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Parasitism, Feeding Rate, and Hydrocarbon Uptake of Pink Shrimp *Pandalus borealis* Fed a Crude Oil Contaminated Diet

A. Moles

Auke Bay Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 11305 Glacier Highway, Juneau, AK 99801, USA

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Pink shrimp, *Pandalus borealis*, are fished commercially in both the North Pacific and North Atlantic Oceans. In the northeast Pacific, commercial fisheries catches have fallen in half over the last two decades and the decline has been attributed to continuously expanding harvests coupled with environmental changes (Wolotira et al. 1990). Pandalid shrimp hatch as males and transform into females after 2-3 yrs. growth. Pink shrimp are opportunistic foragers closely associated with the soft bottom sediments all along the 550 square miles of Alaska's outer continental shelf. This same area, which constitutes over half the United States' coastline, has been the subject of oil leasing, exploration and drilling. Juvenile pink shrimp (males) are found in shallow (<50 m) waters (Wolotira et al. 1990). As nearshore residents, juvenile shrimp are therefore more vulnerable to pollution than are other life stages that are present in deeper water. If hydrocarbons are released into the inshore nursery areas, juvenile shrimp would come in contact with oil contaminated water, sediment, or food.

Shrimp larvae and adults are among the animals most sensitive to oil in water and show cumulative effects following long-term exposure (Stickle et al. 1987). However, epibenthic shrimp are unlikely to be exposed to water-borne oil for long periods because hydrocarbons rapidly evaporate in the water column following an oil spill. Little oil finds its way into the lower subtidal sediments as well (O'Clair et al. 1996). Although pink shrimp do not bury in the sediment, they feed on benthic detritis. Following an oil spill, shrimp or their prey may be contaminated by direct ingestion of colloidal crude oil (Conover 1971). To determine if oiled food would be consumed and incorporated into tissues by shrimp, I determined feeding rates and hydrocarbon uptake of male pink shrimp exposed to oiled food.

MATERIALS AND METHODS

Shrimp for these experiments were collected with a beam trawl at depths of 90-110 meters from southeast Alaska in early February and transported to the Auke Bay Laboratory. Only male shrimp (60- 65 mm total lengths) were retained for use. Shrimp were held in ambient flowing seawater (4°C and 29.5 ppt salinity) and fed mussels on the half shell *ad libitum* for 45 d prior to testing.

Even though mussels are not a prey item for shrimp, mussels were chosen as a logistically simple way of feeding the shrimp in a reproducible manner. Twelve tanks (200 x 50 x 48 cm) with a 6 cm bottom layer of sterilized sediment were prepared for the tests. All tanks were supplied with 2 L/m running ambient seawater and 130 shrimp (average size 62.5±0.7 mm, 2.43±0.08 g). Contaminated food was prepared by immersing live mussels (4-6 cm), *Mytulus trossulus*, in a stable water-soluble fraction (WSF) of Cook Inlet crude oil (Moles et al. 1985). Control mussels had a background level of 1 µg/g (±0.04 SE). We used two concentrations of WSF and two exposure durations to create three stocks of oiled mussels: 3.27±0.19, 20.1±1.15, and 25.1±1.35 µg/g total aromatics in mussel tissue. All four types of mussels were prepared and frozen before feeding experiments began. For the feeding study, shrimp in three replicate tanks were all fed the same concentration of oiled mussels. Hydrocarbon concentrations in the tissues of the WSF exposed mussels were analyzed by gas chromatography/mass spectroscopy. For all technical details on tissue extraction and analytical methods, the reader is referred to Short et al. (1996).

Over the 77 d period, we measured daily food consumption by thawing, draining, and then weighing mussels in the half shell offered and removed after 24 hr. Food was always present in the tanks. Mussels were also placed in tanks with no shrimp to allow corrections for hydration (blanks). The amount eaten in the tank was calculated as the summation of the weight lost for each shell after correction. Data are expressed on a grams/shrimp basis. Shrimp were fed uncontaminated mussels for two weeks before the beginning of the study until feeding rates stabilized and a baseline feeding rate could be determined. Daily feeding rates were averaged for each week. Repeated measures analysis of variance (replicates nested in treatment) was used to test weekly means for differences due to concentration of oil in food or time. Wet weights and total lengths were taken at day 0 and day 77 for 30 shrimp from each tank. All of the shrimp had molted once. A nested analysis of variance was used to detect any differences in initial and final sizes between the treatments or between replicate tanks. Three shrimp were removed from each treatment at 10, 30 and 60 d and muscle and hepatopancreas removed for tissue hydrocarbon analysis. No mortality had been expected. When shrimp began dying, those dying were removed and preserved in 10% buffered formalin for later necropsies to determine the cause of death. Cumulative mortality in each tank was regressed against total hydrocarbons in tissues averaged over the test.

RESULTS AND DISCUSSION

The major difference between the various tanks was an increase in mortality correlated with oil concentration in the tissues. This mortality appeared to be associated with an infestation of the microsporidian Thelohania sp. (Table 1). Of the 192 recorded mortalities among the 1170 shrimp, 157 contained spores in the musculature and the distinctive opaque appearance. The remaining few mortalities were evenly distributed among the tanks and ignored in data analysis.

The greater the concentration of oil in the hepatopancreas, the greater the prevalence of the parasite (r^2= 0.89). The regression of concentration of oil on parasite prevalence was significant as P<0.001 ($F_{1,11}$= 91.3). No mortality occurred in the first six weeks of oiled food ingestion but increased gradually thereafter.

Table 1. Mortalities associated with presence of *Thelohania* sp. among male pink shrimp 77d on an oil contaminated diet.

Concentration (oil/wt)	no. (0d)	Parasitized	Prevalence (%)
1μg/g	390	0	0
3μg/g	390	18	5
20μg/g	390	55	14
25μg/g	390	84	22

Oiled food did not significantly affect feeding rates (Figure 1). The feeding rates ranged from 0.05 - 0.15 grams eaten per shrimp per week. The shrimp in all the tanks ate voraciously when presented with food, consuming an average of 0.1 grams each per week. Examination of daily and weekly feeding rates did not reveal any trends toward reduced or increased feeding rates as a result of contamination. Feeding rates were highly variable, elevated one week and down the next with controls always feeding slightly, but not significantly, better than treatment shrimp. During weeks 2 and 6, shrimp in the control tanks ate significantly (P<0.05) more than shrimp in the oil treatment groups but these differences were not apparent in subsequent weeks.

Mean weights and lengths of shrimp exposed for 77 days to oiled food were not significantly different between treatment and control shrimp and there was no correlation between mean size at experiment's end and exposure concentration. Mean growth rates were 0.035. 0.040, 0.042, and 0.044 mm/d for shrimp held 77 d in 25, 20, 4, and 1 µg/g respectively, an increase of 4-5% as a result of molting. Final mean weights ranged between 2.5 grams and 2.7 grams with the largest mean weight found in the 4µg/g treatment. None of these slight differences was significant.

Shrimp fed oiled food accumulated hydrocarbons in the hepatopancreas but not in the muscle tissue (Fig. 1). For the first 10 d of the study, the levels of parent hydrocarbons (metabolites were not measured) in the hepatopancreas were similar to the exposure concentrations. Shrimp fed 20-25 µg/g hydrocarbon laden food accumulated levels of 15 µg/g aromatics in the hepatopancreas by ten days. Levels in the hepatopancreas fell rapidly after 10 days and were at nearly control levels after 60 days of exposure. Accumulation levels were less than one third the 10 d level by day 30 and virtually non-existent by 77 days. Muscle bioaccumulation appears minimal. The amount of hydrocarbon present in shrimp

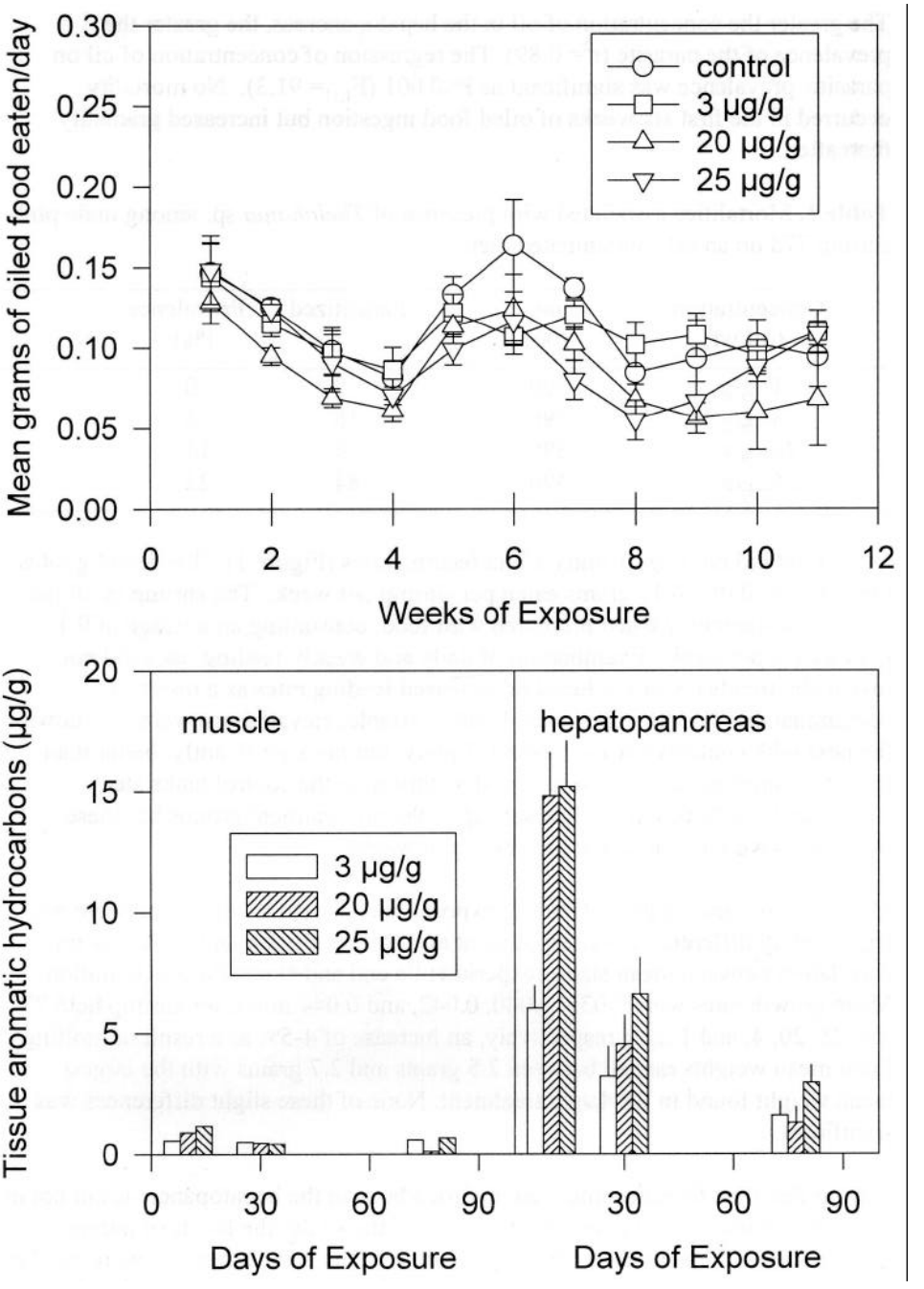

Figure 1. Weekly feeding rates (± SE) and accumulation of aromatic hydrocarbons in tissues of shrimp fed oiled mussels

muscle was not significantly different from the background levels in control muscle or hepatopancreas.

Results of this study demonstrate that direct ingestion of oil-contaminated food can negatively affect the resistance of shrimp to parasitic infection, but rapid depuration of hydrocarbons may preclude some sublethal effects. The most significant finding in our study was the increase in microsporidian infection as a result of crude oil exposure. Increased prevalences of parasites, notably those with a direct life cycle, following long-term exposure to crude oil, has been observed for a wide range of fish species (review by Khan and Thulin 1991). In contrast, the effects of pollutants on invertebrate host/parasite relationships has received little attention. Investigators have advanced a number of theories to explain pollution-induced changes in parasitism including decrease in the immune response, changes in host tissues to create a favorable environment for invasion, and increased stress in the host. Seldom are these alterations in parasite fauna life-threatening, serving instead as biomarkers of pollution in fish. In contrast, *Thelohania* sp. is a pathogenic parasite, attacking and breaking down muscle fibers, thus resulting in an opaque condition and death in shrimp.

This muscle necrosis is similar to an idiopathic necrosis ("tailrot") long noted in penaeid shrimp culture. Under stress such as crowding, handling, or low oxygen, the abdominal muscles will undergo spontaneous necrosis. The shrimp's protective shell is compromised and secondary invasion can occur. These two conditions are too similar to ignore the possibility that *Thelohania* sp. may be a secondary invader following a stress response to an oil contaminated diet.

Caution should be exercised in evaluating the lack of effects on feeding rates. The length of effective bioavailability (and hence exposure) to parent compounds was 10 d rather than 77d due to the rapid depuration of hydrocarbons from the tissues. Aromatic hydrocarbon levels in the hepatopancreas peaked after 10 days just as they do in shrimp exposed to WSF (Stickle et al. 1987). Following an initial period for enzyme induction, shrimp in the present study actively metabolized and excreted hydrocarbons. Although the mussels contained high concentrations of hydrocarbons, less than 0.1 grams of tissue were consumed each day.

Feeding rates, an inherently variable measurement, do suggest that any change in host resistence was more likely due to the added stress of hydrocarbon metabolism than to reduced food intake. The similarities in final size and feeding rates coupled with healthy appetites and positive growth among the treatment groups indicate exposed shrimp processed the same amount of food as control shrimp. There was no evidence of avoidance of tainted food. There was never any expectation of growth effects following a single molt and growth was not an objective of the present study. Information on growth is presented because changes in the relative sizes of test animals and the presence or absence of growth are important factors in evaluating the test data.

Synthesis of enzymes necessary for hydrocarbon metabolism requires energy that might otherwise be available to combat pathogenic organisms and preserve cuticle integrity. Exposure to crude oil also increases respiratory and excretory demands on the organism (Carls et al. 1996). These increased energy needs were apparently not matched by an increase in consumption. Additionally, the combination of parasites and oil can be synergistic, producing effects at intensities or concentrations seldom lethal by themselves (Khan and Thulin 1991).

Other investigators have generally noted little effect of oil ingestion on crustaceans. Lee et al. (1976) found that blue crabs (*Callinectes sapidus* fed tainted mussels showed rapid depuration of the aromatics. King crab, *Paralithodes camtschatica,* fed oiled mussels for six months grew and molted as rapidly as control crabs (Gharrett et al. 1985). Mortality following oil ingestion has previously only been reported from pink salmon fry, *Oncorhynchus gorbuscha* (Carls et al. 1996).

In the event of an oil spill or chronic leaching of hydrocarbons into nearshore waters, *Pandalus borealis* is far more likely to be exposed to oil through ingestion that via the water column or through sediment contamination. Although the 28d LC50 for pink shrimp exposed to WSF is only 28 ng/g (Stickle et al. 1987) as compared with values of 1000-2000 ng/g for other WSF exposed fish and invertebrates (Rice et al. 1979), concentrations of aromatic hydrocarbons in the water column following the Exxon Valdez oil spill were below 6 ppb and declined rapidly (Short and Harris 1996). Therefore, pink shrimp are not likely to have been exposed to toxic concentrations of aromatic hydrocarbons in the water column. Similarly, hydrocarbons are tightly bound to sediments and are biologically unavailable to the epibenthic pink shrimp (Stickle et al. 1987). Zooplankton killed by a spill will, however, sink to the bottom as detritus.

Petroleum hydrocarbons bound to particulate organic matter are easily metabolized by shrimp, but the increased stress of hydrocarbon metabolism and elimination may result in greater susceptibility to secondary parasitic infestation. Energy available for host resistance could have been reduced by metabolic demands. Given the sensitivity of shrimp to petroleum pollution and the potential of a pathogenic response to pollutants, care should be taken in controlling aromatic hydrocarbon discharges into shrimp nursery grounds.

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