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Depression of feeding and growth rates of the seastar *Evasterias troschelii* during long-term exposure to the water-soluble fraction of crude oil

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Abstract

To test the effect of petroleum hydrocarbons on predation by the seastar *Evasterias troschelii* (Stimpson, 1862) on the mussel *Mytilus edulis* (L.), we exposed the predator with the prey to six concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil. Seastars and mussels were collected at Auke Bay, Alaska, in November 1980. During a 28 d exposure in a flow-through system, seastars were more sensitive to the WSF than mussels: the LC_{50} for the seastars was 0.82 ppm at Day 19 and, although no mussels were exposed to WSF for more than 12 d, none died. Daily feeding rates (whether in terms of number of mussels seastar^{-1} d^{-1} or dry weight of mussels seastar^{-1} d^{-1}) were significantly reduced at all concentrations above 0.12 ppm. At 0.20, 0.28 and 0.72 ppm WSF, daily feeding rates (in terms of dry weight of mussels) were, respectively, 53, 37, and 5% of the control rate; at the two highest concentrations (0.97 and 1.31 ppm WSF), the seastars did not feed. Seastars at concentrations greater than 0.12 ppm WSF grew slower than individuals from the control group and the 0.12 ppm-treatment group combined. These laboratory results show that *E. troschelii* is more sensitive to chronic low levels of the WSF of crude oil. The possibility that such oil pollution could reduce predation and permit *M. edulis* to monopolize the low intertidal zone of southern Alaska remains to be studied.

Introduction

Understanding how populations of organisms in marine communities respond to environmental contamination by petroleum hydrocarbons is the goal of many oil pollution studies. Community ecologists have surveyed habitats contaminated by oil, or conducted experiments involving controlled release of oil into microcosms or onto intertidal or supralittoral communities. Results of field surveys are difficult to interpret because natural communities exhibit great spatial and temporal variability, rendering controls difficult to arrange (Mann and Clark, 1978; McIntyre *et al.*, 1978). Controlled experiments on multispecies systems are preferable to field surveys, because environmental variables and the distribution and amount of the toxicant can be closely controlled, and treatments can be replicated. Yet, even when simple multispecies systems are considered, it is hard to decide whether oil is directly affecting the organisms or is altering the outcome of predation or competition. To resolve this ambiguity, strong biological interactions (*sensu* Paine, 1980) important to community structure should be identified, when possible, and the effects of petroleum hydrocarbons on these interactions tested (Lewis, 1972, 1980; Neuhold and Ruggerio, 1975; Spight, 1976). Removal of strongly interacting species or suppression of their interactions could cause cascading changes in a community, involving several trophic levels (Paine, 1980).

Large seastars are frequently strongly interacting species in intertidal and shallow subtidal communities, especially in temperate and subpolar regions (see review by Menge, 1982), because they preferentially prey on species (usually mussels) that dominate in competition for space. The seastar *Evasterias troschelii* is the most common large predatory asteroid in the intertidal regions of inner Prince William Sound in south-central Alaska and the inner waters of southeastern Alaska, where it occurs with *Mytilus edulis*. These two species overlap in range from Saint George Island, Pribilof Islands, to Monterey Bay, California, on the Pacific coast of North America (Fisher, 1930; Soot-Ryen, 1955).

In the rocky intertidal region at Port Valdez and Auke Bay, Alaska, *Evasterias troschelii* prefers to eat *Mytilus edulis*. *M. edulis* made up 81% (number of *E. troschelii* observed feeding, $n = 395$) and 84% ($n = 120$) of the diet of *E. troschelii* at Port Valdez and Auke Bay, respectively. By preferentially preying on *M. edulis*, a dominant competitor for space in Alaska as well as New England (Lubchenco

and Menge, 1978), *E. troschelii* prevents the mussel from occupying and eventually monopolizing space in the lower intertidal zone (O'Clair, unpublished experiments).

Previous studies of the effect of petroleum hydrocarbons on adult predaceous seastars have suffered from two limitations. Exposures have been static (for example, Crapp, 1971; Rice *et al.*, 1979) or, if flow-through, concentrations of oil in the tests were not closely monitored (for example, Cooley, 1976; Ordzie and Garofalo, 1981). Although low levels of oil can change the behavior of seastars (e.g. reduced rates of movement: Cooley, 1976), low concentrations of oil (<4 ppm WSF) neither killed the seastars in these studies nor were the seastars more sensitive to oil than their prey. Evidence on whether oil inhibits feeding of seastars is conflicting. Ordzie and Garofalo (1981) found that *Asterias forbesi* exposed to 0.1–0.5 ppm of Kuwait crude oil assumed a feeding posture more slowly than control individuals. Conversely, Cooley (1976) found no significant difference between the feeding rates of control *A. forbesi* and those exposed to 0.1 ppm of No. 2 fuel oil.

Here we examine the responses (survival, predation rate, growth, and condition) of *Evasterias troschelii* feeding on *Mytilus edulis* during a 28 d flow-through exposure to the water-soluble fraction (WSF) of Cook Inlet crude oil. By focusing on a strong interaction, we are taking the first step in determining how chronic pollution by petroleum hydrocarbons could affect the structure of a community widely distributed in Alaska.

Materials and methods

Evasterias troschelii (Stimpson, 1862) and *Mytilus edulis* (L.) were collected by hand at Auke Bay, Alaska (58°22′N; 134°40′W), in November 1980. The seastars were collected subtidally; mussels were collected intertidally nearby. Both species were acclimated in flowing seawater for 4 d before the experiment. Mussels (length, 3.8 to 6.7 cm) were placed haphazardly on horizontal, perforated polyethylene plates (9.7 cm × 22.8 cm; 8 to 11 mussels per plate), where they attached themselves. The seastars (wet weight 66.5 to 282.4 g) were starved during the acclimation period.

Seastars and mussels were exposed simultaneously to six concentrations (0.12 to 1.31 ppm total aromatic hydrocarbons; Table 1) of the water-soluble fraction (WSF) of Cook Inlet crude oil for 28 d. The concentrations were maintained with a flow-through generation and delivery system (Moles *et al.*, in press). Six exposure tanks were each divided into five stalls (4.2 to 6.6 liters) with topless perforated polyethylene boxes, and two healthy seastars were assigned randomly to each stall. Two control seastars were placed in each of four stalls the same size as the exposure stalls and in a 38-liter stall. The controls were held in clean, flowing seawater throughout the experiment. A perforated plate with attached mussels was hung vertically below the water line on one wall of each stall. The plates were assigned haphazardly to the stalls.

Water entered each exposure tank at the bottom through a long-stemmed funnel and left by a glass standpipe on the opposite side of the container. Flow rates to each tank were maintained at 400 ml min^{-1}. Mean water temperature, recorded daily, was 7.7 °C, range 6.6° to 8.8 °C ($n=94$). The means and ranges of other physical parameters (taken at intervals of 2 to 4 d) were: salinity, 29.7‰, 27.3 to 32.5‰ ($n=36$); pH, 7.8, 7.5 to 8.2 ($n=19$); turbidity, 0.4 nephelometer turbidity units (ntu), 0.1 to 1.2 ntu ($n=36$). Saturation of oxygen (measured daily or twice-daily with a Radiometer oxygen meter) rarely dropped below 70% ($n=472$). Areas of low oxygen concentration that occasionally developed in some of the stalls were eliminated beginning on Day 5 after submersible water pumps were installed in all tanks.

The concentration of total aromatic hydrocarbons in the WSF of the stock solution was estimated from the concentrations of 10 hydrocarbons (Table 2) measured at least daily with gas chromatography (GC). The hydrocarbons were extracted twice into dichloromethane, and the extracts frozen. Within a few days, the frozen extracts were thawed,

Table 1. Concentrations of total aromatic hydrocarbons in six concentrations (excluding control) of water-soluble fraction (WSF) of Cook Inlet crude oil to which *Evasterias troschelii* and *Mytilus edulis* were exposed. SD: standard deviation. *n*: number of measurements of total aromatic hydrocarbons in each concentration

Mean conc (ppm)	*n*	SD	% conc relative to stock solution[a]
1.31[a]	30	0.40	100
0.97	30	0.34	74
0.72	44	0.22	55
0.28	44	0.10	22
0.20	44	0.06	15
0.12	44	0.06	9

[a] Undiluted WSF from oil generator

Table 2. Composition and average daily concentrations of pure compounds in water-soluble fraction of Cook Inlet crude oil to which *Evasterias troschelii* and *Mytilus edulis* were exposed. *n*: number of measurements of the concentration of pure compounds in the stock solution

Compound	Mean (ppm)	SD	% of total aromatics	*n*
Benzene	0.806	0.188	52.8	40
Toluene	0.470	0.170	30.8	54
m- and *p*-xylene	0.100	0.040	6.5	54
o-xylene	0.064	0.025	4.2	54
Cumene	0.006	0.002	0.4	54
Mesitylene	0.005	0.002	0.3	54
Pseudo-cumene	0.026	0.010	1.7	54
Naphthalene	0.028	0.010	1.8	54
1-methyl-naphthalene	0.010	0.004	0.6	50
2-methyl-naphthalene	0.012	0.005	0.8	54

an internal standard (*n*-octane) was added, and analyzed by GC on a splitless capillary column (fused-silica capillary column [OV-101] liquid phase). Before each batch of extracts was analyzed, the gas chromatograph was calibrated by injecting a solution with a known concentration of each of the 10 aromatic hydrocarbons (Rice *et al.*, 1981).

The concentrations of total aromatic hydrocarbons in the water from the treatment tanks were monitored by ultraviolet spectrophotometry following Neff and Anderson (1975). A water sample was collected daily from each tank, extracted with *n*-hexane, and the absorbance measured at 220 nm.

The condition of each seastar was noted daily, and its vitality was tested by prodding its tube feet with a rod. If the tube feet did not move, the seastar was classified as moribund and transferred to clean running seawater for up to 6 d to determine whether it would recover. Dead individuals decomposed rapidly. Four seastars recovered when placed in clean seawater; only individuals that subsequently died are included in the computation of concentrations killing half the seastars (LC_{50}'s). The number of seastars in the stalls was kept constant by regrouping them when dead ones were removed.

Several seastars lost arms or patches of aboral skeleton during the experiment but remained alive. We used logit analysis (Finney, 1952; Berkson, 1957) or the Spearman-Karber method (Hamilton *et al.*, 1977) to estimate LC_{50}'s or concentrations that caused any deleterious change (i.e., loss of patches of skeleton, disarticulation, or death) in half of the individuals (EC_{50}'s).

The number of mussels eaten by seastars in each stall was recorded daily. All empty mussel shells were removed, and the lengths were measured to the nearest 0.01 mm. Feeding seastars (stomach everted inside a mussel) were not disturbed. When less than eight live mussels remained in a stall, a plate having 8 to 11 mussels was added to the stall and the old plate with attached mussels was removed. No mussel was exposed to the WSF for more than 12 d; all uneaten mussels survived the experiment.

The weight of each mussel eaten by a seastar each day was estimated from a regression of dry-tissue weight on shell length. *Mytilus edulis* ($n=135$) were collected from the same site as those used in the laboratory experiment, the shell length of each was measured, and the mussel was shucked. The tissue was dried at 61 °C to a constant weight. The regression equation of log tissue weight (mg) versus log shell length (mm) was $Y=2.8\,X-2$ ($r^2=0.67$).

We estimated growth rates of *Evasterias troschelii* by weighing each seastar 12 to 20 h before the experiment, on Days 9–13, and at the end of the experiment. Seastars were weighed after drip-drying for 5 min on paper towels.

At the end of the experiment, each seastar was dissected and sexed, and the pyloric caeca and gonads were removed, placed on rapid-filtering grade crepe filter paper, and dried for 5 min in a Buchner funnel attached to an aspirator. Wet weights for both organs were determined and recorded separately for each seastar, and hepatic caeca and gonad indices were calculated using the following equation: index = organ weight/seastar weight $\times 100$ (Giese, 1959).

Although seastars were randomly assigned to each stall, average initial weights varied with concentration. Analysis of covariance (ANCOVA) was used to test the effect of initial seastar weight on the responses of seastars to the various concentrations of the WSF. When an ANCOVA could not be used, analysis of variance (ANOVA) was substituted. The models used are as follows:

$$\text{ANCOVA, } Y_{ij}=\mu+\alpha_i+\beta\,(X_{ij}-\bar{X}_i)+E_{ij};$$

$$\text{ANOVA, } \quad Y_{ij}=\mu+\alpha_i+E_{ij},$$

where μ is the grand mean of the treatment populations, α_i is the fixed treatment effect for group i, β is the regression coefficient for the regression of Y on X, and E_{ij} is experimental error. Bartlett's test (Sokal and Rohlf, 1981) was used to evaluate the assumption of homogeneous variance. Heteroscedasticity was corrected with appropriate data transformations (Taylor, 1961). *F*-tests were used to compare means *a priori; a posteriori* comparisons were made with Scheffé's procedure for means adjusted by covariance (Bancroft, 1968). Because sequential feeding measurements on the same individuals are not independent, i.e., the probability that an individual will eat on a given day is not independent of whether it fed the previous day, the statistical tests of the feeding data were performed on means (i.e., feeding rates ÷ seastars in each stall averaged over the entire experiment).

Results

Survival

The effect of the WSF of Cook Inlet crude oil on *Evasterias troschelii* was very rapid. By the second day of the experiment, depending on the concentration of WSF, at least half of the seastars exposed to concentrations $\geqq 0.72$ ppm total aromatic hydrocarbons were lying inverted, apparently narcotized (their tube feet responded slowly to prodding, and they appeared not to be trying to right themselves), on the bottom of their stalls. Most individuals exposed to $\geqq 0.97$ ppm remained inverted during the first 10 d, and their tube feet responded more weakly and slowly than those of individuals exposed to other concentrations. During the first 10 d, a few seastars in the 0.97 ppm concentration and, depending on the date of the observation, from one to seven individuals in the 0.72 ppm concentration, moved about their stalls.

All seastars exposed to concentrations $\leqq 0.28$ ppm and those in the control tanks survived. Moribund individuals were observed for the first time on Day 11 (two seastars each in the 0.97 and 1.31 ppm concentration). By Day 12, all of the remaining seastars in the 0.97 and 1.31 ppm concentrations were moribund and were transferred to clean running seawater. Four seastars in the 0.97 ppm concentration recovered by Day 15 and were discarded on

Day 18. The LC_{50}'s were estimated by the Spearman-Karber method on Day 11 and 12 because deaths occurred only in the 0.97 and 1.31 ppm concentrations on these days and the iterative process of the logit analysis did not converge. Seastars began dying in the 0.72 ppm concentration on Day 15 and continued to do so until Day 19. No deaths occurred after Day 19; the LC_{50}, then, was 0.82 ppm (Fig. 1). This LC_{50} became 0.61 ppm if the seastars in the 0.97 and 0.72 ppm concentrations that showed no tube-foot response and subsequently recovered when transferred to clean seawater were considered as ecologically dead (i.e., the probability of each of them succumbing to predation, competition, or disease in the wild approaches 1) and were treated as mortalities. How these individuals would fare in the wild is unknown; therefore, we adopted 0.82 ppm as the final LC_{50}.

Several seastars exposed to WSF suffered loss of arms or skeletal parts. One individual in the 0.97 ppm concentration and four in the 0.72 ppm concentration lost arms or all or part of the aboral skeleton of the disc. Two of the seastars in the 0.72 ppm concentration subsequently died. The final EC_{50} was 0.71 ppm (87% of the LC_{50}) by Day 19 (Fig. 1).

Feeding rates of *Evasterias troschelii*

The feeding rate of the seastars was inversely related to the concentration of WSF (Table 3; Fig. 2). The 10 control individuals ate a total of 252 *Mytilus edulis* in 28 d, an average of 502.8 mg dry mussel tissue seastar^{-1} d^{-1}. Seastars in the 0.12, 0.2, and 0.28 concentrations fed at rates that were, respectively, 71, 53, and 37% of the control rates. Three seastars in the 0.72 ppm concentration (the highest concentration in which seastars fed) ate (over the course of 6 d) eight mussels during the entire experiment, probably because the exposure concentrations were abnormally low on Day 17 when we changed the barrels supplying oil to the WSF generator. Seastars in the 1.31 and 0.97 ppm concentrations had not fed, appeared moribund, and were removed before the middle of the experiment.

Feeding rate (mussels seastar^{-1} d^{-1}) was correlated ($0.005 > P > 0.001$) with the average initial weight of seastar per stall (Table 4). The mean feeding rate of seastars in the control group (adjusted by covariance for initial weight of seastars) was greater than ($P < 0.001$) those of individuals in the three lowest concentrations combined (Table 4). The feeding rate of *Evasterias troschelii* in the control tanks was not significantly greater ($P > 0.05$) than that for individuals exposed to the lowest WSF concentration (0.12 ppm), but was greater ($0.005 > P > 0.001$) than that for seastars in the 0.2 ppm concentration (Table 4). Results were the same whether feeding rates were measured as mussels eaten seastar^{-1} d^{-1} or mg dry mussel-tissue weight seastar^{-1} d^{-1} (Tables 3 and 4). Feeding rate was significantly reduced, at 24% of the LC_{50} and 28% of the EC_{50}.

We found no consistent relationship between the selection of prey size by our experimental seastars and either seastar size or WSF concentration. The relationship between initial seastar weight and the length of the mussels eaten varied between concentrations of WSF (The *F*-test for a common slope was significant in the ANCOVA; Table 4), but these variables were significantly (negatively) correlated only in the 0.28 ppm concentration (Table 5). The ANOVA of the unadjusted mussel lengths indicated that the WSF of crude oil did not affect the size of mussel eaten by *Evasterias troschelii* (Table 4). Cooley (1976) also found that a low concentration (0.1 ppm) of the WSF of No. 2 fuel oil did not appear to affect prey-size selection of *Asterias forbesi* feeding on *Mytilus edulis*.

We estimated the time seastars spent feeding from the frequency with which individual seastars were observed feeding compared with the total number of observations of

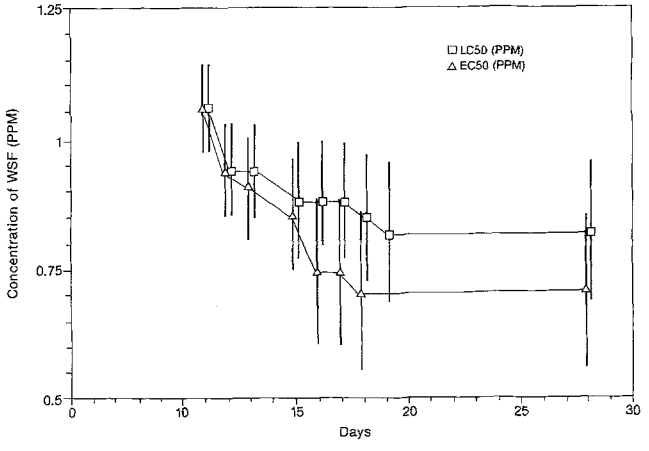

Fig. 1. *Evasterias troschelii.* LC_{50}'s and concentrations resulting in loss of patches of skeleton, disarticulation, or death in half of exposed seastars (EC_{50}) during 28 d exposure to water-soluble fraction (WSF) of Cook Inlet crude oil. Vertical bars are 95% confidence intervals

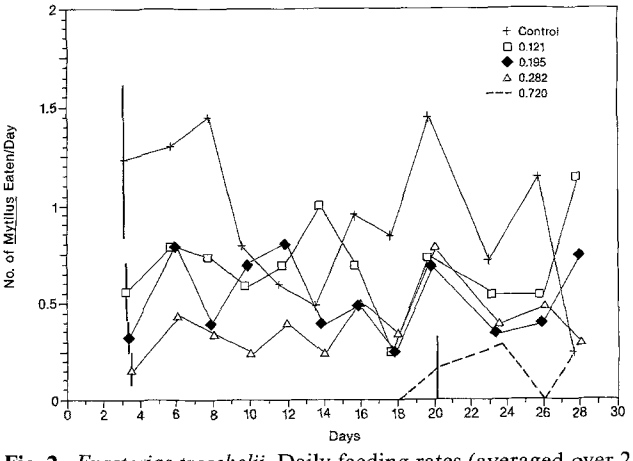

Fig. 2. *Evasterias troschelii.* Daily feeding rates (averaged over 2 d intervals) of seastars eating *Mytilus edulis* in four concentrations of water-soluble fraction of Cook Inlet crude oil. Vertical bars are one standard error of the mean. Data points for WSF concentrations are offset in time and error bars are shown only on the first set of means for clarity

Table 3. *Evasterias troschelii* feeding on *Mytilus edulis*. Data for controls and for seastars exposed to four concentrations of water-soluble fraction of Cook Inlet crude oil for 28 d. Seastars in the 1.31 and 0.97 ppm concentrations did not feed during the experiment and are therefore excluded from this table. $\bar{x}$: mean (unadjusted for covariate); SE: standard error of the mean. n: number of observations in Rows 1–3, number of seastars in Row 4

	Control			Concentration (ppm)[a]											
				0.12			0.20			0.28			0.72		
	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE
Mussels eaten seastar^{-1} d^{-1}	125	0.95	0.08	125	0.69	0.06	125	0.52	0.06	125	0.38	0.04	125	0.04	0.02
Dry tissue wt of mussels eaten seastar^{-1} d^{-1} (mg)	125	502.8	42	125	358.2	33.7	125	264.2	28	125	185.4	20.3	125	25.9	16.2
Length of mussels consumed (mm)	252	50.39	0.40	184	49.98	0.48	137	49.65	0.56	104	48.80	0.60	8	46.79	1.48
No. of observations of feeding ÷ total observations^{-1}	10	0.264	0.04	10	0.268	0.05	10	0.273	0.04	10	0.354	0.03	5	0.052	0.02
No. of feeding seastars (total no. of seastars)	10 (10)			9 (10)			10 (10)			10 (10)			3 (10)		
Total no. of mussels consumed	252			184			137			104			8		

[a] Total aromatics

Table 4. *Evasterias troschelii* and *Mytilus edulis*. Tests of significance (one-way ANCOVAs) for feeding rates of seastars preying on mussels in four concentrations (0.12, 0.2, 0.28, and 0.72 ppm) of water-soluble fraction of Cook Inlet crude oil for 28 d. Independent variables are initial fresh weight of seastars (g) for Row 5 and average initial fresh weight of seastars (g) stall^{-1} for other rows. Feeding rates of seastars in the 0.72 ppm concentration were excluded from the ANCOVA because the rates were zero on all but 5 d, precluding stabilization of the variances were these to be included

	Significance of expt[a]	F-test for common slope	F-test for significance of covariate assuming common slope	Comparisons of means[b]		
				Control versus others[c]	Control versus 0.12 ppm[d]	Control versus 0.2 ppm[d]
Mussels eaten seastar^{-1} d^{-1}[e]	$P<0.001$	$P=0.72$	$0.005>P>0.001$	$P<0.001$	$P>0.05$	$0.005>P>0.001$
Dry tissue wt of mussels eaten seastar^{-1} d^{-1}	$P<0.001$	$P=0.79$	$0.005>P>0.001$	$P<0.001$	$P>0.05$	$0.01>P>0.005$
Length of mussels consumed	$P=0.65$ $P=0.8$[f]	$0.01>P>0.005$ –[f]	– –[f]	– –[f]	– –[f]	– –[f]
No. of observations of feeding ÷ total observations^{-1}	$0.005>P>0.001$	$P=0.38$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

[a] ANCOVA of adjusted Y's
[b] Means adjusted for covariate
[c] *A priori* F-test
[d] Scheffé's test
[e] Transformations of feeding data are as follows: mussels eaten, $Y^{0.3}$; weight of mussels eaten, log $(X+1)$; length of mussels eaten, untransformed; number of feeding observations, untransformed. Transformations derived according to method of Taylor (1961)
[f] ANOVA of unadjusted Y's

seastars stall^{-1}. Individuals in all treatments were observed daily at the same time of day. If the time of day an individual seastar fed were independent of WSF concentration, the frequency seastars were observed feeding compared with the total observations should reflect the time they spend opening and/or digesting each mussel. Seastars in the 0.28 ppm concentration spent more time feeding than individuals in all other concentrations, including controls ($P<0.05$, Scheffé's test); however, pairwise comparisons revealed no differences between controls and seastars in each of the other concentrations (Table 4; $P>0.05$ for control vs 0.28 ppm and control vs 0.72 ppm, Scheffé's test). Seastars in the 0.72 ppm concentration spent less ($P<0.001$) time feeding than the controls taken

Table 5. *Evasterias troschelii* and *Mytilus edulis*. Within-concentration correlations of lengths of mussels consumed and weights of seastar pyloric caeca versus initial seastar weight for controls and after 28 d exposure to four concentrations of water-soluble fraction of Cook Inlet crude oil. *b*: slope; r^2: coefficient of determination

	Control		Concentration (ppm)[a]							
			0.12		0.20		0.28		0.72	
	b	r^2	*b*	r^2	*b*	r^2	*b*	r^2	*b*	r^2
Length of mussels consumed (mm)	0.016	0.33	−0.008	0.03	0.019	0.19	−0.082	0.98**	−0.115	0.84
Pyloric caeca wt (g)	0.60	0.37	0.82	0.78**	0.39	0.22	1.32	0.92**	0.37	0.30

[a] Total aromatics
** $\approx 0.01 > P > 0.001$

Table 6. *Evasterias troschelii*. Weights in various concentrations of water-soluble fraction of Cook Inlet crude oil for 28 d. Values are means ± standard error of the mean

Weights	Control	Concentration (ppm)[a]			
		0.12	0.20	0.28	0.72
Initial wet wt (g)	196.8 ± 17.3	165.7 ± 22.2	176.0 ± 15.4	139.4 ± 15.6	157.1 ± 21.9
Wet wt (g) at Day 12	249.3 ± 18.6	204.4 ± 29.6	214.0 ± 22.3	164.1 ± 17.7	–[b]
% change to Day 12	28.5 ± 4.1	22.0 ± 3.6	20.9 ± 3.6	18.2 ± 2.9	–[b]
Final wet wt (g)	253.2 ± 21.1	224.3 ± 25.7	211.9 ± 21.9	171.9 ± 20.4	167.0 ± 18.4
% change from initial wt	29.8 ± 4.4	39.5 ± 5.5	19.5 ± 4.4	24.0 ± 4.7	6.3 ± 5.9

[a] Total aromatics
[b] Seastars in 0.72 ppm concentration were not weighed at this time because none had fed, and most appeared to be in poor condition

Table 7. *Evasterias troschelii*. Tests of significance of growth rates and weights of pyloric caeca and gonads exposed to various concentrations of water-soluble fraction of Cook Inlet crude oil

	Significance of expt	*F*-test for common slope	*F*-test for significance of covariate assuming common slope	Comparisons of means			
				Control versus others[a]	Control versus 0.12 ppm[a]	Control versus 0.72 ppm[b]	Control and 0.12 ppm versus others[b]
Growth rate	$P < 0.01$[c]	$P = 0.8$	$P < 0.01$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$
Wt of pyloric caeca	$P < 0.025$[d,e]	$P < 0.05$[c]	–	$P < 0.05$[e]	$P > 0.005$[e]	$P > 0.05$[e]	–
Wt of gonads							
Males	$P > 0.05$[c,d]	$P = 0.2$	$P < 0.001$	–	–	–	–
Females	$P = 0.5$[c,d]	$P = 0.2$	$P < 0.001$	–	–	–	–

[a] *A priori F*-test
[b] Scheffé's test
[c] ANCOVA. Covariates: initial wet wt for growth rate; final whole body wt for weights of pyloric caeca and gonads
[d] Weights of pyloric caeca and gonads were log-transformed
[e] ANOVA

together with seastars in the lower concentrations. Frequency of feeding was not correlated with the initial weight of the seastars (Table 4).

Growth rate of *Evasterias troschelii*

Mean growth rates of the seastars decreased with increasing concentration of WSF for individuals exposed to > 0.12 ppm and ≦ 0.72 ppm WSF (Fig. 3). Seastars in the 0.72 ppm concentration were not weighed at the middle of the experiment because none had fed and most appeared to be in poor condition. All individuals for which complete growth information was available gained more weight during the first 12 d of the experiment than during the last 16 d (Table 6). Percent increase from initial to final weight was greatest for seastars in the 0.12 ppm concentration, followed by the controls (Table 6).

Table 8. *Evasterias troschelii.* Data on pyloric caeca and gonads of males and females exposed for 28 d to various concentrations of water-soluble fraction of Cook Inlet crude oil. Values are means ± standard error of mean. Samples sizes are in parentheses

	Control	Concentration (ppm)[a]			
		0.12	0.20	0.28	0.72
Pyloric caeca:					
Males	(4)	(5)	(4)	(7)	(1)
weight (g)	40.2±5.4	27.8±6.8	27.6±2.4	28.5±5.3	14.0
Females	(6)	(5)	(6)	(3)	(4)
weight (g)	42.3±5.9	34.3±2.4	27.6±4.0	21.4±5.7	23.1±1.4
Gonads:					
Males	(4)	(5)	(4)	(7)	(1)
weight (g)	26.3±8.7	21.8±8.3	29.9±5.2	21.2±3.6	15.9
Females	(6)	(5)	(6)	(3)	(4)
weight (g)	29.8±4.0	30.3±8.7	20.6±2.7	13.6±4.8	12.3±3.5

[a] Total aromatics

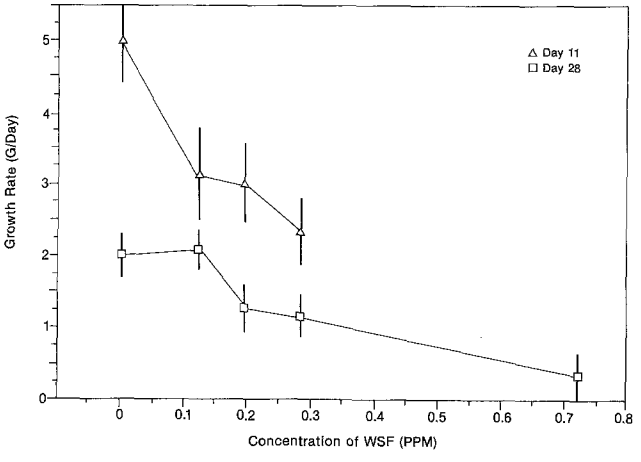

Fig. 3. *Evasterias troschelii.* Growth rates midway and at end of 28 d exposure to water-soluble fraction of Cook Inlet crude oil. Vertical bars are one standard error of the mean

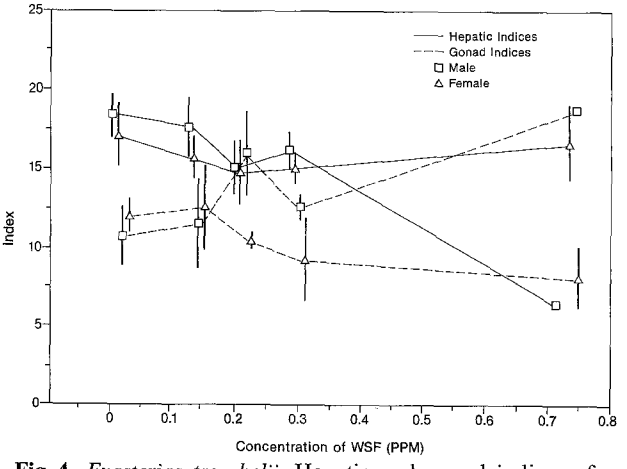

Fig. 4. *Evasterias troschelii.* Hepatic and gonad indices of males and females exposed for 28 d to four concentrations of water-soluble fraction of Cook Inlet crude oil. Vertical bars are one standard error of the mean

Growth rate was correlated with the initial weight of the seastars, and the slope of this relationship did not vary between treatments (ANCOVA, Table 7). Because feeding rates of the controls and seastars in the 0.12 ppm concentration were similar, growth rates should have been similar, as well. The statistical tests confirmed this supposition. Controls and seastars in the 0.12 ppm concentration taken together had higher growth rates than those in the 0.2, 0.28 and 0.72 ppm concentrations taken together. There was no significant difference in growth rates between the controls and seastars in the 0.12 ppm concentration (Table 7).

Hepatic and gonad indices

At the end of the experiment, the controls had the heaviest pyloric caeca, but there was no significant trend of decreasing weight with increasing concentration of WSF in either sex (Table 8). The weights of pyloric caeca of male and female seastars combined were positively correlated with the final wet weight of the seastars in all concentrations (Table 5), but the slopes of the regressions differed (Table 7), and precluded further testing with the ANCOVA. Although controls had greater pyloric caeca weights than individuals exposed to WSF (ANOVA; Table 7), the difference was a result of the greater final weights of the controls. Weights of pyloric caeca expressed as a percent of final whole body weight (i.e., hepatic indices) did not differ between concentrations (ANOVA; $F_{4,40}=0.79$, $P=0.5$; Fig. 4). Similarly, there was no effect of WSF concentration on the gonad indices of either sex (ANOVA: males, $F_{3,16}=1.2$, $P=0.3$; females, $F_{4,19}=0.9$, $P=0.4$; Fig. 4).

Discussion

Evasterias troschelii are sensitive to petroleum hydrocarbons (the final LC_{50} was 0.82 ppm total aromatic hydrocarbons). Asteroids can suffer high mortality during oil spills (North *et al.,* 1964), but published laboratory experiments, although not strictly comparable to ours because of differences in duration, type of oil, or exposure regime, have not shown them to be sensitive to petroleum hydrocarbons. Most *Asterias rubens* exposed to 200 ppm (nominal concentration) of Kuwait crude oil in a static system survived for 8 wk, and one survived > 14 wk in an 18 wk experiment (Crapp, 1971). Crapp, however, did not measure the concentration of the WSF in his experiment. All *A. forbesi* exposed to 0.1 ppm of the water-accommodated fraction (WAF) of No. 2 fuel oil in a flow-through system for 4 wk survived (Cooley, 1976). Survival of *A. forbesi* exposed in a static system to six nominal concentrations of Kuwait crude oil for 6 h followed by 5 d of depuration was similar to the survival of controls and always exceeded 50% even in the stock solution, which measured 1 to 4 ppm total extractable hydrocarbon beneath the surface slick (Ordzie and Garofalo, 1981). *Leptasterias hexactis* exposed to the WSF of Cook Inlet crude oil and No. 2 fuel oil in a static system had a 96 h median tolerance limit measured as total aromatic hydrocarbons of > 10.58 mg l^{-1} and > 3.36 mg l^{-1} for crude oil and fuel oil, respectively (Rice *et al.,* 1979).

In our study, *Evasterias troschelii* exposed to $\geqq 0.2$ ppm WSF of Cook Inlet crude oil ate significantly less than control seastars. *Asterias rubens* exposed to 200 ppm of Kuwait crude oil also stopped feeding for a few days to 2 wk before most of them died (Crapp, 1971), and *A. forbesi* exposed to 0.1 to 0.5 ppm (12.5% of a 1 to 4 ppm stock solution) of Kuwait crude oil took longer to assume the posturing reflex (the assumption of a humped feeding posture) in response to scallop homogenate than controls (Ordzie and Garofalo, 1981). In contrast, Cooley (1976) found that *A. forbesi* exposed to a 0.1 ppm concentration (water-accommodated fraction: WAF) of the presumably more toxic No. 2 fuel oil consumed mussels faster than control seastars and concluded that exposure to low concentrations of WAF over several weeks did not appear to affect predation. The results of Cooley's study are inconclusive, however, because within-treatment variability in the feeding rate of the controls exceeded the variability between exposed and control seastars.

The mechanism by which petroleum hydrocarbons inhibit feeding has received very little attention. Ordzie and Garofalo (1981) concluded that Kuwait crude oil hindered prey recognition by *Asterias forbesi* because exposure to the oil delayed initiation of the posturing reflex. The oil could, however, have interfered with the motor response to a correctly discriminated sensory input. Although we did not examine the effect of oil on chemoreception in *Evasterias troschelii,* we found that the frequency *E. troschelii* were seen feeding increased with increased concentration of WSF up to 0.28 ppm, whereas seastar feeding rate decreased. Seastars in the low-to-intermediate range of concentrations probably spent more time opening and/or digesting each mussel than controls. The WSF may thus have interfered with the feeding of *E. troschelii* by depressing their metabolic rate or disturbing the motor function of the tube feet.

Petroleum hydrocarbons can interfere with motor functions in other echinoderms. *Leptasterias hexactis* exposed to 8 to 12 mg l^{-1} (total aromatic hydrocarbons) of the WSF of Cook Inlet crude oil or No. 2 fuel oil "could not remain attached to the glass exposure containers" (Rice *et al.,* 1979). Sea urchins, *Strongylocentrotus purpuratus,* withdrew their tube feet and could no longer cling to the substrate when exposed to a 0.1% diesel oil emulsion (North *et al.,* 1964). *Asterias forbesi* exposed to 0.1 ppm WAF of No. 2 fuel oil moved significantly slower than control seastars (Cooley, 1976). In the present study, half or more of those *Evasterias troschelii* exposed to concentrations $\geqq 0.72$ ppm WSF were lying on their aboral surface by Day 2 of the experiment, and their tube feet responded sluggishly to prodding. *E. troschelii* and other members of the family Asteridae, including *A. rubens* and *A. forbesi,* commonly open bivalves by force, pulling bivalves apart with their tube feet (Christensen, 1957; Jangoux, 1982). Interference with motor functions, slowing the rate at which *E. troschelii* opened the mussels may have resulted from hydrocarbon narcosis, which has been reported in the adults and larvae of Cnidaria, Annelida, Crustacea, and Gastropoda exposed to petroleum hydrocarbons (see Johnson, 1977, for review).

Mytilus edulis is apparently not sensitive to petroleum hydrocarbons. High concentrations of petroleum hydrocarbons (3 to 1 000 ppm) are required to kill *M. edulis* in the short-term (up to 96 h) (Craddock, 1977). In two studies in which *M. edulis* and predaceous asteroids (*Asterias* spp.) were exposed to petroleum hydrocarbons together (Crapp, 1971; Cooley, 1976), neither author reported on the responses of the mussels to oil. However, Cooley exposed *M. edulis* separately in two additional experiments (long-term and multiple-level) to the same nominal concentration (0.1 ppm WAF) of No. 2 fuel oil as that to which *M. edulis* and *A. forbesi* were exposed. Her three experiments show no obvious differences in the sensitivity of these two species to fuel oil. In the multiple-level exposure, 10% of the mussels exposed to 0.1 ppm WAF for 38 d died. In the long-term exposure to the same concentration, only one mussel died (3% mortality) by Day 47 (4 d after transfer to clean seawater and after a length of exposure about the same as that [49 d] of *A. forbesi*).

Exposure to low concentrations of petroleum hydrocarbons can elicit physiological and cellular responses in *Mytilus edulis,* such as a reduction in the energy available for growth (1 ppm, Gilfillan, 1975; 30 to 36 μg l^{-1}, Widdows *et al.,* 1982; 604 ppb, Stickle *et al.,* in press), reduction in clearance rate, increase in respiration rate, structural and functional changes in the digestive gland, destabilization of lysosomes, and an increase in the flux through the

glycolytic pathway (30 to 36 $\mu g\ l^{-1}$, Widdows *et al.*, 1982). However, the relationship of these responses to measurable ecological parameters that are meaningful at the community level, such as the rate of competitive displacement by *M. edulis* in the absence of predatory control, remains obscure.

Asteroids can greatly influence community structure in low intertidal and shallow subtidal regions. The evidence is strongest for *Pisaster ochraceus* on the outer coast of Washington (Paine, 1966, 1974; Dayton, 1971, 1975), *Stichaster australis* in New Zealand (Paine, 1976), and *Asterias forbesi* in New England (Lubchenco and Menge, 1978). The evidence is weakest for *A. rubens; Leptasterias hexactis* probably does not influence community structure greatly (see review by Menge, 1982).

Although *Evasterias troschelii* has not been shown unequivocally to control intertidal or shallow subtidal community structure, field experiments by one of us (C.E.O.) in the intertidal region at Port Valdez and Auke Bay, Alaska, indicate that this seastar controls the lower limit of distribution of *Mytilus edulis* at least at Auke Bay. At Auke Bay, 2.5 yr after *E. troschelii* had been removed from a stretch of shore, *M. edulis* moved down, creating a sag in the former lower limit of the mussel band 6 m wide and as much as 2 m deep. Elsewhere at the removal site, colonization of the lower intertidal region by *M. edulis* was patchy and seemed to expand from foci of a few individuals. Several of the exclosures at both the removal and control sites were colonized by *M. edulis,* which eventually monopolized the space within them, but control patches adjacent to the exclosures at the experimental site had light and variable colonization and were not significantly different from control patches at the control site. Nevertheless, where *M. edulis* had colonized the patches, the mussel monopolized primary space for at least a year after colonization. A similar sequence of events took place at Valdez, Alaska, but the downward extension of the lower limit of *M. edulis* was more fragmentary, and the results of the exclosure experiments were inconclusive. The results indicate that, at least at Auke Bay, the interaction between *E. troschelii* and *M. edulis* is strong.

If strong interactions determine community structure, the effect of contamination by petroleum hydrocarbons on that structure should depend on the sensitivities to oil of the strongly interacting species. *Evasterias troschelii* are probably more sensitive to crude oil than adult *Mytilus edulis,* and younger stages of *M. edulis* may be even more tolerant to oil than large adults. Using the same WSF generator, analytical methods, and laboratory as used in our study, Stickle *et al.* (in press) exposed *M. edulis* to eight concentrations of the WSF of Cook Inlet crude oil. The 28 d LC_{50} for larger mussels (length, 58 to 71 mm) was 1.42 ± 0.3 ppm. In another experiment, the 28 d LC_{50} for 15 to 20 mm mussels was 1.69 ± 0.42 ppm (Stickle *et al.,* 1984). Plantigrades of *M. edulis* exposed for 40 d to seven concentrations (including control) of the WSF of Cook Inlet crude oil suffered only 18% mortality in the highest concentration (1.4 ppm; O'Clair and Rice, unpublished data). In the present study, the concentration of WSF that reduced the feeding rate of *E. troschelii* was 24% of the LC_{50} for the seastars, 14% of the LC_{50} for large mussels (1.4 ppm, Stickle *et al.,* in press), and 14% of the concentration that killed 18% of the plantigrades of *M. edulis* (1.4 ppm, O'Clair and Rice, unpublished data).

If the abundance of the seastars or their predation rate on mussels is reduced by petroleum pollution, and mussel growth, reproduction, and larval settlement are not significantly reduced, *Mytilus edulis* could expand its population size and boundaries and competitively exclude other species, thereby markedly changing community structure. Although other species in the community could be affected directly by exposure to hydrocarbons, many intertidal species appear to be tolerant to oil pollution (Rice *et al.,* 1979); therefore, species richness would probably be reduced more by competitive exclusion by *M. edulis* than by the direct effects of oil pollution.

In summary, *Evasterias troschelii* is more sensitive to the WSF of Cook Inlet crude oil than *Mytilus edulis* adults. Although the WSF did not measurably affect the hepatic or gonad indices of *E. troschelii* during the 28 d exposure, seastars exposed to concentrations of WSF $\geqq 0.2$ ppm had reduced feeding and growth rates. Whether the reduction in predation on *M. edulis* could result in the colonization and eventual monopolization of the low intertidal zone by *M. edulis* remains to be determined. Because *E. troschelii* probably plays a central role in excluding *M. edulis* from the lower intertidal zone in the inner waters of south-central and southeastern Alaska, a reduced predation rate could allow *M. edulis* to expand its vertical range and exclude other occupiers of primary space in the intertidal region.

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