Journal of the Korean Society of Oceanography
Vol. 37, No. 2, pp. 76 — 81 June 2002

Effects of naval pulp wastes on the growth and feeding rates of a heterotrophic protist and copepods

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I investigated whether US naval pulp wastes (pulverized paper products), which is planned to be dumped into offshore waters, may affect the ecology of major components of marine zooplankton. The presence of slurry (0.6% concentration - wet weight : wet weight) did not significantly affect the population growth rates of the heterotrophic dinoflagellate *Polykrikos kofoidii* fed on *Lingulodinium polyedrum*, but significantly reduced the ingestion rates of the calanoid copepods *Acartia* spp. on *L. polyedrum* and those of the copepod *Calanus pacificus* on *Akashiwo sanguinea* (previously *Gymnodinium sanguineum*). However, *C. pacificus*, originally exposed to 0.6% slurry for 24 hour, can recover its feeding rates when slurry disappears. Therefore, if slurry is diluted quickly due to turbulence after being dumped at 0.6% concentration, its presence may not affect *Calanus*. Chemicals leached from slurry did not affect the feeding rate of *Calanus*. Therefore, mechanical interference by slurry on the feeding and/or swimming of copepods may be mainly responsible for the reduction of the ingestion rates.

Key words: Acartia, Calanus, Dinoflagellate, Polykrikos, Zooplankton

INTRODUCTION

The amount and types of anthropogenic products introduced into marine environments have continuously increased. Usually, when these products are introduced into estuaries and semi-enclosed embayments where water circulation is restricted, food webs in these ecosystems can be significantly affected by these products (Laws, 1981). However, this may not occur in open oceans because of their large water volume and active circulation.

It is planned to dump USA naval pulp wastes (pulverized paper products; paper, cardboard, magazines, and newspapers - approximately a 0.5% slurry of cellulose which have diameters on the order of 10–20 μm and lengths on the order of 1000–2000 μm, a specific gravity of 1.5 g/cm^3, and a low percentage of lignin) into offshore waters, and it must be determined whether these wastes may affect the ecology of any major components of marine organisms.

Zooplankton species play very important roles in food webs as major consumers of bacteria (Azam *et al.*, 1983; Sherr and Sherr, 1994) and phytoplankton (Jeong and Latz, 1994; Kiφrboe and Nielsen, 1994; Jeong, 1999; Jeong *et al.*, 1999a and b, Teegarden *et al.*, 2001; Jeong *et al.*, 2001a and b; Stoecker *et al.*, 2002; Jeong *et al.*, 2002), an important food source for diverse carnivores (particularly the juveniles of most commercially important fish) (Koslow, 1981; Stoecker and Govoni, 1984), and as nutrient regenerators (Paasche and Kristiansen, 1982; Miller *et al.*, 1995). Heterotrophic protists and copepods are among the dominant micro- (20–200 μm in size) and macrozooplankton (>200 μm) in most marine environments (Durbin and Durbin, 1981; Lessard, 1984; Strom and Strom, 1996; Lessard and Murrell, 1996; Grey *et al.*, 1997). Because of their numerical importance and linkages, changes in their abundances or feeding rates can significantly affect the abundances of other marine organisms making up ecosystems (Mullin and Conversi, 1988; Jeong, 1994). I chose *Polykrikos kofoidii* (a heterotrophic dinoflagellate) (Jeong *et al.*, 2001a), and *Calanus pacificus* (a dominant copepod in offshore) (Barnett and Jahn, 1987) and *Acartia* spp. (in estuaries and coastal waters) (Paffenhöfer and Stearns, 1988; Choi and Park, 1993; Plourde *et al.*, 2002) representative of heterotrophic protists

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and copepods, respectively.

Pulp wastes themselves, and/or leached chemicals, may significantly reduce the ingestion rates of copepods on suitable prey by clogging the predators' feeding apparatus or by poisoning them ($H_0$1 and $H_0$3 below) and the population growth rates of heterotrophic protists ($H_0$4). If copepods living and feeding near the surface can survive in dense pulp wastes and then recover their feeding rates on suitable prey after pulp waste has sunk or been dispersed, the wastes will not significantly affect the ecology of copepods ($H_0$2).

To investigate these topics, I tested the following hypotheses:

$H_0$1: The ingestion rate of phytoplankton by copepods is independent of the presence of slurry of pulp wastes. This is a test of an immediate effect.

$H_0$2: There is no effect on ingestion rates in slurry-free water of previous exposure to slurry. This is a test of a residual effect.

$H_0$3: There is no difference in ingestion rates in slurry-free sea water in which slurry had been soaked for 24 hour and then removed by filtration, relative to sea water never contacting slurry. This is a test of a residual effect due to dissolved leachate.

$H_0$4: The population growth rate of a heterotrophic protist is independent of the presence of slurry of pulp wastes.

MATERIALS AND METHODS

Preparation of experimental organisms and conditions

The dinoflagellates *Akashiwo sanguinea* and *Lingulodinium polyedrum*, a ubiquitous heterotrophic dinoflagellate *Polykrikos kofoidii*, and common copepods *Acartia* spp. and *Calanus pacificus* were chosen for these experiments. *A. sanguinea* and *L. polyedrum* are known as prey for *Acartia* spp. and *C. pacificus* (Fernandez, 1979; Jeong, 1994). They were grown in enriched f/4 seawater media (Guillard and Ryther, 1962) without silicate, at room temperature (20–23°C) with continuous illumination of 100 μE $m^{-2}s^{-1}$ of cool white fluorescent lights. Cultures in exponential growth phase were used for feeding experiments.

A dense population of *P. kofoidii*, originally collected at the end of the Scripps pier and maintained in culture with *L. polyedrum* at a 19°C room, was used in this expeiment. Adult female *C. pacificus* were collected from the coastal waters off La Jolla Bay, CA, USA, using a 303 μm mesh net, and adult female *Acartia* spp. from the waters of Misson Bay, CA, using a 54 μm mesh net. Copepods were maintained at a 15°C room in 1 gallon jars with *A. sanguinea* or *L. polyedrum* in filtered sea water for at least two days before experiments.

Experimental designs

The initial densities of the predator and prey, and slurry are given in Table 1, together with the hypotheses each experiment was designed to test.

To set up an experiment, three 1 ml aliquots from a *A. sanguinea* or *L. polyedrum* culture were counted to determine density. The concentrations were then obtained by volume dilution with an autopipette. The wet weight of slurry was measured on a microbalance, and each concentration (ratio of wet weight of slurry to weight of seawater) of slurry was obtained by adding a known weight of slurry into Polycarbonate (PC) bottles. Slurry inside bottles was not

Table 1. Design of experiments.

Experiment No.	time[e]	Slurry (%)[d]	Prey[a] (cells. ml^{-1})	Predator[b] (inds. l^{-1})
1	t=0	0, 0.05, 0.1, 0.3, 0.6	123	10[c]
2	t=0	0, 0.1, 0.6	190	30
3	t=0	0, 0.6	183	10
	t=24h	0, 0	117	10
4	t=0	0, 0[f]	117	10
5	t=0	0, 0.05, 0.1, 0.3, 0.6	84	1.6

a and b: The initial densities of prey and predator (*Akashiwo sanguinea* and *Calanus pacificus* in experiments 1, 3, and 4, *Lingulodinium polyedrum* and *Acartia* spp. in experiment 2, and *L. polyedrum* and *Polykrikos kofoidii* in experiment 5).
c: Incubation bottle size (500 ml in experiments 1, 3, and 4, 270 ml in experiment 2, and 32 ml in experiment 5).
d: ratio of wet weight of slurry to that of filtered seawater.
e: time exposed to 0.6% slurry before measurement of ingestion.
f: water in which slurry had been soaked for 24 hours.

homogeneously distributed, even though bottles were rotated. Such an aggregation of slurry may also be true in nature.

Copepods were rinsed with filtered seawater in a Petri-dish, and 5 healthy female *C. pacificus* (in experiments 1, 3, and 4) or 8 female *Acartia* spp. (in experiment 2) were transferred into each 500 or 270 ml PC bottle, respectively. Duplicate experiment bottles were set up, as were duplicate control bottles containing only *A. sanguinea* or *L. polyedrum* and slurry at all slurry concentrations. Actual initial concentrations of *A. sanguinea* or *L. polyedrum* were measured in one extra control bottle by counting and removing more than 200 individual cells with a Pasteur micropipette. Experimental and control bottles were placed on rotating wheels at 0.9 RPM under dim light at 15°C for 16–20 h. After incubation, 2 ml aliquots from each bottle were transferred into multiwell chambers for counting *A. sanguinea* or *L. polyedrum* cells (after serial dilution where necessary), and *C. pacificus* or *Acartia* spp. were sieved onto a 101 μm net and counted. Ingestion rates (prey ingested copepod^{-1} hour^{-1}) of were calculated, using the equations of Frost (1972), from final concentrations of prey in bottles with and without *Calanus* or *Acartia*.

The slurry concentration of 0.6% was used in experiment 3 because this concentration caused a large reduction in feeding in experiment 1. Two different predator-prey combinations were initially set up in duplicate: (1) 5 female *C. pacificus* (10 *C. pacificus* l^{-1}) and *A. sanguinea* (2) 5 female *C. pacificus*, *A. sanguinea*, and slurry. Duplicate control bottles were similarly set up without copepods. Bottles were incubated for 24 h as described above (in Table 1, t=0). After counting cells, all *C. pacificus* were sieved onto a 101 μm net, counted, and transferred into new bottles containing only new *A. sanguinea* cells without slurry (in Table 1, t=24h). New duplicate control bottles containing only *A. sanguinea* were set up. Bottles were incubated again for 24 h as described above, and cells and *Calanus* were counted.

In experiment 4, 0.6% slurry in filtered sea water was placed in a 15°C room. Twenty-four hours later, the slurry was screened out onto a GF/C millipore filter, and the filtrate sea water was transferred into four PC bottles. *A. sanguinea* was added to all four, and 5 female *C. pacificus* to two of these. Controls were similarly set up using sea water which had not been exposed to slurry. Bottles were incubated for 24 h as described above, and cells and *Calanus* were counted.

In experiment 5, the initial prey concentration was 84 *L. polyedrum* ml^{-1} and concentrations of slurry were 0, 0.05, 0.1, 0.3, and 0.6%. Fifty actively swimming *P. kofoidii* cells, which had been incubated under the similar experimental conditions for 2 days, were transferred into each 32 ml PC bottle after serial rinsing with filtered seawater. Triplicate experiment bottles were set up at all slurry concentrations. Three ml of f/4 media were added into each bottles to keep *L. polyedrum* cells healthy. Actual initial concentrations of *L. polyedrum* were measured in one extra bottle by counting and removing more than 100 individual cells with a Pasteur micropipette. Experimental bottles were placed on rotating wheels at 0.9 RPM under a 13:11 hr light-dark cycle of illumination with approximately 50 μE m^{-2} s^{-1} of cool white fluorescent light at 19°C for 47–53 h. The final concentrations of *P. kofoidii* were measured by counting all cells in multiwell chambers under a dissecting microscope by removal of each cell with a Pasteur micropipette.

The specific growth rate of *P. kofoidii*, μ (day^{-1}), was calculated as:

$$\mu = \frac{ln\left(\frac{P_t}{P_0}\right)}{t}$$

where P_o is the initial concentration of *P. kofoidii* and P_t is the final concentration after time t.

Test of hypotheses

In experiments 1 and 2, the initial concentration of *A. sanguinea* or *L. polyedrum* was fixed, while that of slurry varied (Table 1). Spearman correlation test (Zar 1984) was used to test whether ingestion rates at one slurry concentration were significantly different from those at other slurry concentrations ($H_0$1). Pearson correlation test was used to test whether population growth of *P. kofoidii* on *L. polyedrum* at any slurry concentration was significantly different from those at other slurry concentrations ($H_0$4).

$H_0$2 can be rejected if ingestion rates of *C. pacificus* previously incubated with slurry are significantly different (by two-tailed, two-sample t-test) from those never exposed to slurry.

$H_0$3 can be rejected if ingestion rates in seawater in which slurry had been soaked for 24 hour and then removed by filtration are significantly different (by two-tailed, two-sample t-test) from those in seawater never contacting slurry.

RESULTS AND DISCUSSION

Test of $H_0$1 *(ingestion rate of phytoplankton by copepods is independent of the presence of slurry)*

With increasing slurry concentration, the ingestion rates of *Akashiwo sanguinea* by *Calanus pacificus* exponentially decreased from 205 to 12 prey *Calanus*$^{-1}$ h^{-1} (Fig. 1); this decrease was statistically significant (Spearman correlation test, $p<0.001$; Zar 1984). Therefore, $H_0$1 can be rejected when *A. sanguinea* and *C. pacificus* were prey and predator. Ingestion rates at slurry concentrations of 0.05 and 0.1% were not significantly different from that without added slurry ($p>0.05$), but they were significantly depressed at slurry concentrations $\geq$0.3% ($p<0.05$).

With increasing slurry concentration, the ingestion rates of *Lingulodinium polyedrum* by *Acartia* spp. also significantly decreased from 22 to 5 prey *Acartia*$^{-1}$ h^{-1} (Fig. 2; ANOVA, $p<0.05$). Therefore, $H_0$1 can also be rejected when *L. polyedrum* and *Acartia* spp. were prey and predator. The ingestion rate without added slurry was not significantly different from that at a slurry concentration of 0.1% ($p>0.05$), but was significantly depressed at 0.6% ($p<0.05$).

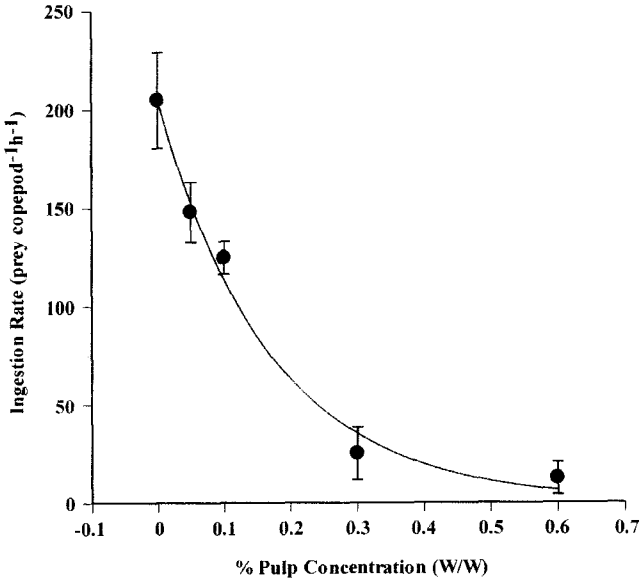

Fig. 1. Ingestion rates of *A. sanguinea* by *Calalnus pacificus* as a function of the slurry concentration. Symbols represent treatment means ± 1 S.E. Relations are fitted by the curvelinear regression. IR (prey eaten *Calanus*$^{-1}$ h^{-1}) $=183\times e^{(-5.42\times SC)}$ ($R^2=0.831$); where SC=slurry concentration.

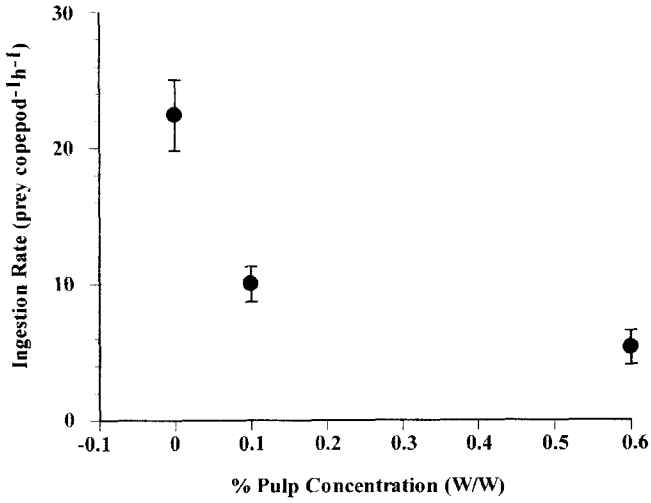

Fig. 2. Ingestion rates of *Lingulodinium polyedrum* by *Acartia* spp. as a function of the slurry concentration. Symbols represent treatment means±1 S.E.

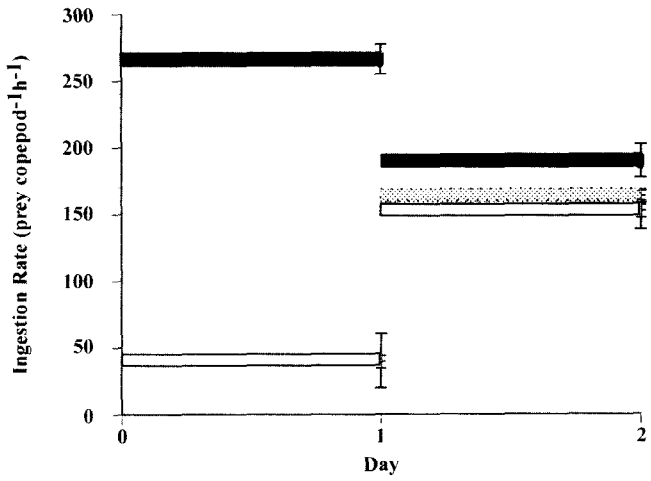

Fig. 3. Ingestion rates of *Akashiwo sanguinea* by *Calalnus pacificus*. Symbols represent treatment means±1 S.E. Black bars - Incubated without slurry in both Day 1 (t=0 in Table 1) and 2 (the initial *Akashiwo sanguinea* concentrations in Day 1 and 2 were 183 and 117 cells ml^{-1}, respectively). Open bars - with 0.6% slurry (wet weight:wet weight) in Day 1 and without slurry in Day 2. Gray bar - in slurry-free sea water in which slurry had been soaked for 24 hour and then removed by filtration.

Test of $H_0$2 *(no effect on ingestion rates in slurry-free water of previous exposure to slurry)*

In experiment 3, after first day incubation, the ingestion rate of *Calanus* on *A. sanguinea* incubated with the slurry concentration of 0.6% was significantly different from that without slurry (Fig. 3; two tailed-t test, $p<0.05$), similar to the result in exper-

iment 1. However, the ingestion rate of *Calanus*, originally incubated with 0.6% slurry for 24 hour and then transferred into new bottles containing *A. sanguinea* without slurry, was not significantly different from that of *Calanus* continuously incubated without slurry. Therefore, $H_0$2 cannot be rejected. The results show that *Calanus* feeding rate recovers when slurry disappears.

Test of $H_0$3 ***(no difference in ingestion rates in slurry-free sea water in which slurry had been soaked for 24 hour and then removed by filtration, relative to sea water never contacting slurry)***

The ingestion rate of *Calanus* in slurry-free sea water in which slurry had been soaked for 24 hour and then removed by filtration (gray bar in Fig. 3) was not significantly different from that in sea water never contacting slurry ($p>0.05$).

Test of $H_0$4 ***(the population growth rate of a heterotrophic protist is independent of the presence of slurry of pulp wastes)***

With increasing slurry concentration, the specific growth rates of *P. kofoidii* on *L. polyedrum* decreased from 0.196 to 0.142 d^{-1} (Fig. 4), but this decrease was not statistically significant (Pearson correlation test, $p>0.2$). Therefore, $H_0$4 cannot be rejected; the presence of slurry does not affect the population of *P. kofoidii*.

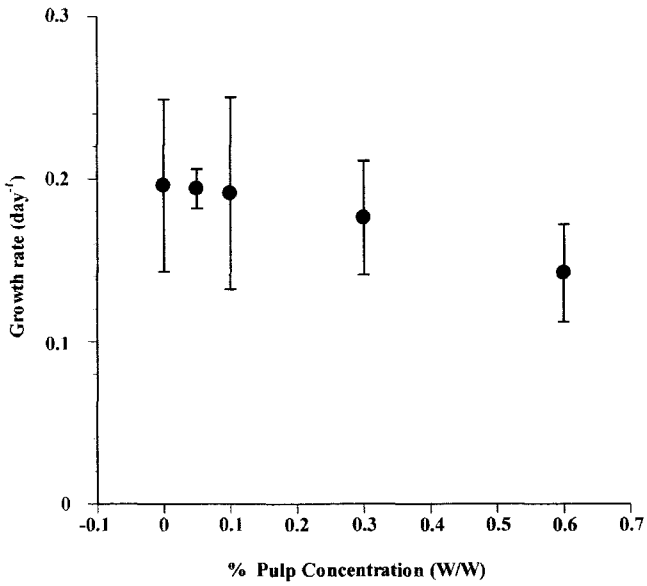

Fig. 4. Specific growth rates of *Polykrikos kofoidii* on *Lingulodinium polyedrum* as a function of the slurry concentration. Symbols represent treatment means±1 S.E.

The presence of slurry significantly reduced the ingestion rates of the copepod *Calanus* on *Acartin's sanguinea* at the slurry concentrations ≥0.3%. However, the ingestion rate of *Calanus*, originally exposed to 0.6% slurry for 24 hour, is restored when slurry disappears (Fig. 3). Therefore, if slurry is diluted quickly due to turbulence after being dumped at 0.6% concentration, its presence may not affect *Calanus*. The presence of slurry also significantly reduced the ingestion rates of *L. polyedrum* by *Acartia* spp. but, the magnitude of the reduction (4 times) was less than that for *Calanus* (17 times). The habitat of *Acartia* spp. (i.e. estuaries or coastal waters) is typically more turbid and polluted than that of *Calanus* (i.e. offshore); *Acartia*'s adaptation to turbid environments may be partially responsible for its greater tolerance of slurry.

Chemicals leached from slurry did not affect the feeding rate of *Calanus* (Fig. 3). Therefore, mechanical interference by slurry on the feeding and/or swimming of copepods, or clogging of the gut may be mainly responsible for the reduction of the ingestion rates.

The presence of slurry (≤0.6%) did not significantly affect the population growth rates of *P. kofoidii* fed on *L. polyedrum*. *P. kofoidii* engulfs, rather than filters (Jeong *et al.*, 2001a), a prey cell, so that mechanical interference of slurry with its feeding behavior may be much less than for copepods.

CONCLUSIONS

The presence of slurry (0.6% concentration) did not significantly affect the population growth rates of *Polykrikos kofoidii*, but significantly reduced the ingestion rates of the copepod *Calanus pacificus* and *Acartia* spp. on phytoplankton prey. However, if slurry is diluted quickly due to water movement after being dumped at 0.6% concentration, its presence may not affect the abundance of the copepods. Chemicals leached from slurry did not affect the feeding rate of *Calanus*.

ACKNOWLEDGEMENTS

I thank late Michael Mullin and Mark Ohman at the Scripps Institution of Oceanography, Stacey Curtis at the US Naval Command Control and Ocean Surveillance Center (NCCOSC) RDTE Division (NRaD) for providing facilities and for comments on the manuscript. I also thank Ronald McConnaughey for collecting copepods. This paper is supported by US Naval Research and Development and MOST and KOSEF (99 RRC).

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Manuscript received May 29, 2002
Revision accepted June 17, 2002
Editorial handling: Hae-Lip Suh