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Larval Settlement of Ilyanassa obsoleta (Gastropoda) and Capitella sp. I (Polychaeta): Responses to Sediment Cues and Effects of No. 6 Fuel Oil

Dianne Rudy '91
Illinois Wesleyan University

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"The difference between men consists, in great measure, in the intelligence of their observations. It is the close observation of little things which is the secret of success in business, in art, in science, and in every pursuit of life."

— *Samuel Smiles*

ABSTRACT

Metamorphosis in response to bottom sediment was measured in two species of marine planktonic larvae. *Ilanassa* , a common mud-snail, and *Capitella sp. I,* a polychaete worm. A sediment control consisted of organic-rich sediment collected from Barnstable Harbor, MA. One sediment treatment, termed muffled sediment, consisted of the sediment control heated at a high temperature in a muffle oven to remove all organic matter. A sharp decrease in percent metamorphosis was observed in larvae exposed to the muffled sediment condition compared to the sediment control. Results confirmed that settlement involves more that physical contact with the sediment and suggest that the metamorphosis-stimulating factor is probably a water soluble substance.

The June 10, 1990 spill of 7,500 gallons of No. 6 fuel oil into Buzzards Bay, Massachusetts, provided oil contaminated samples of salt marsh sediment. Both the sediment control and muffled sediment were contaminated with the oil. The influence of oiled conditions on percent larval metamorphosis varied between species. Ilyanassa obsoleta showed no significant change (ANOVA *= 0.01) in percent metamorphosis between the oiled and the unoiled conditions. In *Capitella sp. I,* larval settlement rates on oiled sediment conditions were significantly lower than the control sediment. This trend suggests that the oil somehow altered the metamorphosis cue detected by larvae of *Capitella sp. I.* However, the variability in results of both species support the need for further studies investigating the specific mechanisms of metamorphosis as well as the impact of No. 6 fuel oil on the development of marine invertebrate larvae.

INTRODUCTION

Many species of marine invertebrates have a free-swimming larval stage which develops in the plankton before becoming competent, or capable of undergoing metamorphosis (Pechenik, 1985). The free-swimming larval stage important as a means of population dispersal for a population of benthic invertebrates. Two processes that larvae undergo in development are settlement and metamorphosis. Settlement is a behavioral term indicating simply that larvae are present in or on the benthos. Unlike settlement, metamorphosis is an irreversible process involving a complete physiological and morphological change to the adult form (Scheltema, 1974; Pechenik, 1980). During this period before competency, the larvae are able to settle but not metamorphose (Chia, 1978). Species can vary in terms of when and how settlement and metamorphosis occur; *Ilyanassa obsoleta* and *Capitella sp. I* are two species which differ in larval development.

Ilyanassa obsoleta is an intertidal gastropod abundant on the mud flats of New England. After initial embryonic development in an egg capsule, the larvae feed in the plankton as free-swimming veliger (Fig. 1). Depending on the amount of food and the temperature, they can swim and grow in the water column for approximately 30 days, or to a shell length of about 600 microns, before becoming competent to metamorphose (Scheltema, 1964, 1967). It is at this point that they are commonly termed "creeping-swimming" and can alternate between settling on the bottom and returning to the water column. The velum, a ciliated structure used for feeding and locomotion, must be shed before the animal is considered to have metamorphosed (Scheltema, 1962).

Capitella sp. I is a benthic polychaete worm that is found in soft-sediment environments rich in organics and sulfides. This particular species is described as one of a complex of several sibling species of *Capitella capitata* (Grassle and Grassle, 1976; Grassle et al., 1987). The trochophore larvae (Fig. 2) are lecithotrophic, and thus do not feed in the plankton but contain a yolk sac. Before the free-swimming larvae hatch, they develop as embryos in brood tubes. These larvae are ideal for settlement experiments because of their short planktonic stage lasting only several hours (Grassle and Grassle, 1976). But with a small amount of organic-rich sediment present, the larvae will settle and metamorphose within minutes. Unlike *I. obsoleta,* settlement and metamorphosis take place con-currrently and are noted to occur when the larvae enter the benthos.

It has been well established that the settlement and meta-morphosis of marine invertebrate larvae are not random processes (Wilson, 1952). Instead, most species of larvae, when competent or able to metamorphose, have the ability to delay metamorphosis until they detect proper environmental cues (Burke, 1983a). This ability is an important adaptation for survival of a species in which free-swimming larvae can metamorphose in response to a favorable cue and thus develop in a opportune environment (Pechenik, 1985). A wide variety of metamorphosis-stimulating cues have been studied: pheromones (Crisp, 1967: Burke, 1986), bacteria (Muller, 1973), decaying organisms (Gerlach, 1977), and algae (Morse et al., 1980). Highly variable among species , the specific stimuli and mechanisms of stimulation by these environmental cues remain unclear (Burke, 1983b, Chia, 1989). Some species are believed to be stimulated by soluble organic substances in the water column, while others require the presence of a bottom sediment to settle (Scheltema, 1986). Cues are believed to be detected by chemoreceptors associated with the nervous system (Burke, 1983a).

There is little data concerning the effects of No. 6 fuel oil (i.e. Bunker C residual oil) on the settlement and metamorphosis of marine larvae. Previous studies have concentrated on lighter oils, such as No. 2 fuel oil, which require frequent measures of concentration due to evaporation, chemical oxidation, and biodegredation because of their high volatility (Johns and Pechenik, 1980; Pechenik and Miller, 1983). Specific aromatic hydrocarbon components, such as naphthalenes or phenathrene, are also frequently used for experimentation (Young, 1977). Number 6 fuel oil has thick coating qualities and is difficult to use in experimentation using water soluble fractions (Neff, 1981).

Many studies concerning the biological effects of oil focus on toxicity (Capuzzo, 1987). Though highly variable among species, the high concentration determined to be lethal termed LC_{50} values, are rarely found in the natural environment (Pechenik, 1987). On the other hand, sub-lethal concentrations provide practical information which can be directly related to oil-polluted communities. Though specific concentration of oil was not measured due to complexity and expense, my study sought to obtain oil concentration which was not lethal to the larvae.

The initial purpose of my study is to confirm results that both *Ilyanassa obsoleta* (Scheltema, 1961) and *Capitella sp. I* (Coumo, 1985) will metamorphose in response to a soluble organic substance without requiring bottom sediment. If the larvae metamorphose in response to a water soluble cue, then larvae over the muffled sediment condition should show a significantly lower percent metamorphosis than larvae over the control sediment. A second purpose of my study is to investigate the effects of No. 6 fuel oil on the rate of metamorphosis of the two species of larvae. Because of its toxic components, the oil may somehow interfere with the metamorphosis-stimulating cue. Thus, larvae should show a decrease in rate of metamorphosis over the oiled sediment condition. Because no metamorphosis-stimulating cue is present in the muffled/oiled sediment, results from this condition will not be significantly different form the muffled sediment condition.

MATERIALS AND METHODS

Larval Cultures

Egg capsules of I. obsoleta were collected form the intertidal flats of Barnstable Harbor Massachusetts in June 1990. Approximately half of the capsules were kept aerated in large 15-liter rearing jars filled with seawater. All seawater used in cultures was obtained from the Woods Hole Oceanographic Institute sea water system and filtered with a spun glass filter. At ambient temperature, approximately 25°C, the larvae underwent normal development with hatching from the egg capsule within a few days (Scheltema, 1962). The remainder of the egg capsules were kept at 15°C so that new cultures could be obtained throughout the summer.

The hatched veligers were placed at ambient temperature in 15-liter jars, aerated, and fed the unicellular algae, *Isochrysis galbana* (tropical strain). Cultures averaged 100 larvae per 15 liter glass container. Algae were grown in mass cultures at 20°C in f/2 media (half strength medium "f" described by Guillard and Ryther, 1962). Every 2-3 days the larvae were put in fresh sea water containing algae at a concentration of approximately 400,000 cells/ml. Larvae were allowed to develop to the creeping-swimming stage, approximately 600 microns in size, for use in experimentation.

Adults of *Capitella sp. I* were obtained form cultures maintained in the lab of Dr. J. P. Grassle (Marine Biological Laboratory, Woods Hole) and maintained at 15°C in large finger bowls. Sippewissett marsh (Sippewissett, MA) sediment, which was used as food in cultures, was washed with sea water, pushed through a 1 mm sieve, and frozen until use. Brood tubes containing developing embryos were isolated and placed in a small glass dish containing sea water without sediment. Dishes were kept at 15°C and checked daily for the hatching of larvae.

Experimental Procedure

All experiments with *I. obsoleta* were done at ambient temperature (25° C) in 10x10x6 cm translucent-white plastic freezer boxes. The dishes were labeled and filled with approximately 300 ml filtered sea water. All sea water used in experimentation was filtered through a Gelman glass fiber filter (variable pore sizes) using a Millipore filter system. About 2 ml of the particular bottom sediment to be tested was added to lightly cover the bottom of the dish and allowed to sit for at least 30 minutes for the sediment to settle. A control condition was also prepared by filling a dish with filtered sea water and without sediment.

Larvae of *I. obsoleta* at the age of 25-30 days, or a size of at least 600 microns, were added to each dish one at a time until the entire culture was used. The larvae were examined individually at 36 hours under a dissecting microscope to determine if the metamorphosis had taken place. Special care was taken to examine crawling larvae for the absence of the velum—the criterion used for determining metamorphosis. Experiments using larvae of *I. obsoleta* were repeated four times.

All experiments with *Capitella sp. I* were done at ambient temperature in glass Stender dishes (50 mm dia., 25 mm height). The dishes were labeled and one-half filled with approximately 12 ml filter sea water. Sediment from one of five conditions was carefully added with a pipette forming a small pile 1 cm in diameter in the center of the dish.

As larvae emerged form the isolated broods, they were used for experimentation within 3 days. Larvae from a single brood were added five to each dish with 2 replicates of each condition. Each experiment included one sea water control. The number of swimming larvae in each dish was counted approximately every six minutes for 60 minutes. Times at the beginning and end of each observation were recorded.

Statistics were computed for both species in order to compare results between sediment conditions. Only the final values for each sediment condition (reading at 60 minutes) in *Capitella sp. I* experiments were used in ANOVA.

Sediment Treatments

1. CONTROL SEA WATER; Every experiment contained one control which consisted of filtered sea water without sediment.

2. CONTROL SEDIMENT; Another condition in every experiment used sediment collected for Barnstable Harbor, MA. Sediment was maintained in a bucket, a few centimeters in depth, using light form a window to maintain algal growth on the surface of the sediment. Sea water was added to cover the top of the sediment and replaced weekly.

Conditions 3-5 involve manipulation of sediment collected from Barnstable Harbor.

3. MUFFLED: Sediment was placed in a muffled oven at approximately 1000°F for three hours. The off-white sediment, resembling beach sand was washed several times with distilled water and air dried for experimental use.

4. OILED: Sediment was oiled from oil-contaminated sediment. Samples were collected at three sites from Naushon Island, MA (Fig. 3): oiled salt marsh and unoiled salt marsh. All samples were kept moist in covered freezer boxes at 15 °C. Initial experiments showed that these samples could not be directly used as substrate conditions because results between unoiled Barnstable sediment and unoiled Naushon sediment were not equal. Therefore, Barnstable sediment was contaminated with oiled salt marsh samples and allowed to sit overnight. The water turned opaque black, smelled of petroleum, and held an oil sheen on its surface. This water was pipetted off with care not to include oiled marsh sediment. This oily water was then added to a dish containing Barnstable sediment. Samples were maintained at 15 °C and used within two days.

5. MUFFLED/OILED: Sediment was oiled as described in #4, except the sediment had been previously placed in the muffle oven (#3) before contaminated with the oil.

RESULTS

Experimental data for *I. obsoleta* are presented in Table I and Figure 4. Means of all *Capitella sp. I* trials are listed in Tables II-V;

trials 7-11 are graphed in Figures 5-10. Values in parentheses are extrapolated values used only when graphing the trend of the means; they are not experimental values and are not used as data points.

Creeping-swimming veligers of *I. obsoleta* showed a significant decrease in settlement on the muffled sediment as opposed to the control sediment (Fig. 4). Larvae in the control sea water consistently maintained the lowest percent metamorphosis with a mean of 8.5% (Table I). Results of the oil treatments did not significantly differ from the corresponding unoiled treatment results. However, larvae exposed to the muffled/oiled sediment treatments showed a 19.9% increase in metamorphosis over larvae of the muffled sediment condition (Fig. 4). Larvae from both the muffled sediment and muffled/oiled sediment conditions demonstrated greater variance and standard error from the mean (S_x*) than did larvae from the other three conditions which had not been treated in the muffle oven (Table 1).

In trials 1-6, *Capitella sp. I* larvae showed a quick and even settlement response to the control sediment and muffled sediment (Tables I, II, III). However, larvae of both oiled conditions demonstrated highly variable results between trials (Tables IV, V). In trials 1-6, larvae were taken from a single brood; a different brood was used in each trial.

Each of trials 7-11 used larvae from a mixture of eleven larval broods. These mixed brood results were much less variable. The closest mean curves from Figs. 5-8 were combined in Figure 9 for comparison. Percent metamorphosis at the final reading of sixty minutes is displayed in Fig. 10. A sharp decrease was evident in percent metamorphosis of larvae exposed to muffled sediment as opposed to the control sediment (Figs. 5,6). Though results were highly variable, the older larvae used in trials 10 and 11 over the muffled/oiled sediment settled more quickly (Fig. 8). Larvae over muffled/oiled sediment from trials 8 and 9, which were 24 hours younger than larvae used in trials 10 and 11, showed a settlement response similar to the larvae over the muffled sediment (Figs. 7, 8). Age did not have a significant effect on results of the other three sediment conditions.

ANOVA results for *I. obsoleta* showed that there was no significant difference between the control sea water and muffled sediment condition. The results of the oiled sediment condition also were not significantly different from the sediment control. All other comparisons were significantly different at *= 0.01, or a 1% degree of uncertainty (Table VI). Larvae of *Capitella sp. I* showed a percent metamorphosis at the final reading at sixty minutes which was significantly different between each sediment treatment (Fig. 10). The oiled sediment results differed from the control sediment results with at *=0.05. All other comparisons were significantly different at *=0.01 (Table VII).

DISCUSSION

In both species of larvae, *Ilyanassa obsoleta* and *Capitella sp. I,* there was a significant difference in the settlement response between the control sediment and the muffled sediment (ANOVA *=0.01) (Table VI). Results tend tó support previous research of Scheltema (1961). The muffled sediment condition in Scheltema's study was obtained by heating sediment over a high flame of a bunsen burner in a crucible for one hour. Results from Scheltema's (1961) experiments show a 64.0% decrease in larval metamorphosis with the muffled sediment condition compared to a difference of 72.2% shown in the current study (Table 1). Larvae of Capitella sp. I showed a percent metamorphosis at the final reading (60 minutes) which was 82.0% less with the muffled sediment condition compared to the control sediment condition (Tables II & III). A previous study supported these results when larvae of Capitella sp. I showed increased settlement when exposed to organic-rich sediment condition over a substrate of abiotic glass beads (Grassle and Butman, 1989).

In the muffled sediment condition, one can safely assume that all of the organic matter was burned away at the extreme temperature of 1000°F. Both of the past studies of Scheltema (1961) and Grassle and Butman (1989) used similar substrate conditions in which the organics were removed. The consistent decrease in larval metamorphosis over the muffled sediment conditions suggest that metamorphosis is not stimulated by just a physical contact with the substrate. It is possible that some organic substance that was present in the sediment before being burned off is detected by the larvae as a stimulus for metamorphosis. If this substance is not present, then the larvae may be capable of delaying metamorphosis until the proper environmental cue is detected (Pechenik, 1984).

There was no significant difference in % metamorphosis of *I. obsoleta* between the oiled sediment and control sediment conditions (ANOVA * =0.01) (Table VI). These results support previous evidence that planktonic larvae of bottom invertebrates are not adversely influenced when the concentration of pollutants is not excessive (Mileikovsky, 1970). It is almost certain that the concentration of oil absorbed on the sediment was high enough to be detected by this species of larvae. Though specific concentrations were not measured, oil could easily be seen and smelled when preparing oiled sediment sample conditions. In addition, many species of larvae have a keen sense of chemical detection and are able to detect the oil at very low concentrations (Scheltema, 1964). Though larval metamorphosis of *I. obsoleta* may not have been delayed by oiled sediment conditions in this study, measurements such as activity levels and respiration rates might have shown more sensitivity to the presence of oil. More studies need to be completed to measure both behavioral and physiological responses to low concentrations of this and other contaminants.

According to oiled sediment results of *I. obsoleta*, the possibility exists that oil could actually be a metamorphic stimulus itself. In this respect, the larvae could have experienced a sub-lethal toxic response. Hydrogen sulfide is believed to cause such a response in *Capitella sp. I larvae* (Dubilier, 1988). This type of reaction involves metamorphosis of larvae as a defense mechanism in response to the presence of oil. In this case, the larvae may metamorphose into a juvenile but die before becoming an adult— thus a sub-lethal response. A prolonged study to examine survival rates of larvae into adulthood would be a good measure of this response. The current study did not pursue this because of the limited time available.

Trials 1-6 with *Capitella sp. I* support the importance of larval brood differences. The first set of experiments used larvae from only a single brood in each trial; thus variability between broods was not accounted for in trials 1-6. Trials 7-11 accounted for brood differences due to the fact that eleven broods were mixed for use in these trials . A mixture of many broods allow for a more accurate comparison of results due to the fact that all experiments used larvae form the same brood mix instead of from different individual broods for each experiment. Therefore, discussion of *Capitella sp. I* results will concentrate on trials 7-11, which account for variability between broods.

Percent larval metamorphosis was significantly altered in *Capitella sp. I* with the addition of oil to the sediment (ANOVA *=0.05) (Table VII). In comparison of the trend of the means between the oiled sediment and the control sediment (Fig. 9), the rate of metamorphosis seems to be slowed with the addition of oil. A possible explanation for this involves a masking of the metamorphic cue(s)—water soluble organic substances present in the control sediment. The oil could somehow alter the organic substance thus, it may not be recognized by the larvae as a proper environmental cue. Another possibility could be that the oil physically covers larval chemoreceptors which are believed to detect the metamorphic stimulus (Burke, 1983a). The specific metamorphic-inducing mechanisms are not yet known and deserve much more research.

Comparison between the muffled sediment condition and muffled/oiled condition results seems puzzling in *I. obsoleta* and *Capitella sp. I* (Figs. 4,10). Because there was a low rate of metamorphosis over the muffled sediment compared with the control sediment, it could be deduced that larvae would also show a low rate of metamorphosis over the muffled/oiled sediment condition as well. In other words, since the muffled sediment condition contained no stimulus to cue metamorphosis in the muffled/oiled sediment condition, then there should be no stimulus to cue metamorphosis in the muffled/oiled sediment condition. Yet, the final percent metamorphosis of larvae of the muffled/oiled condition was significantly greater that the final percent metamorphosis on the muffled sediment (ANOVA *=0.01)(Fig. 10). A probable explanation for the increase in metamorphosis of larvae over the muffled/oiled condition as compared to the muffled condition could involve procedural error. Keeping in mind that the components of the oiled sediment conditions are unknown, it is possible that organics were transferred from the oiled salt marsh samples when preparing oiled sediment conditions. These organic substances could have served as a metamorphic cue to some of the larva.

It is important to note that in the *Capitella sp. I* muffled/oiled condition (Fig. 8), trials 10 and 11 used larvae from the same thirteen-brood culture (trials 8 and 9) except larvae for trials 10 and 11 were 24 hours older. The older larvae settled more quickly than larvae 24 hours younger. These results support the belief that some species of larvae, such as *Capitella sp. I*, that have a short developmental period can only delay metamorphosis for a short period of time (Pechenik, 1984). Since *Capitella sp. I* are competent to metamorphose upon hatching from the brood tube, they cannot delay metamorphosis for a long period of time. Thus, after a delayed period of time, such as 24 hours, larvae will not be as discriminating in accepting a proper environmental cue and therefore, will metamorphose even though a proper stimulus in not detected.

Capitella sp. I is known as an opportunistic species and is often found in polluted areas (Mileikovsky, 1970). *Capitella sp. I* is well-adapted to high stress environments such as the low oxygen and high hydrogen sulfide level associated with salt marshes (Gray, 1981). In fact, after the oil spill of the barge Florida off the coast of Massachusetts in 1969, *Capitella sp. I* had the greatest rate of recolonization of all species studied (Sanders et al., 1980). It might be difficult then to reason why the rate of metamorphosis was slowed by the presence of oil. The current experiment measured the immediate response to the contaminant. Even though *Capitella sp. I* may be an opportunistic organism, it seems probable that any organism would prefer metamorphosis in an uncontaminated environment.

Even with the low number of replicates, the results suggest some important conclusions. Firmly supported are previous theories involving the metamorphic cue— such as the belief that larvae metamorphose in response to an organic water-soluble cue (Scheltema, 1961). More detailed studies of larval development are needed to increase our understanding of the ecology of larval distribution. In addition, my study has great importance in the understanding of how larval populations are affected by No. 6 fuel oil spills. Results from this study suggest that No. 6 fuel oil does not influence the larval metamorphosis of *I. obsoleta* but does affect the metamorphosis of *Capitella sp. I*. Further studies are needed to determine the influence of fuel oils, as well as other pollutants, on the development of marine larvae in order to successfully evaluate the biological impacts of an oil spilland out of the lab due to the fine hospitality of Rudy and his wife, Amelie.

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GRAPHS AND TABLES

Fig. 1 *Ilyanassa obsoleta*, creeping-swimming veliger

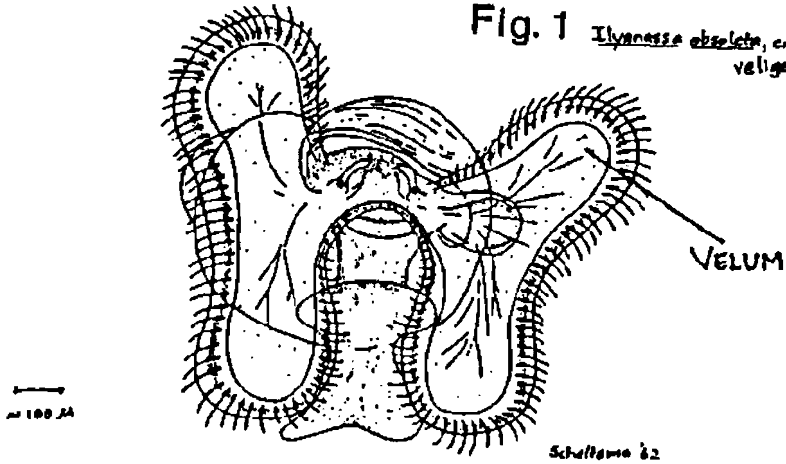


Fig. 2 *Capitella* Sp. I; TROCHOPHORE LARVA

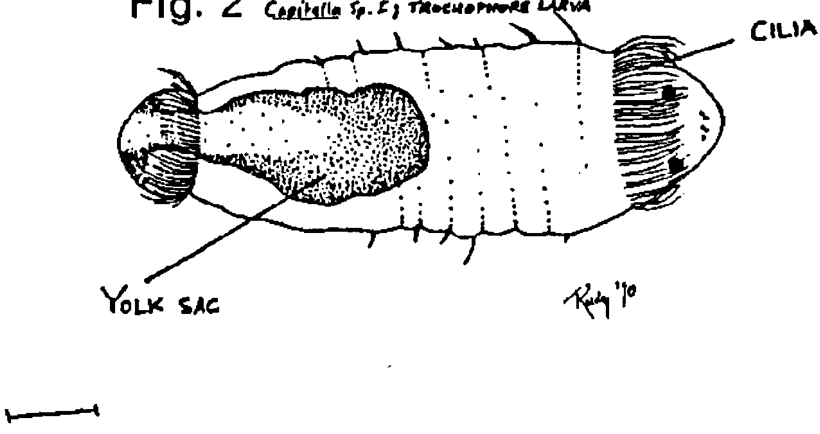
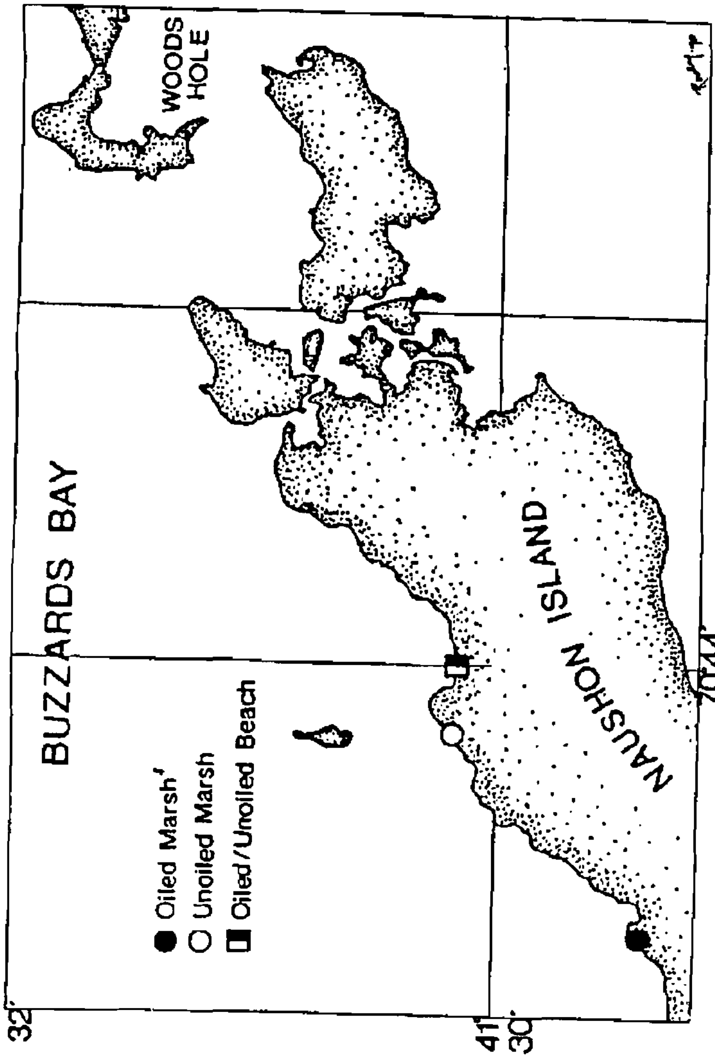


Fig. 3 Bermuda Star Oil Spill Sights; June 10, 1990, Buzzards Bay, MA.



*Samples from this sight used in experiment.

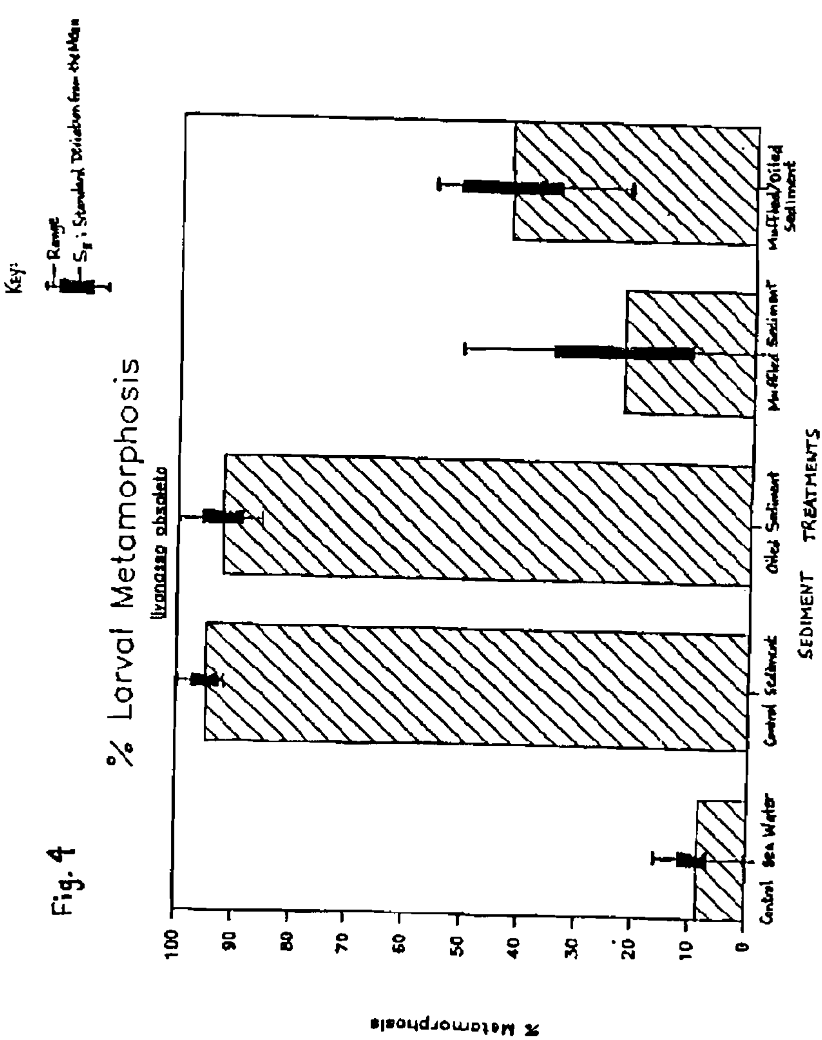


Fig. 4
% Larval Metamorphosis
Ilyanassa obsoleta
Key:
Range
S_x : Standard Deviation from the Mean
% Metamorphosis
100
90
80
70
60
50
40
30
20
10
0
Control Sea Water
Control Sediment
Oiled Sediment
Muffled Sediment
Muffled/Oiled Sediment
SEDIMENT TREATMENTS

Fig. 5 % Settlement, Barnstable Sediment

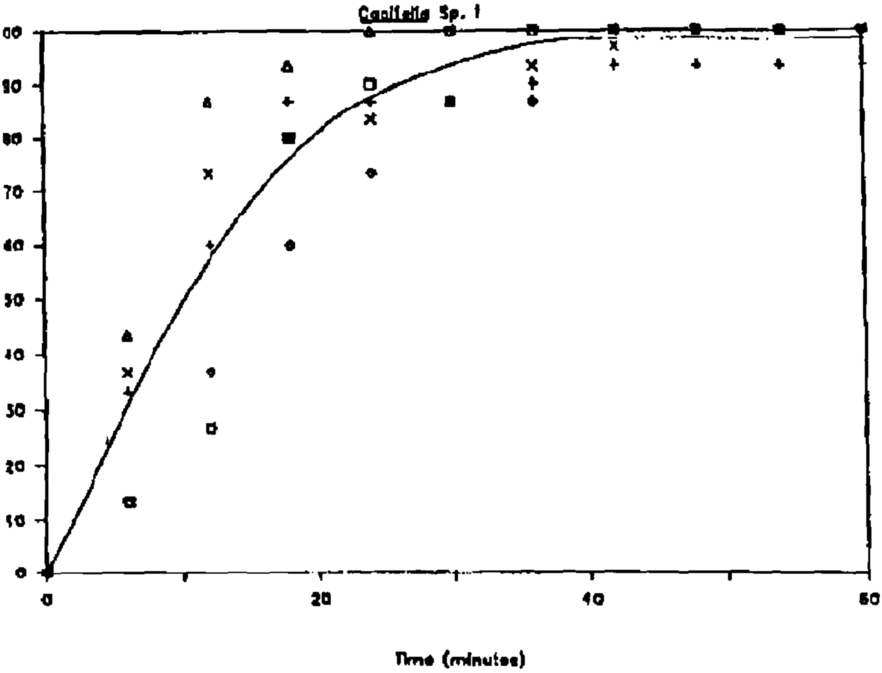


Fig. 6 % Settlement, Barnstable Sediment/Muffle Oven

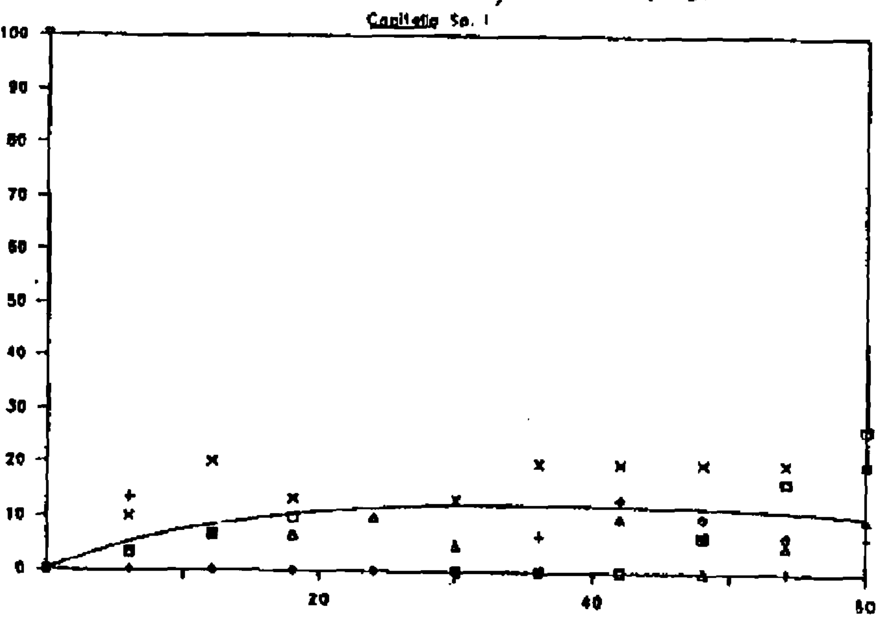


Fig. 7

% Settlement,
Oiled Barnstable Sediment

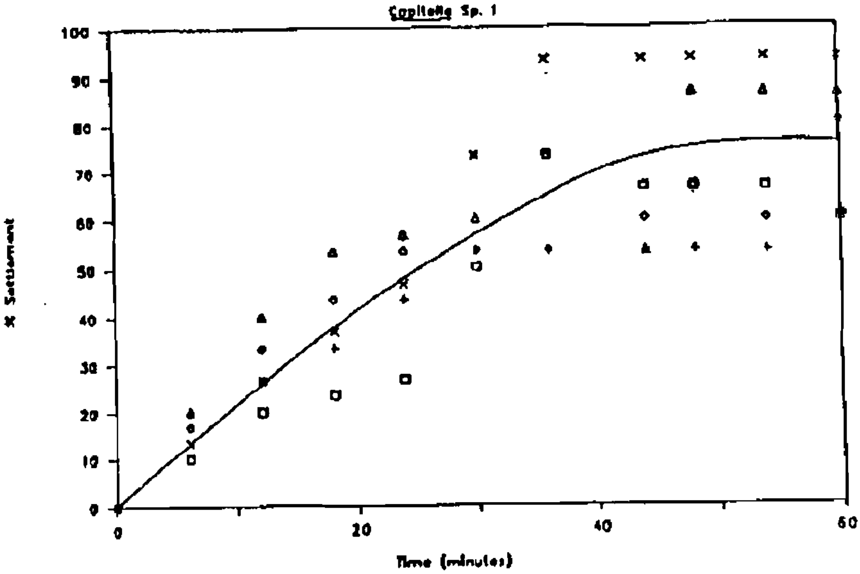
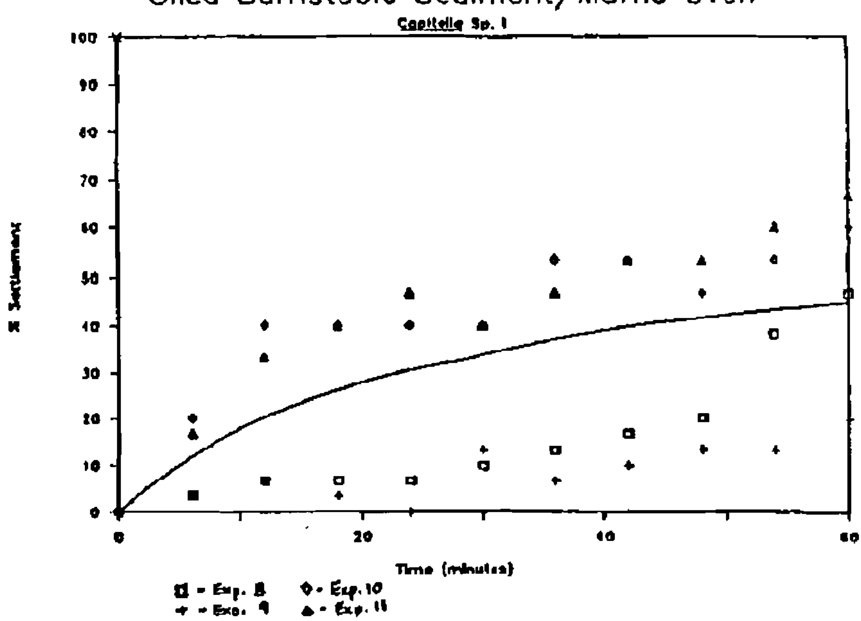


Fig. 8

% Settlement,
Oiled Barnstable Sediment/Muffle Oven



Time (minutes)

Fig. 9

Combination of Means

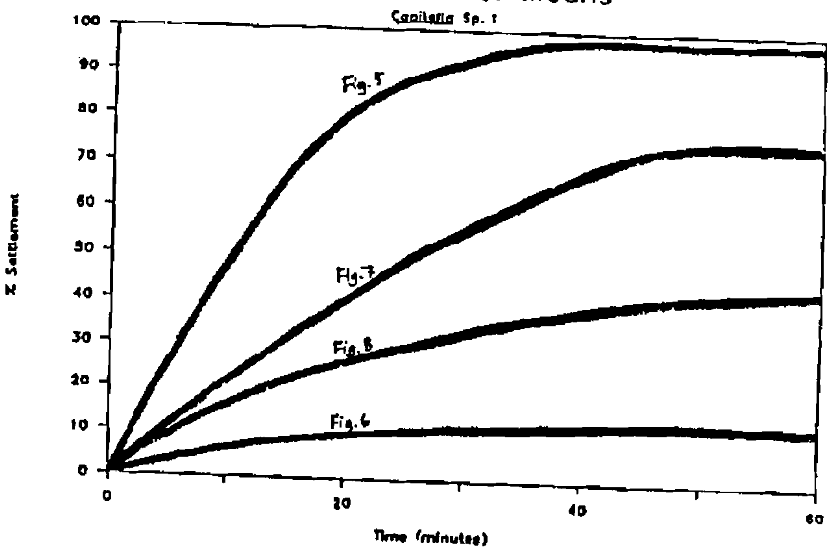


Fig. 5 — CONTROL SEDIMENT
Fig. 7 — OILED SEDIMENT
Fig. 8 — MUFFLED/OILED SEDIMENT
Fig. 6 — MUFFLED SEDIMENT

TABLE I

Ilyanassa obsoleta

% Metamorphosis

Exp. #

Sediment Condition	1*	%	2*	%	3*	%	4*	%	$\bar{x}$ (%)	$S_{\bar{x}}$ (%)
CONTROL SEA WATER	1/16	(6.3)	2/15	(13.3)	0/10	(0)	1/7	(14.3)	8.5	2.9
CONTROL SEDIMENT	14/14	(100)	11/12	(91.7)	12/13	(92.3)	-	-	94.7	2.7
MUFFLED SEDIMENT	-	-	3/17	(17.6)	0/13	(0)	6/12	(50)	22.5	12
OILED SEDIMENT	11/13	(84.6)	15/15	(100)	10/11	(90.9)	-	-	91.8	3.6
MUFFLED/OILED SEDIMENT	-	-	5/22	(22.7)	6/11	54.5	5/10	50	42.4	8.1

*Values presented as fractions represent number of larvae metamorphosed out of total number of larvae in experiment

TABLE II % SETTLEMENT, control

	Time (Minutes)										
EXP.	0	6	12	18	24	30	36	42	48	54	60
*1	0	(25)	50	(55)	60	70	90	90	90	90	90
2	0	(40)	80	86.7	93.3	93.3	93.3	93.3	93.3	93.3	93.3
*3	0	40	(50)	60	60	70	80	80	80	80	80
*4	0	40	40	40	40	50	70	90	90	90	90
*5	0	20	20	50	70	70	100	100	100	100	100
1-5 Av.	0	33	48	58.3	64.7	70.7	86.7	90.7	90.7	90.7	90.7
7	0	(13.4)	26.7	80	(90)	100	(100)	100	100	(100)	100
8	0	33.3	(60)	86.7	(86.7)	86.7	(90)	93.3	93.3	(93.3)	93.3
9	0	13.3	(36.7)	60	(73.4)	86.7	86.7	100	(100)	100	100
10	0	(43.4)	86.7	93.3	100	(100)	100	100	100	(100)	100
11	0	(36.7)	73.3	80	(83.4)	86.7	93.3	(96.7)	100	100	100
7-11 Av.	0	28	56.7	80	86.7	92	94	98	98.7	98.7	98.7

TABLE III % SETTLEMENT, muffled

	Time (Minutes)										
EXP.	0	6	12	18	24	30	36	42	48	54	60
*1	0	(6.7)	(13.3)	20	6.7	23.3	13.3	26.7	20	13.3	13.3
2	0	(6.7)	13.3	13.3	6.7	13.3	6.7	13.3	0	0	13.3
*3	0	0	0	10	10	10	0	0	10	10	10
*4	0	0	20	0	0	0	0	10	0	10	20
1-4 Av.	0	3.4	11.7	10.8	5.9	17.2	5	10	7.5	8.3	14.2
7	0	(3.4)	6.7	(10)	13.3	0	(0)	0	6.7	(16.7)	26.7
8	0	13.3	(6.7)	0	(0)	0	(6.7)	13.3	(6.7)	0	6.7
9	0	(0)	0	0	(0)	0	0	13.3	(10)	6.7	20
10	0	(3.4)	6.7	6.7	10	(5)	0	10	0	(5)	10
11	0	(10)	20	13.3	(13.3)	13.3	20	(20)	20	20	20
7-11 Av.	0	6	8	6	7.3	3.7	5.3	11.3	8.7	9.7	16.7

TABLE IV % SETTLEMENT, *oiled*

EXP.	Time (Minutes) 0	6	12	18	24	30	36	42	48	54
*3	0	(35)	70	70	80	80	80	100	(100)	100
*4	0	(40)	80	90	100	100	100	100	100	100
*5	0	90	100	100	100	100	100	100	100	100
*6	0	90	85	90	90	90	90	90	90	90
3-6 Av.	0	63.8	87.5	87.5	92.5	92.5	92.5	97.5	97.5	97.5
7	0	(10)	20	(23.4)	26.7	(30)	33.1	66.7	(66.7)	66.7
8	0	20	(26.7)	33.3	(43.3)	53.3	(53.3)	53.3	(53.3)	53.3
9	0	(16.7)	33.3	(43.3)	53.3	(53.3)	53.3	(60)	66.7	60
10	0	(20)	40	53.3	(56.7)	60	73.3	53.3	86.7	(86.7)
11	0	(13.4)	26.7	(36.7)	46.7	73.3	93.3	(93.3)	93.3	93.3
7-11 Av.	0	16	27.3	38	45.3	58	67.3	65.3	73.3	81.9

TABLE V % SETTLEMENT, *muffled/oiled*

EXP.	Time (Minutes) 0	6	12	18	24	30	36	42	48	54	60
*3	0	(5)	10	10	10	(10)	10	10	20	20	10
*6	0	100	100	100	100	100	100	100	100	100	100
8	0	(3.4)	6.7	(6.7)	6.7	(10)	13.3	(16.8)	20.3	38.5	46.7
9	0	(3.4)	6.7	(3.4)	0	13.3	6.7	(10)	13.3	13.3	20
10	0	(20)	40	(40)	40	40	53.3	53.3	46.7	(53.4)	60
11	0	(16.7)	33.3	(40)	46.7	40	(46.7)	53.3	53.3	60	66.7
8-11 Av	0	10.9	21.7	22.5	23.4	25.8	30	33.4	33.4	41.3	48.4

TABLE VI

ANOVA for *Ilyanassa obsoleta*

Sediment Condition	Mean difference (%)	LSD α = 0.05	LSD α = 0.01
Control Sea Water vs.			
Control Sediment	86.2**	23.63	33.36
Muffled Sediment	14.0ns		
Oiled Sediment	83.3**		
Muffled-Oiled	33.9**		
Control Sediment vs.			
Muffled Sediment	72.2**	25.27	35.66
Oiled Sediment	2.9ns		
Muffled/Oiled	52.3**		
Muffled Sediment vs.			
Oiled Sediment	69.3**		
Muffled/Oiled	19.9ns		
Oiled Sediment vs.			
Muffled/Oiled	49.4**		

TABLE VII

ANOVA for *Capitella* sp. I

Sediment Condition	Mean Difference (%)	LSD α = 0.05	LSD α = 0.01
Muffled/Oiled Sediment vs.			
Control Sediment	50.3**	19.19	26.94
Muffled Sediment	31.7**		
Oiled Sediment	27.5**		
Oiled Sediment vs.			
Control Sediment	22.8*	18.00	25.40
Muffled Sediment	59.2**		
Muffled Sediment vs.			
Control Sediment	82.0**		

"ns" denotes that percent settlement between the two sediment treatments is not significantly different

"*" denotes a significant difference with a 5% degree of uncertainty
"**" denotes a significant difference with a 1% degree of uncertainty