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Changes in reproductive morphology and physiology observed in the amphipod crustacean, Melita nitida Smith, maintained in the laboratory on polluted estuarine sediments

John T. Tanacredi Ph.D.
Molloy College, jtanacredi@molloy.edu

B. Borowsky

P. Aitken-Ander

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Abstract

An earlier study showed that the amphipod crustacean *Melita nitida* Smith maintained on sediments dosed with waste crankcase oil developed physiological and morphological abnormalities. Most notably, mature females developed abnormal setae along the edges of their brood plates. The present study was conducted to determine whether similar abnormalities might be induced in animals maintained on polluted field sediments containing petroleum by-products among other toxic substances. In the laboratory, heterosexual pairs were maintained on three sediments taken from Jamaica Bay (New York) plus one control sediment and one toxic substratum (*Ulva lactuca* (L.) thalli). The results mirrored the results of the previous study. Under controlled conditions brood production was reduced on polluted sediments by as much as 57% and a greater proportion of females maintained on polluted sediments developed abnormal brood plate setae. In contrast, while brood production was lower in females exposed to *U. lactuca* than on the control sediment, there was no significant difference between the two groups in the number of females that developed abnormal brood plates.

Introduction

Estuaries are of key importance in marine ecosystems. Unfortunately, by virtue of their location they are especially vulnerable to disturbances from human activities (Green, 1968), and this can negatively impact productivity. Jamaica Bay, under the direct management of the National Park Service within Gateway National Recreation Area, is a prime example of this problem because of its proximity to the New York City metropolitan area (Fig. I). Sediment quality is highly variable locally due to street and airport runoff, landfill leachates and combined sewer overflows. Species abundance and species richness also vary significantly at different sites (Franz and Harris, 1988). It is of interest to learn the extent to which sediments contaminated with sublethal concentrations of toxic substances influence estuarine population dynamics. The present study was conducted to determine what effects Jamaica Bay sediments might have on the physiology and morphology of a local resident species, the amphipod crustacean *Melita nitida* Smith.

We used a standard amphipod model for our tests *(Melita nitida,* Borowsky et al., 1993). This was particularly appropriate because amphipods are primary consumers, forming the base of fish food chains in Jamaica Bay (Franz and Tanacredi, 1992), and they are easily maintained in the laboratory. M. *nitida* is common in estuaries along the Western Atlantic as well as in Jamaica Bay. It is typically found in muddy shallow subtidal and low intertidal zones, in salinities of 3-20 ppt (Bousfield, 1973). This species survives and reproduces in the laboratory, but is generally more sensitive to adverse conditions than other amphipods (Borowsky, 1978). In addition, it is closely associated with the benthos because it burrows into and consumes soft sediments.

Materials and methods

2.1. *Substrata tested*

The biological effects of five substrata were tested: three Jamaica Bay sediments (Grassy Bay [G], Pennsylvania Avenue [PI, and Ruffle Bar [R: Fig. 1, bottom]) and two controls. The controls were Easthampton sediment (E), taken from the eastern end of Long Island (Fig. 1, top), and *Ulva lactuca.* Easthampton sediment served as a control for non-toxic sediments, and U. *lactuca* was the control for toxic substances.

Two samples of each Jamaica Bay sediment were analyzed for polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) using standard methods as described in Latimer and Quinn, 1996 (principal congeners shown in Table 1). PCBs were analyzed because they are diagnostic of landfill leachates (there are three abandoned landfills on the shores of Jamaica Bay, and they are thought to be major contributors to sediment pollution in the Bay). PAHs were analyzed because earlier observations demonstrated that waste crankcase oil has detrimental physiological effects on *Melita nitida* (Borowsky et al., 1993), and PAHs are the principal biologically active contaminants of waste crankcase oil (Tanacredi, 1977).

The Pennsylvania Avenue site is on the shore of one of the landfills and Grassy Bay receives runoff from Kennedy Airport. Ruffle Bar is not near a toxic point source, and supports a dense community of animals, principally the amphipod *Ampelisca abdita.* Easthampton sediment, the control for clean sediments, was sampled from Accabonak Harbor, Easthampton, Long Island, NY. This site supports a dense and varied biota, does not receive sewer discharges and is not near a landfill. *Ulva lactuca* was tested because, since fresh samples are toxic (lethal for crab larvae (Johnson and Welsh, 1985), and highly toxic for *Melita nitida* juveniles (over 50% mortality in a pilot study; pers. obs.)), the effects of a known toxic substance could be compared with the polluted sediments.

2.2. Experimental procedures

Tests were divided into two Experiments. Experiment I tested Ruffle Bar and Grassy Bay sediments, and Experiment II investigated Easthampton, Grassy Bay, and Pennsylvania Avenue sediments. *Ulva lactuca* (L.) data was analyzed separately. *Melita nitida* adults were picked from under rocks and debris at the low tide mark near the Cross-Bay Boulevard bridge (Fig. 1, bottom). They were brought to the laboratory immediately, where single heterosexual pairs were placed in individual dishes. Experiment I involved 60 pairs of M. *nitida* collected on June 30, 1989; 30 pairs were placed in Ruffle Bar and 30 were placed in Grassy Bay sediment. Experiment II involved 200 pairs collected on August 12, 1989. These were divided at random into four treatment groups of 50 heterosexual pairs each and placed in either Easthampton, Grassy Bay, *Ulva lactuca,* or Pennsylvania Avenue substrata. In common with other local amphipods, M. *nitida* has two generations a year (pers. obs.). Animals collected in June (Experiment I) were the overwintered adults (broods produced in the fall), while those collected in August (Experiment II) were the summer generation (broods produced in the spring).

Each pair of animals was maintained in a glass culture dish (10 cm diameter) with 150 ml sea water taken offshore, and adjusted to 24 ppt with distilled water. The bottom of each dish was covered with 0.5 cm of test sediment, and the animals were kept at a light-dark cycle of (15 h: 9 h) at 25°C.

Dishes were observed daily, with molts, release of juveniles, and deaths noted. Dishes were maintained until females molted twice. The day after the second molt, all animals (females and juveniles) were preserved in 70% ethanol. Lengths of females were measured along the dorsal surface of the female from the anterior tip of the rostrum to the posterior tip of the

urosome using a binocular microscope with an optical micrometer (as per Barnard, 1969). Males that died before the end of the experiment were replaced with additional males.

Mature female amphipods molt at regular intervals and ovulate a few minutes after each molt. Thus, females that molted two times in the laboratory produced three consecutive broods that could have been analyzed. The first brood (Brood 1) had been fertilized in the field, and juveniles from this brood left the female shortly before her first molt. The second brood (Brood 2) was fertilized at the female's first molt and developed entirely in the laboratory. Juveniles from Brood 2 left the female shortly before her second molt. Brood 3 was fertilized in the laboratory at the female's second molt, and was fixed along with the female 1 day later.

To avoid confusing the number of offspring produced in successive broods, each adult pair was transferred to a new dish with new sediment 2 days after the female's first molt. Juveniles from the first brood were excluded from analysis because some young may have left the female's brood pouch before collection. Juveniles from the second brood and undeveloped eggs from the third brood were counted and included in statistical analyses.

The following data were obtained: (1) mortality rate; (2) female intermolt periods; (3) female body length; (4) number of juveniles from the second brood; (5) number of eggs from the third brood; (6) number of females with abnormal oostegite seta morphology.

2.3. *Oostegite seta morphology*

All female amphipods have a brood pouch comprised of four pairs of thin plates, or oostegites, into which fertilized eggs are released and where the brood develops until hatching. However, only sexually mature females have long fringing setae along the edges of the oostegites. The setae of adjacent oostegites overlap, forming a porous basket which allows water to flow through the brood pouch, but which prevents eggs and juveniles from falling out. Normal setae project from the edge of the oostegite in the same plane as the flat surface of the oostegal plate. In contrast, abnormal setae project in different planes (misalignments, described in Borowsky et al., 1993). Oostegites of mature females in Experiment II were observed at 50 *X* magnification, and scored for misalignments. A female was scored as abnormal if it possessed at least one oostegite (of eight) that was abnormal. The number of abnormal females was used for statistical analyses.

2.4. *Data analysis*

Table 2 shows the fates of females in each treatment group. Females that died before their second molts (81 females) plus females that were immature throughout the experiment (28 females) were excluded from all but mortality analyses (109 females excluded from intermolt period and brood analyses). Of the remaining 15 1 females, some were mature throughout the experiment, but others attained maturity only after their first or their second molts in the laboratory. To avoid harming females, the stage of maturity was determined by examining the oostegite setae of the casts and the females' preserved bodies. Only females that were sexually mature between their first and second molts (113 females; determined by examining second casts) could be included in calculations of second brood production and intermolt periods, and only females that were mature after their second molts could be included in calculations of third brood production (96 females: determined by examining preserved bodies: see Table 2). Experiment I and II females were analyzed separately because the animals came from different generations. Pairs maintained on Pennsylvania Avenue sediments were excluded from intermolt and brood production analyses because so few females (6 of 50) survived past two molts. Data

from pairs maintained on Ulva were not included in statistical analyses and are presented separately, X^2 was used to test whether female mortality differed on different sediments. Because brood sizes are positively correlated with body length in amphipods (Borowsky, 1991), analysis of covariance (ANCOVA) was used to compare brood sizes, using female body lengths as the covariate.

3. **Results**

3.1. Mortality

Female mortality was higher on substrata with higher levels of contaminants in Experiment II (Table 3: $x^2_2 = 78.0$, $P < 0.001$). Mortality was especially high on Pennsylvania Avenue (86.0% of the females died). However, there was no significant difference in mortality in Experiment I, which compared Ruffle Bar and Grassy Bay sediments (Table 3: $x^2_1 = 0.1$, $P > 0.05$). Mortality was significantly higher on *Ulva* than on Easthampton sediments ($x^2_1 = 9.4$ $P < 0.01$) but lower than on the two polluted sediments combined ($x^2_1 = 5.1$, $P < 0.05$).

3.2. Intermolt periods

Although in both experiments average female intermolt periods were longer on more polluted sediments, the differences were not significant (Table 4: Experiment I, $t_{37} = 1.0$; Experiment II, $I_{43} = 0.5$: ps > 0.05). Interestingly, the average intermolt period of winter females maintained on Grassy Bay substrata was about 2 days longer than the intermolt period of summer females on that substratum (Table 4; $t_{42} = 3.3$, $P < 0.05$).

3.3. *Brood production: brood 2 (juveniles)*

In both experiments, more females produced juveniles on less polluted sediments. The difference was significant in Experiment I (Table 5 A: $x^2_1 = 6.3$, $P < 0.01$)) but not in Experiment II ($x^2_1 = 1.0$, $P > 0.05$). In addition, there were more juveniles per brood on less polluted sediments (Table 5 A). Again, the difference was significant in Experiment I (ANCOVA with female body length as the covariate; $F_{(1.36)} = 16.7$, $P <$ O.OOl), but not in Experiment II $F_{(1.43)} = 1$ .O, $P > 0.05$).

3.4. *Brood production: brood 3 (eggs)*

More females produced third broods on the less polluted sediments, but the differences were not significant (Table 5 B: number of females with broods: Experiment 1, x: = 0.6; Experiment II, $x^2_1 = 0.9$, ps > 0.05). In addition, broods produced on the less polluted substrata tended to contain more eggs (number of eggs per brood: not significant in Experiment I, $F_{(1.30)} = 1.9$, $P > 0.05$; but significant in Experiment II, $F_{(1.39)} = 6.7$ $P < 0.05$).

3.5. Oostegite setae

Abnormalities differed significantly among sediment treatment groups (Table 6: $x^2_2 = 20.7$, $P < 0.001$). More abnormal females were found in groups maintained on the more polluted sediments from Jamaica Bay (Grassy Bay [71.4%] and Pennsylvania Avenue [lOO.OO%]). In

fact, all six females maintained on Pennsylvania Avenue sediments, who survived past their second molts, developed abnormal oostegites (Table 6).

Although both Jamaica Bay sediments and *Ulva lactuca* caused high mortalities, U. *lactuca* induced significantly fewer females with oostegite abnormalities (U. *lactuca vs* Grassy Bay and Pennsylvania Avenue combined, $x^2_1 = 8.8$, $P < 0.01$). Further, there was no significant difference between U. *lactuca* and Easthampton sediments (Fisher Exact Probability Test, $P = 0.34$). This suggests that substances present in the sediments, but not in the alga, induced the abnormalities.

3.6. Abnormal oostegite setae and reproductive output

We compared the number of broods produced by normal and abnormal females to determine whether malformed oostegite setae might reduce reproductive output by failing to hold developing broods in the brood pouch. Since oostegite setae were only examined on preserved females, we could only compare observations on third broods. This comparison revealed no significant difference between the abnormal and normal females (all treatment groups combined: normal females, 18 with and 14 without broods; abnormal females, 12 with and 14 without broods: $X^2_1 = 0.3$, *P :> 0.05).*

3.7. *Refining oostegite setae scoring methods*

Characterizing all the oostegites of a single female is tedious and time-consuming. Since preliminary observations showed that the fourth pair of oostegites was more variable than the other pairs, we scored females on the basis of the fourth pair alone, and compared these with the scores of the same females based on all oostegites. There was a high concordance between the scores; 56 of 58 females were classed the same way. Thus, the morphology of the fourth pair of oostegites alone provided about the same information as did the morphology of all the oostegites.

4. **Discussion**

The results of this study show that significant changes in reproductive parameters occurred on some polluted field sediments from Jamaica Bay. Changes in the reproductive physiology and morphology of *Melita nitida* were positively associated with sediment contamination levels. It is interesting that the concentrations of PAH and PCB in Jamaica Bay sediments were relatively low (Effects Range-Low [ER-L], defined as 4 022 ppb of PAHs and 22.7 ppb of PCBs; and Effects Range-Median [ER-M] defined as 44 792 ppb PAH and 180 of PCBs: Long et al., 1995). The amounts at Pennsylvania Avenue, the most contaminated sample tested, were 5 840 ppb PAH and 88 ppb PCB (Table 1). Thus it is possible that the observed physiological changes were caused by other pollutants (such as heavy metals) either acting together with PCBs and PAHs, or acting alone.

Oil pollution may have played a role here. Baden (1990) suggested that the low abundances and fecundity of amphipods she observed at Rixo, Sweden, were caused by the animals' exposure to relatively low levels of oil pollution. In addition, in the present study, experimental females developed abnormal setae, and an earlier study yielded similar results when animals were maintained on neutral sediments spiked with waste crankcase oil (Borowsky et al., 1993).

Support for this working hypothesis comes from the following observations: first, about the same proportion of females maintained on *Ulvu lactuca* thalli developed abnormal oostegite

setae as did females maintained on Easthampton sediments even though mortality on U. *lactuca* was higher than on Easthampton sediments; second, more females developed abnormalities on Pennsylvania Avenue and Grassy Bay sediments than on Ruffle Bar sediments; and third, abnormal oostegite setae did not develop in females maintained in sediments dosed with lead salts (Borowsky et al., in prep). Whether or not deformed oostegite setae cause reduced fecundity needs further investigation. The observations made here were limited to newly ovulated eggs (Brood 3), and these revealed no differences in fecundity.

The principal toxic components of waste crankcase oil are PAHs (Tanacredi, 1977). Since both PAH contaminated sediments and waste crankcase oil induce similar sublethal changes in this species, it is possible that the causal agent(s) are PAHs. But this remains to be tested directly.

A trend in increasing intermolt period with contamination observed in this study was not statistically significant. But the direction of the trend is consistent with two other studies which showed that stress increases intermolt periods in amphipods (correlated with mechanical injury in *Microdeutopus gryllotalpa;* Borowsky, 1980: and with increasing concentrations of waste crankcase oil and lead in *M. nitida;* Borowsky et al., 1993 and in prep., respectively).

This study reveals that, in the laboratory, ambient, polluted sediments reduce productivity in one of the most common amphipods in Jamaica Bay. It is possible that this occurs in the field as well. In addition, the sediments induce an easily recognizable morphological change in females. It would be of great interest to follow up these observations by examining specimens collected at different field sites for the presence of abnormal oostegite setae.

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Figure 1

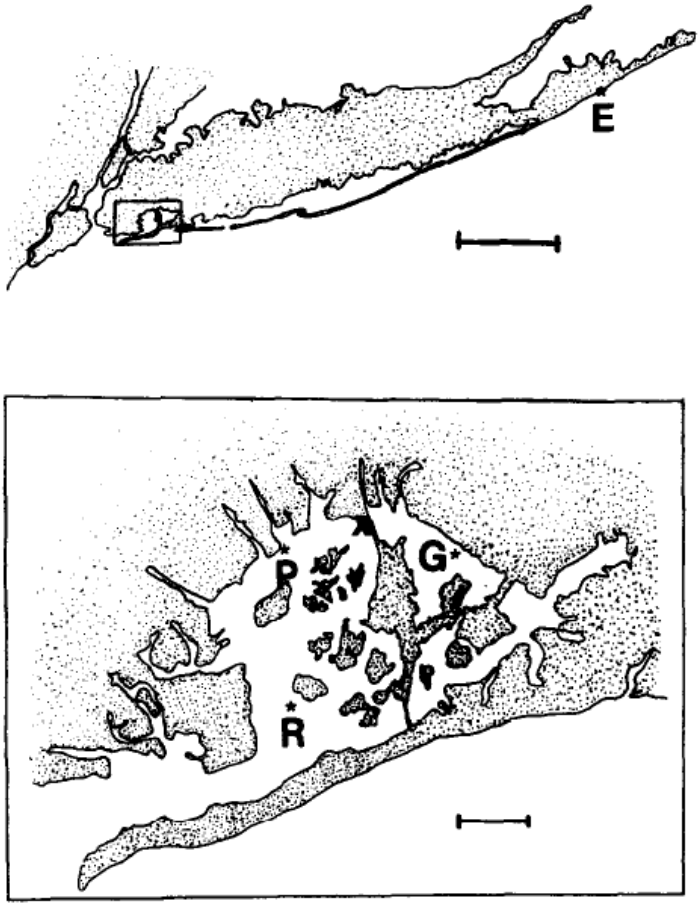

Fig. 1. Location of sediment sample sites. Above: a map of Long Island, New York, USA. Scale line = 25 km (1 cm = about 35 km). The area outlined in the square is Jamaica Bay, which is enlarged below (scale line = 10 km; 2.5 cm = about 3 km). Sediment sample sites: E = Easthampton, P = Pennsylvania Avenue, G = Grassy Bay, R = Ruffle Bar. Animal collection site: X = Cross-Bay Boulevard Bridge.

Table 1

Table 1
Principal polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) congeners in Jamaica Bay test sediments (ng/g dry weight)

Substratum	RB	GB	PA
PAH congener			
2,6-dimethyl-naphthalene	43.8	73.8	91.8
Phenanthrene	54.7	216.0	145.0
Fluoranthene	139.0	575.0	724.0
Pyrene	136.0	466.0	654.0
Benzo(a)pyrene	32.9	149.0	192.0
Sum principal PAHs	406.4	1479.8	1806.8
Total PAHs	840.0	3130.0	5840.0
PCB congener			
2,2′,5-trichlorobiphenyl	0.2	0.5	0.6
2,2′,5,5′-tetrachlorobiphenyl	0.8	2.1	2.6
2,3′,4,4′,5-pentachlorobiphenyl	0.8	3.0	5.1
2,2′,4,4′,5,5′-hexachlorobiphenyl	1.2	7.5	14.2
2.2′.3.4.4′.5,5′-heptachlorobiphenyl	0.9	0.8	12.1
Sum, principal PCBs	7.8	27.8	69.2
Total PCBs	26.8	117.0	156.0

RB = Ruffle Bar, GB = Grassy Bay; PA = Pennsylvania Avenue Landfill.

Table 2
Fates of females placed on different substrata in Experiments I and II

Substratum	Females placed in dishes	Females with short setae, all molts	Mortality analyses		Brood 2 analyses		Brood 3 analyses	
			Females that died (%)	Females that survived (%)	Short setae	Long setae	Short setae	Long setae
Experiment I								
Ruffle Bar	30	1	6	23 (79.3)	8	15	8	15
Grassy Bay	30	2	3	25 (89.3)	1	24	7	18
Experiment II								
E.Hampton	50	8	3	39 (93.9)	13	26	18	21
Grassy Bay	50	6	10	34 (77.3)	14	20	13	21
Penn. Ave.	50	1	43	6 (12.2)	0	6	0	6
U. lactuca	50	10	16	24 (60.0)	2	22	9	15
TOTALS	260	28	81	151	38	113	55	96

Females with short oostegite setae are immature. Females that died before the second molt were excluded from brood analyses. Females with short setae throughout the experiment were excluded from both brood and mortality analyses. Only survivors with long setae at specific molts were included in brood analyses. Only females with long setae on the casts at their second molts were included in brood 2 analyses (juveniles; Table 5A), and only females with long setae on their bodies were included in brood 3 analyses (eggs; Table 5B). Note that the sum of the number of females with long and short setae at each molt equals the number of survivors.

Table 3

Table 3
Number and proportion of females that died before their second molt

Substratum	Survived	Died	Total	% Died
Experiment I				
Ruffle Bar	24	6	30	20.0
Grassy Bay	27	3	30	10.0
Experiment II				
Easthampton	47	3	50	6.0
Grassy Bay	40	10	50	20.0
Pennsylvania	7	43	50	86.0
Ulva lactuca	34	16	50	32.0

Table 4

Table 4
Intermolt periods (in days) of reproductive females maintained on different substrata

Substratum	Experiment I $\bar{x} \pm$SE (n)	Experiment II $\bar{x} \pm$SE (n)
Easthampton	–	6.0±1.2 (26)
Ruffle Bar	7.6±0.2 (15)	–
Grassy Bay	8.3±0.6 (24)	6.2±1.0 (20)
Ulva lactuca	–	6.6±1.1 (22)

$\bar{x}$ = mean; SE = standard error; n = number of females.

Table 5

Table 5
Broods produced at female second and third molts

Substratum	Number of mature females	Number of broods	Juveniles–Eggs/Brood	
			% broods	$\bar{x}\pm$SE
A. Broods produced at female second molts (brood 2: juveniles)				
Experiment I				
Ruffle Bar	15	12	80.0	8.8±2.5
Grassy Bay	24	8	33.3	2.2±1.9
Experiment II				
Easthampton	26	15	57.7	4.5±2.4
Grassy Bay	20	9	45.0	3.3±2.1
Ulva lactuca	22	3	13.6	1.1±2.9
B. Broods produced at female third molts (brood 3: eggs)				
Experiment I				
Ruffle Bar	15	12	80.0	8.1±2.5
Grassy Bay	18	11	61.1	5.2±2.3
Experiment II				
Easthampton	21	12	57.1	7.5±2.8
Grassy Bay	21	8	38.1	2.4±1.9
Ulva lactuca	15	6	40.0	1.7±1.5

Table 6

Table 6
Number of reproductive females in Experiment II with abnormal oostegite setae

Substratum	Number of abnormal females	Number of normal females	Total number examined	% Abnormal
Easthampton	2	16	18[a]	11.1
Grassy Bay	15	6	21	71.4
Pennsylvania	6	0	6	100.0
Ulva lactuca	3	10	13[a]	23.1

Only females with long, intact setae were examined. Abnormal females possessed at least one abnormal oostegite.

[a] Two of the 15 *Ulva lactuca* females, and three of the 21 Easthampton females with long setae on the body died and were partially consumed before they could be examined.

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