stock solution).As various parameters:size,sex,physiological conditions,life history etc,may influence the respiration of the test animals,we performed every experiment with four groups of animals:a)large males(8-10mm length), b)medium males(6-8mm),c) large females(8-10mm) and d)medium females (6-8mm).In addition,all animals used in an experiment (experimental and controls)came from the same source and were hatched and reared under the same conditions.
Respiration rates were determined potentiometrically with a Radiometer PHM 73 Analyser,using 20ml syringes as respiration chambers. The analyser chamber was connected to a Haake constant Temperature Circulator maintained at 22^{o}C throughtout.Artemia were placed in syringes filled with test water.The syringes were kept in a constant Temperature room and tested 5h later.Controls without Artemia exhibited negligible changes in oxygen concentration.Respiration rates in µl O_2 were calculated direcly from the change in percent saturation before and after the experimental period.

RESULTS AND DISCUSSION

Table 2 shows the measured respiration rates of Artemia.Results include the rate of non exposed(control)animals and that of animals exposed to oil,Finasol and the oil/Finasol mixture.

As for the reasons mentionned above,the respiration rates between controls of the various experiments present differences and in order to facilitate the comparison of the results,we expressed the measured values of respiration rates as percentages of the control values(Figure 1).Although results of experiments present differences it is clear that all three solutions elicit changes in the respiratory rates of Artemia.The direction(stimulation or suppression)of the change is the same for the three solutions and seems conc.-dependent.There is a general trend of decrease of respiration rate at the lower and up to LC50 48h conc.At higher conc. a,usually,important stimulation of respiration was observed.Final-

Table 1.Respiration rates and standard deviation (µl O_2/animal/ /hour)of non exposed(controls)and exposed Artemia.

Concentr. (ppm)	(8-10 mm) males	(6-8 mm) males	(8-10 mm) females	(6-8 mm) females
		FINASOL (0 h solution)		
0	90.49±1.58	72.16±2.65	93.07±2.57	72.26±3.16
0.5	87.68±3.7	66.34±8.66	87.13±5.8	50.77±4.16
1	53.09±13.86	-	-	-
20	94.51±12.32	70.42±11.34	92.66±2.75	63.02±6.89
30	36.32±9.17	36.22±9.76	-	35.18±0.51
		FINASOL (48 h solution)		
0	65.57±2.98	38.72±3.42	56.75±5.71	42.75±1.98
0.5	47.83±6.61	35.66±1.65	51.74±2.4	33.03±2.14
10	47.77±9.1	25.27±4.83	-	-
20	75.04±16.6	42.65±7.29	-	-
30	61.43±6.14	37.0±7.5	-	21.9±10.4
		FINASOL (96 h solution)		
0	66.59±6.23	35.17±4.32	70.57±6.36	29.4±1.72
0.5	-	42.11±2.31	74.8±10.3	33.52±9.6
20	-	41.68±11.5	-	29.05±8.2
21	52.06±6.89	33.66±3.32	52.24±3.11	32.75±2.94
30	93.67±2.35	59.05±4.69	-	62.92±3.62
		OIL(0 h solution)		
0	39.85±5.61	23.25±4.16	61.87±13.7	24.73±0.54
20	64.05±1.64	-	66.02±11.8	30.65±3.82
300	-	35.37±4.15	49.51±9.08	28.45±2.15
500	-	49.65±6.93	-	42.53±4.51
		OIL (48 h solution)		
0	62.44±6.69	40.92±5.0	53.14±4.48	28.41±2.13
20	-	14.49±0.35	-	17.63±1.33
500	-	22.41±6.57	76.83±7.63	37.94±3.58
800	69.33±15.6	18.96±3.18	-	23.8±0.03
		OIL (96 h solution)		
0	48.33±3.71	28.39±1.91	65.42±4.98	17.71±6.70
20	47.66±8.8	21.77±6.78	47.91±6.15	24.59±4.81
410	42.31±7.13	18.5±4.00	56.57±19.8	-
500	60.65±6.31	35.47±5.78	-	29.43±7.65
800	-	-	-	16.81±1.56
		OIL + FINASOL (0 h solution)		
0	66.85±16.4	45.1±6.21	81.56±4.55	40.92±4.35
1	70.62±19.51	35.83±6.78	56.85±16.6	34.04±3.88
5.5	55.02±12.8	33.2±5.06	69.28±9.4	44.35±11.3
50	38.99±4.39	22.38±4.74	50.56±1.7	21.44±2.9

ly at the highest tested concentrations the respiration undergoes an important suppression.

Suprisingly in most cases an important respiratory change was noticed in very low toxicant conc. (1/40 to 1/15 of the LC50 48h). The direction of this respiratory change(suppression or stimula-

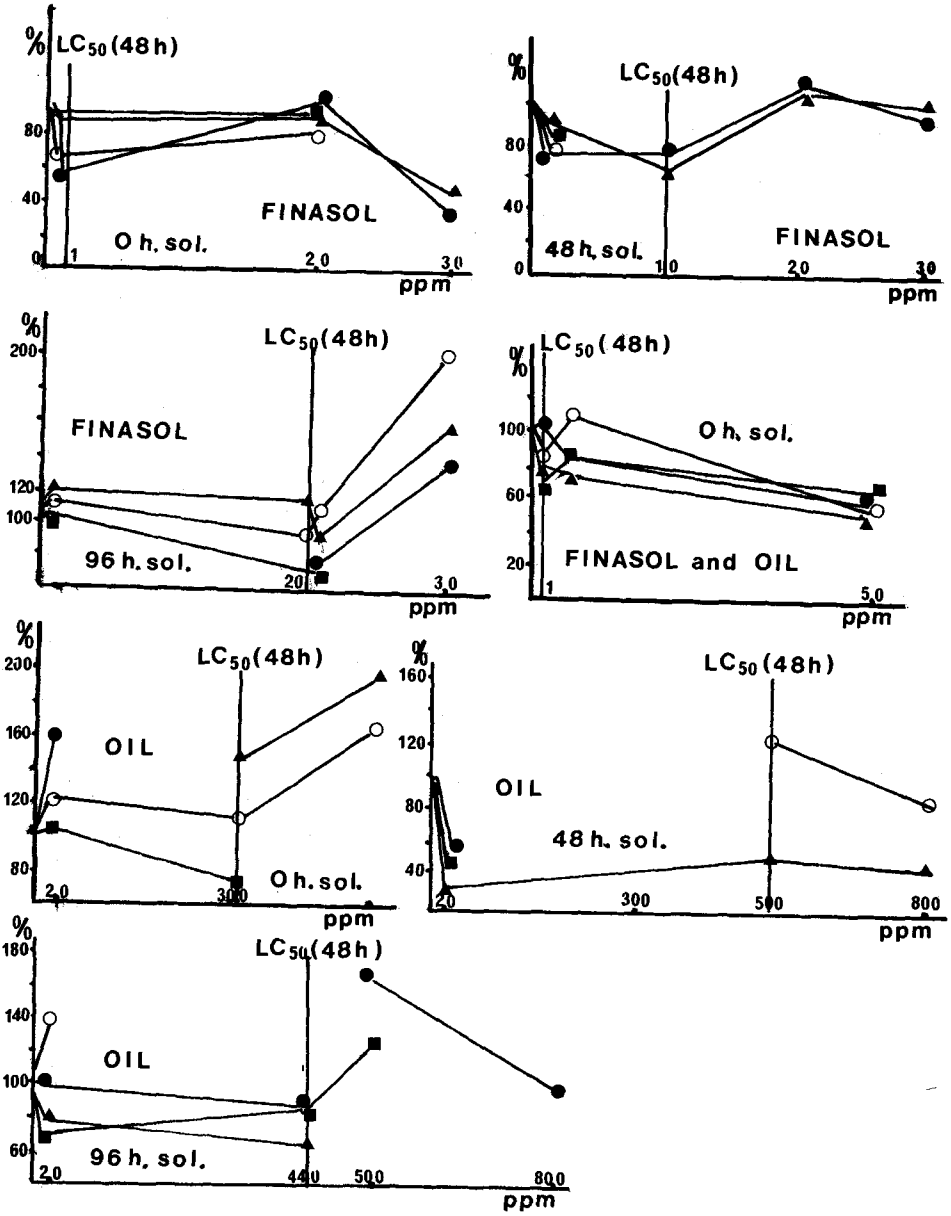

Figure 1.Respiratory rates(% of the controls)of Artemia salina exposed to oil,Finasol and to oil/Finasol solutions of various "ages" O =large females (8-10mm) ■ =large males (8-10mm) ▲ =medium males (6-8mm) ● =medium females(6-8mm)

tion)was not always the same as the one observed at higher and up to LC50 48h conc.

The magnitude of the observed respiration change presents differences not only between the three toxic solutions but also between the various "ages" of solutions of one toxicant and

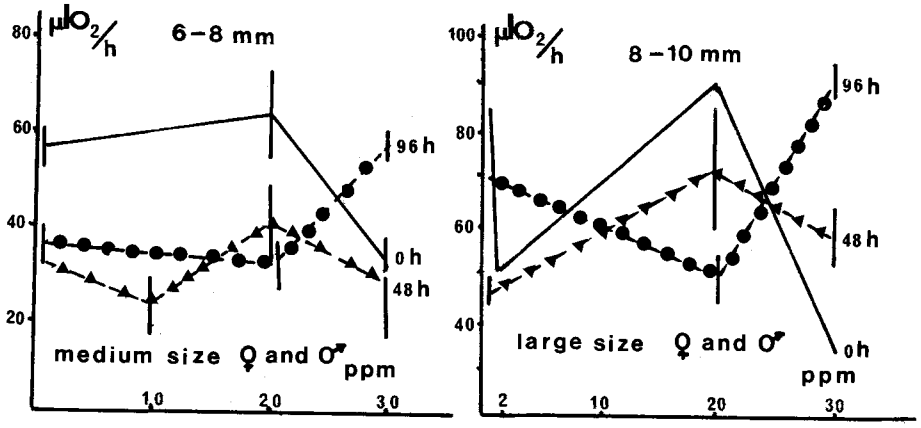

Figure 2.Respiratory rates ($\mu l O_2/h$) of two size classes of Artemia (males and females together)after exposure to three solutions (0h,48h,96h) of Finasol.

even between the various groups of experimental animals.Although non significant differences have been established(by the t-test) between males and females,differences have been found between the two size classes of Artemia.Both males and females of the large size classe were proved sensitive to toxicant stress and presented more important respiratory changes.This is clearly demonstrated in Figure 2. where the respiratory rates of the two size classes (males and females put together)of Artemia after exposure to Finasol is shown.

The respiratory chain is of vital significance for supplying energy to living cells.Respiration represents an important physiological index of an organism because respiratory rates reflect the metabolism of an animal and its overall functional well -being.Physiological stresses causing respiratory responses induced by changes of environmental parameters such as temperature,salinity,dissolved O_2,but also chemical pollutants,have been well documented in the literature.As respiration is sensitive to a whole array of environmental as well as biological variables,respiration was used as a measure of sublethal stress.Exposure to oil and to oil dispersant may also constitute a physiological stress.If this is so Artemia might be expected to show a respiratory response to exposure to oil,oil dispersant and also to oil and dispersant mixture.Our results have demontrated that all three toxic solutions cause physiological changes on the respiration of Artemia.

Significant respiratory responses have been found to occur at high nomimal oil conc.Similarly high values of LC50 48h of oil to Artemia have been noticed previously(Verriopoulos and Moraitou-Apostolopoulou,1982).This is due to the fact that the aque-

ous phase of OWDs usually contains one to two orders of magnitude less than the amount originally added.Furthermore a rapid decline in the conc.of oil hydrocarbons in the aqueous phase is observed. As a result an important decrease of the toxicity of oil,Finasol and oil/Finasol solutions with time (48h old sol.,96h old sol.)has been noticed (Verriopoulos and Moraitou-Apostolopoulou,1982).An analogous decrease of toxicity of the oil and Finasol was also found in our present results.

Sublethal impairment of the capacity of an animal to perform and adapt,as occurs when important respiratory changes are caused,can reduce the chances for survival and the potential for growth and reproduction.The constrains of sublethal stress though possibly slight for individual members of a population,may be highly detrimental to populations with consequences as severe as extinction.The nature of respiratory response(increase or decrease)presents some variability but shows the same pattern for the three solutions and seems conc.-dependent.More variable and less important respiratory reactions are generally noted at the lower conc.up to LC50 48h(with the exception of very low conc.where in some cases important changes have been noted).At conc.higher to LC50 an important increase of respiration has usually been observed and could be mainly attributed to the observed high motility of Artemia at these conc.Similar activity related respiratory increases have been referred to by Zeuthen(1955) and Anderson at al(1974).The observed abrupt lowering of respiration at very high conc.must be the result of a "moribund" state of animals imminent to death.

The respiratory responses to pollutants,as referred to in the literature,present great variability.The nature of the response(increase of decrease)seems to vary not only between the different toxic substances but also between species and often is a function of the conc.of toxicant.As far as heavy metals are concerned Jones (1942) reported that the respiration rate of Gammarus pulex & Polycelis nigra was raised markedly by Cu & Hg as did Hunter (1949)for a gammarid exposed to Cu.Corner and Sparrow(1956) found that these metals depress the respiration rates of Artemia,while Bernard and Lane(1961)suggested that increasing Cu conc.caused barnacle cyprids to increase and then decrease their respiratory rates.Kapoor and Griffiths(1976) have noticed a relatively constant increase of respiration for Phasganophora capitata at low Cu conc.and an exceptionally large increase at the lethal threshold level of Cu.Nereis virens (Raymont and Sheild,1964)and Lemonis macrochirus (O'Hara, 1971)have shown similar results.Reeve et al.(1977)working with plankton found little indication of any systematic effect of Cu and Hg on respiration at conc.up to and beyond those required to produce a lethal effect on 50% of the population in 24h.The same authors conclude that respiration cannot be considered as a sensitive indicator of sublethal stress.Consistent changes in the respiration rate are usually indicative of imminent death.

A great variability of data is also noticed concerning the effects of oil and its constituents.Anderson & al(1974) have found a stimulation of the respiratory rate of Cyprinodon when exposed to WSF of #2 fuel oil while the WSF of the bunker C oil caused a de-

pression of O_2 consumption of this fish.The same authors working with Penaeus found for both oils the same pattern of respiratory responses: lowering of the O_2 consumption at lower conc.,respiratory rates near those of the controls at intermediate WSF conc. Struhsaker and al (1974)found that lower conc.of benzene(a water soluble component of crude oil)accelerated the metabolic rate of larvae of herring and anchovy while at higher conc.the metabolic rate is delayed.Dunning and Major(1974)found that cold sea water extracts of different oils lowered the respiratory rate of Mytilus edulis.
Considering the bulk of published literature dealing with the effects of toxic agents on respiration one remains impressed by the variability of the results.Many authors also refer to strong variations between the experimental animals of a single experiment. Respiration is affected by a great variety of factors including life and nutritional history of animals and stress of handling. Thus an important source of variation is introduced and this becomes particularly important when working with natural populations.In order to overcome this disturbing factor in our experiments,both controls and experimental animals came from the same batch,hatched simultaneously and were reared under the same conditions.In fact we noticed important differences in the respiration rate between the control animals of the various experiments.

In our results but also in other studies the nature of respiratory response has been found to be a function of conc.It is possible that in other studies where only a stimulation or a depression of the respiration rate is refered after exposure to a toxicant,the results concern only the range of the tested conc. and eventually an inverse respiratory reaction could be observed outside the tested conc.range.

Generally consistent respiratory changes have been refered at high conc.and thus respiration could not be characterized as a general sensitive pollutant stress.However in some of our experiments some significant respiratory reactions have been noticed at low conc.Similarly Anderson and al(1974) have found the respiratory responses of Penaeus when exposed to #2 fuel oil to be more pronouced at lower conc.than at an intermediate one. In previous work(Moraitou-Apostolopoulou & al.,1979) we noticed a significant increase of the respiratory rate of A.clausi after exposure to sublethal conc.of Cu,Cr and Cd.Valuable information could therefore be gained from detailed research on the effects of low conc.of toxicants.

On the other hand respiration is relatively easy to monitor in living animalas and,especially,tests are rapid.Respiration therefore could be used at least in urgent screening tests.Finally an interesting observation of our results is that large animals seem more sensitive to respiration changes that smaller ones. Cox and Anderson(1973)have also noticed that the sensitivity of the shrimp Penaeus increases as their size increases.Similarly Anderson et al.(1974)have noticed that the change in the respiratory rate of large individuals of Palaemonetes pugio was more pronounced than that of smaller animals.

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