EFFECTS OF CHRONIC CONCENTRATIONS OF PETROLEUM HYDROCARBONS ON GONADAL MATURATION IN STARRY FLOUNDER (PLATICHTHYS STELLATUS [PALLAS])

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ABSTRACT

Adult starry flounder, during gonadal maturation, were continuously exposed to low concentrations (100 to 200 ppb) of the water-soluble fraction (WSF) of Cook Inlet crude oil for periods of 5, 7 and 21 days. Daily observations of behavior were made and fish were subsampled to determine effects on their gonads and other organs. Accumulations of low-boiling-point aromatic hydrocarbons were measured in the gonad and liver.

Female flounders accumulated a mean concentration of 12.98 µg/g (ppm) monocyclic, cyclohexane and dicyclic components in mature ovaries, 113 times the water column concentrations.

Several changes in behavior and histological damage in ovaries and livers were attributed to chronic doses of WSF. Egg maturation was accelerated and abnormal and dead eggs were observed in both immature and mature ovaries of exposed females. Maturation of male testes was also accelerated. Extensive vacuole formation was found in livers from exposed flounder.

If such effects also occur in the field at chronic levels, low concentrations of oil could have serious consequences on natural populations. If marine organisms prove generally to be more sensitive during their spawning season, fishery management decisions should consider timing of oil-related activities to adequately protect fishery resources.

INTRODUCTION

In recent years, oil spills and tanker accidents have shown a need for greater knowledge of the effects of oil on marine biota. Perhaps of greater concern than catastrophic incidents, however, is increasing chronic oil pollution, particularly in estuaries with continuous boat traffic, recurring minor spills associated with oil transport, and ongoing discharges of municipal and industrial effluents.

Estuaries are important habitats in the life histories of many fishes and sustain important commercial and recreational fisheries. Several species migrate into shallow waters off estuaries (e.g. starry flounder; Orcutt 1950) or through estuaries to spawn (e.g. salmon, striped bass). Estuaries are also important nursery areas for the young stages of many fish. Therefore, many species cannot avoid being exposed to chronic concentrations of pollutants during critical and sensitive life history stages.

Previous work at Tiburon Laboratory showed that prespawning Pacific herring were adversely affected by lower concentrations (100-800 ppb) of benzene than were egg and larval stages (which were adversely affected only at ppm concentrations). Adult herring exposed prior to spawning exhibited erratic spawning behavior, spawned prematurely, and suffered gonadal egg death. Survival of gonadal eggs and larvae to hatching and through yolk absorption was decreased by 50 percent (Struhsaker 1977).

The purpose of the experiments conducted and described here was to examine the effects of low concentrations of the WSF of Cook Inlet crude oil (approximating a chronic field concentration) on female and male starry flounder prior to spawning. An attempt was made to relate these effects to concentrations of the low-boiling-point aromatic and alkyl cyclohexane components accumulated in the gonadal and liver tissues; primary emphasis was given to the low-boiling point, monocyclic and dicyclic aromatic components of the water-soluble fraction (WSF) of crude oil because these components comprise a major portion (50-90%) of the water-soluble fraction (WSF) of several crude and refined oils (Anderson et al. 1974b) and are also relatively toxic.

METHODS

Fish:

Starry flounder, Platichthys stellatus (Pallas) were collected from inshore areas near Bodega Bay, California. Fish were captured with an otter trawl by personnel of the California Department of Fish and Game and were transferred to 1900-liter tanks at Tiburon for acclimation.

Flounder were not fully ripe when captured, and were in varying stages of maturation prior to spawning. Standard lengths, wet weights of adults, lengths and weights of gonads and other morphometric data of the fish used in the experiments are summarized in Table 1. The primary

difference between experiments was in the percentage of adults with maturing gonads. In Experiment 1 (January) females were in earlier maturation stages. In Experiment 2 (February) most females were maturing and in late maturation stages; the males were all ripe. Throughout the experiments we were unable to obtain ripe-running females from the field, so in Experiment 3 (April) an attempt was made to induce spawning. This experiment was unsuccessful, because most females were in a refractory period (resting stage). Males, however, were ripe and some results from this experiment are reported.

Apparatus:

Three experiments were performed in a continuously flowing system using dosing apparatus developed by project personnel (Benville et al., manuscript in prep.). This apparatus (Figure 1) produced a stable outflow of the water-soluble fraction of Cook Inlet crude oil which could be diluted easily to desired concentrations.

Acclimation and Experimental Conditions:

Flounder were acclimated to experimental conditions for at least two weeks prior to experimentation. The experiments were conducted at ambient levels at the time of testing (Table 2). The mean temperatures for the three experiments were 10.7, 13.7, and 17.2°C, respectively. The flounder were fed a diet of squid and bay shrimp during acclimation.

Because they do not feed during spawning and the exposure periods were relatively short (5 and 7 days), flounder were not fed during the first two experiments. In Experiment 3, flounder were fed the last week of the exposure period.

Test groups of starry flounder were placed in each of two 866 liter fiberglass tanks; one tank received a continuous flow of WSF in seawater at a mean concentration from 115 to 221 ppb (depending upon which experiment, Table 2). The other tank received uncontaminated (control) seawater at the same flow rate.

Flounder were continuously dosed during experimentation. Water samples were taken and analyzed daily to determine the concentration of low-boiling-point hydrocarbons in each of the tanks and the dosing apparatus effluent. Temperature, salinity, oxygen, and flow rate were monitored daily.

Daily observations of behavior included ventilation rate (number opercular beats/min), estimation of ventilation volume, regularity of ventilation, estimation of swimming activity, "digging" or escape activity, and feeding behavior. Ventilation rate was measured; other parameters were ranked.

In Experiments 1 and 2, two flounder were taken daily from each tank (exposed and control) for autopsy, chemical analysis, and histology. In Experiment 3, all flounder were autopsied at the end of a 3-week exposure. All fish were weighed, measured and dissected. Macroscopic examinations of all organs were made and the ovaries, testes, liver and gall bladder were removed. Gonads were weighed, measured and examined microscopically

to determine general maturation stage, presence of opaque dead or necrotic eggs, and the gross appearance (color and deliquescense). Maximum egg diameters of 10 eggs from the ovary of each female were measured. Spermatozoa from ripe males were examined under the microscope for motility.

The ventral gonad, gall bladder and half of the liver were placed in clean, preweighed glass culture tubes or glass sample jars. The tubes were sealed tightly with teflon-lined screw caps, the bottles with foil-lined caps. All samples were frozen until they could be analyzed. The dorsal gonad and other half of the liver were preserved in 10% formalin in 1% calcium chloride for histological preparation.

Analytical Procedures:

Analysis of Water Samples. Water samples taken daily from exposure tanks were analyzed for monocyclic aromatic hydrocarbons by extracting one liter twice with 10 ml of TF-Freon and injecting 3.2 microliters of each extract into the gas chromatograph (column packed with 5% SP-1200 and 5% Bentone 34 on 100/120 supelcoport). Liter samples taken daily from solubilizer effluent flowing into the exposure tanks were extracted 3 times with 10 ml of TF-Freon and extracts were injected into the GC. The limit of detectability for monocyclics in water was 0.010 mg/L. In addition, one-liter solubilizer samples were extracted with 80 ml methylene chloride for analysis of dicyclic aromatic compounds.

Extracts were concentrated to about 10 ml and stored in a freezer. Subsequent preparation for analysis followed procedures of MacLeod et al. 1977 and were analyzed on a 10-foot column packed with 5% SP-2100, 1% BMOT on 100/120 supelcoport.

Alkyl cyclohexanes and dicyclics were undetectable in tank water column samples. For results reported here, the concentration was estimated from the concentration in solubilizer effluent and by calculating final tank concentration with the dilution factor.

Analysis of Tissue Samples. Samples analyzed for dicyclic aromatic hydrocarbons and aliphatic compounds were processed according to MacLeod et al. 1977. Samples were analyzed for monocyclic aromatic and alkyl cyclohexane hydrocarbons using procedures developed by project personnel for these experiments (Table 3). In some samples, insufficient weight of tissue (particularly immature gonads) was available, and samples were pooled. In instances where sample weights were below the optimum 10 g, tissue concentrations may be underestimated, particularly of less abundant components. Also, a few liver sample extracts were emulsified and could not be analyzed.

Additional Chemical Analyses. The National Analytical Facility, Seattle, Washington, performed a number of analyses of water, tissue, and crude oil samples by capillary column gas chromatography or mass spectrometry to verify results obtained by in-house analysis and to identify unknown hydrocarbons in tissue and water extracts.

Histological Technique:

Ovaries preserved in 10% formalin in 1% calcium chloride were prepared especially for examination of the lipid distribution in the eggs following the recommendation of Bucke (1972) and using the procedure in Humason (1972). Frozen sections (16 μ) were cut with a cryostat, because routine processing methods would remove lipids. Sections were stained with an oil soluble dye, Oil Red O, counterstained with Harris' Hematoxylin and blued in Scott solution. Staining times were adjusted according to the maturation stages, which varied in stain affinities. One series of sections from each sample was stained with Oil Red O only, another series with Oil Red O and hematoxylin.

Livers were embedded in paraffin and 10 μ transverse and longitudinal sections made. Sections were stained with hematoxylin and eosin according to the procedure in Humason (1972).

Histological Examination:

Each slide was first examined under a low-power (4X or 10X objective) phase contrast microscope and searched for the presence of dead or abnormal eggs. One hundred eggs were counted and closely examined and the percentage dead or abnormal determined. The maximum diameter of the five largest eggs was measured, and the maturation stages ascertained after a complete assessment of all cytological structures occurring at that stage. The maturation stages determined by Yamamoto (1956) for eggs of the flounder, Liopsetta obscura, were found applicable and used in this analysis (11 stages in all). Photomicrographs were taken of control and exposed eggs.

Spawning Induction:

In Experiment 3, an attempt was made to induce the flounder to spawn. Most flounder were adults, but they appeared to be in a post-spawning refractory stage. The technique of Smigielski (1975 a, 1975 b) using carp pituitary (5 mg/454 g total wet weight) in physiological saline solution was employed. Fish were marked with colored thread through the caudal fins so behavior of individual fish could be observed. It was not possible to distinguish sexes in the species with certainty, thus 8 fish were selected at random and injected with carp pituitary, 8 fish were not. The experimental design was as follows:

	Control		Exposed	
	Injected	Not Injected	Injected	Not Injected
Exposed immediately after first injection	2	2	2	2
Exposed 1 week after first injection	2	2	2	2

RESULTS

Morphometrics:

Fish in all experiments were in similar condition. However, more fish in the second experiment were maturing and few females in the third experiment were maturing (Table 1). There were no significant differences between condition for exposed and control fish or length-weight ratios of gonads in the experiments.

The "eyedness" of the flounders was recorded because this genetic feature varies in frequency between populations and with age. For the sample in Experiments 1 and 3, there were approximately 50% right-eyed to 50% left-eyed flounders (Table 1), but more large fish were right-eyed than left-eyed. The differential survival of right-eyed flounder is thought to indicate a physiologically hardier type (Orcutt 1950). In Experiment 3, a higher proportion of the sample was left-eyed; there were more small males in this sample.

Concentrations of Petroleum Components in Test Tanks:

Mean concentrations of petroleum hydrocarbons (WSF) ranged from 115 to 221 µg/L (ppb) in the three experiments (Table 2). Individual component concentrations are given in the Appendix (Table A). Only monocyclic aromatic hydrocarbons were detectable in test tanks, although traces of alkyl cyclohexanes were seen in some samples (Appendix, Table E).

Mortality - Adults:

No mortality occurred at the low exposure concentrations, as expected. In other experiments with flounder, exposed with similar apparatus, mortality was observed at concentrations as low as 1.0 mg/L (ppm) (Yocom et al., manuscript in preparation).

Behavior:

Several behaviors were observed and measured. Usually, individual variation obscures significant treatment differences. In all experiments, however, there was an initial elevation of ventilation rate in exposed fish over that in controls for periods of 2 to 4 days. Eventually, rates of exposed fish dropped and the ventilation rates of exposed and controls were similar. In Experiment 3, the mean ventilation rate of exposed flounder was 45.1 beats/min after the first week and that of controls was 35.7 beats/min. Irregular ventilation rates were also observed in exposed and not in control fish.

Concentrations of Petroleum Components in Tissues:

Tissue concentrations and accumulations of components in ovaries, testes, livers and gall bladders are given in the Appendix (Tables A-E). Means and ranges of monocyclics, alkyl cyclohexanes and dicyclic components are also summarized in Table 4. In Table 4, monocyclics are grouped into monocyclics 1 (M-1) and monocyclics 2 (M-2). The first group (M-1) includes the lowest-boiling-point monocyclics:

benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene. The more substituted monocyclics (M-2) included isopropyl benzene, n-propyl benzene, total C_3-benzenes, total C_4-, and total C_5-benzenes. Alkyl cyclohexanes (CH) identified include methyl cyclohexane, cis 1, 3 dimethyl cyclohexane, 1, 2 dimethyl cyclohexane and a C_3 cyclohexane. Three other unidentified components are probably also C_2 and C_3 cyclohexanes. Dicyclics indentified (D) include naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, and total C_2 naphthalenes.

Monocyclics 2 (M-2) and dicyclics were analyzed only in mature ovaries in Experiment 2. All monocyclics, cyclohexanes, and dicyclics were analyzed in tissues of Experiment 3. Since fewer maturing ovaries occurred in Experiment 1, not all of these samples were analyzed. Monocyclic concentrations in maturing ovaries were similar to those in Experiment 2. Other experiments (Yocom et al, manuscript in preparation) indicated no detectable levels of any components in 120 tissue samples from control flounders. Therefore, only a few controls were analyzed in these experiments. No components were detected in any controls.

Ovaries. Immature ovaries accumulated much lower concentrations of monocyclics and alkyl cyclohexanes (mean total - 0.890 µg/g; Table 4) than mature ovaries (mean total = 8.585 µg/g monocyclics and alkyl cyclohexanes; 12.976 µg/g all monocyclics, cyclohexanes, and dicyclics; Table 4). Uptake of components was rapid, near maximum concentrations occurring after 24 hours of exposure (Figure 2). The maximum accumulation of components in any ovary was 14.618 µg/g.

Proportions of the components remained relatively constant throughout the experiment (Figure 2). The accumulation of alkyl cyclohexanes was of some interest, since these components have not previously been identified as biologically active components of crude oil and yet appear in relatively high concentrations in both ovarian and liver tissues (Yocom et al, manuscript in preparation-b). A gas chromatogram of the freon extract of adult starry flounder ovary exposed for approximately 7 days (Exp. 2) is shown in Figure 3; individual monocyclic and alkyl cyclohexane components are labeled.

The maximum accumulations over water column concentrations are given in the Appendix (Tables A-E), and are summarized in Table 4 and Table 5 (ovaries only). The maximum accumulation was determined by dividing the tissue concentration by the water concentration. In the case of components not detected in tank water samples (monocyclics and alkyl cyclohexanes < 0.010 mg/g; dicyclics < 0.00025 mg/g) the tank concentrations were estimated (see Methods).

Maximum accumulation of all monocyclics, alkyl cyclohexanes, and dicyclics in mature ovaries was approximately 80 to 200X (mean = 112.8X) and of monocyclics (M-1 only) and cyclohexanes was approximately 50 to 160X (mean = 74.6X) the water concentration (Tables 4 and 6). In immature ovaries, the maximum accumulation of monocyclics (M-1 only) and and alkyl cyclohexanes was approximately 3 to 10X (mean = 7.7X) the water concentration.

Testes. There were no immature males in the samples. Testes of mature males accumulated no detectable levels of components after 1 week of exposure (Table 4). However, after 3 weeks of exposure (Appendix, Table D) 0.313 to 1.361 µg/g of toluene accumulated in the testes. No other component was detectable. Uptake in the testes was low and much slower than in ovaries. The maximum accumulation of toluene after 3 weeks of exposure was approximately 3X to 6X the water concentration.

Livers. The concentrations of monocyclics (M-1 only) and alkyl cyclohexanes were determined after 1 week of exposure for livers of both males and females (Table 4). Mean concentrations of these components were 8.450 µg/g in mature males, 15.535 µg/g in mature females, and only 1.346 µg/g in immature females. Data over the 1-week test period indicate an increasing concentration with time, thus uptake does not reach a maximum level rapidly as in the case of the gonads. After 1 week of exposure mean maximum accumulations in mature adults were approximately 70X (males) and 135X (females) the water concentration (Appendix, Tables A-C). After 3 weeks of exposure, maximum monocyclic accumulations were approximately 200X to 400X (immature females) and 225X to 290X (mature males) the water concentration (Appendix, Table D). Maximum accumulation of cyclohexanes was approximately 2600 to 8750X the water concentration (Appendix, Tables B and E).

Gall Bladders. The weight of tissue available for component analysis in gall bladders containing bile was insufficient in most cases, but analysis of monocyclics and cyclohexanes in gall bladders of fish in Experiment 2 (Appendix, Table A) showed that the concentration was approximately the same in gall bladders of males and females and did not significantly increase throughout the 7-day exposure period (0.280-0.469 µg/g). Only toluene was present, no other monocyclics or cyclohexanes were detected. The maximum accumulation in gall bladders was approximately 2 to 4X the water concentration of toluene.

All Tissues. Among the six monocyclics (M-1), the relative accumulation of toluene was the highest, generally followed by m-xylene, benzene, o-xylene, ethylbenzene and p-xylene, respectively. Of the cyclohexanes (CH), methyl cyclohexane reached highest concentrations, followed by cis 1, 3 dimethyl cyclohexane, and 1, 2 dimethyl cyclohexane. Among the more substituted monocyclics (M-2) the highest concentrations were of the C_3-benzenes, followed by C_4-benzenes and C_5-benzenes, respectively. And finally, among the dicyclics (D), naphthalene was highest in concentration followed by 2-methylnaphthalene and 1-methylnaphthalene, respectively. These general proportions were approximately the same in liver tissues of both sexes. In mature ovaries, however, the relative abundance of monocyclics was slightly different than in livers, the concentration of benzene being higher than m-xylene.

Autopsies:

Liver. Some exposed fish showed gross effects in the appearance of the liver. After a few days of exposure, livers appeared to have hemorrhagic areas and were mottled.

There was considerable variation in liver color, from bright yellow to

dark red (Table 1). There was no obvious correlation with sex, size or exposure to WSF. There is an indication that the color variation is associated with gonadal maturation, with fewer fish having yellow livers at later maturation stages (Table 1).

Ovaries. Exposed fish showed some effects in the appearance of the ovary. Exposed ovaries were paler yellow than controls of equivalent maturation stage. These ovaries also contained some opaque white eggs, visible on the surface, appearing to be necrotic foci with several dead eggs when examined microscopically. The capillary network over the ovarian membrane of exposed fish contained blood, but the network appeared paler red than in controls. Microscopic examination indicated hemolysis of red blood cells had occurred, lending to the overall paler appearance of exposed ovaries. There were dead eggs seen in most of the maturing ovaries (Table 6). Dead eggs were not obvious in immature ovaries. Because the differences between control and exposed fish were subtle, further comparison was made histologically (see below).

Testes. No obvious differences were noted in the appearance of the testes. No difference in spermatozoan motility between control and exposed males was measured.

Histological Effects on Eggs:

Abnormalities and Mortality. Abnormal and dead eggs were first observed in immature and maturing ovaries of exposed females by day 2 of the exposure (Table 5, Table 6). These abnormal and dead eggs were observed throughout the remainder of the experiment. Ovaries of fish examined 1 week after the cessation of exposure and depuration had the most dead and abnormal eggs, indicating that effects were not reversible.

The mean percentages of abnormal eggs in exposed were 13.2% in immature and 3.0% in mature females of Exp. 1 and 0.5% in immature and 13.0% in mature females of Exp. 2. The mean percentages of dead eggs were 5.5% in immature and 5.8% in mature females of Exp. 1 and 0.2% in immature and 15.0% in mature females of Exp. 2 (Table 7).

The commonest abnormality observed in both immature and mature eggs was the occurrence of clear vacuoles extruding through the cytoplasm of the egg, usually near the periphery (Figs. 4B, 5B, 6B). The vacuoles were colorless with oil red O staining, indicating that they are not lipid deposits. These vacuoles were never observed in control eggs (Figs. 4A, 5A).

Dead eggs in immature ovaries occurred in necrotic foci, and appeared to be atresic (Figs. 7B, 8A, 8B). By the end of the exposure period, and particularly after the subsequent depuration period, reduced numbers of eggs appeared to occur in exposed immature ovaries compared to controls (Figs. 7A and B), possibly indicating resorption. The first change appeared to be coalescence of nucleoplasm into fewer droplets (Fig. 8A) until only one droplet was present, the nuclear membrane still intact. The coalesced droplet had a different staining affinity, appearing purple rather than blue as do nucleoli of controls and unaffected exposed eggs. Subsequently, the nuclear membrane

disappeared, the droplet remaining, with simultaneous necrosis of the cytoplasm and disintegration of the cell membrane (Fig. 8B).

Dead maturing eggs also occurred in necrotic foci (Fig. 9B). The eggs were disintegrating and appeared to have thicker cell membranes (zona radiata) than the intact controls (Fig. 9A). With red oil O staining, yolk globules appeared a darker red-brown color than the normal red-pink globules of control eggs. Although present in exposed ovaries, the foci of dying and dead mature eggs were difficult to detect histologically with only a few sections from each ovary.

Acceleration of Maturation:

Females. Another apparent effect is the acceleration of egg maturation in exposed flounder over that in controls (Table 7). The stages of Yamamoto (1956; 1-11) were used to assess maturation so that differences between controls and exposed attributable to different maturation stages would not be confused with the effects of exposure. A complete description of egg maturation in starry flounder will be published separately (Bowers and Whipple, manuscript in preparation).

In Experiment 1 (January; Table 5) immature ovaries contained eggs in stages 1 to 3 (1 = chromatin-nucleolus, 2 = early perinucleolus, and 3 = middle to late perinucleolus). Maturing ovaries were in stages 4 and 5 (yolk = yolk vesicle, and 5 = primary yolk stage). In Experiment 2 (February; Table 6) immature ovaries were in stages similar to those in Experiment 1 (stages 1 to 3). In maturing ovaries, however, control eggs were predominantly in stage 6, or 6 to 7 (6 = secondary yolk stage, 7 = tertiary yolk stage). None had reached the full tertiary yolk stage (7). Among the exposed, several ovaries showed eggs in later stages 7 and 8 (7 = tertiary yolk stage and 8 = migratory nucleus stage). Maturing flounder contain eggs in two stages, having eggs in perinucleolus stages as well as the predominant maturation stage.

Table 7 summarizes the means of several parameters showing acceleration of maturation in ovaries of exposed flounder for Experiment 2. An indication of acceleration of maturation in females of Experiment 1 was also noted. Table 7 shows that ovaries of exposed maturing females not only had a higher frequency of more advanced maturation stages, but also that the mean maximum egg diameter was greater. These observations correlate with the higher gonadosomatic index (GSI) in exposed (6.59) than in control (5.64) females. Since the GSI is based on the ratio of ovary weight to body weight and because relatively small females contained advanced maturation stages, the indication is that acceleration in maturation was due to exposure and not a function of the size of the female.

Males. Histological sections of testes were not made. However, the GSI of males also indicated an acceleration of maturation due to exposure. Sample sizes of males in Experiments 1 and 2 were too small. However, the males in Experiment 3 (exposed for 3 weeks) had a higher mean GSI (0.387; n=5) than did controls (0.140; n=7). Further, 80% of the exposed males were ripe, while only 29% of the controls were ripe.

Histological Examination of Livers:

Examination of liver tissue showed that, after 5 to 7 days exposure to 115 ppb WSF, livers were highly vacuolated and the hepatic muralia were disorganized in both male and female flounders (Figs. 10B, 11B). Cytoplasmic vacuolization increased with exposure time. Similar effects were also seen in livers of flounder allowed to depurate for 1 week after the 1 week exposure period, indicating the effects may not be reversible, as the degree of vacuolization was greater. Sloughing of endothelial tissue in blood vessels was found in livers of some exposed flounder. These changes were not seen in livers of control flounder (Figs. 10A, 11A).

CONCLUSIONS AND DISCUSSION

Starry flounder exposed to approximately 100 ppb of the WSF of Cook Inlet crude oil exhibited several effects, including elevated activity and ventilation (respiration), erythrocyte destruction, abnormalities and necrosis in eggs and liver tissue, and acceleration of maturation in both males and females (Table 8).

Flounder were exposed only to WSF components in the water column. In the field, exposure could occur from two other routes; sediments and food. The exposure concentrations in these experiments (approx. 100-200 ppb) are probably higher than in the field. Although measurements of aromatics in natural waters are scarce, available field data show that chronic concentrations of total aromatics in water may reach levels of 1-30 ppb offshore (Clarke et al. 1977b; Hiltabrand 1978). Concentrations are probably higher in polluted estuaries. In addition, an exposure of 7 days is less than flounder would experience in a chronic field situation. Sediment concentrations of aromatic hydrocarbons (Clarke et al. 1977b) reach levels of approximately 1,000-60,000 ppb (wet wt.). Flounder would also be exposed to aromatics in sediments in chronically polluted areas. Field measurements of alkyl cyclohexanes in water or sediments are not available. Flounder could also be exposed to WSF components of petroleum through contaminated food. However, in later prespawning maturation stages, flounder do not feed (Orcutt 1950) and other experiments at our laboratory (Yocom et al., manuscript in preparation) show that when nonspawning adults were fed contaminated littleneck clams (8.0 µg/g M-1+CH), they accumulated 1.72 µg/g in their livers (CH only) compared to flounder exposed through the water column (0.100 mg/L M-1+CH) and fed uncontaminated clams, which accumulated 180.8 µg/g (M-1+CH). Lack of accumulation of the toxic aromatic fraction from food may partially explain the relatively few effects observed in rainbow trout exposed prior to reproduction to petroleum in their diet, as observed by Hodgins et al. (1977a). The major route of accumulation of the more toxic aromatics would appear to be through water column and sediment exposures. The exposure concentration in our experiments is probably a reasonable approximation to chronic exposure of flounder in the field.

No flounders died in these experiments, but behavior was affected. There was an elevation in activity and ventilation, indicating an increased respiration and metabolic rate. A return to control levels occurred subsequently. Similar results were obtained in other studies of larvae and adults of salmon, striped bass, and Pacific herring exposed to sublethal levels of benzene (Brocksen and Bailey 1973; Eldridge et al. 1977) and in other fish and invertebrates exposed to sublethal levels of WSF of crude and refined oil (Anderson et al. 1974a). The behavior of adult herring prior to spawning was greatly affected by exposure to ppb levels of benzene, including rapid ventilation, disequilibrium, and premature spawning (Struhsaker 1977).

Uptake and total accumulation of monocyclics (M-1) and alkyl cyclohexanes (CH) in gonads and livers of flounder varied with sex and maturity (Table 8). Highest mean accumulation occurred in livers of adult females with gonads in the resting stage (2800X; Yocom et al., manuscript in preparation) and lowest in immature females (12X). Mature males accumulated 74X and mature females 135X the concentration of M-1 and CH in the water. Concentrations increased with time in livers of all flounders, reaching much higher levels after 3 weeks than after 1 week of exposure.

Uptake and accumulation of M-1 and CH in gonads also varied with sex and maturity. Rate of uptake was much faster in mature ovaries than in livers, with maximum concentrations in about 24-48 hrs. Concentration levels of all components measured (M-1, CH, M-2, D) remained at an equilibrium level for the remainder of the exposure period. Maximum accumulation was highest in female ovaries (75X), lowest in male testes (not detectable). Ovaries of immature females and resting ovaries of mature females accumulated comparatively low concentrations (7X, 8X respectively).

Variation in accumulation in the liver may be related to feeding and resultant metabolic rates. Adult flounders in the resting stage were fed clams and probably had a faster rate of metabolism and uptake than did sexually maturing male and female flounders and immature females which were not fed. The variation in accumulation of lipid-soluble components in gonads is probably a function of the amount of lipid; maturing ovaries containing far more lipid than immature ovaries and testes containing even less lipid.

Although tissue samples are available for analysis of alkanes, we have not completed these analyses. Anderson (1974a) suggested that although alkanes and aromatic hydrocarbons are both accumulated, the aromatics are concentrated to higher levels and retained longer than the alkanes. Kühnhold et al. (see paper in this proceedings) found that winter flounder accumulated relatively low concentrations of alkanes from low ppb levels of No. 2 fuel oil after long exposures while accumulating higher levels of aromatics. Alkanes are believed to be less toxic than aromatics, although more recent work suggests they may have narcotic effects and cytotoxicity (Clarke et al. 1977a).

The highest total concentrations in ovaries were of M-1, followed by CH, M-2 and D. The proportions of M-1, CH, M-2 and D remained fairly constant in mature ovaries over the exposure period. In livers, however, the relative proportions of M-1 and CH changed, with cyclohexanes increasing relative to monocyclics with time. Concentrations of individual components in mature ovaries after 7 days of exposure were as follows, in decreasing order: methyl cyclohexane, toluene, benzene, C_2 cyclohexane (unidentified #1), m-xylene, Cis 1, 3 dimethyl cyclohexane, ethylbenzene, 1, 2 dimethyl cyclohexane, C_3 cyclohexane, o-xylene, C_3 cyclohexane (unidentified #3) and p-xylene. Mean total accumulation of alkyl cyclohexanes after 7 days of exposure was much higher than of monocyclics-1 (890X, 68X water concentration, respectively). The high accumulation of cyclohexanes in ovaries and livers from undetectable levels in water is of interest since they have not been previously identified as biologically active components. The toxicity of the cyclohexanes is not well known. Some data indicate that they are as toxic to fish as are monocyclic aromatics (Pickering and Henderson 1966), but information is so incomplete that no definite statement can be made. The mean total accumulation of M-2 and D in ovaries after 7 days of exposure was approximately 260X water concentration. Highest concentrations of individual M-2 and D components in mature ovaries were of methylated naphthalenes.

When the relative concentrations of components in both liver and gonadal tissues are compared to concentrations in the water, it is apparent that differential uptake has occurred. Many components are undetectable in the water column but accumulate to very high levels in the liver and gonads, particularly the alkyl cyclohexanes.

A recent review by Clarke et al. (1977b) shows that few laboratory experiments have been done on the accumulation of toxic WSF components in ovaries or testes of fish. Work by Zitko (1971) on the flounder, Pseudopleuronectes americanus, showed a total aromatic concentration of 622 µg/g wet weight in the "guts" of flounder exposed to Bunker C. Previous work at the Tiburon Laboratory, with herring exposed to 100 ppb benzene prior to spawning, showed a concentration of 1.2 µg/g wet weight benzene in mature ovaries after a 48-hr exposure. This is comparable to the uptake of benzene in mature ovaries of flounder (1.27 µg/g) after 48 hrs. In addition, few laboratory experiments measuring accumulation of monocyclics in specific tissues have been made. Korn et al. (1976-1977) measured uptake of radioactive labeled benzene in several tissues (not mature ovaries) of northern anchovy, striped bass and Pacific herring. Residues of benzene and toluene in liver tissue of herring are comparable to those measured here in flounder, with toluene reaching higher concentrations than benzene. Clarke et al. (1977b) summarizes a few other laboratory studies on uptake in fish and tissue levels of other aromatics.

In the review by Clarke et al. (1977b), tissue levels of aromatics measured in field-captured fishes show that from 0.4 to 22 µg/g wet weight occurred in whole tissue. Zitko (1971) measured 21 µg/g aromatics in the "guts" of field specimens of P. americanus exposed to Bunker C. These data indicate that tissue levels of aromatics in field-captured

flounder correspond to those measured in starry flounder exposed to 100 ppb of the WSF of crude oil in this study (liver = 1-20 µg/g in 7 days), and that comparable deleterious effects may also occur.

The effects of exposure are summarized in Table 8. Most effects are similar to those described by Couch (1975), Walsh and Ribelin (1975) and Smith and Cole (1973) for pesticide exposures and are probably generalized sublethal responses of fish to hydrocarbon exposure.

Some effects on the liver, such as disruption of the organization of hepatic muralia and increased lipid deposition could have been accentuated by the 7-day exposure period when flounder were not fed. However, these effects were not observed in control livers. Further, maturing fish normally do not feed. Starvation stress may have interacted with the petroleum stress to produce the pronounced effects on livers of immature fish. Similar tissue effects were observed in livers of English sole exposed to oiled sediments (McCain et al. 1978) and of rainbow trout exposed to oil in food (Hodgins et al. 1977a). Other histopathological effects of oil were reviewed by Hodgins et al. (1977b).

The WSF of Cook Inlet crude oil resulted in both direct and indirect effects on starry flounder in the various maturation stages (Table 8) (see also Kühnhold et al., this proceedings). There were direct effects on eggs (necrosis and abnormalities) which may have indirectly reduced subsequent fertilization, hatching, larval growth and survival. There also appeared to be an indirect effect on hormones promoting gametogenesis, with maturation accelerated in exposed flounder.

There seemed to be no effect of exposure on testes or sperm motility in mature males. However, tissue sections of testes were not made, and subsequent work may reveal effects on a cellular level. Numerous changes in eggs from exposed fish at all stages were noted, including egg death. In immature eggs, the apparent death or disintegration of eggs may have been due to atresia or resorption. A few atresic eggs were observed in control immature females also, although the effect was more pronounced in exposed immature females. As in the liver, atresic eggs in immature females may result from interaction of starvation stress with petroleum stress. Egg abnormalities consisted largely of vacuolization; never observed in eggs from controls. The vacuoles may result from an effect of petroleum hydrocarbons on osmoregulation and the hydration of ovaries.

Maturation was accelerated in exposed flounders (Table 8). Exposed males had higher gonadosomatic indices and a higher percentage of testes undergoing spermiation. Exposed females contained eggs in later maturation stages than control eggs, with increased vitellinogenesis (yolk globules = vitellin = phosolipids; Yamamoto, 1957) and larger egg size. The gonadosomatic indices of exposed females were also higher than controls. An explanation of the acceleration in maturation involves the observation that detoxification of petroleum hydrocarbons requires enzymes of the mixed-function-oxidase (MFO) system which also hydroxylate steroids and are involved in pre-oxidation of lipids. The MFO enzymes, bound to the ribosomes, function in several biochemical pathways of organisms. 17-α-hydroxyprogesterone is the major hormone

inducing maturation of eggs in female winter flounder (Campbell et al. 1976) and normally undergoes hydroxylation to adrenocortical steroids in the MFO system. If aromatic and cyclohexane components competitively inhibit this function, they may potentiate the effect of progesterone thus accelerating egg maturation. Simultaneously the oil components may inhibit the pre-oxidation of lipids and the formation of adrenocortical steroids, affecting water balance and lipid deposition. Similar competitive inhibition of toxicants occurs in mammals (LaDu et al. 1972; Doggett et al. 1977) and may also function in fish exposed to petroleum (Brocksen and Bailey 1973).

The tissue-level effects seen at the end of the exposure period were also present after 7 days of depuration, and appear to be irreversible. The results of Kühnhold et al. (see paper, this proceedings) also indicate that this does occur, with reductions in survival and growth of later stages. A 50% reduction in fecundity, and larval survival through hatching and yolk absorption, was also observed in herring exposed to low ppb benzene prior to spawning (Struhsaker 1977).

A number of studies of the effects of petroleum have shown an initial stimulation of several parameters when organisms are exposed to low sublethal levels, while at higher acute levels a depression or narcosis occurs (Brocksen and Bailey 1973; Anderson 1974a; Eldridge 1978). This phenomenon was described by Smyth (1967) as a sufficient challenge. An example cited was increased growth at low exposure levels of pesticides. However, stimulation by toxicants may not be ultimately beneficial to the organism, as demonstrated here. Also, with chronic exposures, the ability of the organisms to homeostatically adjust may collapse or there may be an energy depletion resulting in decreased growth or reproduction.

The cause-and-effect relationships between the components of crude oil measured in these tissues and the physiological or histological effects observed cannot be ascertained definitely. It is difficult to demonstrate which components or interacting components are exerting the primary effects. That a single component can produce similar effects in herring has been discussed previously (e.g. benzene, Struhsaker 1977). Finally, it is not clear whether the toxic effects are caused by the unchanged components or their metabolites, as pointed out by Malins (1977).

In conclusion, the preliminary results of these experiments indicate strongly that low levels of the WSF of crude oil, approximating chronic exposures, can result in deleterious effects that reduce the fecundity of flounder. The total reduction in survival through spawning, hatching and larval stages cannot be assessed, but results indicate a probable reduction in viable eggs of approximately 15-30%, assuming abnormal eggs will not survive. In herring, the reduction in survival through yolk absorption was estimated as 50% (Struhsaker 1977). These effects occur at concentrations two orders of magnitude less than the acute 96-hr TLMs. Further, if exposure to oil stimulates gametogenesis and spawning, spawning fish may be induced to release eggs in areas of high petroleum concentrations (e.g. oil spill), thus exposing relatively sensitive stages to the effects of the oil.

Although sublethal effects of petroleum on individual organisms in small subsamples have been demonstrated readily in laboratory studies, evidence for these effects occurring in organisms in the field on a population level has been more difficult to provide. Natural variation in population sizes of most species often obscures variation due to a pollutant. Few "fish kills" have been irrefutably linked with a specific pollutant or spill event, particularly in estuarine situations. We suspect, however, that slow population declines of several estuarine-dependent species may be at least in part due to the interaction of chronic pollution with normal environmental stress, such as occurs during spawning. A gradual decline in production of the population may take the form of reduced growth or in the inhibition of reproduction. The latter may occur if spawning years are missed, egg production is reduced, and egg viability decreased. An assessment of the reduction in survival of gonadal eggs by exposing fish in the laboratory prior to spawning enables us to obtain a more direct estimate of the potential reduction in year-class survival attributable to a particular pollutant.

If marine organisms prove generally to be more sensitive during their spawning season, fishery management decisions should consider timing of oil-related activities to adequately protect fishery resources.

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Table 1. Morphometrics of starry flounder subsamples. Experiments 1, 2, 3.

Exp. No. Date	Tot. No.	Fem. No. (%)	Male No. (%)	Sex Ratio (♀:♂)	Eye* L (%)	Eye* R (%)	Females** SL (cm)	Females** WW (g)	Ovaries TL (cm)	Ovaries W (cm)	Ovaries WW (g)	Maturing Females (%)
1 1/19	30	19 (63)	11 (37)	2:1	50	50	39.3 (32.9-50.0)	1140 (652-1877)	10.4 (6.0-18.5)	4.6 (2.0-9.2)	24.20 (2.64-113.30)	32
2 2/12	40	28 (70)	12 (30)	2:1	45	55	35.6 (29.2-41.3)	1042 (549-1707)	13.0 (2.4-23.0)	5.6 (1.9-11.0)	48.8 (1.58-153.00)	67
3 4/25	16	4 (25)	16 (75)	1:3	87	13	35.0 (30.5-38.0)	1172 (710-1360)	7.9 (1.2-16.0)	3.1 (1.0-6.0)	15.7 (0.56-51.22)	25

Exp. No. Date	Liver Color--Both Sexes Yellow (%)	Liver Color--Both Sexes Red (%)	Males** SL (cm)	Males** WW (g)	Testes L (cm)	Testes W (cm)	Testes WW (g)	Ripe Males (%)
1 1/19	76	24	37.1 (32.1-44.1)	911 (686-1300)	4.0 (1.2-10.0)	1.5 (0.5-3.5)	2.54 (0.13-7.50)	38
2 2/12	67	33	32.4 (29.8-35.4)	707 (586-872)	4.5 (2.5-6.0)	2.0 (1.0-3.0)	4.03 (0.28-6.12)	75
3 4/25	100	0	32.9 (29.5-37.2)	915 (540-1270)	3.7 (1.7-6.5)	1.4 (0.5-2.0)	1.93 (0.14-6.53)	58

*Side of fish with most dorsad eye and the dorsal operculum; R = right, L = left.
**Values in parentheses are ranges; other values are means for that experiment.

SL = Standard Length; WW = Wet Weight; TL = Total Length.

Table 2. Experimental conditions. Starry flounder exposed during egg maturation prior to spawning (Experiments 1, 2) and after spawning (Experiment 3). Values in parentheses are ranges, other values are means.

Exp. No.	Dates Days Hrs.	Treatment	Fish No.	Fish Tot. WW (Kg)	Fish WW/L (g)	Mean Water* Concentration (mg/L (ppm)	Oxygen (ppm)	Temperature (°C)	Salinity (ppt)	Volume Water (Liters)	Flow Rate (L/Min)
1	1/18 to 1/23 5 120	Control	15	14.70	16.97	0	6.1 (5.0-8.6)	10.7 (9.5-11.7)	19.7 (19.0-21.0)	866	3 (1-3**)
		Exposed	15	17.57	20.29	0.140 (.041-.326)	5.8 (4.6-8.0)	Same	Same	Same	Same
2	2/12 to 2/19 7 168	Control	20	19.02	21.96	0	8.2 (7.0-8.7)	13.7 (12.5-14.5)	29.1 (29.0-30.0)	866	7 (3-7**)
		Exposed***	20	20.35	23.50	0.115 (0.45-.353)	7.9 (6.4-8.8)	Same	Same	Same	Same
3	4/25 to 5/15 21 504	Control	8	7.15	8.25	0	7.7 (6.1-8.8)	17.2 (15.8-18.5)	18.8 (16.5-19.5)	866	8
		Exposed	8	8.52	9.84	0.221 (.144-.265)	7.7 (6.4-8.6)	17.4 (15.9-18.8)	Same	Same	Same

*Water Concentration = total of 6 monocyclic aromatics in WSF (M-1); benzene, toluene, o-, m-, p-xylene, ethylbenzene. Cyclohexanes (CH), other monocyclics (M-2) and dicyclics (D) undetectable in water samples (<0.010 mg/L).

**Flow rate was too low; increased during first exposure day.

***Remaining fish fed after exposure for 1-wk depuration period.

WW = Wet Weight

Table 3. Procedure for analysis of low-boiling-point hydrocarbons in animal tissues. (Benville et al, MS in prep.)

1. Place 10 grams of tissue in a clean, glass culture tube (with a teflon-seal screw cap).
2. Add 6 ml of 4N NaOH and 4 ml of TF-Freon and cap tightly.
3. Place tube in oven (or water bath) for 18 hours at $30^{o}C$. Shake tube 4 or 5 times during this time.
4. Remove tube from oven and shake vigorously for one minute.
5. Centrifuge tube (while still warm) for 10 minutes at 3000 rpm.
6. If freon layer is clear (not cloudy), draw off with pipette and store in a clean, glass vial with a teflon-seal screw cap until ready to inject on GC.

Emulsions:

If the freon layer is clouded, use following procedure:

7. Freeze clouded sample and recentrifuge while still frozen (20-30 minutes at 2000-3000 rpm).
8. If sample is still cloudy, repeat.
9. If sample has not cleared, add 1-2 ml of 20% H_2SO_4, shake, and recentrifuge. A 15-20% reduction in recovery will result if it is necessary to follow this step.

RECOVERY RATE = 90-96% if tubes remain tightly capped.

LEVEL OF DETECTION = 0.025 µg/g wet weight.

Table 4. Summary of mean concentrations of monocyclic and dicyclic aromatic and cyclohexane components in gonads of immature and maturing starry flounder exposed to the WSF of Cook Inlet crude oil during gonadal maturation. Experiment 2. No immature males in samples. Percentages of total concentrations in parentheses. Tissue concentrations are in µg/g wet weight.
Total Accumulation = Total Mean Tissue Concentration (µg/g)/Mean Water Concentration (0.115 mg/L).

Treatment	Sex	Maturity	Tissue	No.	Mean M-1	Mean CH	Mean M-2	Mean D	Total Mean M-1+CH	Total Mean All	Total Accumulation M-1+CH	Total Accumulation All
Exposed	M	Mature (Ripe)	Testis	5	ND	ND	-	-	ND	-	ND	-
			Liver	4	5.891 (70)	2.559 (30)	-	-	8.450	-	73.5X	-
	F	Immature	Ovary	2	0.181 (20)	0.709 (80)	-	-	0.890	-	7.7X	-
			Liver	2	1.346 (100)	ND (0)	-	-	1.346	-	11.7X	-
	F	Mature	Ovary	6	4.801 (37)	3.784 (29)	2.228 (17)	2.163 (17)	(8.585)	12.976	74.6X	112.8X
			Liver	5	9.708 (63)	5.827 (37)	-	-	15.535	-	135.1X	-
Control	F	Immature	Ovary	1	ND	ND	-	-	ND	-	0	0
			Liver	1	ND	ND	-	-	ND	-	0	0
	F	Mature	Ovary	2	ND	ND	-	-	ND	-	0	0
			Liver	2	ND	ND	-	-	ND	-	0	0

M-1 = Monocyclics-1 = Benzene, toluene, ethylbenzene, p-xylene, o-xylene, m-xylene.
CH = Cyclohexanes = Methyl cyclohexane, Cis 1, 3 dimethyl cyclohexane, 1, 2 dimethyl cyclohexane, C_3 cyclohexane and 3 unidentified compounds, also probably cyclohexanes.
M-2 = Monocyclics-2 = Isopropyl benzene, n-propyl benzene, total C_3 benzenes, total C_4 benzenes and total C_5 benzenes.
D = Dicyclics = Naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, total C_2-naphthalenes.
*Monocyclics-2 (M-2) and dicyclics (D) measured only in mature ovaries.

Table 5. Summary of results from histological examination of ovaries of adult Flounder exposed to the WSF of Cook Inlet crude oil prior to spawning. Experiment 1.

Day Date	Treatment	Mean WSF-M-1 Concen. (mg/L)	Maturity[1]	L (cm)	WW (g)	GSI[2]	Mat. Stage[3] (I-II)	Description	Mean Max Diam. (μ)	% Abn.	% Dead
0.25 1/19	Cont.	ND	Imm.	8.5	6.2	0.58	3	Perinucleolus	120	0	0
	Exp.	0.118	Imm.	7.0	5.2	0.48	3	Perinucleolus	100	0	0
	Exp.		Imm.	7.0	3.8	0.55	3	Perinucleolus	120	0	0
1 1/20	Cont.	ND	Mat.	12.0	27.7	2.26	4 to 5	Yolk Vesicle to Primary Yolk	130 to 280	0	0
	Exp.	0.090	Mat.	10.0	17.1	1.43	4	Yolk Vesicle	260	0	0
2 1/21	Cont.	ND	Imm.	6.2	4.1	0.42	3	Perinucleolus	100	0	0
	Exp.[4]	0.098	Mat.	18.0	91.9	5.40	6	Secondary Yolk	460	3	3
3 1/22	Cont.	ND	Mat.	8.0	8.0	0.73	4	Yolk Vesicle	200	0	0
	Exp.	0.224	Mat.	18.5	113.3	6.04	6	Secondary Yolk	410	5	5
	Exp.		Imm.	5.2	2.6	0.35	3	Perinucleolus	120	21	12
4 1/23	Cont.	ND	Imm.	10.0	6.0	0.62	3	Perinucleolus	100	0	0
	Exp.	0.226	Imm.	7.0	4.4	0.36	3	Perinucleolus	100	32	10
	Exp.		Mat.	14.5	47.2	3.00	4 to 5	Yolk Vesicle to Primary Yolk	150 to 310	4	13

[1] Mat. = Early maturation stages.
Imm. = Not maturing as yet, although adults.

[2] GSI = Gonadosomatic Index = Ovary Wet Weight X 100/Body Wet Weight.

[3] Stages from Yamamoto, 1956.

[4] Total monocyclic (M-1) = 6.470 μg/g (wet weight).

Table 6. Summary of results from histological examination of ovaries of adult flounder exposed to the WSF of Cook Inlet crude oil prior to spawning. Experiment 2.

Day Date	Treatment	WSF-MA-1 Concen. (mg/L)	Tissue Concentrations (µg/g)[1] M-1	CH	M-2	D	Total M1+CH	All	Accumulation M1+CH	All	Maturity	Ovary L (cm)	WW (g)	GSI[2]	Mat.[3] Stage (I-II)	Description	Mean Max Diam. (µ)	% Abn.	% Dead
0.25	Cont.	0	ND	ND	-	-	ND	-	ND	-	Imm.	2.8	0.70	0.07	3	Perinucleolus	100	0	0
2/12	Exp.	0.182	0.363	0.187	-	-	0.550	-	3X	-	Imm.	7.0	4.29	0.46	3	Perinucleolus	100	0	0
1	Cont.	0	ND	ND	ND	ND	ND	ND	0	0	Mat.	21.2	110.0	7.62	6	Secondary Yolk	560	0	0
2/13	Exp.	0.123	4.652	3.646	-	-	8.298	-	67X	-	Mat.	19.0	99.0	7.26	7	Tertiary Yolk	670	0	0
	Exp.	0.123	3.506	2.996	1.977	1.903	6.502	10.382	53X	84X	Mat.	12.5	39.5	3.66	6	Secondary Yolk	460	0	0
2	Cont.	0	ND	ND	-	-	ND	-	0	-	Imm.	7.1	3.33	0.35	3	Perinucleolus	120	0	0
2/14	Exp.	0.119	ND	1.232	-	-	1.232	-	10X	-	Imm.	7.0	3.94	0.51	3	Perinucleolus	120	1	10
3	Cont.	0	ND	ND	-	-	ND	-	0	-	Mat.	23.0	129.0	7.66	6	Secondary Yolk	560	0	0
2/15	Cont.	0	ND	ND	-	-	ND	-	0	-	Mat.	22.0	97.0	8.52	6	Secondary Yolk	560	0	0
	Exp.	0.088	4.877	3.048	2.277	2.138	7.925	12.340	90X	140X	Mat.	20.0	150.0	8.79	7	Tertiary Yolk	670	15	12
4	Cont.	0	ND	ND	-	-	ND	-	0	-	Imm.	5.5	3.50	0.48	3	Perinucleolus	120	0	0
2/16	Cont.	0	ND	ND	-	-	ND	-	0	-	Mat.	14.5	45.0	3.70	6	Secondary Yolk	460	0	0
	Exp.	0.104	5.720	4.528	2.336	2.014	10.248	14.618	98X	140X	Mat.	18.0	82.0	5.37	6	Secondary Yolk	560	28	20
5	Cont.	0	ND	ND	-	-	ND	-	0	-	Mat.	11.5	43.0	4.20	6	Secondary Yolk	460	0	0
2/17	Exp.	0.112	5.043	3.609	2.390	2.327	8.652	13.369	77X	119X	Mat.	21.5	141.0	10.23	7	Tertiary Yolk	670	20	33
	Exp.	0.112	4.136	3.023	-	-	7.159	-	64X	-	Mat.	14.0	43.0	5.26	6	Secondary Yolk	460	5	8
6	Cont.	0	ND	ND	-	-	ND	-	0	-	Mat.	17.0	80.0	6.58	6	Secondary Yolk	510	0	0
2/18	Cont.	0	ND	ND	-	-	ND	-	0	-	Mat.	13.5	34.0	3.12	6	Secondary Yolk	410	0	2
	Exp.	0.075	11.871	(M-1+CH)	-	-	11.871	-	158X	-	Mat.	17.5	66.0	5.40	6	Secondary Yolk	510	32	25
	Exp.	0.075	4.396	3.606	2.009	2.554	8.002	12.565	107X	167X	Mat.	14.0	56.0	5.73	6	Secondary Yolk	460	8	0
7	Cont.	0	ND	ND	ND	ND	ND	ND	0	0	Mat.	15.0	40.0	3.70	6	Secondary Yolk	460	0	0
2/19	Exp.	0.075	5.262	4.918	2.378	2.023	10.180	14.581	136X	194X	Mat.	17.0	63.0	7.63	8	Migratory Nucl.	670	10	28

[1] Tissue concentrations of other monocyclics (M2) and dicyclics measured only in mature ovaries. Mean concentrations in Table 4.

[2] GSI = Gonadosomatic Index = Ovary Wet Weight X 100/Body Wet Weight.

[3] Stages from Yamamoto, 1956.

Table 7. Means of ovarian and egg parameters showing acceleration of maturation in ovaries of exposed flounder. Mature females equally represented in control and exposed samples. Experiment 2.

Treatment	No. Fem. (n)	Maturity	Ovaries			Eggs				
						Predominant Maturation Stage			Max.	Percentage
			Length (cm)	Wet Wt. (g)	GSI[1]	Stage No.	Ovaries (n)	Description	Diam. (μ)	Abnormal (%)	Dead (%)
Control	3	Immature	5.1	2.5	0.30	3	3	Perinucleolus	110	0	0
Exposed	2	Immature	7.0	4.1	0.48	3	2	Perinucleolus	110	0.5	0.2
Control	8	Maturing	17.2	72.3	5.64	6	8	Secondary Yolk only	497 (410-460)	0	0
Exposed	9	Maturing	17.0	82.2	6.59	6.6 (6-8)	5	Secondary Yolk	570 (410-670)	13.0	15.0
							3	Tertiary Yolk			
							1	Migratory Nucleus			

[1] GSI = Gonadosomatic Index = Ovary Wet Weight X 100/Body Wet Weight.

Table 8. Summary of effects observed in immature and mature starry flounder exposed to approximately 100 ppb of the WSF of Cook Inlet crude oil for 7 days. Effects marked with asterisk also observed in flounder after 7 days of depuration. A (+) indicates an elevation of effect above controls; a (-) indicates a reduction of effect below controls. Other effects not observed in controls.

	Behavior-Adult		Liver		Gonads				
Stage-Sex	Activity	VR	Total Accum.[1]	Tissue Effects	Total Accum.[1]	Water Uptake	GSI	Gonad-Gametes Effects	Gametogenesis
Mature Male	+	+	73X	+Vacuolization* +Lipid deposition* +Disruption of hepatic muralia +Sinusoidal congestion	ND	+	+	None Obvious	+Spermiation
Immature Female	+	+	12X	As above	8X	+	+	Pale color Erythrocyte Destruction Vacuolization (0.5-13.2%)* +Nuclear disintegration* +Atresia or egg death (0.2-5.5%)*	-Oocytes-atresia*
Maturing Female	+	+	135X	As above	75X	+	+	Vacuolization (3-13%) +Thicker zona radiata Egg death (5.8-15.0%)	+Egg size +Maturation rate +Vitellinogenesis
Adult Female-Resting stage (Yocom et al., manuscript)	NM	NM	2800X	NM	7X (Toluene only)	NM	NM	----------	----------

[1] Total Accumulation = Mean Tissue Concentration of Monocyclics (M-1) and Cyclohexanes (CH)/Mean Water Concentration of M-1 and CH.

ND = Not detectable; NM = Not measured; GSI = Gonadosomatic Index.

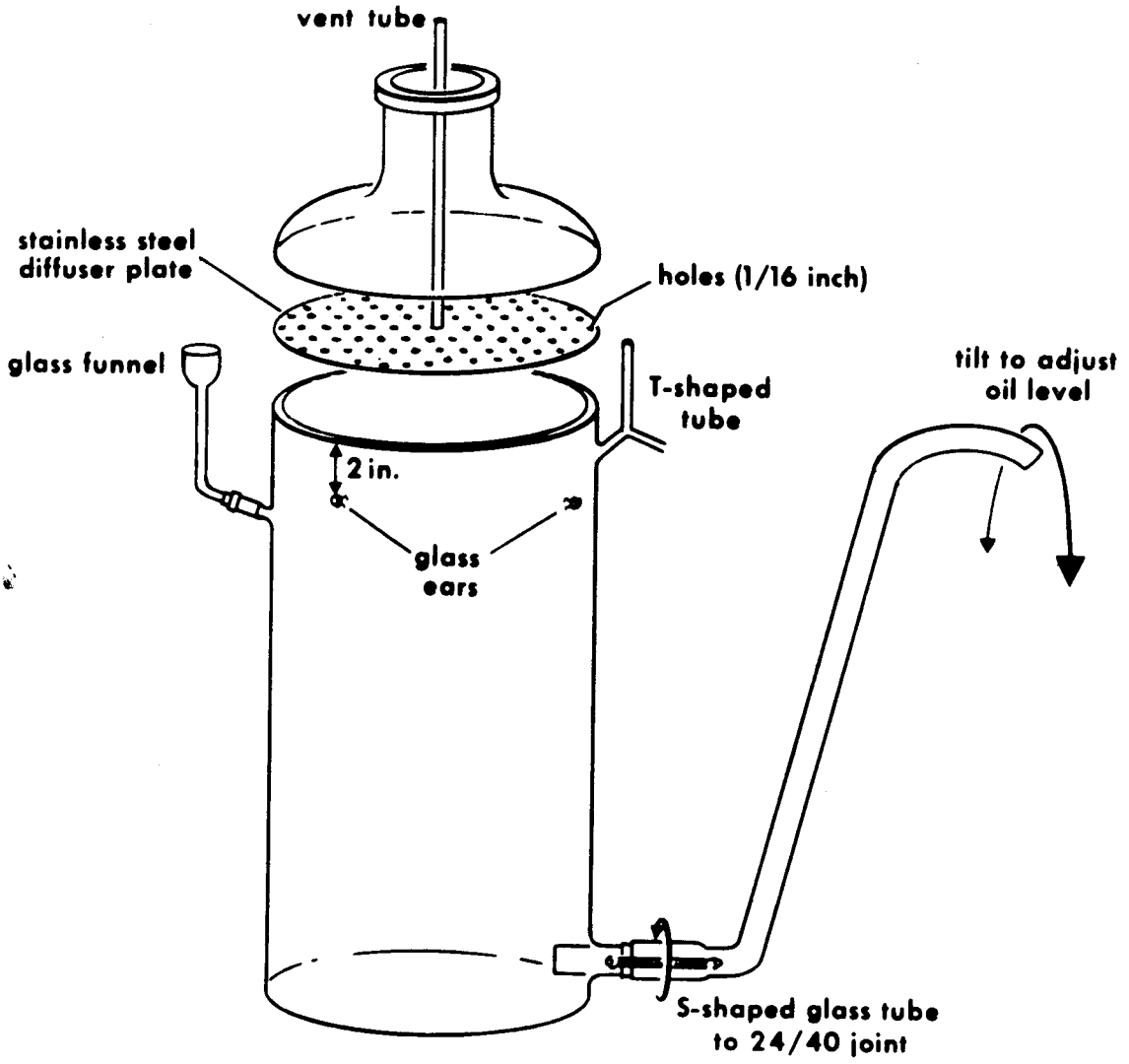

Figure 1. Apparatus (solubilizer) for continuously dosing flounder with the water-soluble fractions (WSF) of crude oil (from Benville et al., manuscript in preparation).

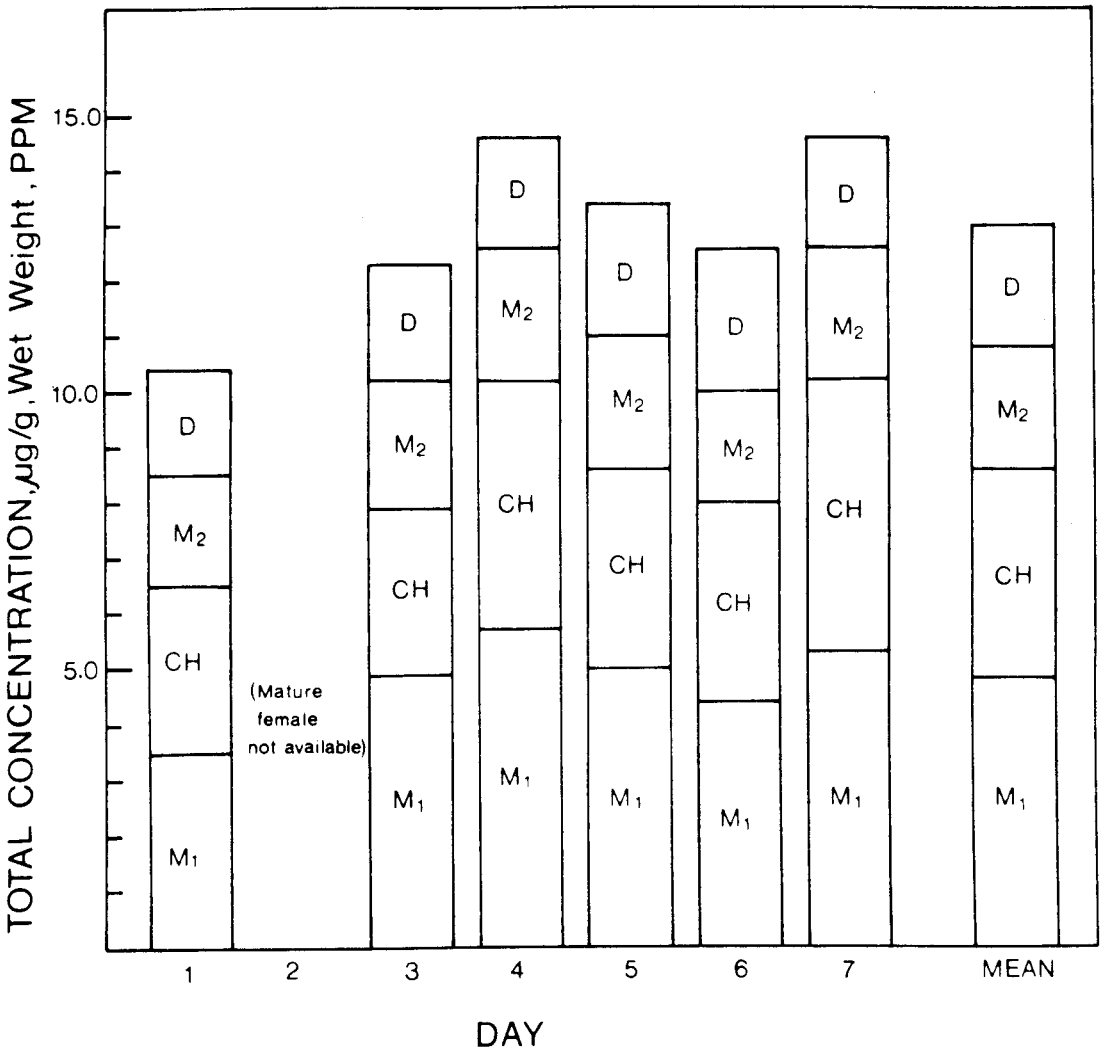

Figure 2. Concentrations of aromatics and alkyl cyclohexanes in maturing ovaries of starry flounder. M1 = Monocyclics 1; CH = Cyclohexanes; M2 = Monocyclics 2; D = Dicyclics. Mean: M1 = 37%; CH = 29%; M2 = 17%; D = 17%.

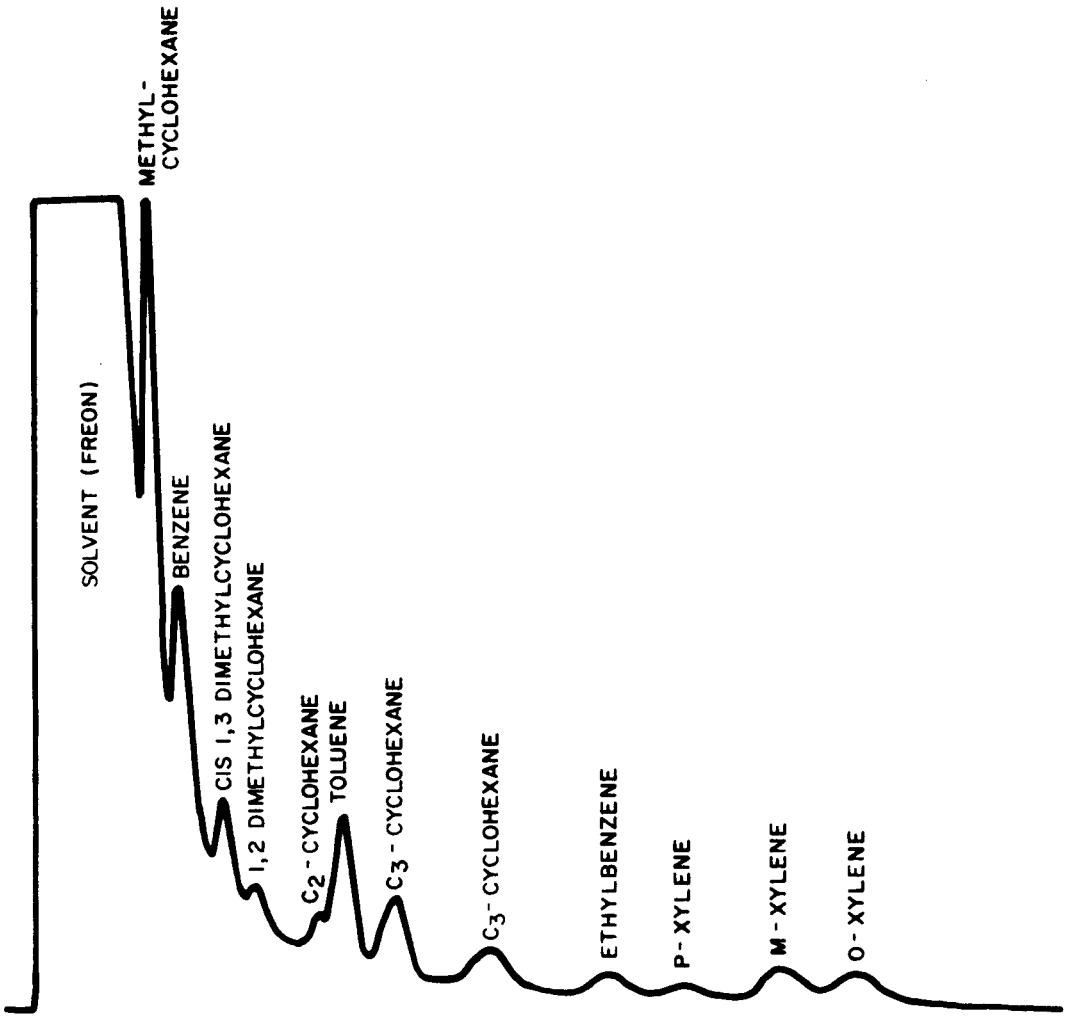

Figure 3. Chromatogram of low-boiling-point hydrocarbons detected in a maturing ovary of a starry flounder exposed to 115 ppb WSF in the water column for 7 days. Total concentration = 8.43 µg/g (ppm).

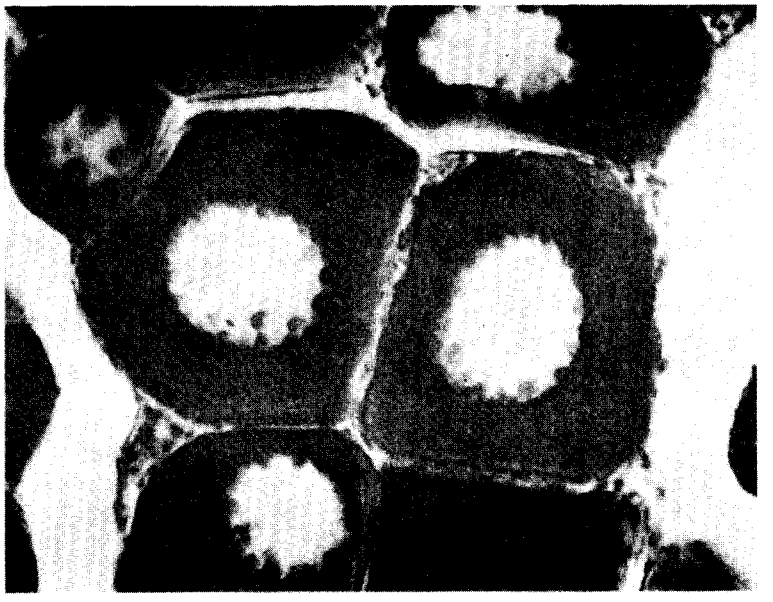

Figure 4A Photomicrograph of normal immature eggs (perinucleolus) stage in control flounder. 400 X.

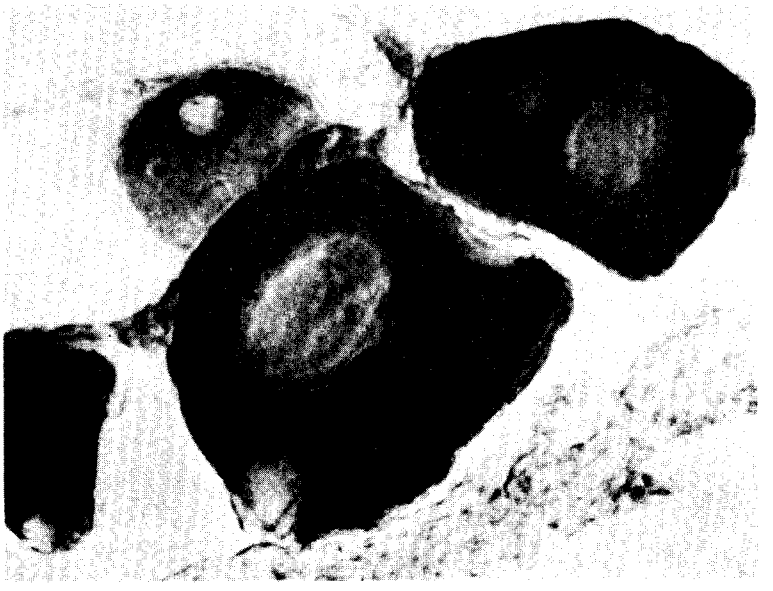

Figure 4B Photomicrograph of abnormal immature eggs (perinucleolus stage) in exposed flounder. Eggs have cytoplasmic vacuoles. 400 X.

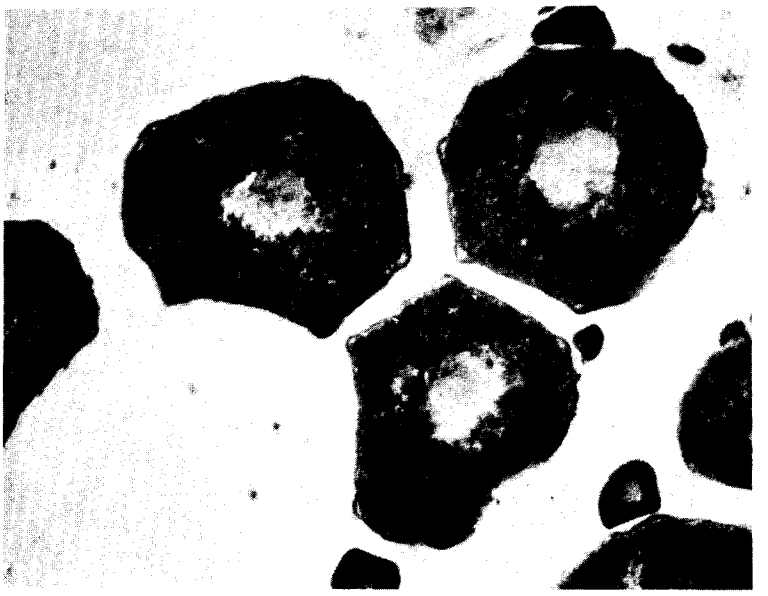

Figure 5A Photomicrograph of normal mature eggs (secondary yolk stage) in control flounder. 100 X.

Figure 5B Photomicrograph of abnormal mature eggs (tertiary yolk stage) in exposed flounder. Eggs have cytoplasmic vacuoles. 100 X.

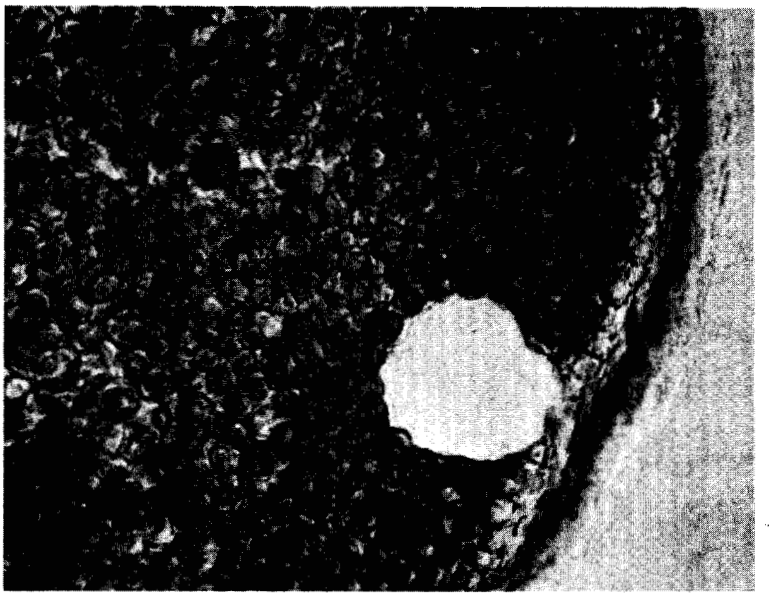

Figure 6A Photomicrograph of abnormal mature egg (tertiary yolk stage) in exposed flounder. Cytoplasmic vacuole in periphery of egg between cytoplasm and zona radiata. 400 X.

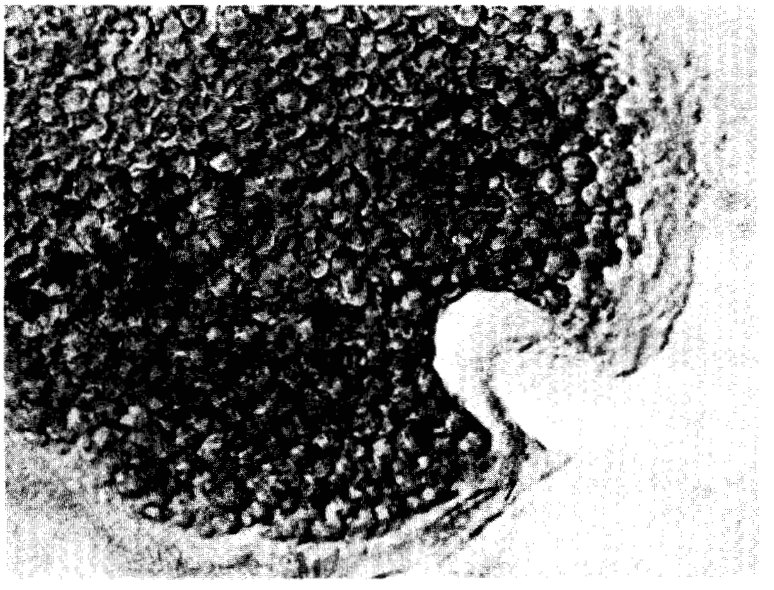

Figure 6B Photomicrograph of abnormal mature egg (tertiary yolk stage) in exposed flounder. Cytoplasmic vacuole in periphery of egg extruding through zona radiata. 400 X.

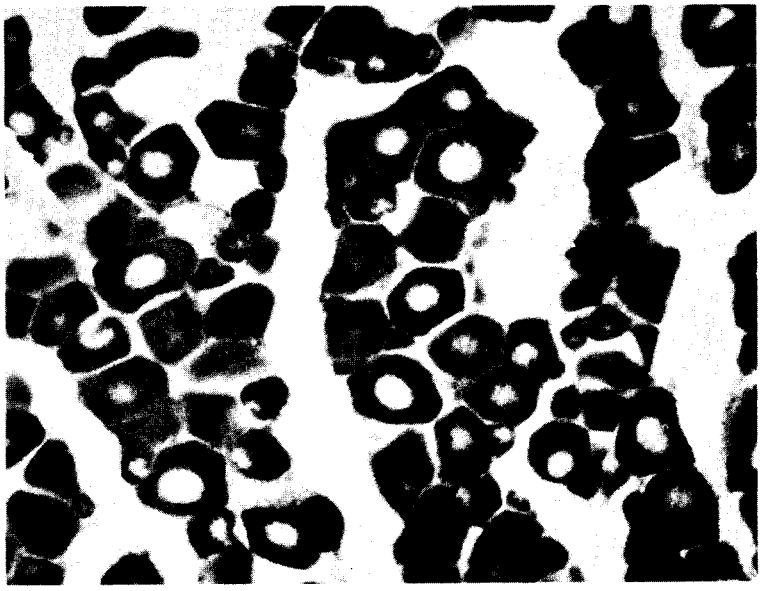

Figure 7A Photomicrograph of normal immature eggs (perinucleolus stage) in control flounder. Note compact groups of many eggs. 100 X.

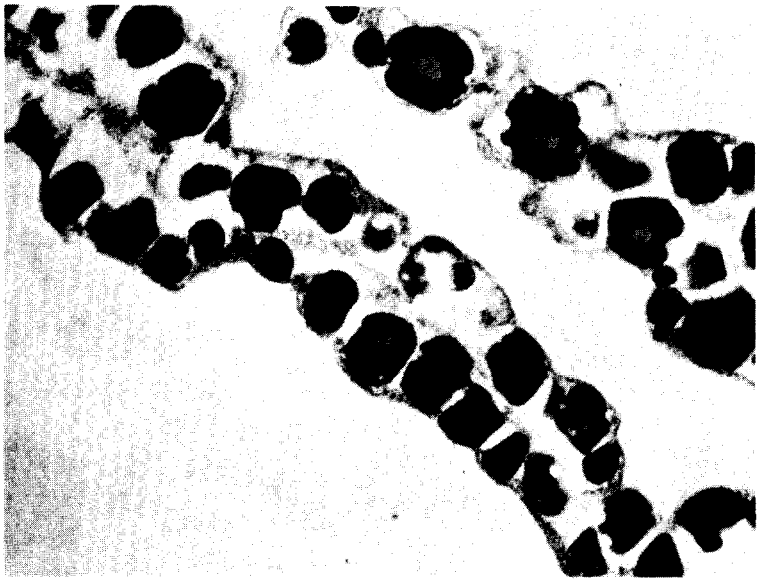

Figure 7B Photomicrograph of immature eggs in perinucleolus stage in exposed flounder. Note relatively small numbers of eggs compared to control. 100 X.

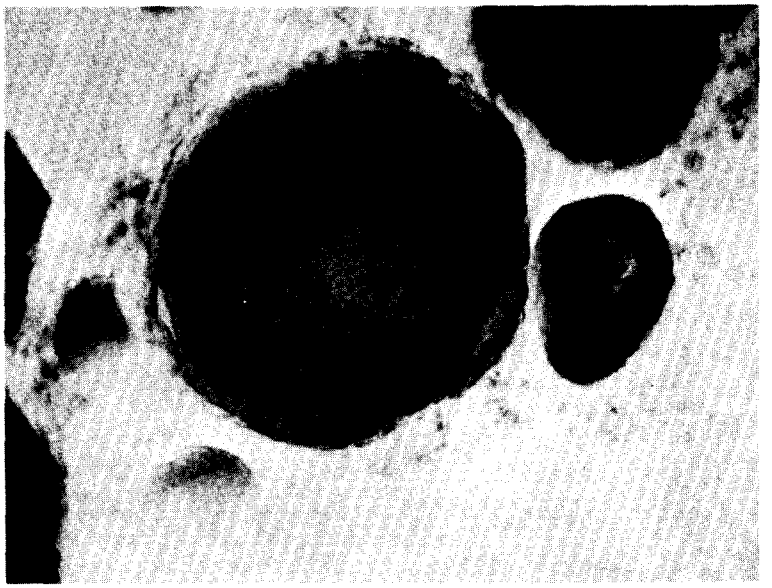

Figure 8A Photomicrograph of dying immature egg (possibly atresic) in exposed flounder. Nucleoplasm starting to coalesce into droplets. 400 X.

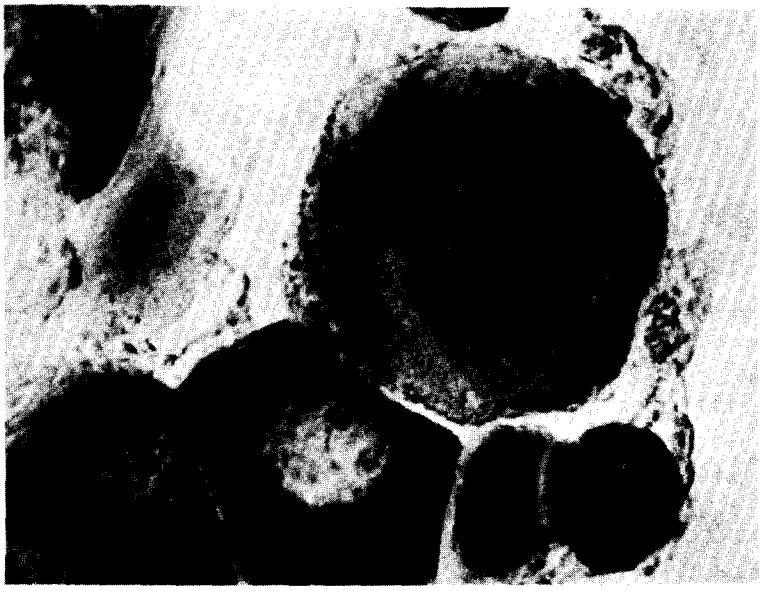

Figure 8B Photomicrograph of dying immature egg (possibly atresic) in exposed flounder. Nucleoplasm coalesced into single droplet, nuclear and cell membranes disintegrated. 400 X.

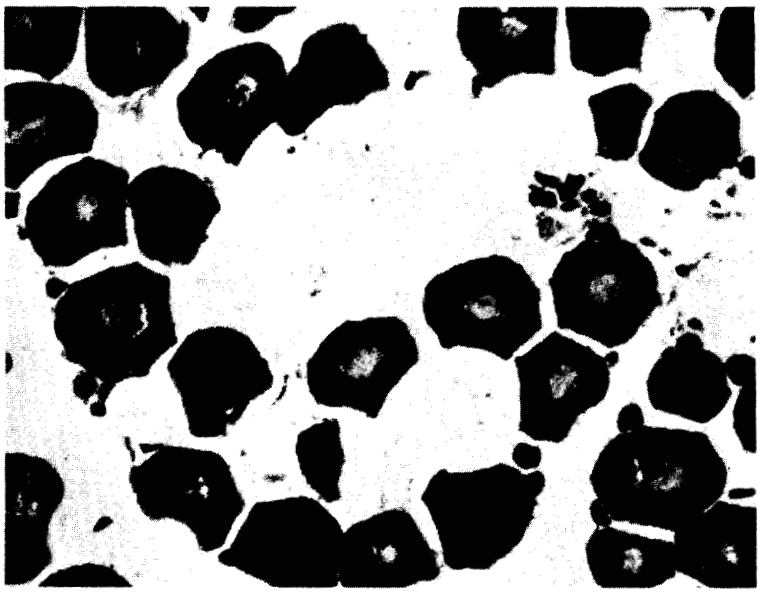

Figure 9A Photomicrograph of normal mature eggs (secondary yolk stage) in control flounder. 40 X.

Figure 9B Photomicrograph of dying mature eggs (tertiary yolk stage) in exposed flounder. Note necrosis of eggs, thickened zona radiata. 40 X.

Figure 10A Section of liver tissue from control flounder. 40 X.

Figure 10B Section of liver tissue from exposed flounder showing heavy vacuolization. 40 X.

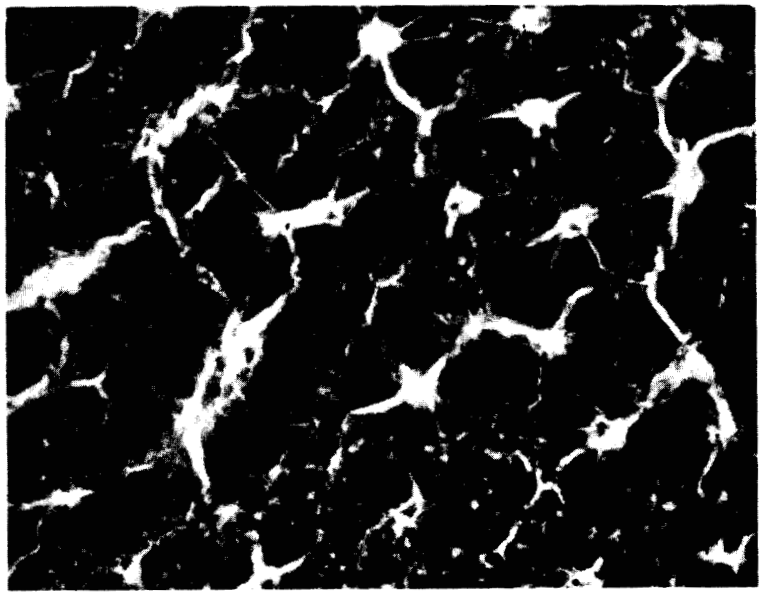

Figure 11A Cross section of hepatic muralia in liver of control flounder showing well-organized hepatocytes surrounding central sinusoids. 400 X.

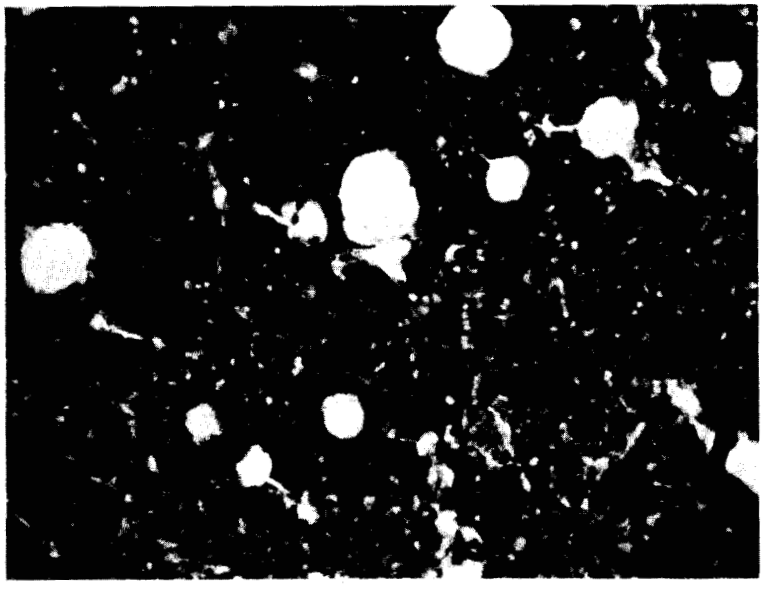

Figure 11B Cross section of hepatic muralia from liver of exposed flounder showing lack of definite structure in muralia and numerous vacuoles. 400 X.

APPENDIX

Table A. Concentrations of monocyclic aromatic components in tissues of starry flounder exposed to the WSF of Cook Inlet crude oil prior to spawning. Experiment 2. Monocyclics not detectable (<0.010 µg/g) in control flounder.

Concentration (ppm) - µg/g (wet weight)
Monocyclics 1 - (M-1)

Treatment Sample	Time (Day-Date-Hrs)	Tissue	Stage	Benzene	Toluene	Ethyl-Benzene	P-Xylene	M-Xylene	O-Xylene	Total Mono-cyclics	Total* Accumu-lation
Seawater	0-1			0.090	0.074	0.005	ND	0.007	0.006	0.182	-
Exposed #1 F	2/12	Ovary	Im-mature	0.309	ND	0.011	0.043	ND	ND	0.363	2.0X
	6	Liver		0.563	1.353	ND	ND	0.495	ND	2.411	13.2X
Exposed #2 M		Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	None
		Liver		0.366	1.024	ND	ND	0.390	ND	1.780	9.8X
Seawater	1			0.046	0.060	0.004	ND	0.007	0.006	0.123	-
Exposed #1 F	2/13	Ovary	Mature	NM	2.734	0.426	ND	0.780	0.712	4.652	37.8X
	24	Liver		0.253	0.852	0.306	ND	0.502	ND	1.913	15.5X
Exposed #2 F		Ovary	Mature	1.633	1.873	ND	ND	ND	ND	3.506	28.5X

ND = Not detectable, but may be present.
NM = Not measured.
M = Male.
F = Female.
Mature = Maturing, but not running ripe.
Immature = Not maturing for spawning in this year.
Ripe = Males with running ripe sperm.

*Total Accumulation = Tissue Concentration (µg/g)/Water Concentration (mg/L).

Appendix - Table A (Contd)

Treatment Sample	Time (Day-Date-Hrs)	Tissue	Stage	Benzene	Toluene	Ethyl-Benzene	P-Xylene	M-Xylene	O-Xylene	Total Mono-cyclics	Total* Accumu-lation
Seawater	2			0.045	0.059	0.004	ND	0.006	0.005	0.119	-
Exposed #1 M	2/14	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	None
	48	Liver		ND	0.291	ND	ND	ND	ND	0.291	2.4X
		GB		ND	0.461	ND	ND	ND	ND	0.461	3.9X
Exposed #2 F	2/14	Ovary	Im-mature	ND	ND	ND	ND	ND	ND	ND	None
	48	Liver		ND	0.281	ND	ND	ND	ND	0.281	2.4X
		GB		ND	0.469	ND	ND	ND	ND	0.469	3.9X
Seawater	3			0.032	0.048	ND	ND	0.005	0.003	0.088	-
Exposed #1 F		Ovary	Mature	1.269	2.284	0.385	ND	0.370	0.569	4.877	55.4X
	2/15	Liver		2.820	8.334	1.740	0.726	3.922	3.262	20.804	236.4X
Exposed #2 M	72	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	None
		Liver		ND	0.524	ND	ND	ND	ND	0.524	5.9X
		GB		ND	0.442	ND	ND	ND	ND	0.442	5.0X

Appendix - Table A (Contd)

Treatment Sample	Time (Day-Date-Hrs)	Tissue	Stage	Benzene	Toluene	Ethyl-Benzene	P-Xylene	M-Xylene	O-Xylene	Total Mono-cyclics	Total* Accumu-lation
Seawater	4			0.041	0.053	0.004	ND	0.006	ND	0.104	-
Exposed #1 F	2/16	Ovary	Mature	1.554	2.846	0.400	ND	0.851	0.069	5.720	55.0X
	96	Liver		0.075	1.966	ND	ND	0.879	ND	2.920	28.1X
		GB		ND	ND	ND	ND	ND	ND	ND	None
Exposed #2 M		Testis	Ripe	ND	0.010	ND	ND	ND	ND	0.010	0.1X
		Liver		1.916	6.783	1.449	0.640	3.977	3.264	18.029	173.4X
		GB		ND	0.306	ND	ND	ND	ND	0.306	2.9X
Seawater	5			0.046	0.057	0.004	ND	0.005	ND	0.112	-
Exposed #1 F	2/17	Ovary	Mature	1.539	2.898	ND	ND	0.612	ND	5.043	45.0X
	120	Liver		0.724	1.709	0.404	ND	0.720	ND	3.557	31.8X
		GB		ND	0.348	ND	ND	ND	ND	0.348	3.1X
Exposed #2 F		Ovary	Mature	1.378	2.495	ND	ND	0.255	0.008	4.136	36.9X
		Liver		2.466	7.112	1.603	0.688	4.188	3.233	19.290	172.2X
		GB		ND	0.280	ND	ND	ND	ND	0.280	2.5X

Appendix - Table A (Contd)

Treatment Sample	Time (Day-Date-Hrs)	Tissue	Stage	Benzene	Toluene	Ethyl-Benzene	P-Xylene	M-Xylene	O-Xylene	Total Mono-cyclics	Total* Accumu-lation
Seawater	6			0.026	0.044	ND	ND	0.005	ND	0.075	-
Exposed #1 F	2/18	Ovary	Mature	NM	4.509	4.356	0.666	1.206	1.134	11.871	158.3X
Exposed #2 F		Ovary	Mature	1.254	2.290	ND	0.007	0.845	ND	4.396	58.6X
Exposed #1 F	2/19	Ovary	Mature	1.038	2.916	0.518	0.050	0.513	0.227	5.262	70.2X
Exposed #2 M		Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	None
		Liver		0.995	1.974	0.594	ND	2.790	2.476	8.829	117.7X
Mean Seawater Concentrations (mg/L)				0.047	0.056	0.003	ND	0.006	0.003	0.115	
(Percentages)				(41%)	(49%)	(3%)	(0%)	(5%)	(3%)		

APPENDIX

Table B. Concentrations of cyclohexane components in tissues of starry flounder exposed to the WSF of Cook Inlet crude oil prior to spawning. Experiment 2. Cyclohexanes and unidentified components not detectable in water column samples or control flounder.

Concentration (ppm) - µg/g (wet weight)
Cyclohexanes - (CH)

Treatment Sample	Time Day Date Hrs.	Tissue	Stage	Methyl Cyclo-hexane	Cis 1, 3 Dimethyl Cyclo-hexane	1, 2 Dimethyl Cyclo-hexane	Uniden-tified #1	Uniden-tified #2	C_3 Cyclo-hexane	Uniden-tified #3	Total Cyclo-hexanes	Maximum* Total Accumu-lation
Exposed #1 F	0-1 2/12 6	Ovary	Im-mature	ND	ND	ND	0.181	0.006	ND	ND	0.187	37X
		Liver		ND	ND	ND	ND	ND	ND	ND	ND	None
Exposed #2 M	0-1 2/12 6	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	ND	None
		Liver		ND	ND	ND	ND	ND	ND	ND	ND	None
Exposed #2 F	I 2/13 24	Ovary	Mature	1.918	ND	ND	1.078	ND	ND	ND	2.996	599X
		Liver		0.447	0.121	ND	ND	ND	ND	ND	0.568	114X
Exposed #1 M	2 2/14 48	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	ND	None
		Liver		ND	ND	ND	ND	ND	ND	ND	ND	None
Exposed #2 F	2 2/14 48	Ovary	Im-mature	ND	ND	ND	1.213	ND	.019	ND	1.232	246X
		Liver		ND	ND	ND	ND	ND	ND	ND	ND	None

ND = Not detectable, but may be present; M = Male; F = Female; Mature = Maturing, but not running ripe; Immature = Not maturing for spawning in this year; Ripe = Males with running ripe sperm.

*Total Accumulation = Tissue Concentration (µg/g)/Water Concentration (mg/L).
Water Concentration less than 0.010 mg/L, not detectable; estimated as 0.005 mg/L total cyclohexanes.

Appendix - Table B (Contd)

Treatment Sample	Time Day Date Hrs.	Tissue	Stage	Methyl Cyclo-hexane	Cis 1, 3 Dimethyl Cyclo-hexane	1, 2 Dimethyl Cyclo-hexane	Uniden-tified #1	Uniden-tified #2	C_3 Cyclo-hexane	Uniden-tified #3	Total Cyclo-hexanes	Maximum* Total Accumu-lation
Exposed #1 F	3 2/15 72	Ovary	Mature, not measured.									
		Liver		9.575	1.464	0.044	1.968	0.009	ND	ND	13.060	2612X
Exposed #2 M	3 2/15 72	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	ND	None
		Liver		ND	ND	ND	ND	ND	ND	ND	ND	None
Exposed #1 F	4 2/16 96	Ovary	Mature	3.255	0.603	0.287	0.383	ND	ND	ND	4.528	906X
		Liver		1.977	0.521	0.248	ND	ND	ND	ND	2.746	549X
Exposed #2 M	4 2/16 96	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	ND	None
		Liver		5.118	1.175	0.590	ND	ND	ND	ND	6.883	1377X
Exposed #1 F	5 2/17 120	Ovary	Mature	2.893	0.544	0.172	ND	ND	ND	ND	3.609	722X
		Liver		1.689	0.386	0.223	ND	ND	ND	ND	2.298	460X
Exposed #2 F	5 2/17 120	Ovary	Mature	2.446	0.419	0.158	ND	ND	ND	ND	3.023	605X
		Liver		8.286	1.727	0.671	ND	ND	ND	ND	10.684	2137X

Appendix - Table B (Contd)

Treatment Sample	Time Day Date Hrs.	Tissue	Stage	Methyl Cyclo-hexane	Cis 1, 3 Dimethyl Cyclo-hexane	1, 2 Dimethyl Cyclo-hexane	Uniden-tified #1	Uniden-tified #2	C_3 Cyclo-hexane	Uniden-tified #3	Total Cyclo-hexanes	Maximum * Total Accumu-lation
Exposed #2 F	6 2/18 144	Ovary	Mature	2.778	0.580	0.248	ND	ND	ND	ND	3.606	721X
Exposed #1 F	7 2/19 168	Ovary	Mature	2.286	0.526	0.134	1.928	ND	0.029	.015	4.918	984X
Exposed #2 M	7 2/19 168	Testis	Ripe	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Liver		2.128	1.160	0.065	ND	ND	ND	ND	3.353	671X

APPENDIX

Table C. Concentrations of monocyclic and dicyclic aromatic components in mature ovaries of starry flounder exposed to WSF of Cook Inlet crude oil prior to gonadal maturation. Experiment 2. Monocyclics-2 (M-2) and dicyclics not detectable in water column samples.

Concentration (ppm) - µg/g (wet weight)[1]
Monocyclics 2 - (M-2) and Dicyclics (D)

Treatment Sample	Time Date-Day	Iso-propyl-benzene	n-propyl-benzene	Total C_3-benzenes	Total C_4-benzenes	Total[2] mono-cyclics	Naphtha-lene	2-methyl-naphtha-lene	1-methyl-naphtha-lene	Total C_2-naphtha-lenes	Total di-cyclics	Total M-2+D	Maximum[3] Total Accumu-lation
Control 1 Female	2/13 1	ND	ND	ND	ND	ND	Contami-nated	ND	ND	ND	ND	ND	None
Exposed 2 Female	2/13 1	0.109	0.138	1.307	0.423	1.977	0.691	0.813	0.272	0.127	1.903	3.880	228X
Exposed 1 Female	2/15 3	0.118	0.163	1.426	0.570	2.277	0.890	0.499	0.428	0.321	2.138	4.415	260X
Exposed 1 Female	2/16 4	0.119	0.149	1.390	0.678	2.336	0.746	0.576	0.407	0.305	2.034	4.370	257X
Exposed 1 Female	2/17 5	0.143	0.182	1.050	1.015	2.390	1.176	0.455	0.455	0.241	2.327	4.717	277X
Exposed 1 Female	2/18 6	0.116	0.155	1.222	0.516	2.009	0.788	0.679	0.489	0.598	2.554	4.563	268X
Exposed 1 Female	2/19 7	0.118	0.152	1.406	0.702	2.378	0.776	0.592	0.407	0.248	2.023	4.401	259X
Control 1 Female	2/19 7	ND	ND	.002	0.013	0.015	ND	ND	ND	ND	ND	0.015	None

[1] Data converted from dry weight to wet weight on basis of % dry weight of total wet weight (analysis by Seattle Analytical Laboratory).

[2] Other monocyclics in Appendix Table A.

[3] Total Accumulation = Tissue Concentration (µg/g)/Water Concentration (mg/L).
Water Concentration less than 0.00025 mg/L, not detectable; estimated as 0.017 mg/L total monocyclics (M-2) and dicyclics (D).

APPENDIX

Table D. Concentration of monocyclic aromatic components in tissues of starry flounder after exposing to the WSF of Cook Inlet crude oil for 3 weeks; post-spawning period. Experiment 3. Monocyclics not detectable (<0.010 µg/g) in control flounder. Some samples pooled.

Concentration (ppm)-µg/g (wet weight)
Monocyclics-1 - (M-1)

Sample	Tissue	Stage	Benzene	Toluene	Ethyl-Benzene	P-Xylene	M-Xylene	O-Xylene	Total Mono-cyclics	Total* accumu-lation
1 Female	Ovary	Immature	ND	0.169	ND	ND	ND	ND	0.169	1.9X T
	Liver		5.011	16.272	10.435	1.283	7.057	5.524	45.582	206X
2 Male	Testis	Ripe	ND	0.352	ND	ND	ND	ND	0.352	4X T
	Liver		8.843	20.030	8.311	5.152	10.807	10.262	63.405	287X
4 Female	Ovary	Immature	ND	0.250	ND	ND	ND	ND	0.250	2.8X T
	Liver		4.049	12.332	2.731	0.939	5.869	4.641	30.561	138X
9 Male + 10 Male	Testis	Ripe	ND	0.313	ND	ND	ND	ND	0.313	3.6X T
9 Male	Liver		8.714	18.099	5.522	2.002	9.418	7.295	51.05	231X
10 Male	Liver		8.254	17.814	5.311	2.127	9.175	7.147	49.828	225X
3 Male + 11 Male	Testis	Ripe	0.359	1.002	ND	ND	ND	ND	1.361	6.2X
11 Male	Liver		15.444	30.016	8.157	2.793	12.404	9.876	78.69	356X
12 Female	Ovary	Immature	ND	0.716	ND	ND	ND	ND	0.716	8.1X T
	Liver		16.349	30.059	11.171	5.644	13.185	12.029	88.437	400X
Mean Seawater Concentration (mg/L)			0.133	0.088	ND	ND	.0003	ND	0.221	

ND = Not detectable but may be present; Immature = Ovaries in resting stage; T = Accumulation of toluene only.

*Total Accumulation = Tissue Concentration (µg/g)/Mean Water Concentration (mg/L).

APPENDIX

Table E. Concentration of cyclohexane components in tissues of starry flounder after exposing to the WSF of Cook Inlet crude oil for 3 weeks; post-spawning period. Experiment 3. Cyclohexanes not detectable (< 0.010 µg/g) in control flounder.

Concentration (ppm)-µg/g (wet weight)
Cyclohexanes - (CH)

Sample	Tissue	Stage	Methyl cyclo-hexane	Cis 1, 3 Dimethyl cyclo-hexane	1, 2 Dimethyl cyclo-hexane	Uniden-tified #1	Uniden-tified #2	C_3 cyclo-hexane	Uniden-tified #3	Total cyclo-hexanes	Total* accumu-lation
1 Female	Ovary	Im-mature	ND	ND	ND	ND	ND	ND	ND	ND	None
	Liver		27.342	8.882	3.857	ND	1.324	ND	ND	41.405	6900X
2 Male	Testis	Ripe	0.284	ND	ND	ND	ND	ND	ND	0.284	47X
	Liver		38.471	8.455	3.803	0.463	1.310	ND	ND	52.502	8750X
4 Female	Ovary	Im-mature	0.134	ND	ND	ND	ND	ND	ND	0.134	22X
	Liver		21.615	5.729	2.578	ND	ND	ND	ND	29.922	4987X
9 Male + 10 Male	Testis	Ripe	0.312	ND	ND	ND	ND	ND	ND	0.312	52X
9 Male	Liver		35.867	7.506	3.541	0.029	0.930	ND	ND	47.873	7979X
10 Male	Liver		33.836	6.792	3.011	0.313	ND	ND	ND	43.952	7325X
11 Male + 3 Male	Testis	Ripe	0.384	ND	ND	ND	ND	ND	ND	0.384	64X
11 Male	Liver		31.744	6.842	3.143	0.561	ND	ND	ND	42.290	7048X
12 Female	Ovary	Im-mature	ND	ND	ND	ND	ND	ND	ND	ND	None
	Liver		31.984	6.863	3.057	0.368				42.272	7045X
Mean Seawater Concentration (mg/L)			ND - 0.006	ND	ND	ND	ND	ND	ND	Max. = 0.006	

ND = Not detectable but may be present. Immature = Ovaries in resting stage.

*Total Accumulation = Tissue Concentration (µg/g)/Maximum water concentration (mg/L).