OCS Study MMS 2004-034

Final Report

The Role of Copepods in the Distribution of Hydrocarbons: An Experimental Approach

by

Switgard Duesterloh

Thomas C. Shirley*
Principal Investigator

Fisheries Division
Juneau Center, School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
11120 Glacier Highway
Juneau, AK 99801

June 2004

*Corresponding author
e-mail: tom.shirley@uaf.edu

Contact information

e-mail: cmi@sfos.uaf.edu
phone: 907.474.7707
fax: 907.474.7204
postal: Coastal Marine Institute
School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
Fairbanks, AK 99775-7220

Table of Contents

List of Tables

List of Figures

Abstract

Copepods may provide a significant pathway for the concentration and transfer of polyaromatic compounds (PAC) to higher trophic level consumers. PAC dissolved from weathered crude oil are more persistent in the environment and have much higher toxicity than the lighter, more volatile fractions of crude oil. Because of their polarity, PAC tend to accumulate in bio-lipids. Subarctic copepod species can contain up to 80% of their body dry weight in lipids and have a high surface-area-to-volume ratio. Thus, PAC accumulation is rapid and bioaccumulation factors are in the order of 500–8000, depending upon species and lipid content. While direct toxic effects of oil on copepods have been reported in the order of 10 mg L^{-1}, toxicity increases substantially in the presence of natural ultraviolet radiation (UV). Phototoxic effects on the copepods *Calanus marshallae* and *Metridia okhotensis* were observed at concentrations of ~2µg L^{-1} total dissolved PAC followed by 4–8 hours of exposure to ambient daylight. Responses included mortality, immobilization and discoloration of lipid sacs. Further experiments were conducted to test the interaction effects of various concentrations of PAC dissolved from weathered Alaska North Slope crude oil and subsequent exposure to sunlight with and without the UVB component on the copepods *Neocalanus flemingeri* and *N. plumchrus*. Phototoxicity was found to be a linear function of the product of light intensity and PAC concentration. High natural variability in egg production rates precluded significant results of the toxicity of oil to copepod reproduction. This work has shown that copepods could potentially provide a mechanism for the concentration of dissolved PAC from the water and its transfer into pelagic and benthic food chains.

Chapter 1. Introduction

Overview

The last 15 years have changed the understanding of oil toxicity mechanisms, in part achieved by numerous studies propagated by the *Exxon Valdez* oil spill in Prince William Sound (PWS) in 1989. Among the significant advances are a better understanding of the persistence and long-term toxicity of larger polyaromatic compounds (PAC), which were previously thought to be of minor importance, compared to the lighter, more volatile but narcotic 1- and 2-ringed polyaromatic hydrocarbons (PAH). Polyaromatic compounds investigated in this study include PAH and dibenzothiophenes. PAC were reported to cause genetic damage in fish when early developmental stages were exposed to oil. Also, significant deposits of *Exxon Valdez* oil persisted on some beaches and continued to leak oil into the water for more than a decade after the spill. Concurrently with advances in oil toxicity studies, the relatively new field of phototoxicity received increasing scientific attention. The toxicity of specific polyaromatic hydrocarbons to various biota was found to increase up to 50,000-fold with ultraviolet radiation (UV) interaction. Phototoxicity of oil was also reported for several crude and fuel oils under light regimes likely encountered by biota in their natural environment.

PAC tend to accumulate in bio-lipids because of their polarity. As an abundant component of the pelagic community with a high surface-area-to-volume ratio and a high bio-lipid content, copepods may provide a significant pathway for the concentration and transfer of PAC to higher trophic level consumers. *Neocalanus* copepods are the most abundant taxon of the zooplankton and can constitute over 60% of the biomass during the spring and summer months in the Gulf of Alaska (GOA) and adjacent coastal regions. Many copepod species in polar and subpolar regions accumulate internal lipid stores of 60 to >80% of their body dry weight. As an adaptation to the seasonally fluctuating supply of phytoplankton, which is their predominant food source, the late copepodite stages accumulate large lipid reserves April to June in surface waters. Egg production and spawning can be delayed for several months and occur at

depth or during the spring ascent, timed to ensure food abundance for the offspring during growth and fat storage. The potential of *Neocalanus* copepods for accumulation and transfer of PAC and a possible correlation to total lipid content was investigated.

Accumulated PAC may act as internal photoreceptors, causing photo-oxidation in surrounding tissue. We studied the synergistic effect of exposure to dissolved PAC (~2μg L^{-1}) from Alaska North Slope crude oil and ultraviolet radiation in ambient daylight on the copepods *Calanus marshallae* and *Metridia okhotensis*. These were the first phototoxicity tests with translucent organisms that are at risk of exposure to dissolved PAC and UV radiation in Prince William Sound and the Gulf of Alaska. Responses included mortality, impairment of swimming ability and discoloration of lipid sacs. The interaction of the effect of PAC and UV radiation was highly significant ($P < 0.005$) in two experiments. Further experiments were conducted to test the interaction effects of various concentrations of PAC dissolved from weathered Alaska North Slope crude oil and subsequent exposure to sunlight with and without the UVB component on the copepods *Neocalanus flemingeri* and *N. plumchrus*. The results confirmed that phototoxicity is a linear function of the product of light intensity and PAC concentration. The observed sensitivity to photoenhanced oil toxicity may have implications for the role of copepods in the transfer of hydrocarbons to other trophic levels: Local populations could be subject to increased mortality if oil exposure is accompanied by or followed by sunny weather. This would cause food depletion for zooplanktonivorous fishes and may introduce PAC to the benthic food chain through sedimentation of dead copepods. The resulting reduction of energy flow from primary production to higher trophic levels may have adverse effects on commercial fisheries.

An attempt was made to assess the toxicity of oil to copepod reproduction. The feasibility of culture experiments to compare egg production rates and survival of oiled and unoiled female *Calanus marshallae* and *Pseudocalanus* spp. copepods was investigated. However, egg production rates varied greatly between females and between subsequent days. From these pilot experiments we concluded that the sample size needed to detect a significant difference between egg production of oiled and unoiled females was larger than could be obtained with the available methods.

While this research has demonstrated that copepods could potentially provide a mechanism for the concentration of dissolved PAC from water and its transfer into pelagic and benthic food chains, it does not attempt to assess the magnitude of this pathway. The retention time of oil in copepods and how dietary uptake of copepod-accumulated PAC affects predators remains to be investigated. This research further provided new evidence that oil toxicity increases in the presence of UV light and adverse effects on plankton organisms are evident at levels of PAC and UV concentrations possibly encountered in nature. However, we did not attempt to quantify the possible extent of injury to copepod populations.

Background

Photoenhanced toxicity of oil is a function of numerous interplaying variables: a) the composition of the oil, b) the specific spectral composition of sunlight and its energetic implications, and c) PAH concentrations in tissue and light intensity. Each variable is subject to numerous influencing factors, which each in itself is subject to ongoing research efforts directed at understanding the mechanisms, which ultimately define the nature and degree of the observed toxic effects.

Traditional assays of the toxicity of oil were conducted with fresh oil, which in comparison to weathered oil has a greater number of lighter fractions (benzene, toluene, ethylbenzene, xylene). These lighter compounds are more volatile and cause higher immediate toxicity through a narcosis mechanism. In short-term toxicity tests the heavier fractions of the oil, which contain more aromatic ring-structures and are more persistent, caused little effect on the test organisms and were thus considered to be of minor toxicity. Within the last decade of studies on oil toxicity this view has changed: Long-term studies found

that toxicity increases with the number of ring structures of the molecules; thus weathered oil with a higher proportion of multi-ring structures is more toxic than unweathered oil, with a lower proportion of the heavier compounds. The mechanism responsible for the toxicity of multi-ring PAH is the destruction of DNA, and to a lesser degree, other biomolecules. At the same time, larger PAH are more persistent in the environment, leaching bioavailable contaminants years after an initial pulse perturbation.

In context with the study of photoenhanced toxicity, these persistent PAH have structural properties to enable catalysis of tissue damage by absorption and transformation of solar energy. The advancing resolution of the structural properties of some known phototoxic PAH results in the publication of absorption spectra. These indicate not only that the inherent phototoxic potential of PAH depends on molecular structure, but also that every molecule has a specific spectral region in which it can be activated. Thus, a photosensitizing molecule in an organism may be latent until the specific activating wavelength is encountered and initiates the phototoxic reaction.

The damaging influence of UV radiation, particularly the shorter wavelength UVB radiation (280–340 nm), received considerable attention after the discovery of the decreasing trend of ozone content of the earth's stratosphere and the correlated increase in UVB transmittance. Sensitivity to increased levels of UVB radiation was found in virtually all biological systems, from primary producers to sensitive life stages of vertebrates and plants. Fundamental changes in productivity were predicted for some ecosystems, while others were believed to be relatively buffered from harmful UVB penetration. While this UV sensitivity may have influenced the evolution of responses like pigmentation and vertical migration in copepods and has been instrumental in the evolution of community structures, accurate determination of exposure levels requires local underwater UV measurements. However, if UVB is a limiting factor in plankton distribution, the presence of internal PAC may cause mortality at natural light levels: Phototoxicity is the product of UV exposure dose and PAC tissue concentration.

Also, phototoxicity has been observed with the exclusion of UVB radiation, suggesting that the lower energy contained in longer wavelengths is sufficient to catalyze the chemical reactions involved. Nevertheless, greater phototoxic effects of oil on fish were observed, when both UVB and UVA were used, indicating a possible interplay of the different light spectra.

Phototoxic effects are a product of total PAH concentration in the test organism and exposure light intensity. In aquatic organisms with rapid molecular exchange over the outer surface or the gill tissue, accumulation of PAH is correlated with the total concentration of the chemical in the water. The sensitivity of biological organisms may depend on metabolic pathways that place photosensitive chemicals in more or less harmful positions. These sensitivities are apt to change over the course of a lifetime as detoxifying mechanisms develop (in fish and mammals) or chemical composition changes (e.g., increased fat storage in late stage copepods). The lipophilic nature of PAH favors tissues that are rich in bio-lipids for PAH deposition. Organisms with large fat deposits may be less susceptible to immediate damage than those which deposit the toxic compound in structural membranes, but accumulation potential may increase with fat content. Despite limited knowledge of the specific phototoxic compounds, there is a consistent trend of increasing phototoxicity with increasing total PAH concentration in test organism tissue, indicating that measurement of total PAH concentration is a good substitute for phototoxic compound analysis.

The past 15 years of research in phototoxicity have promoted our understanding of the chemical processes involved and our ability to predict the phototoxicity of specific compounds. Phototoxicity is of environmental concern in numerous aquatic systems at exposure levels present today. The first legal consequences of these findings were manifested in the ban of recreational 2-stroke carbureted motorcraft from Lake Tahoe. However, knowledge about ecosystem responses to increased PAH contamination or increased levels of UV radiation is limited and may be the central aspect of ecological studies related to phototoxicity in the coming decades.

Chapter 2. Bioaccumulation of Polyaromatic Compounds from Oil Relative to Lipid Content in the Copepods *Neocalanus flemingeri* and *N. plumchrus*[1]

Abstract

The bioaccumulation potentials of copepods for polyaromatic compounds (PAC) from aqueous solution in relation to total lipid content were investigated. Neocalanus flemingeri *and* N. plumchrus *were sampled from four locations in Prince William Sound and the Gulf of Alaska between mid-April and early June and experimentally exposed to low concentrations (0.5–12 µg of total PAC L^{-1}) of dissolved Alaska North Slope crude oil. Total lipid content, lipid class composition and tissue accumulation of PAC were analyzed. Accumulation of PAC was passive and unselective. A positive correlation existed between total lipid content and bioaccumulation factors. In two samples with a co-occurrence of the two* Neocalanus *species, no difference in lipid content or composition between species was found.* Neocalanus *copepods may aid in the concentration and transfer of PAC from the water column to higher trophic level consumers.*

Introduction

The increased use of fossil fuels inevitably heightens the risk of accidental spills of oil into the marine environment. Since many polyaromatic compounds (PAC) are known to have carcinogenic properties [Arfsten et al. 1996], the fate of PAC in the environment, sites of bioaccumulation, and identification of transport mechanisms are important. Bioaccumulation describes the augmentation of a substance in the tissue of an organism compared to its concentration in the surrounding environment. Bioaccumulation factors, as reported in this study, describe the ratio of PAC concentrations in copepod tissue to that of the exposure water. Also discussed is the potential role of copepods in transferring accumulated PAC to higher trophic levels. Because of the polar attraction of oil-derived PAC to biogenic lipids, I hypothesized that copepods may accumulate dissolved PAC from the water and that the concentration factor is correlated with the total lipid content. While this correlation has been suspected [Corner 1975], chemical analysis of PAC concentrations in the exposure water and the tissue of exposed copepods, and the corresponding lipid class analysis have never been presented.

The congeneric and sympatric copepods *Neocalanus flemingeri* and *N. plumchrus* dominate the macrozooplankton biomass of the subarctic Pacific during spring and early summer [Miller 1988] and are a major component in the diet of many commercially important fish species [Cooney 1993]. Perhaps as adaptations to the seasonality of high-latitude systems, both species migrate vertically and accumulate large lipid stores during times of phytoplankton abundance in surface waters. Total lipid contents are among the highest measured in copepods and reach more than 80% of dry weight (this study), with an average of >60% during April–June and consist predominantly of wax esters [Sargent and Henderson 1986]. These large wax ester stores are synthesized de novo by calanoid copepods, principally to fuel reproduction [Sargent and Henderson 1986]. Gonad development occurs in midsummer and fall, respectively, and spawning peaks in January for *N. flemingeri* and September for *N. plumchrus* at depths below 400 m [Miller and Clemons 1988]. Thus, large amounts of energy in the form of stored lipids in copepods are available to surface-feeding predators during April to June, and this food source then shifts to deeper layers of the water column for several months until lipids are diminished during gonad development and spawning [Sargent and Falk-Petersen 1988].

[1] Duesterloh, S. Bioaccumulation of polyaromatic compounds from oil relative to lipid content in the copepods *Neocalanus flemingeri* and *N. plumchrus*. Prepared for publication in the Journal of Plankton Research.

Bioaccumulation of PAC by *Neocalanus flemingeri* was investigated experimentally. Copepods were sampled between mid-April and the end of June 2001 at different locations in Prince William Sound (PWS) and the Gulf of Alaska (GOA) to obtain test organisms that would vary in lipid content. While *N. flemingeri* dominated the copepodite V size class in the spring, an approximately equal abundance of *N. flemingeri* and *N. plumchrus* occurred in early June, and a species comparison was conducted.

Methods

Four separate, but identically designed, experiments were conducted between mid-April and early June 2001 at the University of Alaska Fairbanks/Institute of Marine Science (UAF/IMS) Seward Marine Center in Seward, Alaska. Copepods were exposed to three levels of total PAC (TPAC) concentration in the exposure water (high, low, control) and then tested for PAC concentration in their tissue. Each copepod sample consisted of n = 10 specimens, and 3 replicates were collected from each exposure dose. With each experiment 3 replicate samples of 10 copepods each were also collected and analyzed for lipid content. For experiments 1–4, *Neocalanus flemingeri* copepodite stage V (CV) were used; experiment 4 was also conducted with *N. plumchrus* CV.

Sample collection

Zooplankton samples were collected with 200-µm mesh, open-ring nets equipped with altered design cod ends to minimize breakage of setae and towed from 50 m depth to the surface. All samples were collected in PWS and the GOA (Table 1) and were kindly provided by researchers of the GLOBEC (GLOBal ocean ECosystems dynamics) Gulf of Alaska Monitoring Program cruises, diluted if dense, and kept at ambient water surface temperature until processed. In the laboratory, storage, sorting and experiments were conducted in a constant-temperature walk-in chamber at 6–8 °C. Copepods were pipetted into 1-ml culture wells for microscopic species and life stage identification and quickly transferred to beakers, with 5 copepods per 50-ml beaker until the start of the experiment. Dry weight samples were immediately frozen and stored at –20 °C. The lipid samples from experiments 1 and 2 were initially frozen at –80 °C. Due to a temporary unavailability of the super cold freezer, storage of these samples and freezing of the samples for experiments 3 and 4 had to be moved to a freezer set at –20 °C for about 7 weeks, before all samples were again stored at –80 °C. Freezing caused little damage to the lipids, but prolonged storage at –15 °C did; rapid freezing followed by storage below –70 °C is recommended [Ohman 1996].

Species identification

Neocalanus flemingeri and *N. plumchrus* were identified using a microscope according to visual criteria [Miller 1988]: Living CV of *N. flemingeri* bear patches of bright red, while *N. plumchrus* has a more red-orange color. In addition, the pigmentation of the first antenna in *N. flemingeri* is restricted to the base of the left antenna, while *N. plumchrus* has pigment in both antennae [Miller 1988]. However, I observed that pigmentation was variable, especially between samples from different locations, and diminished under stress. I observed many individuals with no coloration in the first antenna. The size of the second maxilla was proportionately smaller in *N. flemingeri* than in *N. plumchrus* and was used as an additional clue. Specimens that did not fit the criteria for either species were not used. To verify species identification by these visual criteria, 8 randomly chosen copepods of each species were preserved and a scatter plot of cephalosome length against prosome length was plotted [see Figure 18 in Miller 1988]. From this I deduced that the error due to false species identification by visual criteria was <15%; small individuals of *N. plumchrus* can be mistaken for *N. flemingeri* if coloration is not distinct. In addition, a reference sample of 10–20 specimens was preserved at the time of sorting and 2–3 randomly selected copepods were dissected for microscopic inspection of the ventral tooth of the mandibular gnathobase, which bears 4–5 teeth in *N. flemingeri* while only 2–3 teeth are present in *N. plumchrus*.

Table 1. Summary of analytical data and derived bioaccumulation factors (BAF). N.f. = *Neocalanus flemingeri*; N.p. = *N. plumchrus*; PWS = Prince William Sound, GOA = Gulf of Alaska, GAK1 = GOA oceanographic station at the mouth of Resurrection Bay; TPAC = total polycyclic aromatic compounds; *dry weight derived from different live sample than test organisms (see text for further explanation).

Experiment	**1**	**2**	**3**	**4**	**4**
Date	16 April 01	5 May 01	14 May 01	1 June 01	1 June 01
Species	N.f.	N.f.	N.f.	N.f.	N.p.
Location	PWS	GOA	Cape Cleare	GAK1	GAK1
dry wt • ind^{-1} (mg)	0.716*	0.392	0.124*	0.317	0.313
% lipid (dry wt)	17	88	80	41	40
Water TPAC (ng L^{-1})					
High	15384	10223	10861	7587	7587
Low	8376	6214	6215	4950	4950
Control	2024	797	625	495	495
Tissue TPAC (ng g^{-1})					
High	12729	19618	12505	13391	15090
Low	7317	11071	5834	7952	4152
Control	616	368	642	553	951
BAF					
High	827	1919	1151	1765	1989
Low	874	1782	939	1607	839
Control	304	462	1028	1117	1922

Oil exposure and PAC analysis

The Alaska North Slope crude oil was weathered by heating and overnight stirring at 80 °C to 20% weight loss, which removed most monocyclic aromatic compounds, and then added to 2- and 3-mm-diameter glass beads at an application rate of 2.6 g oil kg^{-1} of beads. The oiled beads were tumbled for approximately 24 h, spread to a single layer and left under a hood for 4 days at 25 °C to allow the oil to harden onto the beads, and then were stored at –20 °C until use.

A detailed description of the generating columns that produced the aqueous solutions of PAC dissolved from crude oil is provided in Chapter 3/Duesterloh et al. [2002]. For the low-dose treatments, one generating column was filled with 100 ml of 3-mm-diameter oil-coated glass beads; for the high-dose treatments two generating columns were filled with 100 ml of 2-mm-diameter oil-coated glass beads each and connected. In the control treatments the generating columns were filled with 100 ml of PAC-cleaned 3-mm-diameter glass beads. Fresh columns were constructed for each experiment, except for experiment 3, which was conducted in close succession to experiment 2 with the same columns. Prior experience indicated that there was no loss in total PAC concentration from the columns within 96 h.

Seawater was directed from the laboratory supply line into an overhead tank of approximately 80 L capacity. It was then pumped through a generating column containing glass beads at a flow rate of

5 ml min^{-1} into a 2-L Erlenmeyer filtration flask in which the hose connector served as an overflow. Each column was flushed with seawater for 22 h before the peristaltic pump was activated and the flow rate in all columns was adjusted to 5 ml min^{-1}. The experiment was started within 20 h after activation of the pump, at which time 0.9 L of the water in the Erlenmeyer flask was collected for PAC extraction and copepods were added to the remaining water volume in the flask. A small screen of 330-µm plankton mesh covered the outflow opening of the flasks to prevent loss of copepods. After 24 h, copepods were collected and frozen (–20 °C) and 0.9 L of the exposure water was extracted with dichloromethane and then frozen for later PAC analysis at the Auke Bay Laboratory (National Marine Fisheries Service /National Oceanic and Atmospheric Administration [NMFS/NOAA]) in Juneau, Alaska.

Procedures for the quantitative determination of PAC in water and in tissues were described previously [Short et al. 1996]. Seawater samples (0.9 L) were extracted two times with 50–60 ml dichloromethane. Copepod samples (n = 10) were macerated in a glass grinder twice, each time with 1 ml dichloromethane. Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath, followed by fractionation and purification by alumina/silica gel chromatography. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). PAC analytes, including dibenzothiophenes and polyaromatic hydrocarbons containing 2–5 rings, plus the alkylated homologues, are listed in Table 2. A method blank, spiked method blank and 2 reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision and absence of laboratory-introduced artifacts and interferences. Detection limits were determined experimentally [Glaser et al. 1981] for PAC and generally were 5–20 ng PAC L^{-1} seawater at the 95% confidence level. For tissues, an 80% confidence level was chosen. Concentrations below the detection limit were treated as zero.

Table 2. Polyaromatic compounds measured in exposure water and copepod tissue.

1.	naphthalene	22.	anthracene
2.	1-methylnaphthalene + 2-methylnaphthalene	23.	fluoranthene
3.	C-2 naphthalenes	24.	pyrene
4.	C-3 naphthalenes	25.	C-1 fluoranthenes/pyrenes
5.	C-4 naphthalenes	26.	benz[a]anthracene
6.	biphenyl	27.	chrysene
7.	acenaphthylene	28.	C-1 chrysenes
8.	acenaphthene	29.	C-2 chrysenes
9.	fluorene	30.	C-3 chrysenes
10.	C-1 fluorenes	31.	C-4 chrysenes
11.	C-2 fluorenes	32.	benzo[b]fluoranthene
12.	C-3 fluorenes	33.	benzo[k]fluoranthene
13.	dibenzothiophene	34.	benzo[e]pyrene
14.	C-1 dibenzothiophenes	35.	benzo[a]pyrene
15.	C-2 dibenzothiophenes	36.	perylene
16.	C-3 dibenzothiophenes	37.	indeno[1,2,3-cd]pyrene
17.	phenanthrene	38.	dibenzo[a,h]anthracene
18.	C-1 phenanthrenes/anthracenes	39.	benzo[g,h,i]perylene
19.	C-2 phenanthrenes/anthracenes		
20.	C-3 phenanthrenes/anthracenes		
21.	C-4 phenanthrenes/anthracenes		

To test whether there was a difference between the start and end total PAC concentrations in the exposure water, a paired comparison t-test was conducted. In this test the variation introduced by possible differences between the experiments is eliminated by testing the mean difference and standard deviation of the difference in concentrations between start and end rather than by pooling of the means of all start and all end concentrations.

Lipid content and composition analysis

With each experiment, a corresponding sample of 3 replicates of *Neocalanus flemingeri* (n = 10) was measured for lipid content and composition. In experiments 3 and 4, lipid content and composition were also measured in *N. plumchrus*.

The lipid extraction method was modified from Christie [1982]. The copepod sample was homogenized with 3 ml of a 2:1 chloroform:methanol mixture. Twenty-five percent of the total volume of 0.88% KCl in distilled water was added, and after thorough mixing the top layer was discarded. After the addition of 25% of the remaining volume of 1:1 distilled water:methanol and thorough shaking, the mixture was allowed to separate. The purified lipid layer was volume reduced to 1 ml under nitrogen and 0.5 ml was dried and weighed. The remaining 0.5 ml was stored in a –20 °C freezer until analysis for lipid class composition with a high pressure lipid chromatograph (HPLC) equipped with an evaporative light scattering detector (ELSD).

For calculation of the total lipid percentage, mean dry weights were obtained from separate, corresponding samples (3 replicates, n = 10 copepods), which were thawed, weighed, and dried at 60 °C to a constant weight (36 h). For experiments 1 and 2, dry weight samples could not be obtained from the same live sample because of limited numbers of *N. flemingeri*. For experiment 1, I assumed that the dry weight was similar to that of copepods sampled in PWS 2 weeks later, and for experiment 2, dry weights from copepods sampled at the same time of the month in a different location in the GOA were used. For experiments 3 and 4, dry weights were measured from 3 replicate samples of n = 10 copepods from the same live sample as the copepods used in the exposures.

Bioaccumulation factors

Bioaccumulation factors (BAF) were calculated as follows [Barron 1995]:

$$BAF = \frac{PAC_{Tissue}[ng\ g^{-1}] \times 1000}{PAC_{Water}[ng\ L^{-1}]}$$

Note that in this equation, the tissue PAC concentration is weighted by the water PAC concentration. Tissue PAC concentrations are reported on a wet weight basis.

For each treatment, the BAF were regressed against the total lipid content of the corresponding copepod sample and the regression equation and correlation factors were calculated (Figure 1).

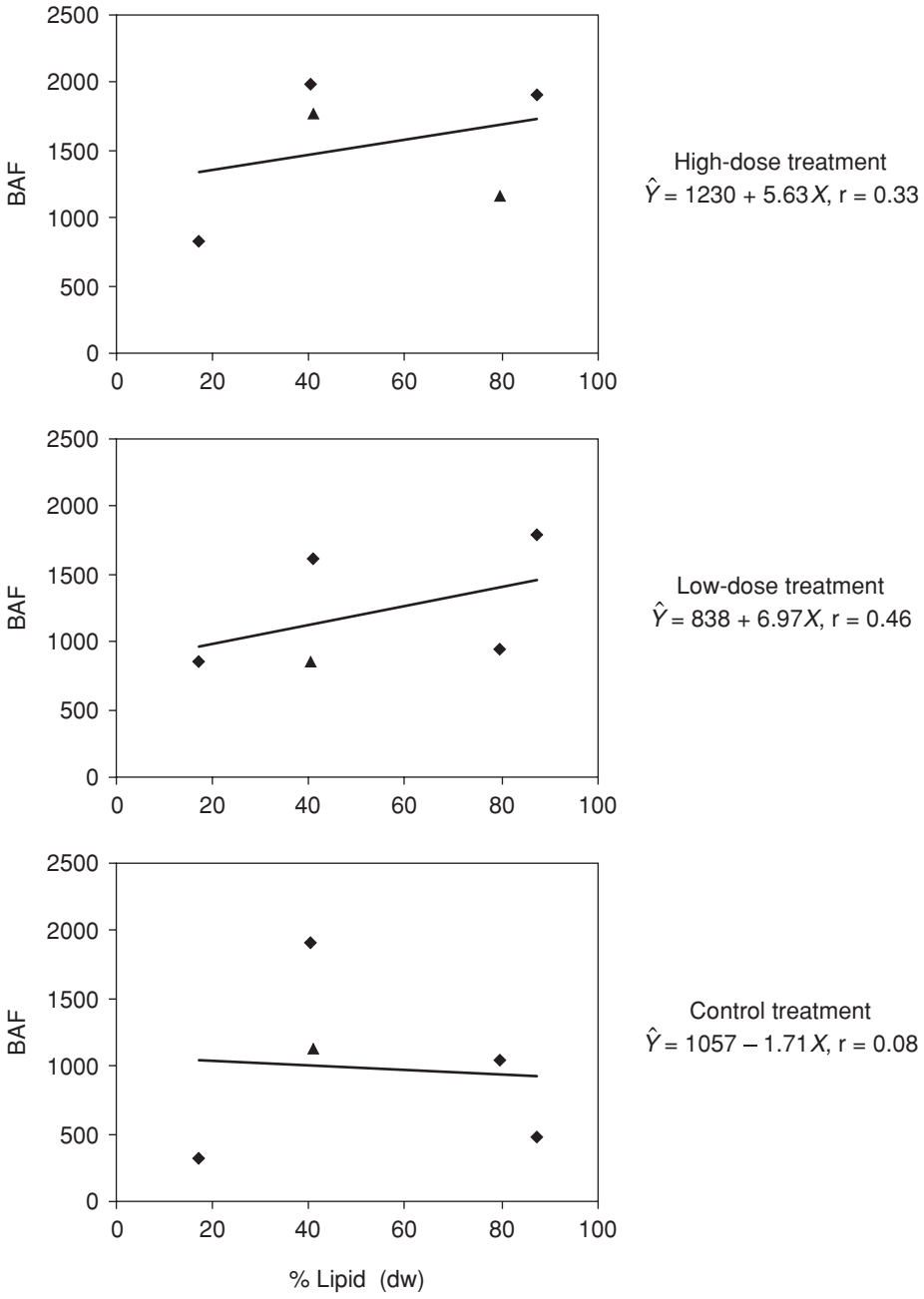

Figure 1. Correlation between bioaccumulation factors (BAF) and total lipid content in *Neocalanus* copepods in high-dose, low-dose and control treatments. Triangle data points are *N. plumchrus*; diamond data points are *N. flemingeri.*

Results

PAC exposures

The mean total PAC concentrations in the exposure seawater and their standard deviations (in parentheses) at the start and end of the 24-hr exposures (n = 4) were as follows:

TPAC concentration (μg L^{-1})	Start	End
High	10.98 (±3.15)	11.05 (±3.37)
Low	6.89 (±1.95)	5.98 (±1.05)
Control	1.25 (±1.07)	0.72 (±0.35)

The paired comparison t-test at the 95% significance level resulted in no difference between start and end concentrations. Consequently, the mean value of the start and end concentrations was used in all subsequent calculations.

All control water samples had a distinctive PAC signature which was identified as typical for creosote contamination. Total PAC concentrations ranged from 0.42 to 2.84 μg L^{-1} and averaged 0.91 μg L^{-1}. However, concentrations of individual PAC were at least 10 times lower than experimental PAC concentrations (Figure 2). The background creosote signature in the test water originated most likely from old railroad ties and pilings that washed into Resurrection Bay during the 1964 earthquake and tsunami.

In copepod tissues, total PAC concentrations in the high-dose samples were approximately twice those in the low-dose treatments. The variation between experiments was greater than in the water total PAC. As in the water samples, 3-ring naphthalenes and monoaromatic pyrenes seemed to accumulate in slightly higher proportions compared to the other analytes. Small amounts of C-2 and C-3 naphthalenes and acenaphthene were present in the tissue controls, but these were near the method detection limits of the individual analytes for the small sample weights used.

The patterns of concentrations of individual PAC analytes in the exposure water and the corresponding copepod samples are nearly identical, as would be expected for unselective and passive uptake (see, for example, Figure 2). A regression of total water PAC concentrations to total tissue PAC concentrations yielded a correlation coefficient (r) of 0.58. The proportions of individual analytes were consistent between high and low doses and between experiments, with the possible exception of 3-ring naphthalenes and monoaromatic pyrenes, which seemed to be present in slightly higher proportion in the high-dose compared to the low-dose treatments.

Lipid content and composition

There was no consistent trend of increasing lipid content with sampling date. Lipid contents obtained with the gravimetric method ranged between 17 and 88% of copepod dry weight (Table 1).

There was no difference in total lipid content (%) between co-occurring *N. flemingeri* and *N. plumchrus*:

	GOA, 16 May	Cape Cleare, 1 June
N. flemingeri	84.10	41.01
N. plumchrus	84.64	40.21

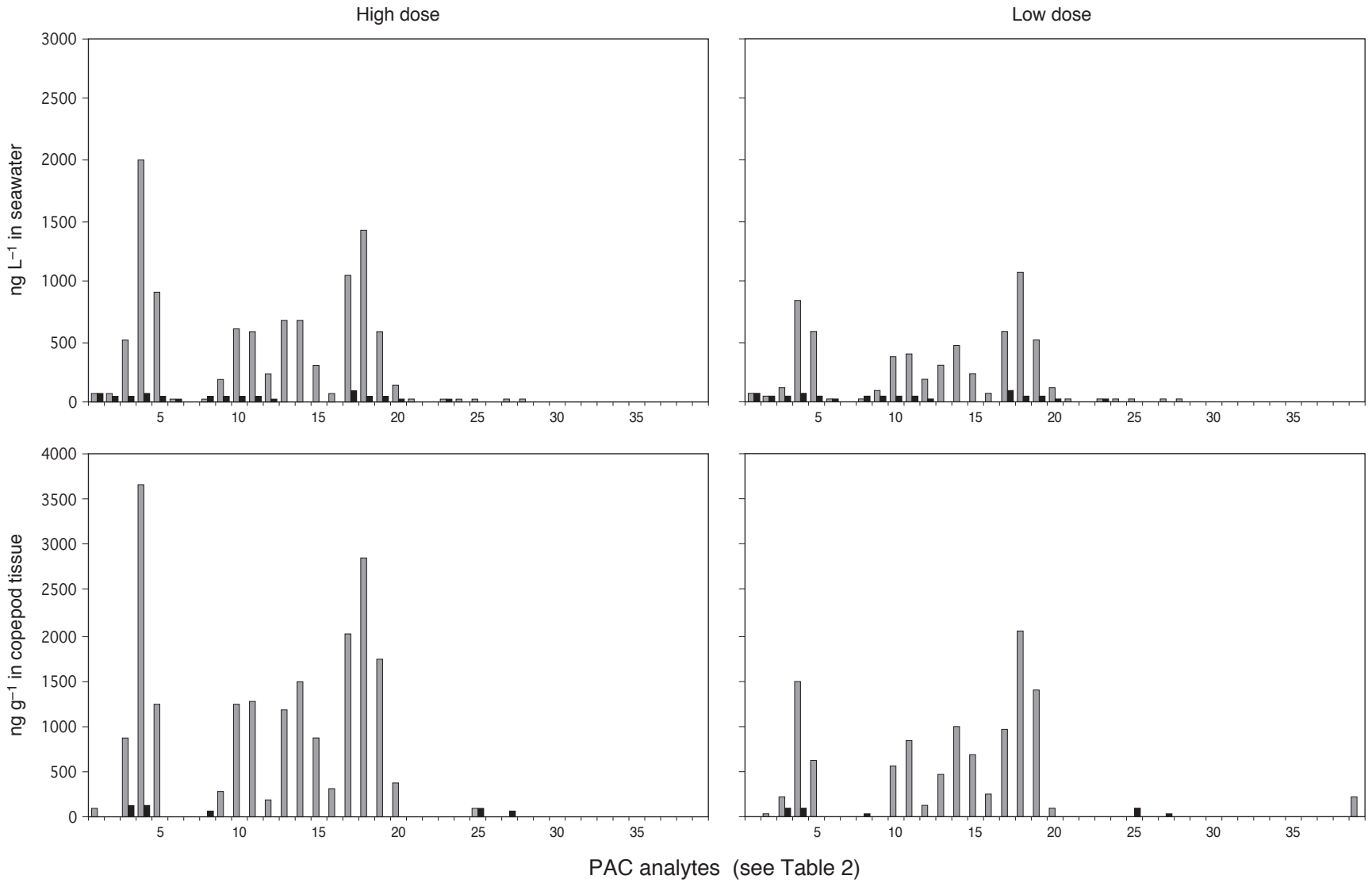

Figure 2. Concentrations of PAC analytes in exposure seawater (n = 2) and copepod tissue (n = 3) on a dry weight basis in experiment 2. Gray bars indicate concentrations in the high- and low-dose treatments; black bars are concentrations in the respective controls. Categories on the X-axes are the PAC analytes listed in Table 2 in sequential order from left to right.

On average, lipids were composed of 84 ± 10.3% wax esters, 8 ± 7% cholesterol, 9 ± 5% free fatty acids and no triacylglycerol (mean ± 1 standard deviation) (Figure 3). The difference in lipid composition between *N. flemingeri* (n = 15, sampled at 5 locations and dates) and *N. plumchrus* (n = 6, sampled at 2 locations and dates) was significant for the wax ester/cholesterol ester group and the cholesterols, but it was not significant when the Cape Cleare samples were excluded from the test. Copepods of both species from the Cape Cleare sampling location had a significantly higher free fatty acid component than those from the other sampling locations (t-test, $\alpha = 0.05$). If copepods from the same samples were compared, there was no difference between species.

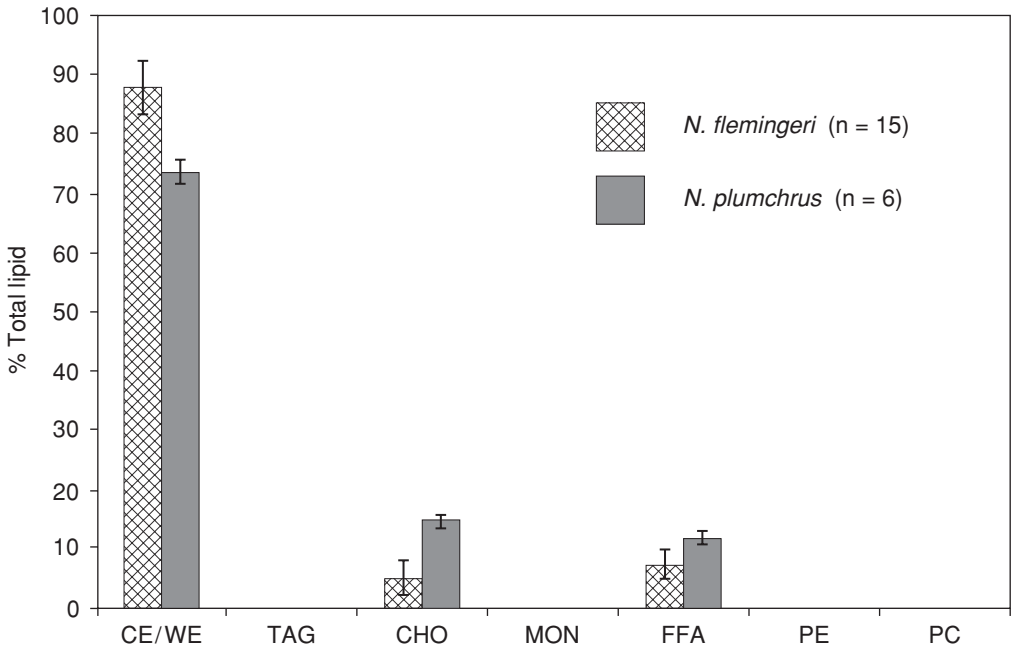

Figure 3. Proportions of the major lipid classes in *Neocalanus flemingeri* (n = 15) and *N. plumchrus* (n = 6). Bars depict standard deviations. See text for discussion of the significance of the difference between species. CE/WE = cholesterol esters, wax esters; TAG = triacylglycerides, CHO = cholesterol; MON = monoacylglycerides; FFA = free fatty acids; PE = phosphatidylethanolamine; PC = phosphatidylcholine.

Statistical analysis

The regression equations of bioaccumulation factors and total lipid content had a positive slope for the high- and low-dose treatments (Figure 1). These slopes were significantly different from zero (P = 0.59) in the high-dose treatment but not in the low-dose treatment. In the control treatment, the correlation coefficient was low (r = 0.08), compared to r = 0.33 in the high- and r = 0.46 in the low-dose treatments. Plots of jackknife residuals against the predicted value showed no violations of regression assumptions in any of the regressions.

Discussion

Bioaccumulation of dissolved PAC from the surrounding water by copepods was correlated with total lipid content. However, a positive linear relationship was significant only in the high-dose treatment. The correlation coefficients were 0.33 and 0.46 for the high-dose and low-dose treatments, respectively, and reflect the large spread of the data around the regression line. In contrast, there was a poor correlation ($r = 0.08$) and a slightly negative slope of the regression line in the control treatment. The similar slopes in the low- and high-dose regressions indicate that uptake occurred below saturation concentrations: BAF are expected to be constant at lower concentrations and decrease when saturation levels or lethal doses are approached. Lethal doses of oil to copepods were reported at seawater concentrations of 5–10 mg PAC L^{-1} [Spies 1987]. In comparison, PAC exposure concentrations in this study were lower by a factor of 1000. The formula for BAF considers both the exposure water concentration and the accumulated tissue concentration of PAC. BAF were not significantly different (t-test, $\alpha = 0.05$) between the low- and high-dose treatments, while a significant correlation existed between water and tissue PAC concentrations. Consequently, higher tissue concentrations in copepods can be expected when PAC exposure concentration, lipid content, or both, increase.

The spread in the data is largely a result of variation in copepod weights. Both the weights and the lipid contents varied greatly between sampling dates and locations. An assumed increase in lipid content of copepods between April and June was not supported by data. However, Russell Hopcroft (University of Alaska Fairbanks, unpublished data) observed that over all life stages, *N. flemingeri* sampled in PWS in May were consistently heavier than those sampled in April. While the assumption of seasonally increasing total lipid content may hold true for a local population, differences between sampling locations in the study region were more pronounced. For example, Cape Cleare copepods were smaller (mean prosoma length 4.16 mm, n = 10) and only 20% of the dry weight of PWS copepods (mean prosoma length 5.01 mm, n = 10). The great variation in weights and lengths was also reflected in datasets collected independently by researchers of the GLOBEC program (Chris Stark, University of Alaska Fairbanks, unpublished data). High interannual variation in dry weights and also in lipid-free dry weights of *N. flemingeri* in the GOA and a rapid increase in stored lipid between 8 and 18 May 1988 were reported earlier [Miller 1993].

The agreement in total lipid content and composition between co-occurring copepods of the two *Neocalanus* species in this study indicates no energetic difference. In the assessment of oil spill effects on copepods and the role of copepods in the possible transfer of oil to higher trophic levels, the two species could be treated as one. However, I caution against a general assumption of equal lipid content and composition for the two, because my conclusion is based only on two sampling locations, and differences may exist in other geographical regions. *Neocalanus* abundance shifted from exclusively *N. flemingeri* in spring to co-occurrence of *N. flemingeri* and *N. plumchrus* in mid-May and early June. This was in accordance with the reported life history analysis [Miller and Clemons 1988]. Total lipid content in *N. flemingeri* during May was measured and values ranged from 12 to 44% of dry weight [Miller 1993]. While the low value was attributed to an early developmental stage, the high values were believed to be low relative to other years and calanoid copepods in general [Miller 1993]. In comparison, total lipid content of *N. flemingeri* in this study ranged from 17 to 88%. However, in the calculation of lipid content, a higher copepod dry weight results in a lower estimate of total lipid content. If copepod weight increased in the two weeks between collection of the lipid and dry weight samples for experiment 1 (see methods), the calculated low value of 17% lipid may be slightly underestimating the true lipid content of this sample. Similarly, it is possible that the high value of 88% is a slight overestimation. However, two additional samples of *N. flemingeri* and *N. plumchrus*, obtained in the GOA during May, were in the 80–85% range. The lipid class composition of co-occurring *N. flemingeri* and *N. plumchrus* in this study was not significantly different. However, the ratio of wax esters to free fatty acids and triacylglycerides changes during gonad development and egg production [Sargent and

Falk-Petersen 1988]. Consequently, because of the differently phased life histories of the two species, a dissimilarity in lipid class composition might be expected during other times of the year.

Neocalanus copepods may provide an important mechanism for the transfer of dissolved PAC from oil to higher trophic level consumers like fish. This research has demonstrated that *Neocalanus*, due to their large lipid stores, aid in the concentration of dissolved oil from the water. Early research has identified copepods as relatively insensitive to oil compared with other plankton organisms [Capuzzo 1987]. This insensitivity might be explained by the association of the oil-derived PAC with the wax esters in the lipid stores of the copepods, which remain metabolically inactive until onset of gonad development and egg production. When freshly oiled plankton were fed to pink salmon, negative effects on growth and survival of the fish were observed [Carls et al. 1996]. The retention time of passively accumulated oil from aqueous solution in copepods remains to be investigated.

Acknowledgments

Funding for this project was provided by the University of Alaska Coastal Marine Institute and the Oil Spill Recovery Institute. Substantial in-kind support and professional advice were contributed by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory: We particularly thank Stanley Rice and Jeff Short for their assistance. Thanks also to Marie Larsen, Larry Holland and Josephine Lunasin for many hours of training in the chemical analysis. Russell Hopcroft from the University of Alaska Fairbanks helped with crucial logistics and is thanked for sample collection and for sharing his data on *Neocalanus* length/weight regression. Chris Stark from the University of Alaska Fairbanks shared his collection of *Neocalanus* wet weight data. The UAF Institute of Marine Science Seward Marine Station, is thanked for laboratory space, housing and technical support on short notice. Rebecca Zeiber was a tireless helper during the experimental phase.

Chapter 3. Photoenhanced Toxicity of Weathered Alaska North Slope Crude Oil to the Calanoid Copepods *Calanus marshallae* and *Metridia okhotensis*[1]

Abstract

This study investigated the synergistic toxicity of aqueous polyaromatic compounds (PAC) dissolved from crude oil and ultraviolet radiation (UV) in natural sunlight to the calanoid copepods Calanus marshallae *and* Metridia okhotensis. *These copepods were first exposed to low doses (~2 μg of total PAC L^{-1}) of the water-soluble fraction of weathered Alaska North Slope crude oil for 24 h and subsequently to low or high levels of natural sunlight. Responses included mortality, impairment of swimming ability and discoloration of lipid sacs. There was 80–100% mortality and morbidity of* C. marshallae *exposed to UV and oil, compared to a less than 10% effect in oil-only or UV-only treatments. In* M. okhotensis *100% mortality occurred in the UV and oil treatment, 43% mortality and 27% morbidity in the UV-only treatment, and a less than 5% effect in the oil-only treatment. Bioaccumulation factors were ~8000 for* C. marshallae *and ~2000 for* M. okhotensis. *The interaction of the effect of PAC and UV radiation was highly significant ($P < 0.005$) in both experiments.*

Introduction

Toxicological studies used to define the hazards of polycyclic aromatic compounds (PAC) and oil have usually been conducted under laboratory lighting with minimal ultraviolet radiation (UV) [Arfsten et al. 1996]. Recent studies have established that PAC derived from petroleum sources cause toxicity to aquatic fauna that is enhanced 2-fold to greater than 100-fold by exposure to UV [Pelletier et al. 1997; Cleveland et al. 2000]. Photoenhanced toxicity occurs when organisms are exposed to the UV component of sunlight following tissue accumulation of PAC (photosensitization) or when dissolved PAC are photochemically transformed to compounds of higher toxicity and subsequently absorbed by the organism (photomodification). Some PAC can catalyze the production of electronically excited molecular oxygen by transfer of energy initially absorbed by the PAC from UV [Landrum et al. 1987; Choi and Oris 2000]. The excited oxygen may then increase rates of nonspecific oxidation within tissues. Translucent biota inhabiting the upper water column or the intertidal and shallow subtidal epibenthos are exposed to UV in sunlight and may encounter PAC dissolved from chronic or catastrophic oil pollution sources.

Previous laboratory studies have used laboratory-cultured test species, lengthy durations of UV exposure (e.g., 30–100 h) and aqueous PAC extracts prepared by slowly stirring freshwater or seawater beneath thick surface slicks of oil where the ratio of surface area to volume of the oil $(S/V)_{oil}$ is relatively low (~2 cm^{-1}). These mixing conditions favor PAC extracts that are especially enriched in smaller PAC that dissolve more rapidly (e.g., naphthalene homologues [Short and Heintz 1997]) but are not phototoxic. In contrast, the $(S/V)_{oil}$ of oil slicks resulting from oil spills is usually much higher (~200 cm^{-1}) than those typically used to prepare laboratory test solutions. This higher relative surface area accelerates the dissolution rates of PAC, which can lead to higher concentrations of 3- and 4-ring PAC, some of which are phototoxic. We therefore conducted experiments reported below to investigate the photoenhanced toxicity of PAC extracted from weathered crude oil under conditions of high $(S/V)_{oil}$ to two ecologically

---

[1] Duesterloh, S., J.W. Short and M.G. Barron. 2002. Photoenhanced toxicity of weathered Alaska North Slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. Environmental Science and Technology 36(18):3953–3959.

Jeffrey W. Short, Auke Bay Laboratory, Alaska Fisheries Science Center, NMFS/NOAA, 11305 Glacier Highway, Juneau, AK 99801-8626; Mace G. Barron, P.E.A.K. Research, 1134 Avon Lane, Longmont, CO 80501

important and vulnerable marine species of calanoid copepods under environmentally realistic exposure conditions.

Calanoid copepods occupy an important niche in marine food webs because they ingest a substantial proportion of annual primary production in the temperate and subarctic waters of the North Atlantic and North Pacific Oceans [Parsons and Lalli 1988] and so account for the majority of the secondary production on a biomass basis in these waters. Copepod abundance also influences the density and composition of phytoplankton through grazing and nutrient recycling [e.g., Mauchline 1998]. As secondary production, they are prey for most of the higher trophic level species, either directly or indirectly; for example, forage or juvenile fishes are usually zooplanktivorous and are themselves prey for piscivorous fishes and marine mammals. The most ecologically important genera of the calanoid copepods in this respect include *Calanus*, *Neocalanus*, *Metridia*, and *Pseudocalanus* and can constitute >60–70% of zooplankton biomass [e.g., Mauchline 1998]. These copepods are all translucent, and the advanced life stages of many are exposed to UV light while grazing on phytoplankton blooms near the sea surface during daylight [Hays 1995]. Many of these copepods, especially in the genera *Calanus* and *Neocalanus*, accumulate stores of lipids in their later life stages that may exceed 60% of their dry weight [Kattner et al. 1994; Evanson et al. 2000; Sargent and Falk-Petersen 1988] and may bioaccumulate substantial burdens of lipophilic pollutants such as PAC through equilibrium partitioning. Calanoid copepods thus include ecological key-role species that may be especially vulnerable to photoenhanced toxicity of PAC derived from petroleum products.

In this study we evaluated the photoenhanced toxicity of low doses (~2 μg of total PAC L^{-1}) of weathered Alaska North Slope crude oil to two species of calanoid copepods, *Calanus marshallae* and *Metridia okhotensis*, field-collected in Alaska waters. A new continuous-flow PAC-exposure system was developed for these exposures. Exposure durations to PAC solutions and subsequently to natural sunlight at a subarctic latitude were less than 24 and 8 h, respectively, and included one test where the natural sunlight was attenuated by cloud cover during a rainy day. These exposure conditions and test species all occurred concurrently during the *Exxon Valdez* oil spill in Prince William Sound, Alaska, so our experiments simulate conditions that may be encountered in the field.

Materials and Methods

We performed two successive experiments, which were identical in all respects except for the UV exposure and the number of copepod species used. A 24-h exposure to oiled seawater treatments in an indoor flow-through system was followed by a static outdoor UV exposure. Each experiment included three treatments: (1) exposure to UV radiation but no exposure to PAC (denoted as UV-only) (Figure 4, treatment 1a + 1b), (2) exposure to PAC dissolved from crude oil but no exposure to UV radiation (denoted as oil-only) (Figure 4, treatment 2a + 2b), and (3) exposure to PAC followed by exposure to sunlight radiation (denoted as oil+UV) (Figure 4, treatment 3a + 3b). In each experiment one composite sample of copepods was frozen immediately following the PAC exposure for later evaluation of tissue PAC content (Figure 4, treatment 4a + 4b). Copepods in the oil treatments were exposed to about 2 μg of total PAC L^{-1} in both experiments. In experiment 1 (high-UV experiment) we exposed 15–16 *C. marshallae* per flask to bright natural sunlight for 3.8 h. In experiment 2 (low-UV experiment) we exposed 22–23 *C. marshallae* per flask and 31 and 40 *M. okhotensis* per flask to 8.2 h of cloud-attenuated sunlight. The number of copepods per flask differed because of variable availability of freshly caught organisms. After the UV exposure all copepods were evaluated for their biological response, then transferred to clean seawater and checked for delayed effects the following day.

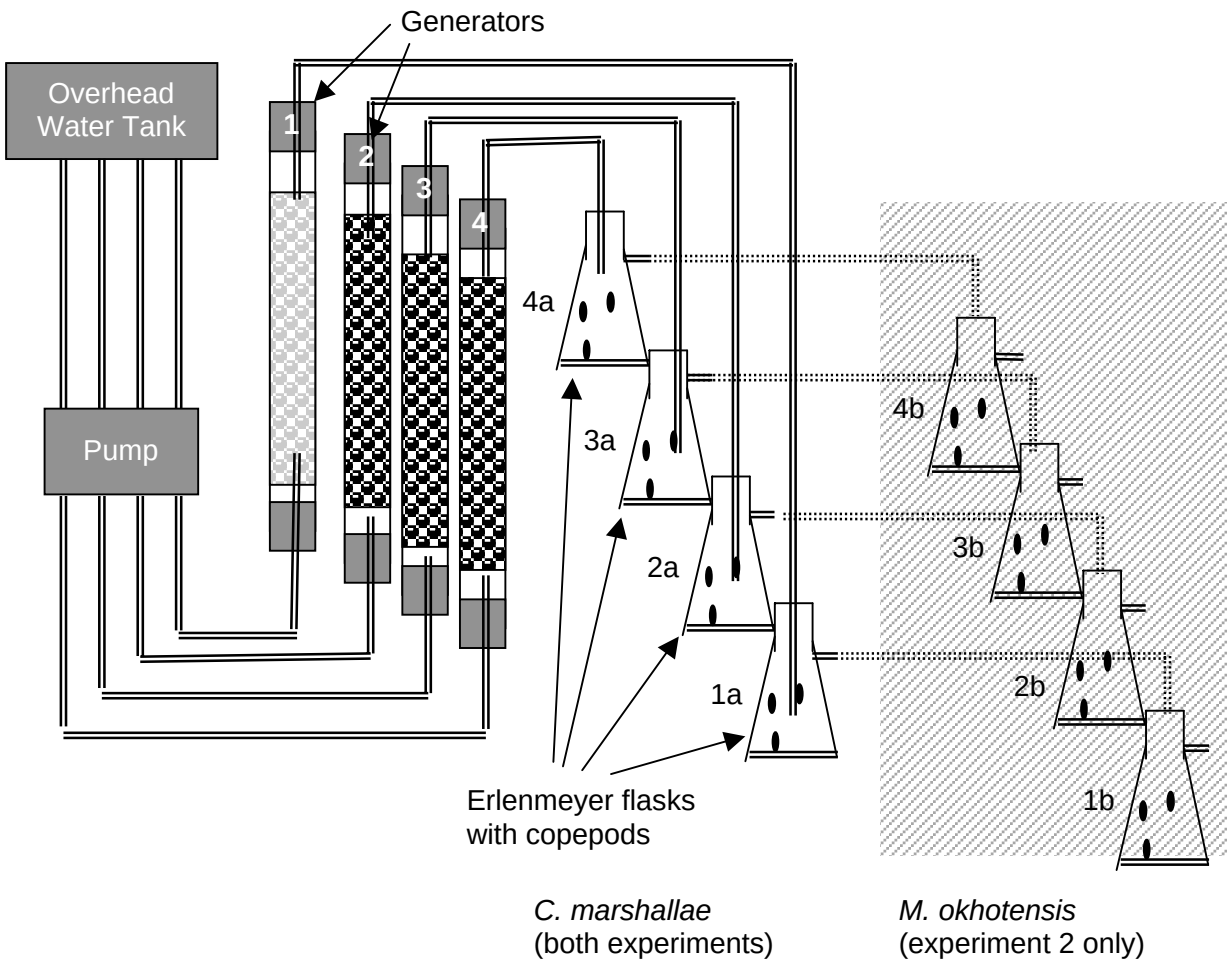

Figure 4. Diagram of the flow-through oil exposure system. Seawater is pumped from an overhead tank through generators filled with oil-coated glass beads to achieve consistent low-dosage exposure of organisms in Erlenmeyer flasks. Generating columns 2–4 contain oiled glass beads; column 1 contains unoiled beads for control treatment. In experiment 2 the effluent from Erlenmeyer flasks 1a, 2a, 3a and 4a is directed into Erlenmeyer flasks 1b, 2b, 3b and 4b, respectively.

Animal collection

C. marshallae and *M. okhotensis* were collected in Lynn Canal and adjacent Barlow Cove in southeastern Alaska. They were pipetted from zooplankton collected with a 330-µm mesh plankton net towed vertically from a maximum depth of 120 m. The fifth copepodite stage (CV) of *C. marshallae* was identified by microscopic examination and selected for testing. Live specimens of *M. okhotensis* were not distinguished to life stage, but the population sampled contained copepodites at least 3.5 mm long (total length) and consisted mostly of stage CV and adults. Copepods were subsequently stored at 6 °C for about 24 h until start of the oil exposure.

Oil exposure

The Alaska North Slope crude oil was weathered by heating and overnight stirring at 80 °C to 20% weight loss, which removed most monocyclic aromatic compounds, and then added to 3-mm-diameter glass beads at an application rate of 2.6 g oil kg^{-1} of beads. The oiled beads were tumbled for approximately 24 h, spread to a single layer and left under a hood for 4 days at 25 °C to allow the oil to harden onto the beads, and then were stored at –20 °C until use.

Three generating columns that produced aqueous solutions of PAC dissolved from crude oil were constructed by placing 100 ml of the oiled beads inside 25-cm long by 2.5-cm inner diameter (i.d.) glass columns stoppered at each end by a glass plug and a piece of plankton mesh (505 μm) followed by a neoprene stopper penetrated by a 2.8-mm i.d. glass tube. For UV-only treatments a separate column was constructed in the same way except that the glass beads were not coated with oil. A dilute solution of PAC was prepared by pumping natural seawater (30‰, 10 ± 1°C) through the columns at a 5 ± 0.5 ml min^{-1} flow rate by peristaltic pump. The effluent from the columns was directed through glass tubing into 2-L Erlenmeyer filtration flasks for 20 h to rinse the exposure apparatus, followed by introduction of 15–40 copepods to each flask, depending on species and experiment. The ratio of total copepod wet weight and the exposure volume was less than 0.05 g L^{-1}. The exposure flasks were fitted with a small nylon screen to prevent copepod escape, and samples of flask effluents were collected at the start and end of each experiment to measure PAC doses. At the end of the oil exposure, copepods collected for analysis of tissue PAC content (Figure 4, treatment 4a + 4b) were immediately frozen at –20 °C. Oil exposures and all handling procedures were conducted under fluorescent indoor lightning with negligible UV.

UV exposures

For the UV exposures, test flasks were placed in an outdoor water bath (10 ± 1°C). In experiment 1, the temperature in the flasks was 10 ± 1°C; in experiment 2, the temperature was 14 ± 1°C. The difference corresponded to the difference in water temperature in Auke Bay. Dissolved oxygen in the flasks was not measured in experiment 1; in experiment 2 it was 17 ± 0.01 mg L^{-1} for all treatments of *C. marshallae* and 13.2–14.6 mg L^{-1} in the *M. okhotensis* flasks. The dissolved oxygen content of saturated seawater was 18.4 mg L^{-1}. Water levels in the flasks and waterbath were approximately equal. Attenuation of light by the borosilicate glass flasks was 3% for visible light, 16% for UVA, and 64% for UVB. The percentage attenuation from the flask was determined from the difference in UVA, UVB and visible light measured by an Optics S2000 photodiode array spectrometer (Ocean Optics, Inc.; Dunedin, FL) inside and outside the flask. The fiber optic cable with a cosine correcting diffuser was inserted through a small hole drilled in the bottom of the flask so that the surface of the diffuser was slightly above the bottom of the flask. Measurements were made in rapid succession under natural sunlight during cloudless conditions to ensure constant solar radiation. The oil-only exposure flasks were wrapped in aluminum foil to exclude all light. High-UV exposures (experiment 1) were conducted on 13 July (12:38 to 16:27), and low-UV exposures (experiment 2) were conducted on 19 July (11:38 to 19:47) 2000 in Juneau, Alaska. Thirteen July was cloudless, and 19 July was heavily overcast with 1.4 cm of rainfall. The duration of the low-UV exposure was longer, to provide approximately 50% of the high UV exposure. UV intensity was continuously monitored at the water bath level with a 5-channel radiometer (GUV-511: Biospherical Instruments; San Diego, CA) linked to a computer. Average UVA (320–400 nm) and UVB (280–320 nm) intensities (μW cm^{-2}) during sunlight exposures were estimated by summing the measured UV intensity for each channel and the interpolated UV intensity outside of each bandpass. The total UV dose (μW • h • cm^{-2}) was determined from the average UV intensity and the duration of sunlight exposure.

Biological response measurement

Immediately following UV exposure, all copepods were individually evaluated. If the initial escape response was slow, the copepods were investigated microscopically with regard to swimming behavior and mobility of appendages. Copepods were categorized as unaffected, impaired in their swimming

ability, or dead. Impaired copepods were unable to move their antennules or had no use of antennules and pleiopods. Affected individuals were examined microscopically for evidence of tissue damage. Subsequently, all live specimens were transferred to filtered seawater. The evaluation procedure was repeated 17.5 h after the end of the high-UV exposure and 22.5 h after the end of the low-UV exposure.

Chemical analysis

Procedures for the quantitative determination of PAC in water and in tissues were described by Short et al. [1996]. Seawater samples (0.9 L) were extracted twice with 100 ml of dichloromethane. Copepods were pulverized in a porcelain grinder three times each with 1 ml of dichloromethane. Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath, followed by fractionation and purification by alumina/silica gel chromatography. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). PAC analytes included dibenzothiophenes and polyaromatic hydrocarbons (PAH) containing 2–5 rings, including the alkylated homologues listed in Figure 5.

All tissue concentrations above method detection limits are reported on a dry-weight basis. A method blank, spiked method blank, and two reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision and absence of laboratory-introduced artifacts and interferences. Detection limits were determined experimentally [Glaser et al. 1981] for PAC and generally were 5–20 ng of PAC L^{-1} seawater or 0.05–0.2 ng of PAC copepod^{-1}. Concentrations below the detection limit were treated as 0.

Data analysis

Concentrations of total PAC were calculated by summing the concentrations of each of the PAC above the method detection limit. The relative concentrations of PAC were calculated as the ratio of each respective PAC concentration to the total PAC concentration. Water samples were collected at the beginning and end of the exposure period from each generating column in each experiment. Means of PAC in these samples are presented in Figure 5a, where samples from columns 2–4 (Figure 4) were treated as replicates (total of 12 samples). The differences in total PAC measured at the beginning and end of each experiment were evaluated by Student's t-test. Biological response data were tabulated as proportions, and 95% confidence limits for proportions were derived from binomial confidence limit tables (Figure 6) [Freund 1979]. The use of the binomial distribution requires the assumptions that each copepod has an equal probability of death from the treatment and that copepod deaths are independent of each other. Significance of the interaction of the effects of oil and UV exposure was tested with multivariate contingency tables using χ^2 statistics; the hypothesis of independence was rejected at the $P < 0.05$ significance level [Dixon and Massey 1957]. For the purpose of this test, biological response data were grouped into "unaffected" and "affected" (impaired or dead).

The concentrations of PAC in copepods were calculated as the ratio of the amount of analyte per individual and the dry weight of individuals given in the literature. We used 175 μg per individual for the dry weight of *C. marshallae* CV [from Mauchline 1998]. For *M. okhotensis*, we assumed a mean stage of CV and used a wet weight per individual of 1.48 mg [Coyle et al. 1990] and a ratio of dry to wet weight of 18.8% [Table 4 in Omori 1969] to estimate a dry weight of 278 μg per individual.

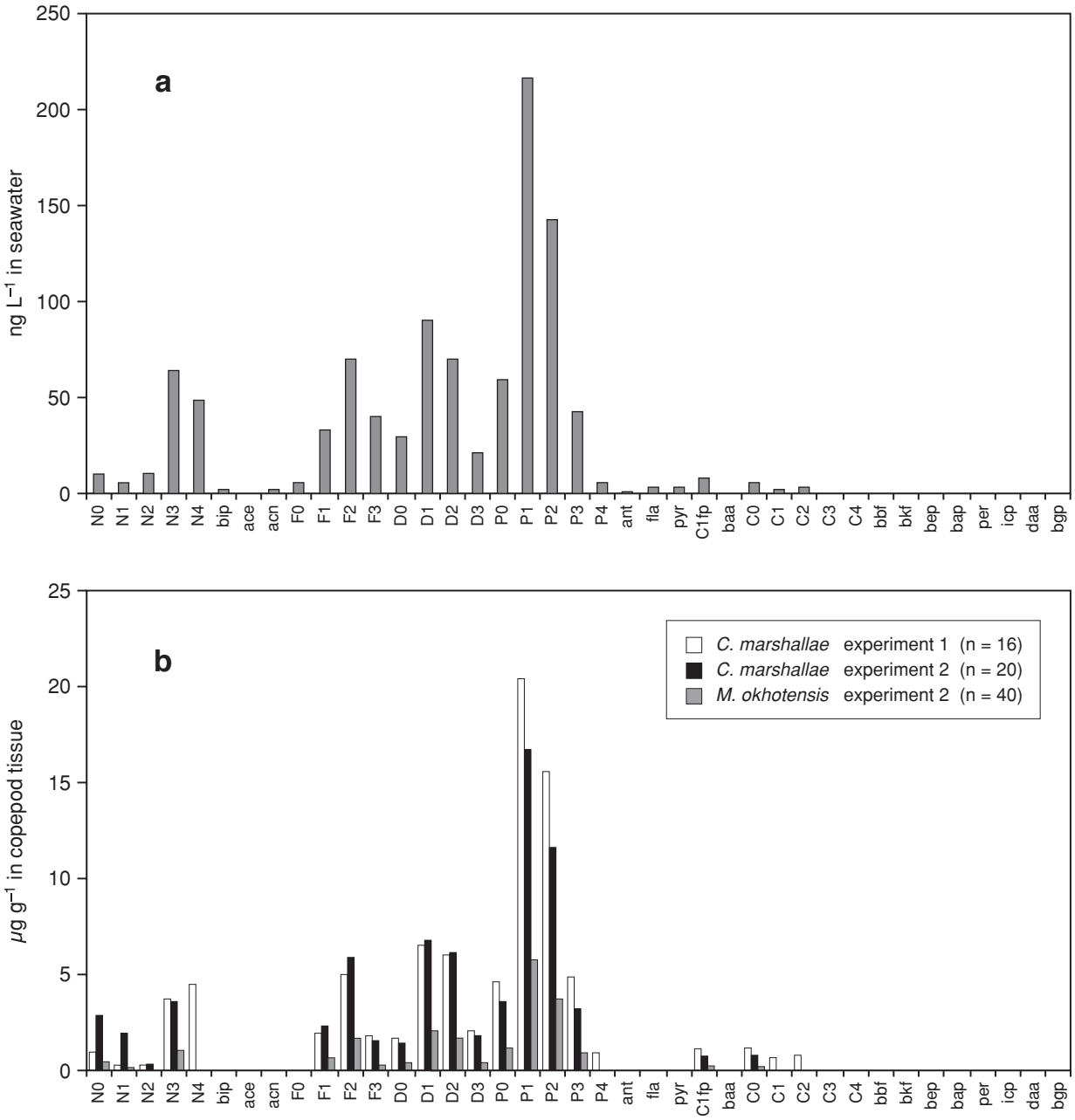

Figure 5. a) Proportions of PAC analytes in exposure seawater (n = 6 replicate samples; these were collected at the start [n = 3] and end [n = 3] of the 24-hr exposure time from generators 2, 3 and 4 [see Figure 4]). Ranges are depicted by bars but are very small (between 0 and 4%). b) Proportions of PAC analytes in 2 samples of *C. marshallae* (white bars = high UV, black bars = low UV) and 1 sample of *M. okhotensis* (gray bars). Samples were collected before UV exposure: n = sampling units in samples; N, F, D, P and C refer to naphthalene, fluorene, dibenzothiophene, phenanthrene and chrysene, respectively; and the numbers following these letters indicate the number of alkyl-substituent carbon atoms. Other PAC are abbreviated as follows: bip = biphenyl, ace = acenaphthylene, acn = acenapthene, ant = anthracene, fla = fluoranthene, pyr = pyrene, C1fp = C1 fluoranthenes/pyrenes, baa = benz[a]anthracene, bbf = benzo[b]fluoranthene, bkf = benzo[k]fluoranthene, bep = benzo[e]pyrene, bap = benzo[a]pyrene, per = perylene, icp = indeno-1,2,3-c,d-pyrene, daa = dibenzo[a,h]anthracene, bgp = benzo[g,h,i]perylene.

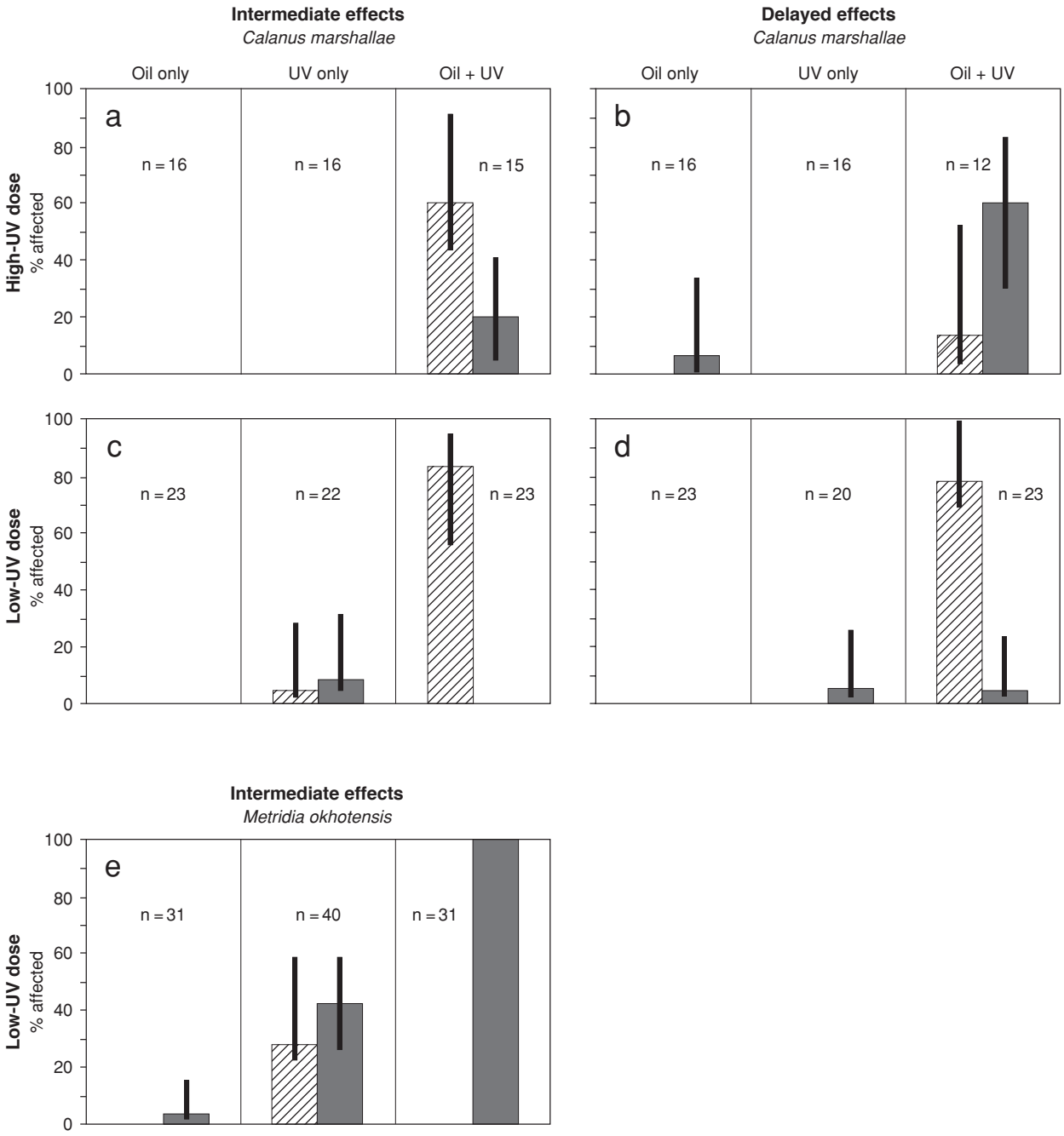

Figure 6. Biological responses in phototoxicity experiments: dark gray bars, dead; cross-hatched bars, impaired (unable to move swimming appendages). Bars depict binomial confidence limits. n = sampling units in sample; a) *Calanus marshallae*: 24-h oil exposure followed by 3.8-h UV exposure on a sunny day; b) as in panel a but after 17.5-h depuration. c) *C. marshallae*: 24-h oil exposure followed by 8.2-h UV exposure on a rainy day; d) as in panel c but after 22.5-h depuration. e) *Metridia okhotensis*: 24-h oil exposure followed by 8.2-h UV exposure on a rainy day.

Results

PAC exposures and uptake

The solution of PAC in seawater produced by the oil exposure apparatus (Figure 4) consisted mainly of three-ring PAC (Figure 5a), at total PAC concentrations near 2 μg L^{-1}. The total PAC concentration increased from 1.55 ± 0.65 μg L^{-1} (n = 3) at the beginning of experiment 1 to 2.64 ± 0.38 μg L^{-1} (n = 3) at the end, a marginally significant (P = 0.051) change. During experiment 2 the total PAC concentration change was insignificant (P = 0.77) and averaged 2.08 ± 0.43 μg L^{-1} (n = 6). In the aqueous-phase exposures, known phototoxic PAC such as anthracene, fluoranthene and pyrene had low concentrations relative to the measured total PAC.

Both *C. marshallae* and *M. okhotensis* accumulated high concentrations of total PAC from the exposure seawater. The histogram in Figure 5b shows the PAC proportions in tissues in one sample of *M. okhotensis* and two samples of *C. marshallae* taken at the end of the 24-h oil exposures. The total PAC concentrations in *C. marshallae* were 85.4 and 71.7 μg g^{-1} dry weight at the end of the oil exposure in experiments 1 and 2, respectively, compared to a concentration of 21.3 μg g^{-1} dry weight in *M. okhotensis* at the end of the oil exposure in experiment 2. The composition of the PAC accumulated by the copepods was nearly identical with the PAC composition of the exposure seawater (Figure 5). The apparent bioaccumulation factor was approximately 8000 on a wet weight basis for *C. marshallae* (averaged for the two samples) and approximately 2000 for *M. okhotensis.*

UV exposures

Experiment 1 (high-UV exposure) had 4–5 times the UVA and UVB intensity of experiment 2 (low-UV exposure) and approximately 2 times the UV dose because of the shorter exposure duration (Table 3).

Table 3. Summary of UVA and UVB intensity and total dose (duration × intensity) in the high and low sunlight exposures (measured in air).

Experiment	Environmental Conditions	Exposure Duration (h)	Average Intensity μW cm^{-2}		UV Dose μW • h • cm^{-2}	
			UVA	UVB	UVA	UVB
High-UV	sun, no clouds	3.8	3731	60.9	14253	233
Low-UV	clouds, haze, rain	8.2	761	16.8	6202	137

Photoenhanced toxicity to C. marshallae

In experiment 1 (high-UV exposure), more than 80% of the *C. marshallae* copepods exposed to oil+UV were affected. Of these, 60% were impaired or immobile and 20% were dead. In contrast, none of the copepods were affected when exposed to oil-only or UV-only (Figure 6a). Two-thirds of those copepods impaired or immobile at the end of the UV exposure died by the following day in this experiment (6 animals), while one other impaired copepod recovered (Figure 6b). In experiment 2 (low-UV exposure), no immediate mortality was observed for *C. marshallae* in the oil+UV treatment, but about 85% were immobile by the end of the UV exposure (Figure 6c). Only one copepod immobile at the end of the UV exposure died by the following day, while three immobile copepods recovered the ability to move their swimming appendages but not their antennules (Figure 6d). In contrast, less then 15% of the

copepods were affected when exposed to oil-only or UV-only, similar to the high-UV experiment (Figure 6a, c). *C. marshallae* in the oil+UV treatment that were impaired or immobile had opaque rather than transparent lipid sacs, whereas copepods in the oil-only or UV-only treatments had transparent lipid sacs (Figure 7).

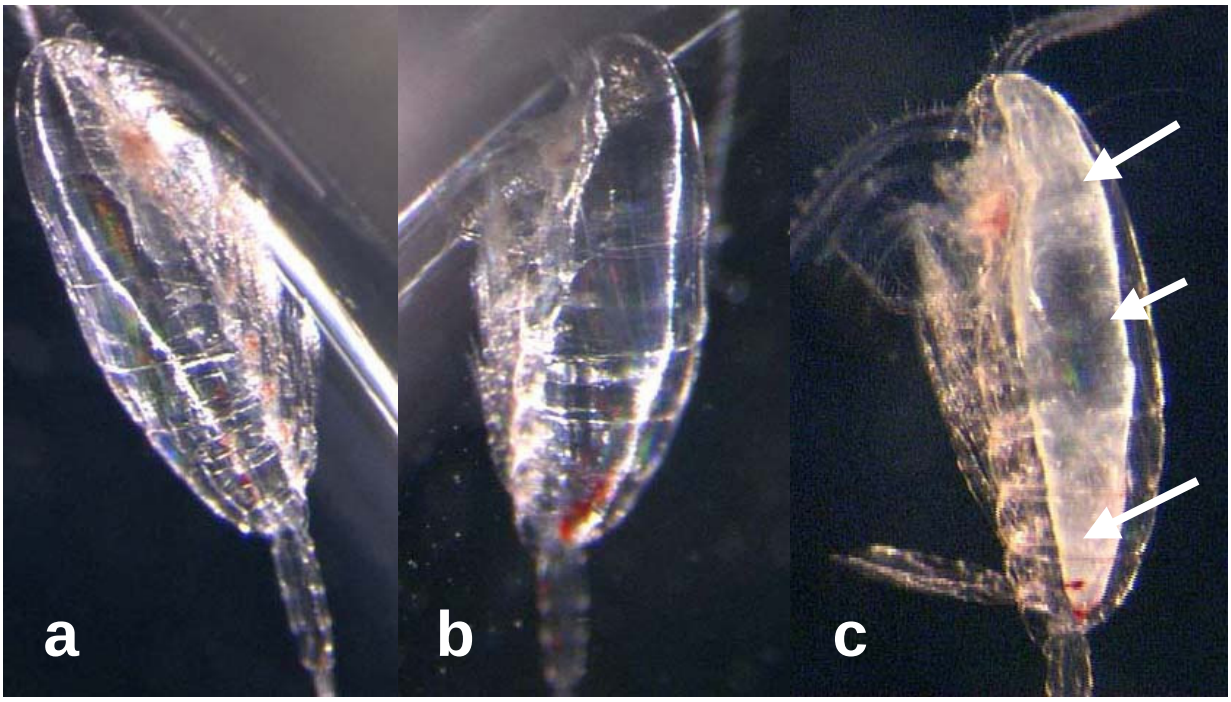

Figure 7. Photos of *Calanus marshallae* oil sacs: a) after 24-hr exposure to oil only, b) after 8.2-hr exposure to low UV only, c) after 24-hr exposure to oil followed by 8.2-hr low UV exposure. Lipid sacs appear clear in a and b, opaque in c (arrows point to coloration of lipid sac).

Photoenhanced toxicity to M. okhotensis

The pattern of results for *M. okhotensis* was similar to that of *C. marshallae*, except a higher UV sensitivity was observed (Figure 6e). All copepods (n = 31) in the oil+UV treatment were dead by the end of the UV exposure. In the UV-only treatment (n = 40) 70% were affected (17 died and 11 were either immobile or impaired in their swimming ability), with 12 copepods being unaffected. Five percent were affected in the oil-only treatment (n = 31). For all experiments, interactions between oil and UV exposures were highly significant (χ^2 test, $P < 0.005$), clearly indicating photoenhanced toxicity.

Discussion

Phototoxicity of Alaska North Slope crude oil

Our results demonstrate that PAC dissolved from crude oil are phototoxic to subarctic marine copepods at aqueous PAC concentrations that would likely result from an oil spill and at UV radiation intensities that would often be encountered in nature. Although our experimental treatments were not replicated within the high- or low-UV experiments, the results of the two sequential experiments with *C. marshallae* may be considered as a duplicated experiment in which one of the dosage treatments (UV exposure) is affected by a random variable (sunlight intensity). The highly significant interaction between oil exposure and subsequent UV exposure in both experiments is compelling evidence of photoenhanced toxicity to *C. marshallae*. The similar pattern of results with *M. okhotensis* is additional confirmation of these effects and suggests that different species of translucent copepods may be vulnerable to photoenhanced toxicity if exposed to environmentally realistic concentrations of PAC and UV radiation in the upper water column. For example, total PAC concentrations exceeding 2 $\mu g\ L^{-1}$ have been measured beneath oil slicks following catastrophic oil spills [Short and Harris 1996; Neff and Stubblefield 1995; Law 1978], and the UV intensities in the present study were within the expected ranges for the environmental conditions, time of year, and latitude of the exposures: The average UVA intensity of the high-UV exposure (3700 $\mu W\ cm^{-2}$) is equivalent to 18% attenuation of surface sunlight at solar noon on a clear day in Prince William Sound, Alaska [Barron and Ka‘aihue 2001]. The low-UV exposure (average UVA intensity of 761 $\mu W\ cm^{-2}$) is comparable to UVA levels reported for highly attenuated aquatic habitats [Barron et al. 2000]. UVA appears to be the most active region of the light spectrum for photoenhanced toxicity, whereas UVB appears responsible for the majority of biological injury from UV-only exposure [Diamond et al. 2000].

Photoenhanced toxicity has been reported from single-compound studies for several aquatic organisms [e.g., Pelletier et al. 1997; Landrum et al. 1987; Little et al. 2000; Ren et al. 1994; Oris and Giesy 1987; Bowling et al. 1983; Morgan and Warshawsky 1977], but limited literature is available on phototoxicity of crude or refined oils [Calfee et al. 1999]. While lethal concentrations of total PAC to some copepod species are reported on the order of 0.05 to 9.4 $mg\ L^{-1}$, we found phototoxic concentrations to be lower by a factor of 23 to >4000 [Spies 1987]. Phenanthrenes were the most abundant PAC analyzed in copepod tissue in this study and, like fluorenes and naphthalenes, are not photosensitizers [Arfsten et al. 1996; Ankley et al. 1994; Veith et al. 1995]. Photoproducts of phenanthrenes, however, were more toxic than the parent compound in tests with the diatom *Phaeodactylum tricornutum* [Wiegman et al. 1999]. Fluoranthenes and pyrenes are highly phototoxic to bivalves and mysids [Pelletier et al. 1997], but their concentrations were below the method detection limit in copepods (Figure 5). The phototoxicity of dibenzothiophenes is uncertain, and chrysenes are the only known phototoxic agents detected in copepod tissue in this study. Clearly more work is needed to identify the phototoxic compounds in crude oil.

Bioaccumulation of PAC

Organisms with a high ratio of surface area to volume rapidly accumulate PAC [Southworth et al. 1978]. The similarity of the PAC compositions in the exposure water and in the copepod tissue at the end of the exposure indicates that the PAC were passively accumulated from the water without selective uptake (Figure 5). Copepods may thus form an important and largely unrecognized ecological compartment for the accumulation of PAC from the water and the transfer of PAC to higher trophic level consumers. The higher PAC concentrations found in *C. marshallae* (accumulation factor 8000) compared with *M. okhotensis* (accumulation factor 2000) may reflect different sizes of lipid pools. Bioaccumulation of polyaromatic hydrocarbons in *Daphnia pulex* was described in terms of lipid/water PAH partitioning and a linear relationship between the n-octanol–water partition coefficient and the concentration factor for several PAH was reported [Southworth et al. 1978]. Consistent with the results for the marine copepods, accumulation factors of PAH in *D. pulex* ranged from 100 to 10,000 and equilibrium concentrations were approached within 24-h exposure periods [Southworth et al. 1978]. Both copepod species have high

surface-area-to-volume ratios and would therefore approach equilibrium rapidly. But *Calanus* and *Neocalanus* copepods have higher bioaccumulation capacities for PAC because of their characteristically higher total lipid content. Most estimates for lipid content in *Daphnia* fall in the 1–3.5% range [Southworth et al. 1978], while total lipid content in high-latitude copepod species varies seasonally from 8% to 73% [Mauchline 1998].

Tissue damage

In many copepods lipid is accumulated during the spring feeding season and stored internally as wax esters [Hagen 1988]. The highly lipophilic PAC are accumulated by organisms and absorbed into fat tissue [Landrum et al. 1987]. The opaque appearance of the lipid sac of *C. marshallae*, observed in the oil+UV treatment, may be indicative of severe lipid damage resulting from lipid peroxidation caused by singlet oxygen as has been shown in fish [Choi and Oris 2000]. In comparison, copepods in the oil-only and UV-only treatments had transparent lipid sacs (Figure 7). Light scattering occurs when molecular clusters in the path of the light are larger than the wavelength of the penetrating light. Thus, formation of larger molecular structures must have occurred in the lipid sac or its surrounding membrane to cause the observed change from a transparent to a cloudy appearance. This observation may hold promise for a method to assess damage from photoenhanced oil toxicity to surface-feeding copepod swarms in their natural environment. More research is necessary to determine whether the tissue damage from lipid peroxidation is consistent and discernible in preserved samples. Also, although this interpretation suggests a photosensitization mechanism, our experiments were not designed to explain the mechanism of phototoxicity involved. Photosensitization was identified as the main mechanism causing photoenhanced toxicity in various invertebrates [Pelletier et al. 1997; Bowling et al. 1983; Ankley et al. 1994; Allred and Giesy 1985], but several authors described photomodification as a cause of increased toxicity of PAC to plants [Wiegman et al. 1999; Huang et al. 1995; Marwood et al. 1999].

Ecological implications

Phototoxic effects on copepods could conceivably cause ecosystem disruptions that have not been accounted for in traditional oil spill damage assessments. Particularly in nearshore habitats where vertical migration of copepods is inhibited due to shallow depths and geographical enclosure, phototoxicity could cause mass mortality in the local plankton population. This lack of primary consumers could trigger initial increases in phytoplankton, but food depletion in juvenile or forage fish populations (e.g., salmon fry). The observed sublethal response of copepods to phototoxicity was an impairment of the escape response which may cause increased vulnerability to predators. The potential for photoenhanced toxicity to fish larvae from transfer of PAC from prey is currently unknown, although feeding on oil-contaminated prey in pink salmon fry has been reported to reduce growth rates [Carls et al. 1996]. Areas of future research should include identifying phototoxic compounds and quantifying injury to copepod populations.

Acknowledgments

This work was supported by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory; Oil Spill Recovery Institute; and University of Alaska Coastal Marine Institute. We would like to thank three anonymous reviewers for helpful comments on the manuscript. We thank Patrick Disterhoft and colleagues at the Surface Radiation Research Branch of NOAA, Boulder, CO, for use of the UV radiometer and their advice and assistance.

Chapter 4. The Interaction of Polyaromatic Compound (PAC) Concentration and Ultraviolet Radiation Dose in Phototoxic Effects on *Neocalanus* Copepods in the North Pacific[1]

Abstract

Phototoxicity of dissolved Alaska North Slope crude oil to Neocalanus *copepods was tested in experiments with concentrations ranging from 0.5 to 10 μg L^{-1} of total polyaromatic compounds (PAC) and ultraviolet radiation (UV) from ambient daylight exposures. The copepods were clearly sensitive to photoenhanced toxicity at these concentrations. The interaction of PAC and sunlight was highly significant ($P < 0.001$). No significant difference in effects existed between the full spectrum and the UVB-exclusion light treatments, indicating that UVA was the phototoxic region of wavelengths in these experiments. Phototoxic effects, recorded as the frequency of test copepods dead or impaired at the end of the UV exposure, were closely correlated with PAC concentration in the exposure water and the product of PAC concentration and light dose (intensity × exposure duration) in a linear relationship. This correlation was only slightly better when tissue residue concentrations were used ($r = 0.998$) than for water PAC concentrations ($r = 0.991$).*

Introduction

Photoenhanced toxicity of oil in the presence of ultraviolet radiation (UV) has been shown for many single compound polyaromatic hydrocarbons (PAH) and some crude oils and may occur at concentrations encountered in the environment [e.g., Oris and Giesy 1987; Pelletier et al. 1997; Little et al. 2000]. However, the extent to which the effect occurs in waters contaminated with polyaromatic compounds (PAC) and the ecological consequences are not well understood. Most studies of phototoxicity have been conducted under artificial laboratory UV light and with oil solutions with a relatively high proportion of lighter PAC compared to those created by weathered oil in the environment [Chapter 3/Duesterloh et al. 2002]. In this study we tested the interaction effects of various concentrations of dissolved weathered Alaska North Slope crude oil, high in phototoxic PAC, and subsequent exposure to sunlight with and without the UVB component, on the copepods *Neocalanus flemingeri* and *N. plumchrus*.

Copepods of the genus *Neocalanus* dominate the zooplankton biomass in the North Pacific, Gulf of Alaska (GOA) and adjacent inlets during several months of the year [Cooney 1988]. Their natural range includes clear ocean waters with maximum UV penetration properties, to highly productive inlets with ongoing oil exploration and high risk of PAC encounter. Many secondary consumers depend on *Neocalanus* as a large seasonal energy source, which is reflected in life history and behavior patterns that are geared towards the maximum chance of encountering the production peaks of these copepods. At 4–5 mm in prosoma length, late stage copepodites and adults of *Neocalanus flemingeri* and *N. plumchrus* are among the largest copepods, and they can accumulate >60 % of their body dry weight in lipid stores [Evanson et al. 2000]. Because of the lipophilic nature of oil-derived PAC, they accumulate in the lipid tissue of organisms and the high surface-area-to-volume ratio of copepods accounts for a high bioaccumulation potential [Chapter 3/Duesterloh et al. 2002].

The toxicity of photoactivated PAC should be a direct function of chemical (PAC) dose and light intensity [Veith et al. 1995]. Ankley et al. [1995] investigated the specific toxicity of fluoranthene to the benthic oligochaete *Lumbriculus variegatus* at three different light intensities; a linear relationship between the product of light intensity and initial tissue PAC residue and the median time to death was

[1] Duesterloh, S., and T.C. Shirley. The interaction of polyaromatic carbon (PAC) concentration and ultraviolet radiation dose in phototoxic effects on *Neocalanus* copepods in the North Pacific. Prepared for submission to Photochemistry and Photobiology.

reported [Ankley et al. 1995]. The results supported the Bunsen–Roscoe photochemical law of reciprocity, which states that in the absence of "complicating" side reactions, the product of light intensity and reaction time is constant for a fixed concentration of the sensitizer (PAC). In a similar approach, we tested four PAC doses, with the light intensity and exposure duration the same for each of the two experiments. Instead of reporting the time to death, we chose to record effects at approximately the time when half of the copepods in the medium PAC dose were affected. In this approach, the proposition was tested that the product of exposure duration and light intensity times PAC residue would follow a linear relationship when plotted against the percentage of affected organisms.

Known phototoxic PAC absorb in the UVA wavelength range (320–400 nm) [Diamond et al. 2000]. However, phototoxicity of weathered crude oil to larvae of Pacific herring was increased when the exposures also included a UVB component [Barron et al. 2003]. The role of UVB in phototoxicity to copepods was examined by including a light treatment with selective UVB exclusion to compare the phototoxic effectiveness of full spectrum sunlight and that of sunlight without the UVB component.

Materials and Methods

Two separate experiments, each with 3 light spectra (no light, daylight without UVB, full spectrum daylight) and 4 concentrations of water-dissolved PAC were conducted. Oil exposures took place in the laboratory in 24-h flow-through exposures, and light exposures followed immediately after transfer to an outdoor water bath. The oil exposure for experiment 1 was started on 14 May 2001, with daylight exposures from 15:20 to 19:10 on 15 May and 10:10 to 13:30 on 16 May. For experiment 2, the oil exposure started on 4 June 2001, followed by daylight exposure from 11:45 to 16:25 on 5 June. In experiment 1, 20 *Neocalanus flemingeri* were used per treatment. In experiment 2, 16–18 copepods were used per treatment, half of which were *N. flemingeri* and the others *N. plumchrus*.

Copepod collection

Copepods were collected with 200-µm mesh open-ring nets, equipped with altered design cod ends to minimize breakage of setae, and towed vertically from 50 m depth to the surface. The live samples were kindly provided by researchers of the GLOBEC (GLOBal ocean ECosystems dynamics) Gulf of Alaska Monitoring Program cruises, diluted if dense, and kept at ambient water surface temperatures until processed. In the laboratory, storage, sorting and experiments were conducted in a constant-temperature walk-in chamber set at 6–8 °C. Copepods were pipetted into 1-ml culture wells for microscopic species and life stage identification and quickly transferred to beakers, with 5 copepods per 50-ml beaker until the start of the experiment.

Oil exposure and PAC analysis

The Alaska North Slope crude oil was weathered by heating and overnight stirring at 80 °C to 20% weight loss, which removed most monocyclic aromatic compounds, and then added to 2-mm-diameter glass beads at an application rate of 2.6 g oil kg^{-1} of beads. The oiled beads were tumbled for approximately 24 h, spread to a single layer and left under a hood for 4 days at 25 °C to allow the oil to harden onto the beads, and then were stored at –20 °C until use.

A detailed description of the generating columns that produced the aqueous solutions of PAC dissolved from crude oil is provided in Chapter 3/Duesterloh et al. [2002]. The columns were filled with 20–100 ml of oil-coated glass beads, and unoiled beads were added to fill up excess volume. For the high-dose treatments several generating columns were connected. In the control treatments the generating columns were filled with 100 ml of PAC-cleaned glass beads. Prior experience showed that there was no loss in total PAC concentration from the columns within 96 h. Seawater was directed from the laboratory supply line into an overhead tank of approximately 80-L capacity. Water was then pumped through the

generating column at a flow rate of 5 ml min^{-1} into a 2-L Erlenmeyer filtration flask in which the hose connector served as an overflow. Each column was flushed with seawater for 22 h before the peristaltic pump was activated and the flow rate in all columns was adjusted. The experiment was started within 20 h after activation of the pump, at which time 0.9 L of the water in the Erlenmeyer flask was collected for PAC extraction and copepods were added to the remaining water volume in the flask. A screen of 330-µm plankton mesh covered the outflow opening of the flasks to prevent loss of copepods. After 24 h, copepods were collected and frozen (–20 °C) and 0.9 L of the exposure water was extracted with dichloromethane and then frozen for later PAC analysis at the Auke Bay Laboratory (National Marine Fisheries Service /National Oceanic and Atmospheric Administration [NMFS/NOAA]) in Juneau, Alaska.

Procedures for the quantitative determination of PAC in water and in tissues were described by Short et al. [1996]. Seawater samples (0.9 L) were extracted twice with 50–60 ml dichloromethane. Tissue samples were collected in experiment 1 but not in experiment 2, because of lower *Neocalanus* abundance in the later sample. Copepods were macerated in a glass grinder twice, each time with 1 ml dichloromethane. Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath, followed by fractionation and purification by alumina/silica gel chromatography. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). PAC analytes included dibenzothiophenes and polyaromatic hydrocarbons containing 2–5 rings. A method blank, spiked method blank and two reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision and absence of laboratory-introduced artifacts and interferences. Detection limits were determined experimentally [Glaser et al. 1981] for PAC and generally were 5–20 ng PAC L^{-1} seawater at the 95% confidence level. For tissues an 80% confidence level was chosen. Concentrations below the detection limit were treated as 0.

UV exposures

For the UV exposures, a water bath was installed outside and supplied with seawater from the laboratory intake lines. Temperatures were 7 ± 1°C (mean ± 1 standard deviation) during experiment 1 and 8 ± 1°C during experiment 2. Erlenmeyer flasks were placed in the water bath so that the water level inside the flask was approximately the same as in the water bath and arranged to avoid shading. For the flasks used for the UVB exclusion treatment, a Mylar-D cylinder was constructed to cover the entire flask. This reduced the UVB radiation by 68% when measured in air compared to a reduction of UVA and visible light by 25%. When measured in water and under the flask (measurements inside the flask were not possible because of the small neck), UVB was reduced by >99%, compared to a reduction of UVA and visible light of 15–17%. All recordings were made with a Macam Photometrics UV meter at 10 cm depth in the waterbath. The attenuation of 10 cm water was determined at 32, 33 and 21% for UVB, UVA and visible light, respectively. Levels of UVA, UVB and visible light were recorded every 10 min, then integrated to estimate the total UV dose over the exposure period. The exposure was terminated when ~50% immobilization or mortality was observed in the medium dose treatment. Copepods were then microscopically evaluated and biological responses were categorized into unaffected, impaired and dead. For all further analysis the categories impaired and dead were pooled into one category: affected.

Statistical analysis

To test for a difference between start and end PAC concentrations in the exposure water, a paired comparison t-test was used. For experiment 2, a t-test ($\alpha = 0.05$) was employed to test for a difference in effects on *Neocalanus flemingeri* and *N. plumchrus*.

Frequencies of affected and unaffected copepods were tabulated in three-dimensional contingency tables with the factors PAC concentration, light spectrum, and affected or unaffected [Zar 1999]. The two experiments were analyzed independently of each other. First, a test for mutual interdependence was conducted. When the null hypothesis of no interaction between the factors was rejected, a test for partial

interdependence between PAC concentration and light spectrum was performed. For all tests the chi-square statistic at the 95% significance level was used.

To test the proposition of a linear relationship between the product of light dose (intensity × exposure duration) and PAC concentration, light dose times PAC concentration in copepod tissue was plotted against the percentage of affected copepods and the correlation coefficients were calculated for both light spectra in experiment 1. Then, with pooled data from the two experiments, the PAC concentrations in water were plotted against the percentage of affected copepods and correlation coefficients were calculated. Finally, the total light dose was multiplied by the total PAC content in water and plotted against the percentage of copepods affected in the corresponding treatment; correlation coefficients were compared to those obtained from water PAC concentrations alone.

A Student's t-test was used to test for a difference in effects of the UVB-exclusion and full spectrum treatments in both experiments ($\alpha = 0.05$).

Results

An increase of impairment and mortality with PAC concentration was observed in treatments with a combination of PAC and sunlight exposure in both experiments, clearly indicating dose-dependent photoenhanced toxicity of oil on *Neocalanus* copepods (Figure 8). No difference could be detected between the phototoxic effects on the two species *N. flemingeri* and *N. plumchrus* in experiment 2 (t-test, $\alpha = 0.05$, $P < 0.001$).

UV doses and PAC concentrations

The total light doses and ranges of light intensity are given in Table 4. In experiment 1, the total dose was ~30% higher in UVA and visible light and ~15% higher in UVB compared to experiment 2. Mean PAC concentrations in the exposure water were between 0.6 and 9 μg L^{-1}. The difference between start and end concentrations (Figure 9) was insignificant (paired comparisons t-test, $\alpha = 0.05$). Exposure concentrations were approximately 3 orders of magnitude less than reported LD_{50} (dose at which 50% of test organisms die) concentrations of oil on copepods [Spies 1987]. In all further calculations the mean concentration was used (Table 4). The first two concentrations listed in Table 4 are the no-oil control treatments. The low but prevalent total PAC concentrations measured in the no-oil controls do not reflect experimental cross-contamination but rather a background signature of creosote that was in the intake water. Concentrations of any individual PAC from this background signature were ≤0.01 times those of the measured phototoxic agents.

Table 4. Total light doses and ranges of light intensity of visible light, UVA and UVB measured during the two experiments.

Experiment		Visible Light		UVA		UVB	
	Exposure Duration	Total Dose ($W \cdot m^{-2} \cdot min^{-1}$)	Range Intensity ($W \cdot m^{-2}$)	Total Dose ($W \cdot m^{-2} \cdot min^{-1}$)	Range Intensity ($W \cdot m^{-2}$)	Total Dose ($W \cdot m^{-2} \cdot min^{-1}$)	Range Intensity ($W \cdot m^{-2}$)
1	230(day 1)	27400	21–216	4508	4.2–30	113	0.1–0.8
	200(day 2)	36438	103–322	5096	15.8–42.2	138	0.4–1.0
	430 (total)	63838	21–322	9604	4.2–42.2	251	0.1–1.1
2	280	44812	102–241	7027	16–36	212	0.5–1.1

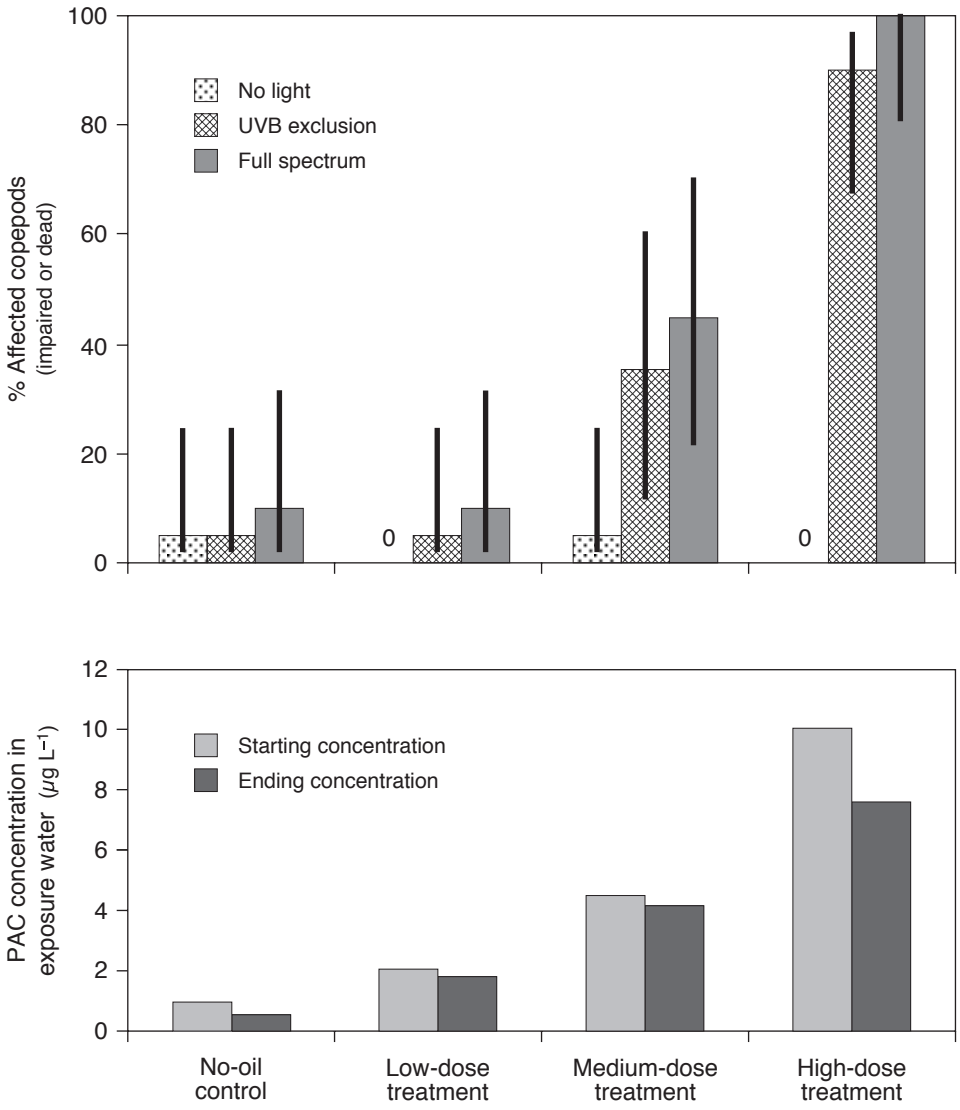

Figure 8. Phototoxic effects (impaired or dead) of dissolved oil on copepods in experiment 1 (*Neocalanus flemingeri*; *n* = 20, top panel) and total PAC concentrations in the exposure water at the start and end of the oil exposure (bottom panel). Bars indicate binomial confidence intervals.

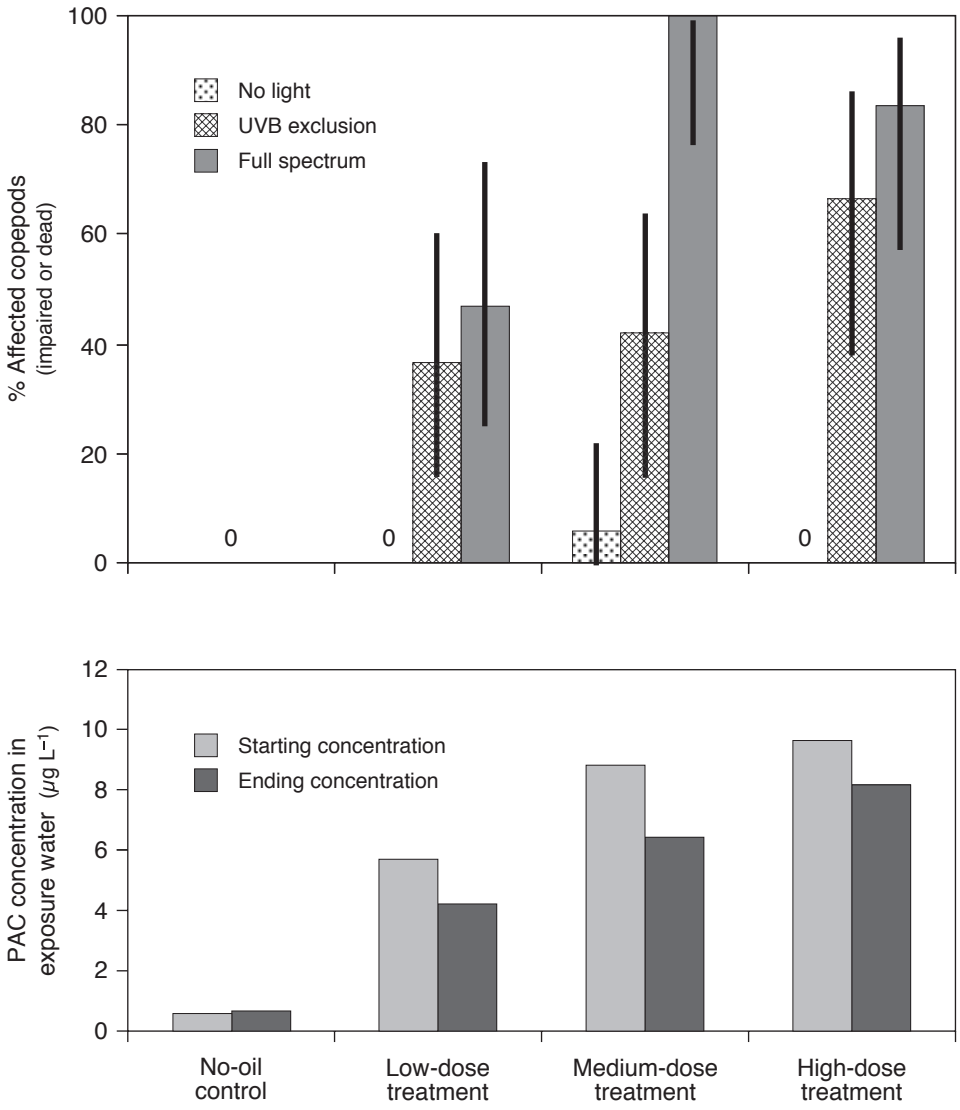

Figure 9. Phototoxic effects (impaired or dead) of dissolved oil on copepods in experiment 2 (*N. flemingeri* and *N. plumchrus*; *n* = 15–19, pooled data, top panel) and total PAC concentrations in the exposure water at the start and end of the oil exposure (bottom panel). Bars indicate binomial confidence intervals.

Test for interaction between light spectrum and PAC concentration

The interaction between PAC concentration and light spectrum was highly significant, indicating that the toxicity of dissolved weathered Alaska North Slope crude oil is enhanced by photoactivation. The null hypothesis of no interaction between any of the variables was rejected ($P < 0.001$). Also, the partial independence test [Zar 1999] between light spectrum and PAC concentration resulted in rejection of the null hypothesis of no interaction ($P < 0.001$).

Test for linearity of PAC concentration × light dose versus effect

The product of PAC concentration in copepod tissue and the light dose (intensity × exposure duration) was a good predictor of effects on copepods (Figure 10). The correlation coefficient r was > 0.99 in both light treatments (Figure 10e, f). This was not true for the product of PAC in water concentration times light dose, which was slightly less correlated with the percentage of affected copepods (r = 0.93) than the PAC concentration in water alone (r = 0.97) in the full spectrum treatment (Figure 10a, c). In the UVB exclusion treatment, however, the product of PAC concentration in water and light dose had a slightly higher correlation coefficient (r = 0.97) than PAC in water alone (r = 0.95) (Figure 10b, d).

Phototoxicity of full spectrum light versus UVB exclusion

The full spectrum treatment consistently had slightly higher frequencies of affected copepods than the UVB exclusion treatment (Figures 8, 9). However, this trend was not significant in t-tests comparing the means of the two treatments ($\alpha = 0.05$). No effect was observed in the no-oil treatments, which indicates that all light exposures were below toxic levels of UV radiation alone.

Discussion

We observed a dose-dependent, significant interaction of PAC dissolved from weathered Alaska North Slope crude oil and UV radiation in sunlight with *Neocalanus* copepods. In one experiment conducted on 4–5 June, there was no difference (t-test, $\alpha = 0.05$) in effects on the species *Neocalanus flemingeri* and *N. plumchrus*. The significant increases in frequencies of affected copepods with increasing PAC concentrations are in accord with results obtained in studies with other test organisms and under artificial UV light sources [e.g., Pelletier et al. 1997; Cleveland et al. 2000]. The observed dose-dependent interaction of dissolved PAC at these low concentrations (0.5–10 $\mu g\ L^{-1}$) and the natural sunlight source confirm and extend earlier results with the copepods *Calanus marshallae* and *Metridia okhotensis* [Chapter 3/Duesterloh et al. 2002].

A reciprocal relationship between PAC concentration measured in tissue residue and light dose is supported by our data (r > 0.99). In two independent experiments, each with four PAC exposure concentrations, the response was tightly correlated with the product of light dose and PAC concentration in the exposure water, and also with PAC water concentration. PAC accumulation by copepods can be assumed to be rapid and proportional to the PAC water concentration at the levels tested, but tissue concentrations had slightly closer correlations with phototoxicity effects than water PAC concentrations (Figure 10). Confirmation of the reciprocal relationship and application of the Bunsen–Roscoe photochemical law was provided by Ankley et al. [1995]. Because of the difficulty in accurately defining the time of death or unrecoverable damage in copepods, we chose to terminate the experiment when approximately 50% of the effects were observed in the medium dose treatment. In this approach the factor "exposure time" becomes fixed and the relationship to be tested is that of PAC concentration and light dose (intensity × exposure duration). This implies a possible overestimation of the lethal dose (100% affected) in the high PAC concentration treatments, because the experiment was continued past the test organism's time of death. However, the lowest PAC concentration in experiment 1 had no significant phototoxic effect. Thus, we can define a lower and upper phototoxicity threshold in terms of the product of light dose ($W \cdot m^{-2} \cdot min^{-1}$) and the PAC concentration in water ($\mu g\ L^{-1}$) between ~100–600.

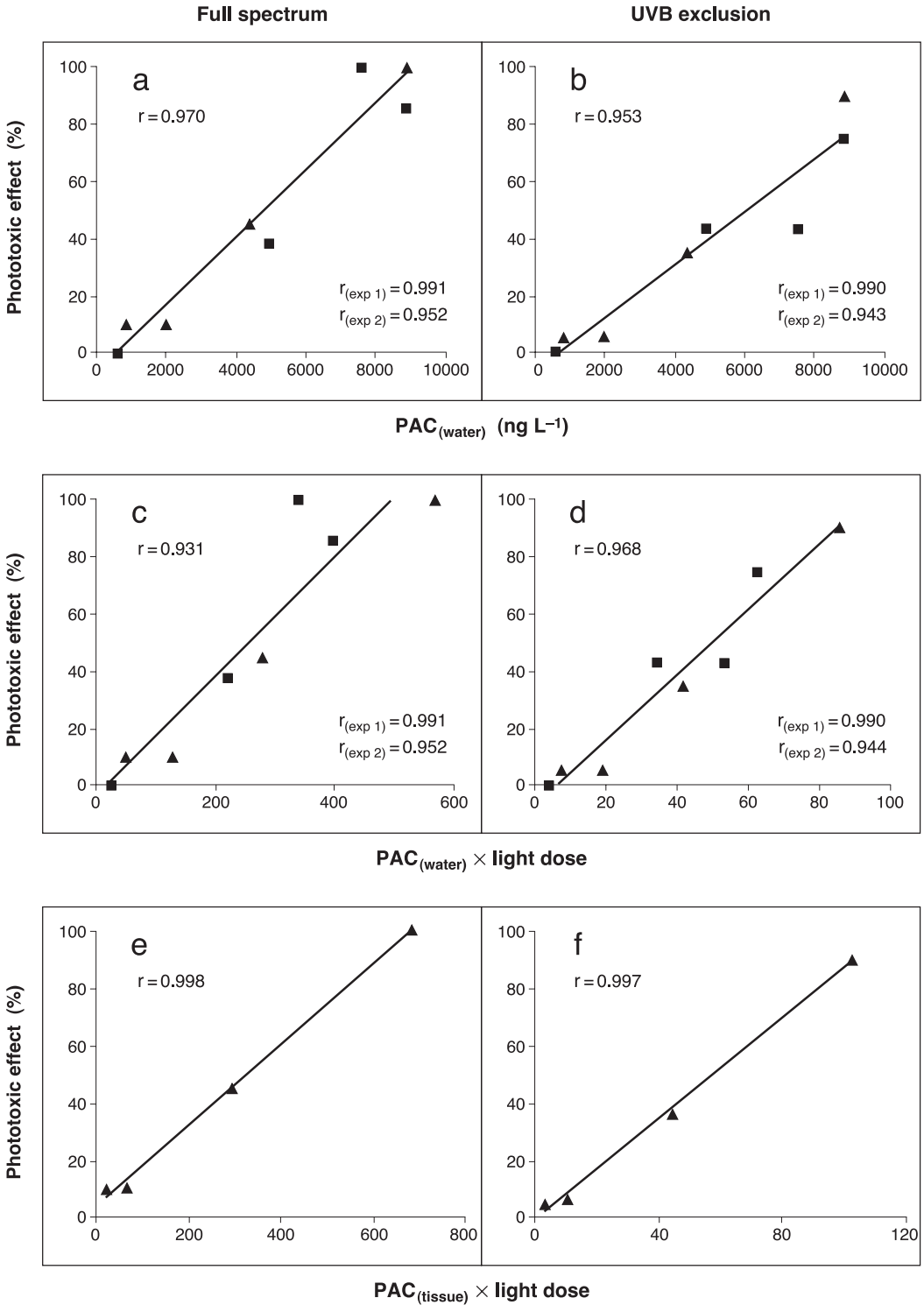

Figure 10. Correlations of phototoxicity, PAC concentration and light dose. Regression of phototoxic effect in full spectrum sunlight (left panel) and in sunlight with UVB exclusion (right panel). Top: PAC concentration in the exposure water (a, b); middle: the product of PAC concentration in the exposure water and the exposure light dose (c, d); bottom: the product of PAC concentration in copepod tissue and the exposure light dose (experiment 1 only) (e, f). triangle = experiment 1, square = experiment 2.

The results of the UVB exclusion experiment were not significantly different from those of the full spectrum light treatment, indicating that the observed phototoxicity was induced in the UVA wavelength range. However, there was a tendency for the frequencies of affected copepods to be slightly lower in the UVB exclusion treatment compared to the full spectrum treatment. This may be explained by the ~30% UVA attenuation of the Mylar foil. An alternative explanation is that the interplay of UVA and UVB wavelengths increases the total phototoxic effects. Photoactive agents differ in peak absorbance spectra [Diamond et al. 2000], and phototoxicity of some agents increases in the presence of shorter (UVB) wavelengths [Huovinen et al. 2001]. A significantly higher phototoxicity of sunlight compared to a UVA treatment was reported for herring larvae in experiments using ~10 times higher concentrations of dissolved weathered Alaska North Slope crude oil [Barron et al. 2003]. More research is needed to resolve the role of UVB radiation in phototoxic effects of oil on copepods, particularly at higher PAC concentrations.

The UV exposures of both experiments were conducted on days with intermittent disk visibility. For comparison, the visible light curves for experiment 2 and a clear sky day, measured at the same location and under the same conditions, are shown in Figure 11. The light doses used in our experiments were well below the total irradiation on a sunny day. In experiment 1, the light exposure was on two subsequent days, interrupted by a 13-h dark period. Repair mechanisms could reduce damage caused by UVB radiation in some copepod species during periods of visible light exposure [Zagarese et al. 1997]; however, no such repair occurred during 24 h of incubation in the dark [Zagarese et al. 1997]. Thus, we assume that the effects observed at the end of the exposure on day 2 reflect the accrued phototoxicity of the total light dose received on two subsequent days.

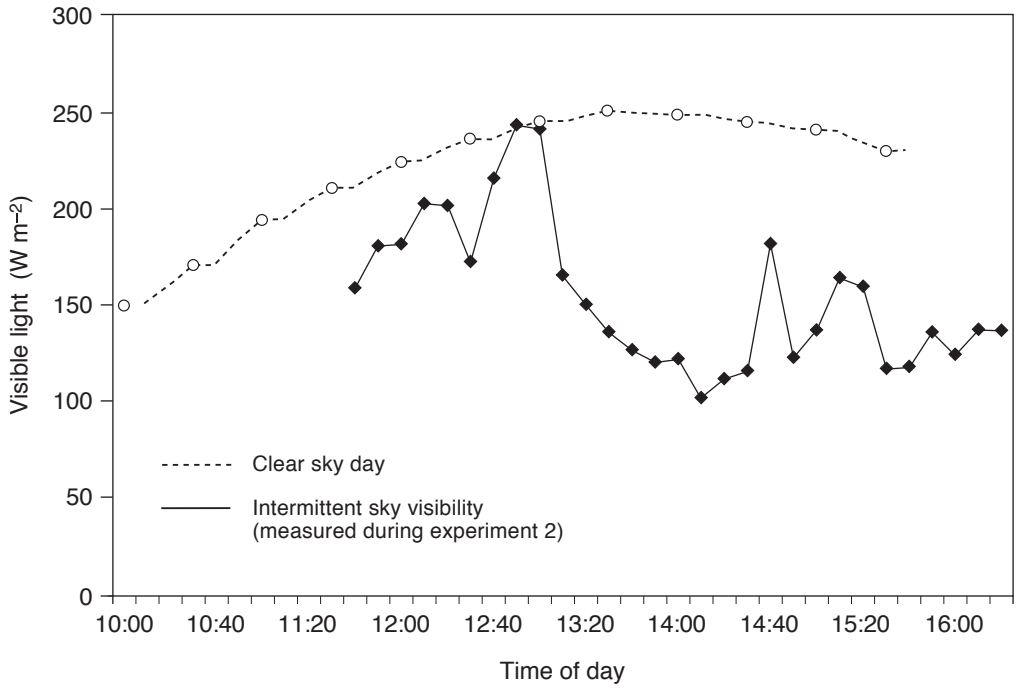

Figure 11. Comparison of total light intensities measured on a clear day (11 May 2001) and a day with intermittent disk visibility, measured during experiment 2 (5 June 2001) in Seward, Alaska. All measurements were taken under a 10-cm water column.

Oil exploration and transportation in Alaska have been accompanied by much concern about environmental risks. Numerous studies, mostly generated by the *Exxon Valdez* oil spill in 1989, have investigated ecological implications of catastrophic oil spills and chronic contamination in these highly productive marine ecosystems [Rice et al. 2001; Peterson 2001]. The potential for phototoxicity as a complicating and accelerating factor was only recently recognized [Barron and Kaʻaihue 2001]. The present study clearly demonstrates the potential for dominant, subarctic copepods to accumulate dissolved PAC from the water and their sensitivity to photoenhanced toxicity. Because copepods are important forage species and many fishes and invertebrates directly or indirectly depend on their abundance as a vital energy source, population shifts in copepods may change ecological patterns on a large scale. However, it is presently unknown to what extent phototoxicity could threaten copepod populations that have encountered dissolved PAC from chronic or accidental contamination.

Acknowledgments

Funding for this project was provided by the University of Alaska Coastal Marine Institute and the Oil Spill Recovery Institute. Substantial in-kind support and professional advice were contributed by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory. We would particularly like to thank Stanley Rice and Jeff Short for their assistance. Russell Hopcroft from the University of Alaska Fairbanks helped with crucial logistics and is thanked for sample collection. The Institute of Marine Science, University of Alaska Fairbanks, is thanked for laboratory space, housing and technical support on short notice. Rebecca Zeiber was a tireless helper during the experimental phase.

Chapter 5. Egg Production of Starved *Calanus marshallae* and *Pseudocalanus* spp. and the Potential for the Distribution of Polyaromatic Compounds in Copepod Eggs[1]

Abstract

This study investigated the feasibility of culture experiments to compare egg production rates and survival of oiled and unoiled female Calanus marshallae *and* Pseudocalanus *spp. copepods. Twenty* C. marshallae *were cultured and egg production and mortality were monitored over 28 days. Sixty* Pseudocalanus *spp. were maintained in culture wells, and egg production and mortality were monitored over four days. Copepods in cultures were unoiled and not fed.* C. marshallae *had little mortality for the first two weeks; after four weeks in culture 50% survived. Spawning activity and mean egg production declined rapidly after the third day in culture. In* Pseudocalanus *spp. mortality increased from 5% on the first day in culture to 36% on day four. Accordingly, spawning activity and egg production declined with each subsequent day. Egg production data from these experiments were used to calculate a sample size for determining a significant difference in mean egg production between two treatments. For* C. marshallae *the experimental design was rejected based on high natural variability in egg production and the difficulty in obtaining large numbers of gravid females. High mortality and strong decline in egg production early in the experiment made* Pseudocalanus *spp. unsuited for the intended comparison of egg production rates between previously oiled and unoiled females, because of the duration of the oil exposure (24 hours prior to the start of the incubation for egg counts). However, mortality increased more rapidly in 11 oiled female* C. marshallae *than in unoiled females after 19 days of starvation.*

Introduction

Eggs of previously oiled female copepods may receive polyaromatic compounds (PAC) when lipids are incorporated into egg tissue. Copepods accumulate, store and release PAC when exposed to the water soluble fraction of oil [Lee 1975; Chapter 3/Duesterloh et al. 2002]. Because of the lipophilic properties of PAC, uptake has been suspected to be associated with lipid stores in copepods [Corner 1975]. The seasonal lipid stores of large copepods in higher latitudes are mainly used for gonad development and egg production [Sargent and Falk-Petersen 1988; Hagen and Schnack-Schiel 1996; Evanson et al. 2000]. A high lipid content of eggs was reported for *Euchaeta japonica* and *Calanus pacificus* [Sargent and Falk-Petersen 1988].

If toxic PAC are passed from females to eggs, effects on viability, hatching rates and survival of offspring may occur. Reductions in reproduction rates in response to exposure to anthracene were observed with the water flea *Daphnia magna* [Holst and Giesy 1989]. Depressed copepod populations may negatively affect survival of higher trophic level consumers like fish, birds and marine mammals and influence the length and productivity of the phytoplankton bloom and sedimentation rates [Parsons and Lalli 1988]. Conversely, concentration and composition of the phytoplankton can influence copepod production rates [Ban et al. 1997; Frost 1985; Peterson 1988]. Also, vertically migrating copepods carrying PAC may introduce these toxic compounds at depth when spawning. Differences in life history patterns and the number of generations produced per year determine the timing and depth of egg release. For example, *Pseudocalanus* spp. have continuous egg production throughout 6–7 months of the year while they are actively feeding in surface water (0–50 m) [Frost 1985; Paul et al. 1990]. In contrast, spawning of *Neocalanus plumchrus* is limited from January to mid-April at depths below 300 m and is entirely dependent on internal lipid stores [Fulton 1973; Evanson et al. 2000].

[1] Duesterloh, S. Egg production of starved *Calanus marshallae* and *Pseudocalanus* spp. and the potential for the distribution of polyaromatic compounds in copepod eggs. Prepared in the format of Journal of Plankton Research.

Toxic effects of oil may become evident only at times of mobilization of storage lipids. Thus, toxicity of oil to copepods may be underestimated in short-term toxicity studies. Lethal concentrations (LD_{50}) of oil on copepods vary widely from 1 to 1350 µg L^{-1} and are higher than for most other plankton taxa tested [Capuzzo 1987; Lee et al. 1977]. Some of the variation is due to differences in oil composition and test conditions. However, female *Eurytemora affinis* treated with ^{14}C-labeled naphthalene retained about 10% of the radioactivity originally taken up and egg production and life span were reduced by 25% [Corner 1975]. The feasibility of a comparison of egg production of oiled and unoiled females of *Calanus marshallae* and *Pseudocalanus* spp. was tested in this study by evaluating the underlying natural variability in egg production and survival in cultures under conditions of food limitation. A comparison of survival of oiled and unoiled female *C. marshallae* is presented and implications of delayed oil toxicity to copepods are discussed in this chapter.

Methods

Zooplankton collection

Calanus marshallae samples were collected on 25 July 2000 in Lynn Canal, southeastern Alaska, with a 330-µm mesh open-ring plankton net towed vertically from a maximum depth of 100 m. *Pseudocalanus* spp. were collected in Auke Bay on 25 August 2000 with a 155-µm mesh open-ring plankton net towed vertically from a maximum depth of 20 m.

Egg production experiments

Females of *C. marshallae* and *Pseudocalanus* spp. were identified by microscopic examination and pipetted individually into test chambers containing filtered seawater.

Twenty *C. marshallae* were cultured in glass beakers with approximately 50 ml of seawater in a constant-temperature walk-in chamber set at 6 °C. Egg production was monitored on days 1, 3, 4, 6, 8, 10, 13, 15, 17, 19, 24 and 28 by microscopic examination. If eggs were present, they were counted and removed. The water in the test chambers was partially exchanged at every examination and completely exchanged twice weekly.

Sixty *Pseudocalanus* spp. were cultured in 1-ml culture wells and maintained in a flow-through water bath at ambient Auke Bay water temperature (10–12 °C). Monitoring for survival and egg production was conducted microscopically every 24 h. At this time, eggs were removed and the water in the test chambers was partially exchanged.

Egg counts of *C. marshallae* were used to estimate a sample size that would be sufficient to detect differences in egg production between the means of two treatments (oiled and unoiled). Egg production values are presented as mean ± 1 standard deviation. The experiment was assumed to resemble a simple random sample of spawning events in the natural population, and the number of egg clutches in the population was assumed to be large. The sample variance was calculated from all clutches in the experiment and used as an unbiased estimator for the population variance. Confidence limits were set at 0.95 and the maximum allowable difference *d* between the estimate and the true value was 1.7 eggs, resembling approximately 10% of the maximum mean egg production in this experiment [Thompson 1992].

Post-oil survival experiment

Eleven female *C. marshallae* were exposed to a low-dose preparation (total PAC of ~2 µg L^{-1}) of the water-soluble fraction of weathered Alaska North Slope crude oil for 24 h. The flow-through exposure system and PAC composition of the exposure water were reported in Chapter 3/Duesterloh et al. [2002]. The exposure water contained primarily 3- and 4-ring PAC, including naphthalenes, fluorenes,

dibenzothiophenes, phenathrenes and trace concentrations of chrysenes. Following the oil exposure, females were sorted into individual beakers containing ~50 ml of filtered seawater and maintained at 6 °C. As a control, 11 females that had not been treated with the oil preparation were cultured in the same way. Survival and egg production were checked daily until death; water was exchanged approximately every 48 h.

Results

Egg production experiments

C. marshallae had less than 10% mortality for the first two weeks. In the third and fourth week mortality increased, but after four weeks in culture 50% of the initial females were still alive (Figure 12a). Egg clutches contained between 1 and 54 eggs. For most females only one spawning event was observed, but 3 females laid eggs twice during the experiment. The highest spawning activity was measured on day 3 (over 50% of all females), then spawning activity dropped to <5–15% until day 13, after which spawning ceased entirely (Figure 12b). The time of cessation of spawning activity was concurrent with an increase in mortality. Mean daily egg production varied from 17.6 (± 18.5) on day 3 to 0.05 (± 0.2) on day 10 (all females included) (Figure 12c).

Over the course of the experiment with 20 females, a total of 22 egg clutches were recorded; 4 females did not spawn. Sample variance was calculated and used as an unbiased estimator for the population variance. With and the assumption that n = ∞, the equation [Thompson 1992]:

$$n = \frac{1}{\left(\frac{d^2}{z^2\sigma^2}\right) + \left(\frac{1}{N}\right)}$$

resolves to 138 egg clutches.

In this equation n is the sample size, d is the maximum allowable difference between the estimate and the true value, and z denotes the upper $\alpha/2$ point of the standard normal distribution.

Consequently, 138 egg clutches are needed per treatment to determine the mean daily egg production within the allowable range defined by d (± 1.7) with 95% confidence. Since 20 females produced 22 egg clutches, 126 females are needed to produce 138 egg clutches and 252 females would be needed for the two treatments (oiled and unoiled) of the experiment. Limited availability of females in the study area precluded an experiment of that magnitude.

Pseudocalanus spp. had 5% mortality on day 1 and 12.3%, 18% and 36.6% on days 2, 3 and 4, respectively (Figure 12d). Fecundity was between 1 and 16 eggs per day per female, and females were observed to lay eggs on subsequent days. Mean egg production overall was 2.8 (± 3.9) on day 1, 0.8 (± 1.7) and 0.1 (± 0.5) on days 2 and 3, respectively, and dropped to zero on day 4 (Figure 12f). If only producing females were included in the calculation, mean egg production was 4.5 (± 4.1) eggs per female per day on day 1, 2.9 (± 2.4) on day 2, and 2 (± 1) on day 3. Over 60% of females were spawning initially, but spawning activity dropped to 28% and less than 10% on days 2 and 3, respectively (Figure 12e).

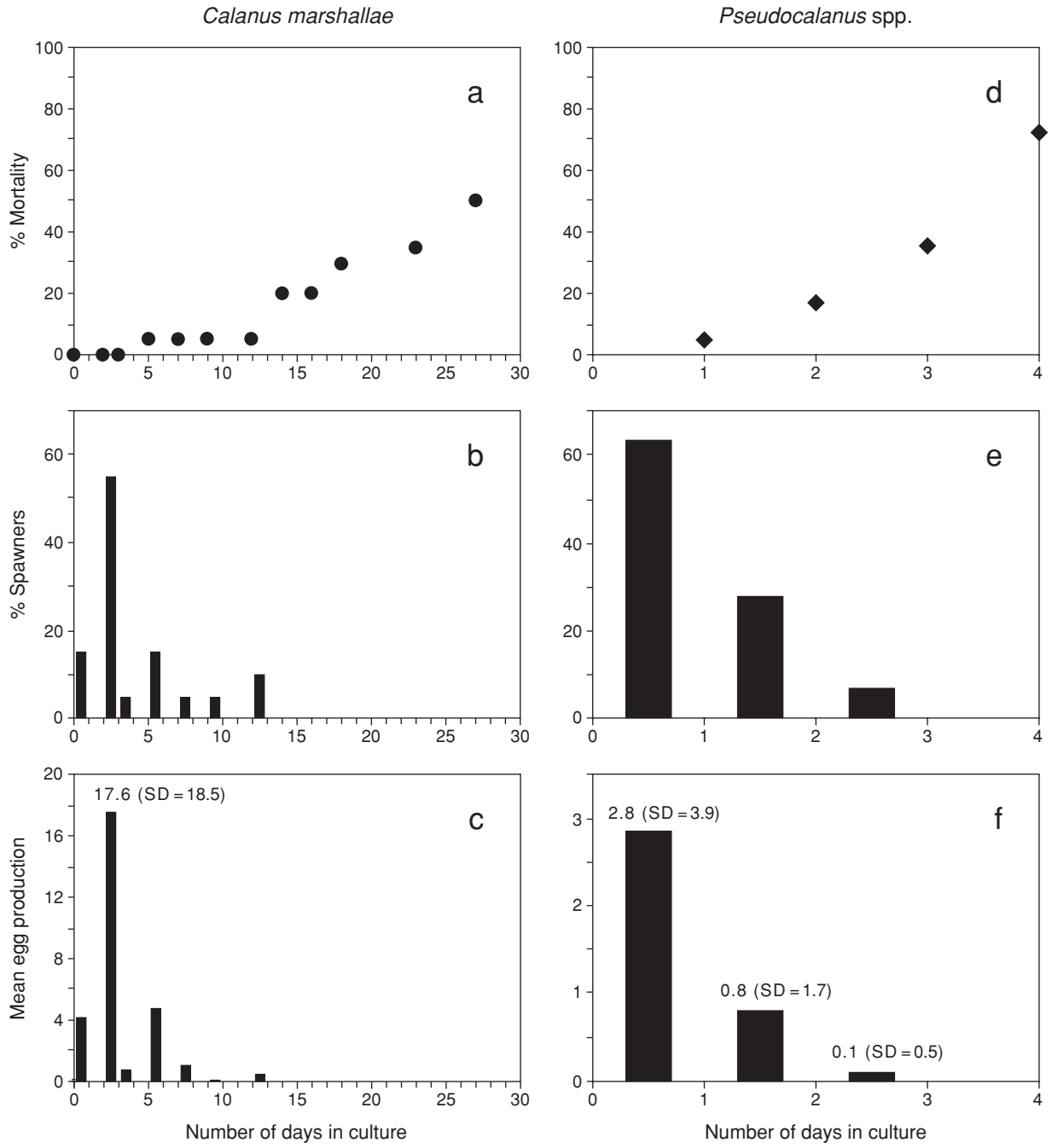

Figure 12. Comparison of mortality, spawning activity and mean egg production in females of 20 *Calanus marshallae* and 60 *Pseudocalanus* spp. cultured without food for 28 (*C. marshallae*) and 4 (*Pseudocalanus* spp.) days. Standard deviations of mean egg production, which are referred to in the text, are given in parentheses.

Post-oil survival experiment

For the first 17 days in culture, mortality in the oiled and unoiled treatments was low (1 female died in each treatment). Between day 18 and day 37 the mortality rate (%) in the oiled treatment was twice as high as in the unoiled treatment. On day 37 the experiment was terminated with a total of 10 deaths in the oiled treatment and 7 deaths in the unoiled treatment (Figure 13).

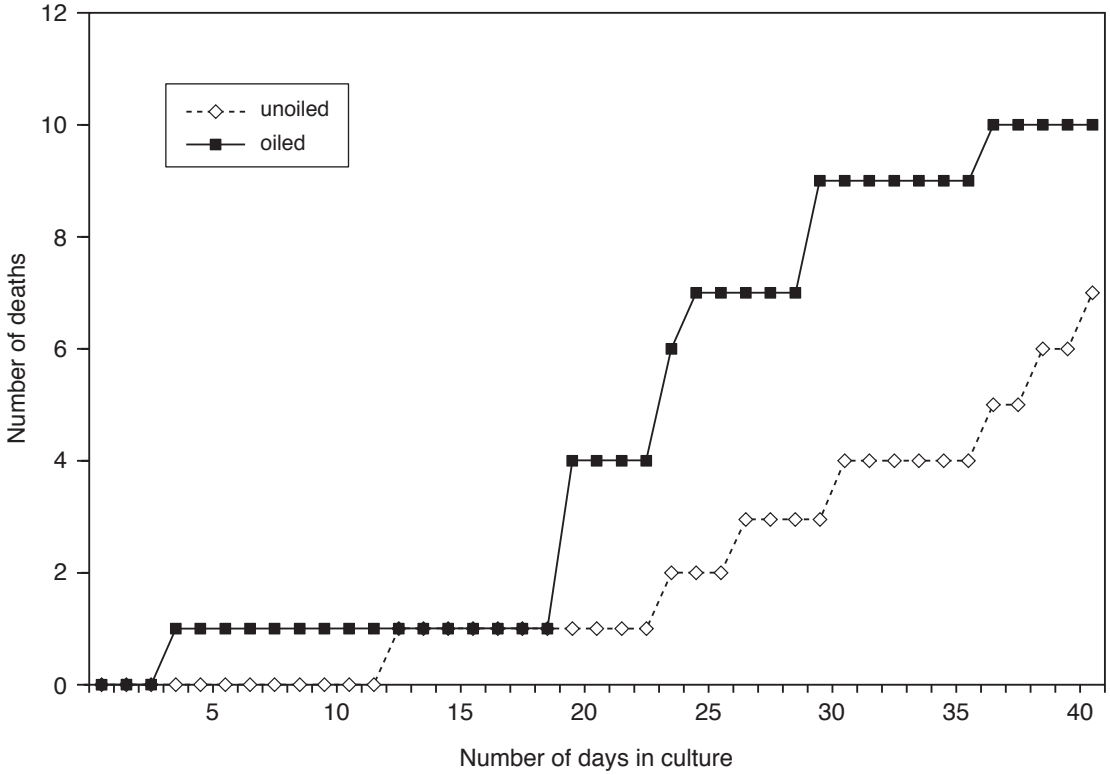

Figure 13. Mortality rates of 11 oiled and 11 unoiled *Calanus marshallae* females in cultures without food. The 24-h oil exposure occurred on day 1 at a concentration of ~2 μg L^{-1} total PAC.

Discussion

Mean egg production in *C. marshallae* in this study was lower than reported from laboratory cultured females fed excess amounts of *Thalassiosira weissflogii* [Peterson 1988], but spawning continued for 14 days without food. The range of clutch sizes (1–54 eggs) coincides with the reported range of 1–61 eggs per clutch [Peterson 1988]. However, the highest mean daily egg production of 17.6 (± 18.5) measured in this study is approximately half of the values reported for the months of June to August (31.3–32.2 eggs per clutch) [Peterson 1988]. Cessation of egg production in starved females was observed 24 h after the start of the incubation without food [Peterson 1988]. Inconsistent with this finding, I observed the highest egg production during the 48-h period between the first and third day in culture, and egg production continued at low rates until day 14. These differences in mean egg production and reproductive timing may be in part caused by temperature differences: incubations were at 6°C in this study and at 10°C in the former. The size of egg clutches and the interval between clutches vary seasonally and between

latitudes within species of *Calanus* [Mauchline 1988]. Based only on the variance observed in this experiment, a sample size to detect a difference in mean egg production between two treatments was estimated. Most *C. marshallae* in the net samples were stage V copepodites and females were rare in comparison. Thus, it was not feasible to conduct an experiment comparing effects of oil on egg production with this species.

The genus *Pseudocalanus* contains at least seven distinct species, four of which (*P. minutus, P. moultoni, P. newmani, P. mimus*) may occur in southeast Alaska [Frost 1989]. Morphological differences between the species are slight, and no attempt was made in this study to identify *Pseudocalanus* beyond genus. Mean egg production rates in *Pseudocalanus* spp. were studied in Auke Bay in 1987 and 1988 in 24-h egg production assessments [Paul et al. 1990]. Thus, data gathered on day 1 in the present study were directly comparable to the previous research. Egg production in this study was slightly higher when egg-producing and non-producing females were considered, but standard deviation was also greater (2.86 ± 3.9 and 2.6 ± 1.1). If non-producing females were not included in the calculation, mean egg production in this study was lower (4.53 ± 4.1) than reported for 1987 (7.4 ± 3.7) and 1988 (8.3 ± 4.5) [Paul et al. 1990]. Female *Pseudocalanus* spp. in June 2000 produced fewer eggs than Paul et al. [1990] found in April and May 1987 and 1988, but the percentage of spawners was higher (~60%). This is consistent with a proposed decline in egg production per female toward the end of a season due to aging and a steady increase in the percentage of active spawners toward the later season [Paul et al. 1990].

Food availability is the principal factor influencing egg production rates in copepods [Mauchline 1998]. High mortality and strong decline in egg production early in the experiment made *Pseudocalanus* spp. unsuited for the intended comparison of egg production rates between previously oiled and unoiled females, because of the duration of the oil exposure (24 h prior to the start of the incubation for egg counts). The high mortality in the incubations with *Pseudocalanus* spp. was assumed to be an effect of starvation. Generally, egg production increases as food concentration increases to achieve an asymptotic level [Mauchline 1998]. However, egg production may be affected by food availability in some species and not in others; phytoplankton bloom conditions accelerated egg production in *Calanus pacificus* but not in the co-occurring *Pseudocalanus* sp. in Dabob Bay, Washington [Frost 1985]. Inconsistent with my observation of high starvation mortality and reduction in egg production, *Pseudocalanus elongatus* females incubated in filtered seawater resumed egg production for up to 6 days [Frost 1985]. I conclude that starvation may have acted in synergy with another unidentified stress factor to cause the responses observed in these experiments with *Pseudocalanus* spp.

Low-dosage oil exposure may adversely affect long-term survival in copepods. Consistent with the reduced life span in oiled *Eurytemora affinis* [Corner 1975], mortality in previously oil-exposed *C. marshallae* females in this study occurred about 3 days sooner and at a higher rate than in unoiled females. In the current experiment, egg production rates were not compared because of the small sample size. *Calanus marshallae* has previously been reported to have no immediate mortality in response to low-dosage oil treatments as used in this experiment [Chapter 3/Duesterloh et al. 2002]. However, due to their high lipid content these copepods readily bioaccumulate PAC from aqueous solution (accumulation factor ~8000). Although no data were collected on long-term retention of PAC in this study, a 10% retention of naphthalene in female *Eurytemora affinis* and retention of PAC by nauplii lasting throughout several life stages has been reported [Corner 1975]. Like many high-latitude copepod species, late stages of *C. marshallae* accumulate a lipid store for gonad development, egg production and, to a lesser extent, metabolic needs during the winter months [Evanson et al. 2000; Sargent and Falk-Petersen 1988]. Because of the lipophilic properties of PAC the majority of retained PAC may be assumed to be associated with the seasonal lipid stores in the lipid sac. I speculate that toxic PAC are not harmful to the copepods as long as they are stored in a metabolically inactive form. However, at times of metabolic activation of the lipid stores, the toxic PAC may be reactivated and cause reduction in reproductive output and premature mortality.

Bioaccumulation and retention of toxic PAC and a reduction of reproductive success in copepods may impact trophic interactions in marine communities. Copepods concentrate PAC from a dilute solution in their environment and deposit them in their lipid stores, thus making them accessible to higher trophic level predators. Copepods are prey to many planktivores including forage fish and the juveniles of numerous commercial species (e.g., salmon, pollock, herring) as well as some invertebrates. Feeding on oil-contaminated prey by pink salmon fry has been reported to reduce growth rates [Carls et al. 1996]. More research is necessary to assess the magnitude and possible impact of this transport mechanism on natural fish populations.

Depressed copepod populations resulting from decreased reproduction rates in response to oil exposure may interrupt the energy transfer from primary production to higher trophic level consumers. Copepods form an important link in the food chain by transforming primary production from phytoplankton into animal protein, accessible to predators such as fish. Exposure of several copepod species to industrial waste discharge resulted in reductions in feeding rates, respiration rates and production rates. Toxic waste exposure was energetically equivalent to food limitation [Capuzzo 1985]. Total egg production of a population and other variables determine the population size of the next generation. Low copepod abundance resulting from reduced reproductive success may cause food limitation in those predators with limited mobility (e.g., larval fish), while mobile predators disperse in search of better food resources. The dependence of larval fish recruitment on zooplankton stocks was demonstrated in Auke Bay, Alaska [Haldorson et al. 1993; Coyle and Paul 1992]. In *Pseudocalanus* spp. female abundance at the time of the onset of the spring phytoplankton bloom was a more important factor for nauplii production and larval fish recruitment than the strength of the phytoplankton bloom [Paul et al. 1990].

The proposed association of PAC with the lipid stores of copepods suggests that when lipid stores are utilized for egg production, PAC are incorporated in egg tissue. Ontogenetic vertical migration in copepods may transport PAC contained in egg tissue to depth, possibly introducing them into pelagic and benthic food chains. The synthesis of storage lipids from non-lipid dietary precursors and the utilization of lipids for gonad development and egg production were studied in detail for the copepods *Calanus finmarchicus* and *Metridia longa* [Sargent and Falk-Petersen 1988]. Oil droplets were observed in freshly laid eggs of *C. pacificus* [Fulton 1973]. *C. pacificus* is taxonomically very similar to *C. marshallae*, but females have reduced mouth parts and do not feed, so that egg production is entirely dependent upon the lipid stores, whereas in *C. marshallae* egg production is dependent on food availability [Fulton 1973; Peterson 1988]. This suggests that *C. marshallae* lay eggs within the upper 50 m of the water column, which is their spring and summer habitat, while *C. pacificus* spawn at depths below 300 m [Fulton 1973]. The life cycles of the larger species of the genus *Neocalanus*, which dominate the biomass in high-latitude offshore plankton communities [Cooney 1986], are generally similar to that of *C. pacificus* in that only one generation is produced per year and spawning occurs at depth during the winter months [Miller and Clemons 1988; Miller 1993]. Vertical transport of PAC in eggs thus depends on the predominant species of copepods and their life cycle, and may be of importance in some regions but not in others.

Acknowledgments

Funding for this project was provided by the University of Alaska Coastal Marine Institute and the Oil Spill Recovery Institute. In-kind support and professional advice were contributed by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory. Antje Klawon was a tireless helper with the experimental work.

Final Report Acknowledgments

Editor's comment:
This report is essentially Switgard Duesterloh's Ph.D. dissertation, with a small amount of additional text requested by the Minerals Management Service, and formatting and other minor changes dictated by the style of the University of Alaska Coastal Marine Institute final report series. Chapters 2 through 5 were prepared for journal publication and have separate acknowledgment sections. Below are the acknowledgments Dr. Duesterloh made in her dissertation.

Behind every thesis there are the contributions of many people who have shared their time, experience, expertise and good will, each contributing a piece to the construction of the final work. I want to express my heartfelt gratitude to all who have helped me throughout the process from proposal writing to field, experimental and laboratory work to thesis writing and publishing.

I would like to thank my committee chair and advisor Dr. Thomas C. Shirley at the Juneau Center School of Fisheries and Ocean Sciences, University of Alaska Fairbanks for his continuous support, advice and dedication. Many thanks are also extended to my committee members Drs. Terrance J. Quinn II and Lewis Haldorson, both Juneau Center School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Dr. Stanley D. Rice, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory and Dr. Gary C. Thomas, Prince William Sound Science Center, Cordova.

Many students, researchers and volunteers have assisted in the collection of plankton samples. Lynette and Cecil McNutt loaned their private boat to research and were tireless at night sampling efforts. Special thanks are in order to Dr. Russell Hopcroft, University of Alaska Fairbanks for his help in logistics and the provision of live plankton samples. I thank Tom Smith and the staff at the Institute of Marine Science in Seward for providing an excellent working platform and logistic assistance. Rebecca Zeiber helped in the experimental phase of Chapters 2 and 4.

I also thank Marie Larson, Larry Holland and Josephine Lunasin, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory for many hours of training and assistance in the chemical analysis of oil and lipid samples.

I am especially thankful to my co-authors of the publication of Chapter 3, Jeff Short of the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory and Dr. Mace Barron, P.E.A.K. Research, for sharing their expertise in the fields of oil chemistry and phototoxicity and for review and improvements of the manuscript.

Thanks also to all who have lent their time and insights for the interpretation of the data and the improvement of the manuscript: Dr. Thomas C. Shirley, Dr. Terrance J. Quinn II, Dr. Nicola Hillgruber, Juneau Center School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Jeff Short and Marc Carls, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory.

Financial support for this research was provided by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratory, the Oil Spill Recovery Institute and the University of Alaska Coastal Marine Institute.

Finally, I would like to thank my parents and Ms. Sarah Werner for financial support during the starting phase and for their confidence in me. My husband, Stephen E. Bodnar and our son Lars are thanked for their love and support throughout.

Study Products

Duesterloh, S. 2000. Part 1: Copepod reproduction in culture experiments, and Part 2: Synergistic effects of oil and ultraviolet radiation (UV) on copepods. Seminar summarizing research results presented at the Juneau Center School of Fisheries and Ocean Sciences public seminar series, December 2000, Juneau.

Duesterloh, S. 2001. Photoenhanced toxicity of Alaskan North Slope crude oil to copepods. Presentation at the American Fisheries Society Student Chapter, November 2001, Juneau.

Duesterloh, S. 2001. Photoenhanced toxicity of Alaskan North Slope crude oil to copepods. Presentation at the American Fisheries Society Alaska Chapter, November 2001, Sitka.

Duesterloh, S. 2002. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons. University of Alaska Coastal Marine Institute Annual Research Review, February 2002, Fairbanks.

Duesterloh, S. 2002. The role of copepods in the distribution of hydrocarbons. Seminar summarizing research progress at the Juneau Center School of Fisheries and Ocean Sciences public seminar series. February 2002, Juneau.

Duesterloh, S. 2002. The role of copepods in the distribution of hydrocarbons: An experimental approach. Seminar in partial fulfillment of dissertation requirements presented at the Juneau Center School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, October 2002, Juneau.

Duesterloh, S. 2002. The role of copepods in the distribution of hydrocarbons: An experimental approach. Ph.D. Dissertation, University of Alaska Fairbanks, 182 p.

Duesterloh, S., and T.C. Shirley. 2001. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons. University of Alaska Coastal Marine Institute Annual Research Review, February 2001, Fairbanks.

Duesterloh, S., and T.C. Shirley. 2003. The Role of Copepods in the Distribution of Hydrocarbons: An Experimental Approach. Final Report. OCS Study MMS 2004-034, University of Alaska Coastal Marine Institute, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region, 53 p.

Duesterloh, S., J.W. Short and M.G. Barron. 2002. Photoenhanced toxicity of weathered Alaska North Slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. Environmental Science and Technology 36(18):3953–3959.

Shirley, T.C. 2001. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons. Seminar summarizing research progress at Minerals Management Service office. April 2001, Anchorage.

Shirley, T.C. 2001. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons. Seminar to the National Science Foundation Research Experience for Undergraduates Symposium. June 2001, Juneau.

Shirley, T.C. 2002. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons. Seminar summarizing research progress at Minerals Management Service office. April 2002, Anchorage.

Shirley T.C., and S. Duesterloh. 2000. The role of zooplankton in the distribution of hydrocarbons, p. 77. *In* University of Alaska Coastal Marine Institute Annual Report No. 6. OCS Study MMS 2000-046, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Shirley T.C., and S. Duesterloh. 2000. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons, p. 51–54. *In* University of Alaska Coastal Marine Institute Annual Report No. 7. OCS Study MMS 2000-070, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Shirley T.C., and S. Duesterloh. 2002. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons, p. 39–47. *In* University of Alaska Coastal Marine Institute Annual Report No. 8. OCS Study MMS 2002-001, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Shirley T.C., and S. Duesterloh. 2003. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons, p. 7–11. *In* University of Alaska Coastal Marine Institute Annual Report No. 9. OCS Study MMS 2003-003, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Shirley T.C., and S. Duesterloh. 2004. An experimental approach to investigate seasonal differences in the role of zooplankton in the distribution of hydrocarbons, p. 7. *In* University of Alaska Coastal Marine Institute Annual Report No. 10. OCS Study MMS 2004-002, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

References

Allred, P.M., and J.P. Giesy. 1985. Solar radiation-induced toxicity of anthracene to *Daphnia pulex*. Environ. Toxicol. Chem. 4:219–226.

Ankley, G.T., S.A. Collyard, P.D. Monson and P.A. Kosian. 1994. Influence of ultraviolet light on the toxicity of sediments contaminated with polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 13:1791–1796.

Ankley, G.T., R.J. Erickson, G.L. Phipps, V.R. Mattson, P.A. Kosian, B.R. Sheedy and J.S. Cos. 1995. Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. Environ. Sci. Technol. 29:2828–2833.

Arfsten, D.P., D.J. Schaeffer and D.C. Mulveny. 1996. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: A review. Ecotox. Environ. Safe. 33:1–24.

Ban, S., C. Burns, J. Castel, Y. Chaudron, E. Christou, R. Escribano, S.F. Umani, S. Gasparini, F.G. Ruiz, M. Hoffmeyer, A. Ianora, H.K. Kang, M. Laabir, A. Lacoste, A. Miralto, X. Ning, S. Poulet, V. Rodriguez, J. Runge, J. Shi, M. Starr, S. Uye and Y. Wang. 1997. The paradox of diatom–copepod interactions. Mar. Ecol. Prog. Ser. 157:287–293.

Barron, M.G. 1995. Bioaccumulation and bioconcentration in aquatic organisms, p. 652–666. *In* D.J. Hoffman, B.A. Rattner, G.A. Burton, Jr. and J. Cairns, Jr. [eds.], Handbook of Ecotoxicology. Lewis Publishers, Boca Raton, Florida.

Barron, M.G., M.G. Carls, J.W. Short and S.D. Rice. 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. Environ. Toxicol. Chem. 22:650–660.

Barron, M.G., and L. Ka‘aihue. 2001. Potential for photoenhanced toxicity of spilled oil in Prince William Sound and Gulf of Alaska waters. Mar. Pollut. Bull. 43:86–92.

Barron, M.G., E.E. Little, R. Calfee and S. Diamond. 2000. Quantifying solar spectral irradiance in aquatic habitats for the assessment of photoenhanced toxicity. Environ. Toxicol. Chem. 19:920–925.

Bowling, J.W., G.J. Leversee, P.F. Landrum and J.P. Giesy. 1983. Acute mortality of anthracene-contaminated fish exposed to sunlight. Aquat. Toxicol. 3:79–90.

Calfee, R.D., E.E. Little, L. Cleveland and M.G. Barron. 1999. Photoenhanced toxicity of a weathered oil on *Ceriodaphnia dubia* reproduction. Environ. Sci. Pollut. Res. 6:207–212.

Capuzzo, J.M. 1985. The effects of pollutants on marine zooplankton populations: Sublethal physiological indices of population stress, p. 475–491. *In* J.S. Gray and M.E. Christiansen [eds.], Marine Biology of Polar Regions and Effects of Stress on Marine Organisms. Proceedings of the 18th European Biology Symposium, University of Oslo, Norway, 14–20 August 1983. John Wiley & Sons, Chichester, England.

Capuzzo, J.M. 1987. Biological effects of petroleum hydrocarbons: Assessments from experimental results, p. 343–410. *In* D.F. Boesch and M.N. Rabalais [eds.], Long-Term Environmental Effects of Offshore Oil and Gas Development. Elsevier Applied Sciences, London.

Carls, M.G., L. Holland, M. Larsen, J.L. Lum, D.G. Mortensen, S.Y. Wang and A.C. Wertheimer. 1996. Growth, feeding, and survival of pink salmon fry exposed to food contaminated with crude oil, p. 608–618. *In* S.D. Rice, R.B. Spies, D.A. Wolfe and B.A. Wright [eds.], Proceedings of the *Exxon Valdez* Oil Spill Symposium. American Fisheries Society Symposium 18, Bethesda, Maryland.

Choi, J., and J.T. Oris. 2000. Anthracene photoinduced toxicity to PLHC-1 cell line (*Poeciliopsis lucida*) and the role of lipid peroxidation in toxicity. Environ. Toxicol. Chem. 19:2699–2706.

Christie, W.W. 1982. Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids. Pergamon Press, Oxford, 207 p.

Cleveland, L., E.E. Little, R.D. Calfee and M.G. Barron. 2000. Photoenhanced toxicity of weathered oil to *Mysidopsis bahia*. Aquat. Toxicol. 49:63–76.

Cooney, R.T. 1986. The seasonal occurrence of *Neocalanus cristatus*, *Neocalanus plumchrus* and *Eucalanus bungii* over the shelf of the northern Gulf of Alaska. Cont. Shelf Res. 5:541–553.

Cooney, R.T. 1988. Distribution and ecology of zooplankton in the Gulf of Alaska: A synopsis. Bull. Oceanogr. Res. Inst. Univ. Tokyo 26:27–41.

Cooney, R.T. 1993. A theoretical evaluation of the carrying capacity of Prince William Sound, Alaska, for juvenile Pacific salmon. Fish. Res. 18:77–87.

Corner, E.D.S. 1975. The fate of fossil fuel hydrocarbons in marine animals. Proc. Roy. Soc. Lond. B. 189:391–413.

Coyle, K.O., and A.J. Paul. 1992. Interannual differences in prey taken by capelin, herring, and red salmon relative to zooplankton abundance during the spring bloom in a southeast Alaskan embayment. Fish. Oceanogr. 1:294–305.

Coyle, K.O., A.J. Paul and D.A. Ziemann. 1990. Copepod populations during the spring bloom in an Alaskan subarctic embayment. J.Plankton Res. 12:759–797.

Diamond, S.A., D.R. Mount, L.P. Burkhard, G.T. Ankley, E.A. Makynen and E.N. Leonard. 2000. Effect of irradiance spectra on the photoinduced toxicity of three polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 19:1389–1396.

Dixon, W.J., and F.J. Massey. 1957. Introduction to Statistical Analysis. McGraw-Hill, New York, 488 p.

Duesterloh, S., J.W. Short and M.G. Barron. 2002. Photoenhanced toxicity of weathered Alaska North Slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. Environmental Science and Technology 36(18):3953–3959.

Evanson, M., E.A. Bornhold, R.H. Goldblatt, P.J. Harrison and A.G. Lewis. 2000. Temporal variation in body composition and lipid storage of the overwintering, subarctic copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia (Canada). Mar. Ecol. Prog. Ser. 192:239–247.

Freund, J.E. 1979. Modern Elementary Statistics. Prentice-Hall, Englewood Cliffs, New Jersey, 510 p.

Frost, B.W. 1985. Food limitation of the planktonic marine copepods *Calanus pacificus* and *Pseudocalanus* sp. in a temperate fjord. Arch. Hydrobiol. Beih. Ergebn. Limnol. 21:1–13.

Frost, B.W. 1989. A taxonomy of the marine calanoid copepod genus *Pseudocalanus*. Can. J. Zool. 67:525–551.

Fulton, J.D. 1973. Some aspects of the life history of *Calanus plumchrus* in the Strait of Georgia. J. Fish. Res. Board Can. 30:811–815.

Glaser, J.A., D.L. Forest, G.D. McKee, S.A. Quave and W.L. Budde. 1981. Trace analyses for wastewaters. Environ. Sci. Technol. 15:1426–1435.

Hagen, W. 1988. On the significance of lipids in Antarctic zooplankton. Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany, 129 p.

Hagen, W., and S.B. Schnack-Schiel. 1996. Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction? Deep-Sea Res. I. 43:139–158.

Haldorson, L., M. Pritchett, D. Sterritt and J. Watts. 1993. Abundance patterns of marine fish larvae during spring in a southeastern Alaskan bay. Fish. Bull. 91:36–44.

Hays, G.C. 1995. Ontogenetic and seasonal variation in the diel vertical migration of the copepods *Metridia lucens* and *Metridia longa*. Limnol. Oceanogr. 40:1461–1465.

Holst, L.L., and J.P. Giesy. 1989. Chronic effects of the photoinduced toxicity of anthracene on *Daphnia magna* reproduction. Environ. Toxicol. Chem. 8:933–942.

Huang, X.-D., D.G. Dixon and B.M. Greenberg. 1995. Increased polycyclic aromatic hydrocarbon toxicity following their photomodification in natural sunlight: Impacts on the duckweed *Lemna gibba* L. G-3. Ecotox. Environ. Safe. 32:194–200.

Huovinen, P.S., M.R. Soimasuo and A.O.J. Oikari. 2001. Photoinduced toxicity of retene to *Daphnia magna* under enhanced UV-B radiation. Chemosphere 45:683–691.

Kattner, G., M. Graeve and W. Hagen. 1994. Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanus propinquus, Calanoides acutus* and *Rhincalanus gigas*. Mar. Biol. 118:637–644.

Landrum, P.F., J.P. Giesy, J.T. Oris and P.M. Allred. 1987. Photoinduced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms, p. 304–318. *In* J.H. Vandermeulen and S.E. Hrudey [eds.], Oil in Freshwater: Chemistry, Biology, Countermeasure Technology. Pergamon Press, New York.

Law, R.J. 1978. Determination of petroleum hydrocarbons in water, fish and sediments following the Ekofisk blow-out. Mar. Pollut. Bull. 9:321–324.

Lee, R.F. 1975. Fate of petroleum hydrocarbons in marine zooplankton, p. 549–553. *In* Proceedings of the 1975 Conference on Prevention and Control of Oil Pollution, 25–27 March 1975, San Francisco. American Petroleum Institute, Washington D.C.

Lee, R.F., M. Takahashi, J.R. Beers, W.H. Thomas, D.L.R. Seibert, P. Koeller and D.R. Green. 1977. Controlled ecosystems: Their use in the study of the effects of petroleum hydrocarbons on plankton, p. 323–342. *In* F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg [eds.], Physiological Responses of Marine Biota to Pollutants. Academic Press Inc., New York. (Takahaski in the book)

Little, E.E., L. Cleveland, R. Calfee and M.G. Barron. 2000. Assessment of the photoenhanced toxicity of a weathered oil to the tidewater silverside. Environ. Toxicol. Chem. 19:926–932.

Marwood, C.A., R.E.H. Smith, K.R. Solomon, M.N. Charlton and B.M. Greenberg. 1999. Intact and photomodified polycyclic aromatic hydrocarbons inhibit photosynthesis in natural assemblages of Lake Erie phytoplankton exposed to solar radiation. Ecotox. Environ. Safe. 44:322–327.

Mauchline, J. 1998. The Biology of Calanoid Copepods. Advances in Marine Biology, Vol. 33. Academic Press, San Diego, California, 710 p.

Miller, C.B. 1988. *Neocalanus flemingeri*, A new species of Calanidae (Copepoda: Calanoida) from the subarctic Pacific Ocean, with a comparative rediscription of *Neocalanus plumchrus* (Marukawa) 1921. Prog. Oceanogr. 20:223–273.

Miller, C.B. 1993. Development of large copepods during spring in the Gulf of Alaska. Prog. Oceanogr. 32:295–317.

Miller, C.B., and M.J. Clemons. 1988. Revised life history analysis for large grazing copepods in the subarctic Pacific Ocean. Prog. Oceanogr. 20:293–313.

Morgan, D.D., and D. Warshawsky. 1977. The photodynamic immobilization of *Artemia salina* nauplii by polycyclic aromatic hydrocarbons and its relationship to carcinogenic activity. Photochem. Photobiol. 25:39–46.

Neff, J.M., and W.A. Stubblefield. 1995. Chemical and toxicological evaluation of water quality following the *Exxon Valdez* oil spill, p. 141–177. *In* P.G. Wells, J.N. Butler and J.S. Hughes [eds.], *Exxon Valdez* Oil Spill: Fate and Effects in Alaskan Waters. ASTM STP 1219, American Society for Testing and Materials, Philadelphia.

Ohman, M.D. 1996. Freezing and storage of copepod samples for analysis of lipids. Mar. Ecol. Prog. Ser. 130:295–298.

Omori, M. 1969. Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. Mar. Biol. 3:4–10.

Oris, J.T., and J.P. Giesy, Jr. 1987. The photo-induced toxicity of polycyclic aromatic hydrocarbons to larvae of the fathead minnow (*Pimephales promelas*). Chemosphere 16:1395–1404.

Parsons, T.R., and C.M. Lalli. 1988. Comparative oceanic ecology of the plankton communities of the subarctic Atlantic and Pacific oceans. Oceanogr. Mar. Biol. Ann. Rev. 26:317–359.

Paul, A.J., K.O. Coyle and D.A. Ziemann. 1990. Variations in egg production rates by *Pseudocalanus* spp. in a subarctic Alaskan bay during the onset of feeding by larval fish. J. Crustacean Biol. 10:648–658.

Pelletier, M.C., R.M. Burgess, K.T. Ho, A. Kuhn, R.A. McKinney and S.A. Ryba. 1997. Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environ. Toxicol. Chem. 16:2190–2199.

Peterson, C.H. 2001. The *Exxon Valdez* oil spill in Alaska: Acute, indirect and chronic effects on the ecosystem. Adv. Mar. Biol. 39:1–103.

Peterson, W.T. 1988. Rates of egg production by the copepod *Calanus marshallae* in the laboratory and in the sea off Oregon, USA. Mar. Ecol. Prog. Ser. 47:229–237.

Ren, L., X.-D. Huang, B.J. McConkey, D.G. Dixon and B.M. Greenberg. 1994. Photoinduced toxicity of three polycyclic aromatic hydrocarbons (fluoranthene, pyrene, and naphthalene) to the duckweed *Lemna gibba* L. G-3. Ecotox. Environ. Safe. 28:160–171.

Rice, S.D., R.E. Thomas, M.G. Carls, R.A. Heintz, A.C. Wertheimer, M.L. Murphy, J.W. Short and A. Moles. 2001. Impacts to pink salmon following the *Exxon Valdez* oil spill: Persistence, toxicity, sensitivity, and controversy. Rev. Fish. Sci. 9:165–211.

Sargent, J.R., and S. Falk-Petersen. 1988. The lipid biochemistry of calanoid copepods. Hydrobiologia 167/168:101–114.

Sargent, J.R., and R.J. Henderson. 1986. Lipids, p. 59–108. *In* E.D.S. Corner and S.C.M. O'Hara [eds.], The Biological Chemistry of Marine Copepods. Oxford University Press, Oxford.

Short, J.W., and P.M. Harris. 1996. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound after the *Exxon Valdez* oil spill, p. 29–39. *In* S.D. Rice, R.B. Spies, D.A. Wolfe and B.A. Wright [eds.], Proceedings of the *Exxon Valdez* Oil Spill Symposium. American Fisheries Society Symposium 18, Bethesda, Maryland.

Short, J.W., and R.A. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the northwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31:2375–2384.

Short, J.W., T.J. Jackson, M.L. Larsen and T.L. Wade. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill, p. 140–148. *In* S.D. Rice, R.B. Spies, D.A. Wolfe and B.A. Wright [eds.], Proceedings of the *Exxon Valdez* Oil Spill Symposium. American Fisheries Society Symposium 18, Bethesda, Maryland.

Southworth, G.R., J.J. Beauchamp and P.K. Schmieder. 1978. Bioaccumulation potential of polycyclic aromatic hydrocarbons in *Daphnia pulex*. Water Res. 12:973–977.

Spies, R.B. 1987. The biological effects of petroleum hydrocarbons in the sea: Assessments from the field and microcosms, p. 411–467. *In* D.F. Boesch and M.N. Rabalais [eds.], Long-Term Environmental Effects of Offshore Oil and Gas Development. Elsevier Applied Sciences, London.

Thompson, S.K. 1992. Sampling. John Wiley & Sons, Inc., New York, 343 p.

Veith, G.D., O.G. Mekenyan, G.T. Ankley and D.J. Call. 1995. A QSAR analysis of substituent effects on the photoinduced acute toxicity of PAHs. Chemosphere 30:2129–2142.

Wiegman, S., P.L.A. van Vlaardingen, W.J.G.M. Peijnenburg, S.A.M. van Beusekom, M.H.S. Kraak and W. Admiraal. 1999. Photokinetics of azaarenes and toxicity of phototransformation products to the marine diatom *Phaeodactylum tricornutum*. Environ. Sci. Technol. 33:4256–4262.

Zagarese, H.E., M. Feldman and C.E. Williamson. 1997. UV-B–induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanoida). J. Plankton Res. 19:357–367.

Zar, J.H. 1999. Biostatistical Analysis. 4th ed. Prentice Hall, Upper Saddle River, New Jersey, 929 p.