Bull. Environ. Contam. Toxicol. 31, 152–157 (1983)

Feeding Behavior of *Daphnia pulex* in Crude Oil Dispersions

C. Kim Wong,[1] J. Rudi Strickler,[2] and F. Rainer Engelhardt[3]

[1]*Department of Zoology, University of Toronto, Erindale Campus, Mississauga, Ontario, Canada, L5L 1C6;* [2]*Australian Institute of Marine Sciences, P.M.B. No. 3, Townsville M.S.O., Q4810, Australia;* [3]*Canada Oil and Gas Lands Administration, 355 River Road, Ottawa, Ontario, Canada, K1A 0E4*

Crustacean zooplankton forms an important component of most aquatic ecosystems and constitutes the major food source for many fishes. Previous work has demonstrated that crude oil reduces the survival and fecundity of zooplankton (BARNETT & KONTOGIANNIS 1975; BERDUGO et al. 1977; KONTOGIANNIS & BARNETT 1973; LEE & NICOL 1977; O'BRIEN 1978; WONG et al 1981). Since zooplankton grazers use chemosensory input in their feeding behavior (POULET & MARSOT 1978) it is possible that chemically toxic hydrocarbons may suppress feeding in zooplankton (BERMAN & HEINLE 1980). Furthermore, physical entanglement of oil may slow down the movements of the feeding appendages. It is recognized that wave action can break oil up into dispersed particles to a size range of 10–100 μm (FORRESTER 1971), which is similar in size to edible phytoplankton (CONOVER 1971). Zooplankton grazers display an upper limit for the volume of food ingested per unit time (MARSHALL 1973). Thus, even if oil particles are chemically acceptable, the animals may still enter a starvation mode due to reduced algal intake.

The present investigation was conducted to examine the effects of a crude oil on the feeding behavior pattern of Daphnia pulex , a species which is commonly used for toxicity tests. Previous studies have shown that crude oils are toxic to Daphnia (O'BRIEN 1978; WONG et al. 1981, ENGELHARDT et al. 1982). Feeding behavior was selected as an effects index because it is important to survival and reproduction and its pattern has been studied in detail (BURNS 1968, MCMAHON & RIGLER 1963).

MATERIALS AND METHODS

The Daphnia pulex were laboratory cultures of animals originally collected from Heney Lake, Quebec. Food consisted of Chlamydomonas sp.. Algal cells were centrifuged and resuspended in Millipore (0.45 μm) filtered lakewater before being fed to the daphnids. Food concentrations were predetermined by counting cells in a hemacytometer. Feeding behavior was observed by microscopic observation of the animals in a 10x10x1.5 cm glass chamber containing Millipore (0.45 μm) filtered lakewater. For behavioral observation gravid adult D. pulex of 2 mm body length were tethered dorsally to the end of a human hair with a polymer adhesive (ALCARAZ et al. 1980). This allowed the animals to maintain a normal upright orientation and facilitated continuous

observation. All experiments were conducted at 20+2 C. Light entered the chamber from above. A red filter was used to ensure that the animals were minimally disturbed (SCHEFFER et al. 1958). The rates of movement of the various appendages were recorded manually with the use of a stop watch. The component pattern of feeding behavior in D. pulex was characterized in a range of concentration of Chlamydomonas, from 0 to $5x10^4$ cells /ml. To prepare the oil medium, 1 ml of fresh Norman Wells crude oil was placed on 200 ml of Millipore (0.45 µm) filtered lakewater in a beaker and the mixture was dispersed with a Polytron homogenizer without sonication. The emulsion was allowed to stand for 1 h permitting a partial loss of the most volatile hydrocarbons and the movement of large oil particles to the surface. The portion below the surface layer was used for the exposure experiments.

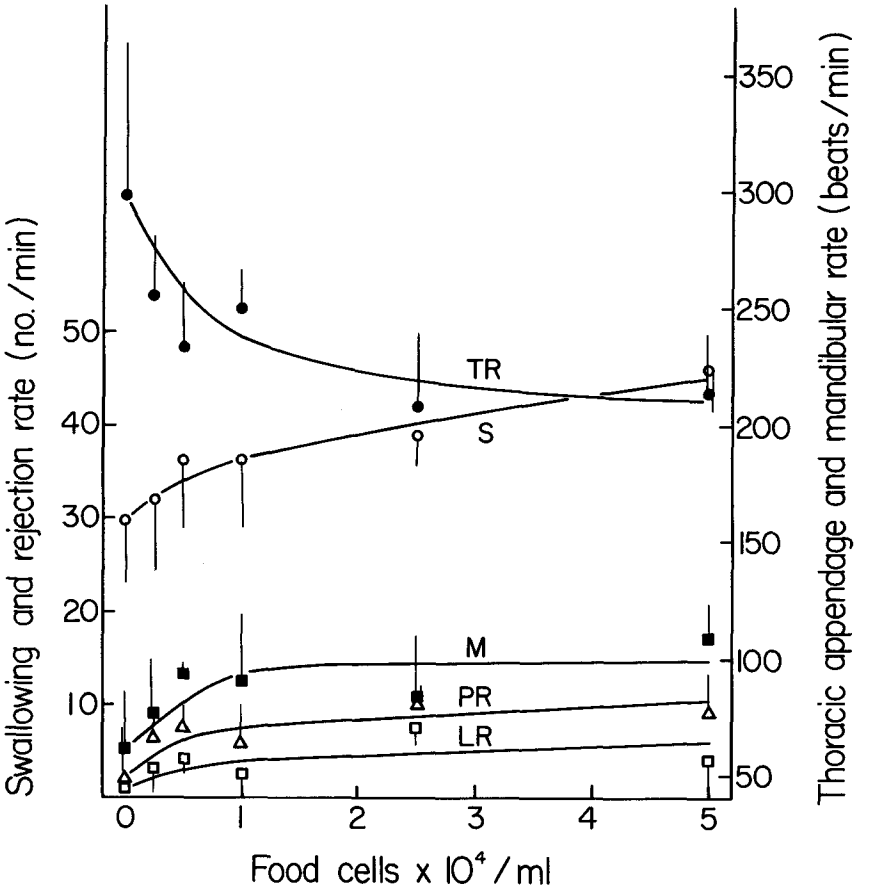

Figure 1. Feeding behavior of D. pulex in different concentrations of Chlamydomonas. TA= thoracic appendage, S= swallowing, M= mandibles, PR= postabdomen rejection, LR= labral rejection. Vertical bars represent 1 S.D.

Oil particles of sizes from less than 1 µm to 20 µm, and averaging at 5 µm, were determined in the dispersion with the use of a microscope fitted with a calibrated ocular grid. Non-toxic paraffin oil dispersions were prepared in the same way. For each experiment, D. pulex was allowed an acclimation period of 45 min before feeding behavior was recorded. Then aliquots of oil dispersion were pipetted into the chamber to a concentration of 50 ppm. Since much of the oil had floated to the surface, this calculated concentration must be regard as a nominal value. A particle density of $2x10^4$ particle/ml was determined by counting at least 500 particles in a hemacytometer. Behavioral recordings were made for three hours, starting immediately after the addition of oil. A similar volume of uncontaminated filtered lakewater was added to controls.

RESULTS AND DISCUSSION

Chlamydomonas cells were collected by each Daphnia into its food groove by rhythmic pumping movements of the thoracic appendages. Food to be ingested was then passed between the mandibles and passed into the midgut by peristaltic waves of the oesophagus. Excess or unacceptable food was removed from the food groove and the mouth region by movements of the postabdomen and the labrum.

Figure 1 shows the responses of D. pulex to changes in food cell concentration. Thoracic appendage rates were at a maximum in filtered lakewater and decreased as cell concentrations increased, becoming stable at $2.5x10^4$ cells/ml of medium. The frequencies of mandibular beat, swallowing, and rejection increased with increasing cell concentration up to a critical maximum concentration which was close to the $2x10^4$ cells/ml reported by STARKWEATHER (1978) for D. pulex on Chlamydomonas. Beyond the critical concentration, little change occurred in feeding behavior.

Feeding behavior changed immediately after crude oil particles were introduced into the medium. The frequencies of thoracic appendage movement, mandibular beat, and swallowing decreased while the rates of rejection by both the postabdomen and labrum increased (Fig.2). The combined effect of these was a reduction in ingestion rate. Similar behavioral changes were observed over all the food concentrations tested, including tests in the absence of food cells. The decrease in mandibular rate and the increase in rejection rates were most pronounced (Table 1).

Some small but significant changes were also observed in control animals and animals exposed to inert paraffin oil (Table 1). The consistency and the magnitude of change were greater, however, in animals presented with crude oil.

The feeding pattern of D. pulex observed in this study was similar to that described in other studies (BURNS 1968; MCMAHON & RIGLER 1963). More specifically, the mandiblar rates and critical

concentration we observed agree with those determined by STARKWEATHER (1978) for the same species.

The feeding behavior of D. pulex changed immediately after the addition of crude oil to the medium. Since thoracic appendage movements were halted during postabdominal rejection, the drop in thoracic appendage rate was probably the result of increases in postabdominal rejections. Mandibular beats were interrupted during labral rejection; but since mandible movement slowed down before the first labral rejection, it is unlikely that the reduced mandibular rate could have been accounted for entirely by an increase in labral rejection rates. Lowered swallowing may have

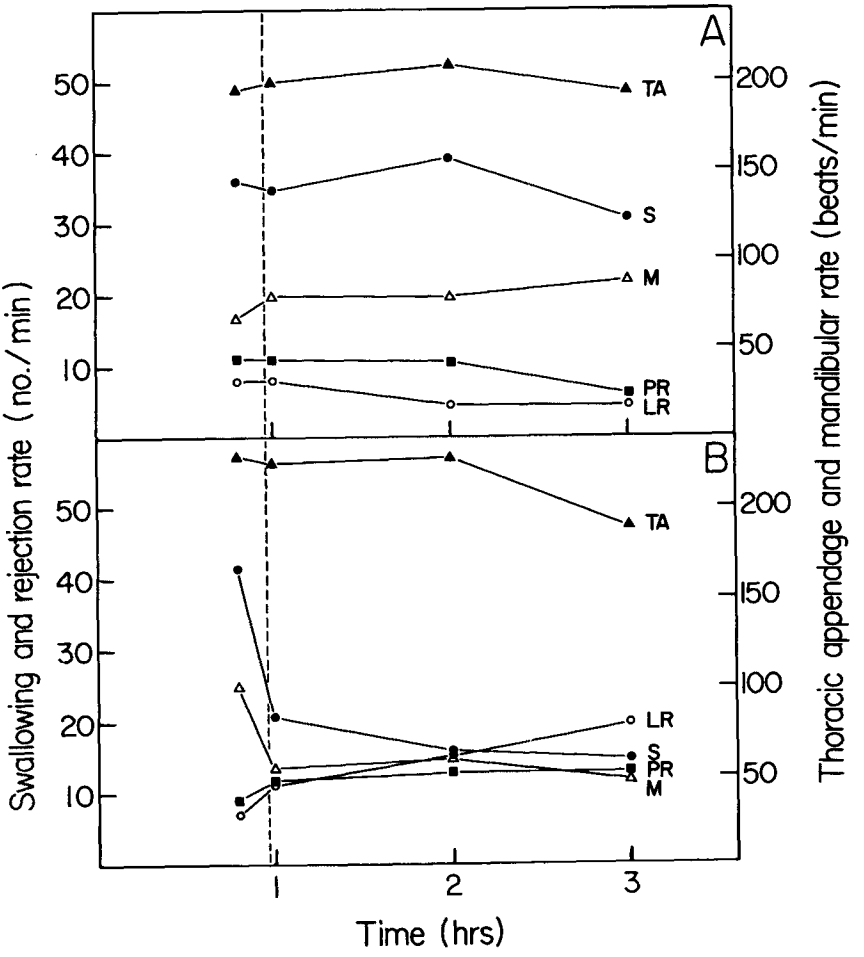

Figure 2. Feeding behavior of D. pulex following the introduction of crude oil, showing mean values of 3 animals. The food medium consisted of Chlamydomonas at 2.5×10^4 cells/ml. The broken line indicates the time when oil dispersion added. (A) control, (B) crude oil. (See abbreviations in Fig. 1).

Table 1. Effects of crude oil and paraffin oil on the feeding behavior of D. pulex in different food concentrations. Sample sizes are given in parentheses. Asterisks indicate significance at 95% level based on the Chi-square goodness of fit text.

Behavior Component	No. Food Cells $x10^4$/ml	Component Rate (beat/min) Control before	Control after	Oil before	Oil after	Paraffin before	Paraffin after
TA	0	246	213*(3)	322	251* (6)	297	296 (5)
	2.5	256	261 (2)	241	217* (4)	266	252* (2)
	5.0	207	216 (2)	211	191* (3)	187	199 (2)
M	0	61	58*	80	54*	86	86
	2.5	90	96*	69	28*	82	71
	5.0	96	105	114	55*	86	66*
S	0	27	25	34	31*	50	54
	2.5	35	34	31	23*	33	38*
	5.0	50	41*	47	43*	37	39
PR	0	5	3	3	9*	5	4
	2.5	4	2	8	13*	10	4*
	5.0	12	13	8	10	13	10
LR	0	2	1	2	5*	1	2
	2.5	2	1	5	7*	3	2
	5.0	5	4	5	5	4	3

TA = thoracic appendage, M = mandibles, S = swallowing, PR = postabdomen rejection, LR = labral rejection

been due to change in food concentration (BURNS 1968), whereas the change in thoracic appendage rate occurred over a period of 12-20 min (MCMAHON & RIGLER 1963). This suggests that different feeding behavior components may be under different sensory controls. The differential feeding response of those components in the presence of oil also supports the supposition that the different neural component units may have shown a different time to onset of effect. The resultant asynchrony of the feeding components expresses an early toxicity effect of dispersed petroleum. Metabolism in D. pulex was found to increase up to two times of normal following exposure to both dispersed and water-soluble components of crude oil (ENGELHARDT et al. 1982). As a consequence, the interference with feeding patterns recorded in this study, probably resulting in decreased food intake, may be especially critical during the stress of oil exposure.

Acknowledgements. This study was supported by operating and strategic Grants from the Natural Sciences and Engineering Research Council of Canada to F.R.E. and J.R.S.

REFERENCES

ALCARAZ,M., G.A.PAFFENHOFER, and J.R.STRICKLER: 3rd Special Symposium, Amer. Soc. Limnol. Oceanogr. (1980).
BARNETT,C.J., and J.E.KONTOGIANNIS: Environ. Pollut. 8,45 (1975).
BERDUGO,V., R.P.HARRIS, and S.C.O'HARA: Mar. Pollut. Bull. 8,138 (1977).
BERMAN,M.S., and D.R.HEINLE: Mar. Biol. 56,59 (1980).
BURNS,C.W: Int. Revue Ges. Hydrobiol. 53,83 (1968).
CONOVER,R.J: J. Fish. Res. Bd. Can. 28, 1327 (1971)
ENGELHARDT,F.R., R.G.TRUCCO, C.K.WONG, and J.R.STRICKLER: In "Land and water issues related to energy development", P.J.RAND eds., Ann Arbor Science Publ. Inc. (in press).
FORRESTER,W.D: J. Mar. Res. 29,151 (1971).
KONTOGIANNIS,J.E., and C.J.BARNETT: Environ. Pollut. 4,69 (1973).
LEE,W.Y., and J.A.C.NICHOL: Environ. Pollut. 12, 279 (1977).
MARSHALL,S.M: Adv. Mar. Biol. 11,57 (1973).
MCMAHON,J.W., and F.H.RIGLER: Can. J. Zool. 41,321 (1963).
O'BRIEN,W.J: Arctic 31,219 (1978).
POULET,S.A., and P.MARSOT: Science 200,1403 (1978).
SCHEFFER,D., P.ROBERT, and J.MEDIONI: C.R. Seances Soc. Biol. Fil. 152,1000 (1958).
STARKWEATHER,P.L: Limnol. Oceanogr. 23,307 (1978).
WONG,C.K., F.R.ENGELHARDT, and J.R.STRICKLER: Bull. Environ. Contam. Toxicol. 26,606 (1981).

Accepted March 16, 1983