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Effects of Prudhoe Bay Crude Oil on Molting Tanner Crabs, *Chionoecetes bairdi*

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ABSTRACT

Premolt and postmolt juvenile male Tanner crabs, Chionoecetes bairdi, *from Alaska waters were exposed to Prudhoe Bay crude oil in static bioassays in the laboratory. Crabs in both stages were similarly susceptible to crude oil; the estimated 48-hour TLm (median tolerance limits) values were 0.56 ml oil/liter. Molting success decreased with increasing exposure of crabs to oil, and newly molted crabs autotomized limbs during exposure to oil. Relating the results of our study to the known behavior of crabs and the documented behavior of oil spills in the ocean suggests that oil spilled in Alaska waters would harm the Tanner crab resources. The impact on all crab resources of chronic low-level oil pollution from the ballast water discharged into Prince William Sound is unknown. This study further illustrates our present state of ignorance concerning the biological effects of oil in the marine environment.*

Alaska has an abundance of both living marine resources and petroleum and faces the problem of their compatible utilization. The proposed transport of Prudhoe Bay oil to Valdez in Prince William Sound via pipeline requires subsequent transfer to tankers for marine shipment, which poses a potential for contact between oil and marine animals. Even if catastrophic spills are avoided, chronic rates of oil pollution discharged at depth from the ballast-water treatment plant at Port Valdez could approximate 13 barrels of oil per day plus incidental surface spillage during loading operations (U.S. Department of Interior, 1972).

Prince William Sound is a major fishing area for several commercial species, e.g. salmon, *Oncorhynchus* spp.; king crabs, *Paralithodes camtschatica;* and Dungeness crabs, *Cancer magister,* and has recently become a major producing area for Tanner crabs, *Chionoecetes bairdi*. More than 12 million pounds of Tanner crabs were taken in Prince William Sound between January and June 1973—a catch second only to the Kodiak area in volume landed. Prince William Sound is also a nursery area for Tanner crabs; juveniles were abundant and widespread in a trawl survey covering the eastern and northern parts of the sound (Van Hyning and Cooney, 1972). In addition to their commercial value, Tanner crabs are representative of the large group of marine crustaceans valuable in themselves as part of the food web, both as larvae and adults.

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The effects of oil on marine life in subarctic marine ecosystems like Prince William Sound are unknown and are difficult to predict because of a lack of appropriate research. That massive quantities of oil would have catastrophic effects on marine life is quite apparent, but the effects of chronic exposure to sublethal concentrations of oil are less certain. A growing body of evidence indicates that chronic exposure to low levels of oil may be more significant to the conservation of the fishery resources than occasional catastrophic spills (Blumer, 1970; Kuhnhold, 1970; Whittle and Blumer, 1970; Rice, 1973). Blumer, Mullin, and Guillard (1970) and Blumer, Sousa, and Sass (1970) showed that hydrocarbons, including unsaturated ones, are remarkably stable in the marine food chain. Therefore, it is clearly important to determine the effects of persistent and nonpersistent hydrocarbon pollutants on marine organisms, especially during sensitive stages in the organism's life history.

Molting, a requisite of growth for an animal with an exoskeleton, is a vulnerable period in the life history of crabs and all other crustaceans. Molting occurs many times during the life cycle of an individual, beginning with the larva and ceasing only when growth of the adult stops. The molting cycle is chemically controlled by hormones, and it is therefore likely that chemical pollutants may affect molting. Wells (1972) noted that when lobster larvae were exposed to crude oil, the resulting deaths generally occurred when the larvae were molting. He also showed that sublethal concentrations of oil delayed molting in the early larval stages (1 to 4) during exposures up to 30 days.

Although the study by Wells (1972) was limited in scope, the results suggested that oil has specific effects on the molting process. Because molting is an integral part of crustacean growth and crustaceans are key links

in the marine ecosystem, any pollutant having a detrimental effect on molting should have a far-reaching effect on the ecosystem. Therefore, in the spring of 1973, we initiated a study to determine if short-term static exposure to Prudhoe Bay crude oil affected molting of Tanner crabs. This species was selected because of the combination of its availability to us and its commercial and ecological significance. Specific questions we investigated were: (1) How toxic is Prudhoe Bay oil to premolt and newly molted Tanner crabs? (2) Does short-term exposure of premolt crabs to oil at sublethal levels cause molt arrest or delayed mortality after molting? (3) Does oil interfere with the hardening of the exoskeleton or have other observable effects?

Figure 1.—Water bath and aerated containers used in static acute bioassays with crabs. Aeration tubes are glass-tipped to prevent contact of oil-water solution with tubing.

METHODS

Male Tanner crabs were collected by scuba divers in March 1973 from a large group of molting crabs near the head of Auke Bay, lower Lynn Canal, southeastern Alaska. The crabs were held in flowing seawater in the laboratory for 1 to 3 days before each oil exposure test. No attempt was made to separate mature from immature crabs, but most of the crabs were 73 to 115 mm in carapace width, a size range in which male Tanner crabs are usually sexually immature (Brown and Powell, 1972). Test and control animals were handled similarly. None of the crabs were fed before or after exposure to oil, because crabs do not usually feed for several days before or after the molt. The tests were basically of two types: (1) acute toxicity bioassays (includes lethal dosages) with premolt and postmolt crabs to determine differences in median tolerance limits (TLm), and (2) subacute bioassays in which premolt crabs were exposed to oil to study effects on molting. In the latter experiment, the premolt crabs were exposed to lower dosages of oil and placed in holding pens for several weeks to study molting success.

Acute Bioassays with Premolt and Postmolt Crabs

In the acute bioassays with premolt crabs[1] (76 to 110 mm in carapace width), each crab was placed in a glass jar of about 20-liter capacity containing 15 liters of aerated seawater. Water temperatures were maintained at 4.0° to 5.0°C by placing the jars in a circulating cold-water bath (Fig. 1). A measured quantity of oil[2] was put into each jar, and the mixture of water and oil was vigorously agitated with a mechanical stirrer for 1 min at 2,250 rpm. The mixing procedure created a fine emulsion almost immediately which settled up to form a slick within 1 to 2 min after the mechanical stirring stopped. It is assumed that this procedure approximates the standardized shaking procedure of LaRoche, Eisler, and Tarzwell (1970). Twelve test crabs were exposed at each of four doses of oil (0.18, 0.32, 0.56, and 1.00 ml/liter), and 12 control crabs were placed in jars containing only seawater. Forty-eight-hour TLm values for acute bioassays were determined graphically according to the method of Doudoroff et al. (1951). One crab was placed in each jar and the weight of each crab was about 180g, resulting in an average mass to water volume ratio of 12 g/liter. This ratio is three times the recommended ratio of 4 g/liter for static tests; however, in a separate test four crabs in one control jar survived for more than a week (48 g/liter), which indicated that the ratio of 12 g/liter was not so great as to substantially influence the results by inducing physiological stress. Test and control solutions were aerated continuously during the bioassay.

Acute bioassays with postmolt crabs (83 to 115 mm carapace width) were performed in the same way as those

[1]Premolt Tanner crabs that have not molted for a year or more are easily recognized and separated from new-shelled postmolt crabs by the color, wear marks, and epifauna on their carapaces. All premolt crabs used in this study had molted during the previous spring molting season.

[2]Prudhoe Bay crude oil for the experiments had been supplied by Atlantic Richfield Company in a sealed 55-gal. barrel. When the barrel arrived at the laboratory, the contents were transferred to 1-quart cans sealed with Teflon-lined lids to prevent loss of volatile components.

with premolt crabs. The oil concentrations were 0.32, 0.56, and 1.00 ml/liter; 12 crabs were exposed to each concentration in the first experiment and 11 crabs in the second. Crabs of comparable size were used as controls in each experiment.

Subacute Bioassays Involving Molting and Autotomy of Limbs

In the tests of possible subacute or delayed effects of oil on molting, premolt crabs (73 to 114 mm in carapace width) were exposed for 48 hours to three sublethal concentrations of crude oil in seawater[3] (0.32, 0.18, and 0.056 ml/liter) by the same procedures described for the acute bioassays. The exposed crabs and a control group were then transferred to holding pens in the natural environment, where molting success was monitored until all of the control crabs had molted—1½ to 4½ weeks (Fig. 2). The numbers of premolt, postmolt, and dead crabs were recorded every 3 to 5 days, and the growth increment of molted crabs was determined. The hardness of the exoskeleton of the molted crabs in the holding pen was subjectively determined. Three major trials were completed: 40 crabs were exposed to 0.32 ml oil/liter, 30 to 0.18 ml, and 33 to 0.056 ml. The control group contained 32 crabs.

Many surviving postmolt crabs lost limbs (apparently autotomized) while exposed to oil during the 48-hour subacute bioassays, but only a few premolt crabs lost limbs. Because the loss of several legs or chelae is probably detrimental to crab survival in nature, we repeated the test with crabs of similar postmolt age (about 1 week after molting) to confirm that they were autotomizing their limbs. The crabs were subjected to exposures of oil (0.32 to 1.00 ml/liter for 24 hours) during which the shed legs were counted in the undisturbed jars in the cooling bath (Fig. 3). The jars were smooth inside and contained nothing that could snag a leg or provide a base for the crab to pull against. Tests to evaluate leg loss were also repeated 4 weeks after the peak molt period for Tanner crabs.

[3]The highest concentration of oil (0.32 ml/liter was lethal to 25 percent of the crabs over three trials.

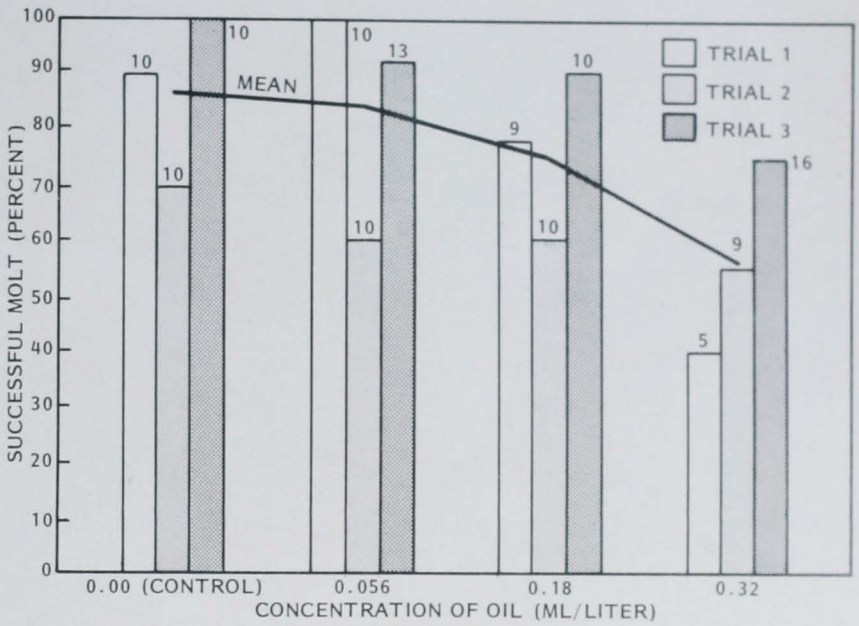

Figure 2.—Molting success of Tanner crabs that survived 48-hour exposure to various concentrations of Prudhoe Bay crude oil. Three successive trials were conducted. The numbers on top of each bar are numbers of crabs surviving the 48-hour exposure and then held to observe their molting success. The curve represents the unweighted mean of the three trials at each concentration.

TOXICITY OF CRUDE OIL TO PREMOLT AND POSTMOLT CRABS

The concentration of oil tolerated by 50 percent of both premolt and postmolt crabs (TLm) for 48 hours at about 5°C was estimated from the bioassays to be 0.56 ml/liter (Table 1). The 24- and 48-hour TLm values of premolt crabs were not apparently different, but the 24-h TLm of postmolt crabs was higher—0.83 ml oil/liter. However, the statistical precision and significance of these TLm values were not examined. Because of the high ratio of tissue mass to water volume (and tissue mass to toxic materials), the TLm estimates may be higher than would have been obtained under ideal conditions (i.e., material is probably more toxic than the tests indicate).

Crabs responded to all concentrations first by increased activity, followed within 60 min at the high oil concentrations by a contraction of the legs; then within 2 to 3 hours complete relaxation and death. Relaxation prior to death may be indicative of narcosis. At the lowest oil concentrations, the crabs remained normally active.

EFFECTS OF CRUDE OIL ON MOLTING SUCCESS

Results of the study in which premolt crabs were exposed to subacute concentrations of crude oil in seawater (Fig. 2) suggest that brief exposure to small amounts of crude oil (0.056

Table 1.—TLm values at 5°C of Prudhoe Bay crude oil for premolt and postmolt juvenile male Tanner crabs. Values are for one bioassay with premolt crabs and the mean of two assays for postmolt crabs.

Hours of exposure	TLm value (ml oil/liter)	
	Premolt crabs	Postmolt crabs
24	0.56	0.83
48	0.56	0.56

Figure 3.—Examples of leg loss in live crabs at various crude oil concentrations. Oil layer has been removed from two highest concentrations.

to 0.32 ml/liter) may have a delayed detrimental effect on molting. Most of the crabs (75 percent) survived the 48-hour exposure to oil at 0.32 ml/liter, and the molting success figures are based on these crabs. The trials were made successively over a period of 2 weeks, and the closer the crabs were to molting, the less sensitive they appeared to be to oil—the percentage that molted and lived after exposure to 0.32 ml/liter increased with each successive experiment (Fig. 2). The experimental crabs were from a group rapidly approaching ecdysis, i.e., trial 1 crabs were further from molting than trial 2 crabs, which were further from molting than trial 3 crabs. It is possible that the progressive physiological changes associated with the molt cycle (Passano, 1960) are accompanied by a change in sensitivity to oil. Although the percentage of crabs that molted and survived a given oil exposure varied considerably among the three trials, survival definitely declined as concentrations of oil increased (Fig. 2). The 32 control crabs (no exposure to oil) had the best overall survival, and all had molted when the experiment was terminated. Failure to molt successfully was usually manifested in incomplete molt and death, but 6 percent of the crabs exposed to oil simply did not molt during the experiment.

The new shell of molted crabs that survived the exposure appeared normal with respect to both morphology and rate of exoskeleton hardening. Growth as indicated by the percent incremental increase in width was the same (27 percent) for exposed and control crabs; however, the effect of limb loss was not considered for either group. That limb loss may reduce body growth as well as stimulate ecdysis was reaffirmed in recent studies by Bennett (1973) and Skinner and Graham (1972), respectively.

Statistical examination of the survival and molting data in Figure 2 by a nonparametric test appropriate for a randomized block design (Friedman, 1937) showed that the proportion of crabs molting in at least one of the treatments, the high-level oil dose, was smaller than the control or lowest oil dose. Therefore, brief exposure of premolt Tanner crabs to crude oil 1 to 4 weeks before molting probably has a detrimental effect on molting. The indication that crabs appeared less sensitive to oil as they approached the molt was not statistically significant.

AUTOTOMY OF LIMBS INDUCED BY EXPOSURE TO OIL

Although we found no difference in survival between postmolt and premolt crabs exposed to oil, the postmolt crabs lost many of their limbs whereas the premolt crabs lost none of theirs. The number of limbs lost by postmolt crabs varied between crabs, but the average increased with increasing oil concentrations both for the 24-hour (11 April) and the 48-hour exposures (29-30 March).

To eliminate mechanical disturbance as a possible factor in the leg loss associated with exposure to oil, the jars containing crabs were not disturbed, moved, or handled in the experiment in which crabs were held for 24 hour until the autotomized legs had been counted. Substantial loss of legs occurred in crabs exposed to 0.56 ml oil/liter (Table 2). At this dose, 64 percent of the crabs were alive after 24 hours of exposure, and these live crabs as a group had lost an average 3.3 legs per crab (range 1 to 8) (Fig. 4). Clearly, the exposure to oil caused the loss—crabs began losing legs within 3 hours. The crabs that died during the 24-hour test had lost an average of 5.5 legs each (range 2 to 7). In several instances, crabs lost all legs on one side, including the cheliped; in other instances both chelipeds plus several other legs were lost. That crabs autotomized several limbs either simultaneously or with little movement was indicated in several instances by detached legs still in place around the bodies of live crabs (Fig. 5). Crabs did not lose regenerated legs as readily as normal legs.

It is not known how long after molting that Tanner crabs continue to be susceptible to dropping legs during exposure to oil. We repeated the tests on 1 May, about 4 weeks after the peak of molting, and seven crabs exposed to 0.56 ml oil/liter for 24 hours dropped only three legs. Apparently, crabs become less vulnerable to oil-induced leg loss with increasing time after molting.

The fact that oil stimulated autotomy in post molt Tanner crabs suggested to us that other crab species and crustaceans may react similarly. However, we exposed premolt and postmolt juvenile king crabs (11.4 g average weight) to sublethal and lethal doses of oil (0.1 to 1.00 ml/liter), and no crabs in either group lost legs.

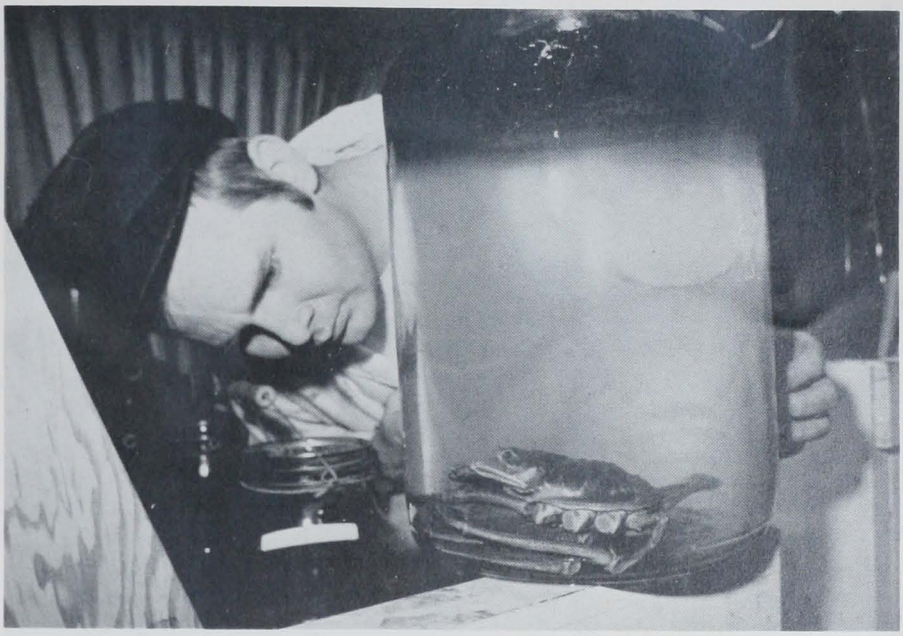

Figure 4.—A common occurrence was the loss of three or more adjacent legs, which seriously hampers the crab's mobility.

DISCUSSION

Molting Tanner crabs were affected in three observable ways by short-term exposure to Prudhoe Bay crude oil under static laboratory conditions. High concentrations killed crabs outright, but lower concentrations had two less obvious effects—reduction in molting success and autotomizing of limbs. Thus even among Tanner crabs that survived an initial exposure to oil, their mortality in the natural environment may be increased, depending on the time of year that exposure occurs and the stage of their molt. Crabs, which constitute about 25 percent of the total number of crustacean species on a worldwide basis, may be particularly susceptible to oil pollution because most occur in shallow waters for part or all of their life cycles. In Alaska, the four major commercial crab species (*Paralithodes camtschatica, P. platypus, Chionoecetes bairdi,* and *Cancer magister*) all enter shallow water in spring and summer to molt, mate, and release their larvae.

The method used in our experiments to mix the oil with the seawater and to introduce the crabs to the oil-seawater mixture makes it impossible to determine whether the water-soluble fractions of the oil are causing the damage or whether exposure of the crab to emulsified oil particles is responsible for the observed effects. Some exposed crabs acquired a very thin film of oil on their exoskeletons, and it is possible that oil passed directly through their gill membranes or body integuments. The 48-hour TLm for juvenile male Tanner crabs was quite high (0.56 ml oil/liter) with respect to total oil; however, the water-soluble fractions, which approximate only 0.1 percent of this value, may be the agents causing damage instead of total oil.

Regardless of what the toxic components are, observations on the behavior of oil from past spills make it reasonable to assume that our experiments approximate the type of oil exposure which crabs could experience under conditions of a spill in estuarine waters.

Campbell and Martin (1973) discuss several instances of formation of extremely stable oil-water emulsions—the oil spilled by the *Torrey Canyon*, the oil spilled in the wreck of the *World Glory* off the coast of South Africa, the oil leaked from offshore wells in Santa Barbara, and the oil spilled from the wreck of the *Arrow* in Chedabucto Bay, Nova Scotia. The emulsions formed over a wide temperature range in these spills contained from 36 to 80 percent water and resulted from several types of oils (Kuwait crude to bunker C fuel).

Table 2. — Autotomy of limbs resulting from exposure of recently molted juvenile male Tanner crabs to different concentrations of crude oil for 48 and 24 hours.

Concentration of oil (ml/liter)	48-hour exposure (29-30 March)					24-hour exposure (11 April)				
	Crabs alive		Average number of limbs lost per crab			Crabs alive		Average number of limbs lost per crab		
	Number	Percent	Alive	Dead	Combined	Number	Percent	Alive	Dead	Combined
0.00	8	100	0.0	0.0	0.0	11	100	0.0	0.0	0.0
0.32	7	58	0.0	0.6	0.25	9	82	0.1	0.0	0.1
0.56	6	50	1.1	3.0	2.0	7	64	3.3	5.5	4.0
1.00	1	8	9.0	4.7	5.0	2	18	3.5	5.1	4.8

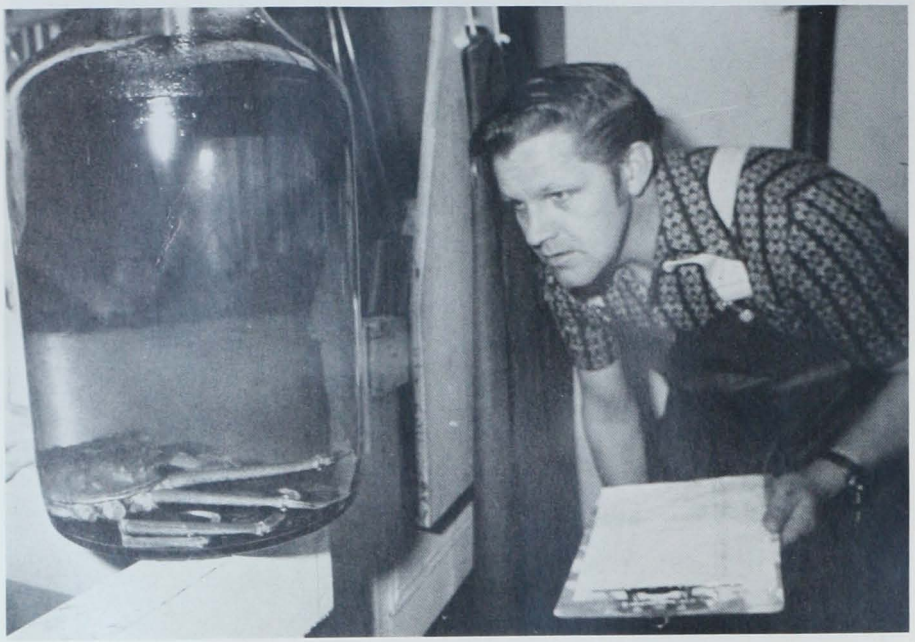

Figure 5.—Crabs autotomized several limbs, either simultaneously or with little movement, as legs remained in place around the body in several instances.

Oil from the *Arrow* (108,000 barrels of bunker C in seawater at temperatures between 0° and 2°C) formed highly stable emulsions and ". . . within 2 weeks of the spill, oil particles were observed down to a depth of 50 m throughout the entire area of the bay (approximately 900 km^2). Oil particles were also found outside the bay in a tongue approximately 10 km wide which extended eastward from the mouth of the bay for a distance of 70 km." (Campbell and Martin, 1973.)

Since extremely stable oil-water emulsions were formed over a wide temperature range (spanning temperatures which occur in Alaska waters), we expect that highly stable emulsions will form after oil spills in Alaska and that oil will reach depths occupied by Tanner crabs. There is little doubt that oil spills would have adverse effects on Alaska's Tanner crabs, and that crabs would be especially vulnerable to oil spills in the spring when they move into shallow water to molt and mate; however, it is difficult with available data to predict the magnitude and persistence of these effects.

The effects on marine life of chronic exposure to sublethal concentrations of oil are even more difficult to predict than the effects from large oil spills. It is possible that the large volume-low hydrocarbon concentration effluent (1.3 million barrels per day at 10 ppm oil, according to the U.S. Department of Interior, 1972) from the proposed ballast-water treatment facility in Port Valdez will adversely affect (decreased molting success and increased autotomy) Tanner crabs and other organisms. This effluent will be introduced at depth and will consist of the water-soluble hydrocarbon components from Prudhoe Bay crude oil.

The fact that oil reduces molting success in Tanner crabs and induces newly molted Tanner crabs to autotomize limbs suggests that oil pollution may have adverse effects on crustaceans in general. Because molting is an integral part of crustacean growth and crustaceans are important in the marine environment, any extensive disruption of the molting process of important marine species would be disastrous. Data are needed to measure the effect of oil on molting in other crabs and crustaceans.

Autotomy is a phenomenon generally associated with molting in the crustaceans, where it permits dropping damaged legs that will not slide out of the old exoskeleton. Autotomy of limbs other than at molting is usually associated with mechanical stimulation, although it has been known for some time that autotomy is also stimulated by noxious preserving fluids (Wood and Wood, 1932). The high degree to which autotomy has developed in Tanner crabs, especially during and shortly after the molt, may be detrimental to their survival if they are exposed to chemical pollution. We have observed autotomy within 3 hours of initial exposure to oil, and in nature a sublethal dose could cause crabs to autotomize some limbs. Pollutant-stimulated autotomy could be very significant if it could be induced by the accumulated effects of chronic low-level exposure to oil. The implication is clear: crabs with fewer legs and chelae would be less successful in feeding, defending, migrating, and mating.

The lack of oil-induced autotomy by postmolt Tanner crabs tested 4 weeks after molting, and lack of autotomy in juvenile king crabs in our tests indicates that extrapolation of either of these observations to other species, other life stages, and other crude oil and refined products is not warranted until verified by further specific research.

Our laboratory experiments indicate that oil pollution may cause significant biological damage other than immediate death of the affected organisms. Of immediate need is research to (1) identify and determine the persistence of oil fractions which affect molting and induce leg loss, (2) determine the exposure necessary to affect molting and leg loss, (3) determine the effects of acute exposures of oil on molting and autotomy of other crustaceans, and (4) determine the chronic effects of exposure to oil on molting success and autotomy.

SUMMARY

This study investigated the effects of Prudhoe Bay crude oil on juvenile Tanner crabs during the molting process. High doses of oil caused mortality, while lower doses decreased molting success and induced autotomy of limbs. Because the concentrations of oil used in the experiments were within the concentration range and depth distribution that could occur in real situations in nature, it is quite possible that these and other adverse effects could result from oil spills or from chronic low-level pollution. Specific results of the study are:

1. Median tolerance limits for 48 hours for both premolt and postmolt Tanner crabs was estimated to be 0.56 ml oil/liter.
2. Molting success of premolt crabs after exposure to 0.32 ml oil/liter was significantly lower than molting success of control crabs.
3. Observations of autotomy in recently molted crabs that survived acute oil exposures suggested that delayed and indirect mortality may occur among crabs that survive a short exposure to oil.

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