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Effects of Chronic Exposure to the Water-Soluble Fraction (WSF) of Hibernia Crude Oil on Capelin *(Mallotus villosus)* Embryos

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EFFECTS OF CHRONIC EXPOSURE TO THE WATER-SOLUBLE FRACTION (WSF) OF HIBERNIA CRUDE OIL ON CAPELIN (*Mallotus villosus*) EMBRYOS

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ABSTRACT

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Capelin (Mallotus villosus) embryos obtained from intertidal aggregations of running-ripe capelin during June/July 1987 in Bryants Cove, Newfoundland were exposed to five concentrations (0, 0.5, 1.3, 2.7, 5.3 ppm total hydrocarbons) of the water-soluble fraction (WSF) of Hibernia crude oil, with exposures beginning at 0 and 5 days after fertilization. Lowest observed effect concentrations (LOECs) for lethal effects were 2.7 ppm for embryos exposed at age 0 days and 5.3 ppm for embryos exposed at age 5 days. At concentrations below these lethal levels, there was an inverse parabolic relationship beween age at hatch and oil concentration. In all sublethal concentrations, embryos were significantly smaller at hatch, with significantly larger yolks, than control embryos. The intensity of eye pigmentation also declined with increasing concentration, but oil exposure did not affect the number of cartilaginous skeletal elements or cause skeletal or other deformities. This report provides the first quantitative results of the lethal and sublethal effects of Hibernia crude oil to capelin embryos. The effects documented suggest that the components of Hibernia WSF (primarily mono- and diaromatics) act as general stressors, inhibiting metabolism. The universality of these effects enables us to examine the ecological implications of oil pollution for capelin, even though the chemical and exposure conditions resulting from a unique event such as an oil spill are difficult to reproduce in any laboratory experiment. Capelin may be particularily susceptible to lethal effects on early life history stages. There is indirect evidence that the abundance of early stages of a year class affects later abundance; the spawning and harvested population in any local inshore fishery consists almost entirely of one or two age classes (3- and 4-year olds). Therefore, if stocks are discrete enough to home to natal spawning areas, a spill could have significant effects on local harvests 3 years later. Sublethal effects on age and size at hatch will also be important if they affect emergence from beach sediments and the subsequent transition to exogenous feeding.

RÉSUMÉ

Paine, M.D., W.C. Leggett, J.K. McRuer, and K.T. Frank. 1988. Effects of chronic exposure to the water-soluble fraction (WSF) of Hibernia Crude oil on capelin (Mallotus villosus) embryos. Can. Tech. Rept. Fish. Aquat. Sci. 1627: iv + 25 p.

Des embryons de capelan (Mallotus villosus) obtenus de concentrations intertidales de capelans pleins en frai à Bryants Cove (Terre-Neuve) en juin et juillet 1987 ont été exposés à cinq concentrations (0, 0.5, 1.3, 2.7 et 5.3 ppm d'hydrocarbures totaux) de la fraction soluble dans l'eau (FSE) du pétrole brut du champ Hibernia. Les oeufs ont été exposés de 0 à 5 jours après leur fécondation. Les concentrations les plus faibles donnant un effet observable (CFEO) étaient, dans le cas d'un effet létal, de 2.7 ppm pour les embryons exposés à l'âge 0 et de 5.3 ppm pour ceux exposés à l'âge de 5 jours. On a noté, aux concentrations inférieures à ces niveaux létaux, une relation parabolique inverse entre l'âge à l'éclosion et la concentration du pétrole. A toutes ces concentrations, les embryons étaient, par rapport aux témoins, significativement plus petits à l'éclosion et les vitellus étaient significativement plus gros. L'intensité de la pigmentation des yeux diminuait aussi avec l'augmentation de la concentration, mais l'exposition au pétrole n'a pas modifié le nombre des éléments cartilagineux du squelette ou provoqué d'autres anomalies du squelette. Ce rapport est le premier où l'on trouve des données quantitatives sur les effets létaux et sublétaux du pétrole brut du champ Hibernia chez les embryons de capelan. Les effets décelés portent à croire que les composantes de la FSE du pétrole du champ Hibernia (surtout des mono et des diaromatiques) agissent comme facteurs de stress généraux que inhibent le métabolisme. Le caractère universel de ces effets a permis aux auteurs d'examiner les implications écologiques, pour le capelan, de la pollution par le pétrole, ceci même si les paramètres chimiques et les conditions d'exposition liés à un événement unique, tel un déversement de pétrole, s'avèrent difficiles à reproduire dans le cadre d'une expérience de laboratoire. Le capelan pourrait s'avérer particulièrement sensible aux effets létaux au cours des premières étapes de sa vie. Selon certains indices indirects, l'abondance des individus aux premiers stades de développement d'une classe annuelle pourrait avoir une incidence sur l'abondance ultérieure. La population de géniteurs recoltée dans une pêche locale donnée est presqu'entièrement composée d'une ou de deux classes d'âge (individus de 3 ou 4 ans). Si les stocks sont suffisamment discrets pour que les géniteurs retournent à leur lieu de naissance, un déversement pourrait avoir des effets importants sur des pêches locales trois ans plus tard. Les effets sublétaux sur l'âge et la taille à l'éclosion pourront aussi avoir des incidences importantes s'ils influent sur l'émergence des sédiments des plages et la transition ultérieure à l'alimentation exogène.

INTRODUCTION

Increased concern about the possible effects of oil pollution has been expressed as a result of the recent development of offshore oil fields such as Hibernia in the western Atlantic. Oil from spills or chronic inputs tends to concentrate in shallow waters and along the shoreline (Mann and Clark 1978). These are spawning and incubation areas for a significant number of marine fish species, including capelin (Mallotus villosus). In Canada, capelin support an inshore fishery with an annual landed value as high as $20 million. They are also a major food source for other commercially important Northwest Atlantic fish species, marine mammals, and sea birds (Bailey et al. 1977).

In 1987/88, the Panel for Energy Research and Development (PERD) provided financial support for a project entitled "Hydrocarbon effects on capelin eggs and larvae" in order to provide technical information on the direct and indirect effects of Hibernia crude oil on the early life stages of capelin, one of the most ecologically important fish stocks on the Grand Banks and coastal Newfoundland. The project has been proceeding on two fronts by examining 1) hydrocarbon effects on embryos and free embryos from beach-spawning stocks and 2) hydrocarbon effects on older capelin larvae from the offshore spawning stock centred on the Southeast Shoal of the Grand Banks. This report summarizes a major portion of the results of hydrocarbon effects on the embryos and free embryos from the beach-spawning stock initiated during the summer of 1987. In this report, we follow Balon's (1975) terminology for developmental intervals. Thus the embryonic period includes the interval between hatch and onset of exogenous feeding (=free embryo phase). The larval period does not begin until exogenous feeding commences; in capelin, this would follow shortly after emergence from beach sediments. Thus, the boundary dividing the embryonic and larval periods conveniently separates the life history intervals studied between the inshore and offshore stocks of capelin.

In the summer of 1987, the following experiments were carried out on beach-spawning stocks from Bryant's Cove, Nfld.:

(1) effects of early and continuous exposure to Hibernia crude oil on survival and time to hatch, and growth of capelin embryos,

(2) effects of later and continuous exposure on time to hatch and subsequent starvation, and growth of capelin free embryos, and

(3) effects of short term exposure to Hibernia crude oil on survival of capelin free embryos.

This report provides the results of the first experiment. Objectives of this experiment were to provide baseline information on the composition of Hibernia crude oil and its soluble fraction; to determine the nature of oil toxicity to capelin and the effects of age at exposure; to determine which response variables were most sensitive to oil effects; and to examine variance in toxicity at several different levels (individual fish, rearing container, family).

MATERIALS AND METHODS

The experiment consisted of 4 runs spaced 1 day apart; a different family of fish was used for each run. In run 1, four oil concentrations (10, 25, 50, 100% WSF) were tested on embryos exposed at age 0 days. This run was initially intended to test two methods of preparing oil WSFs. In runs 2-4, the four oil concentrations were each tested at two ages at exposure (0 and 5 days). Oil-free controls (sea water filtered sequentially through 20, 5, 2, and 1um filters and sterilized with ultraviolet light) were included in each run. Embryos in control solutions could not be exposed at any age >0 days because later exposure simply involved replacing sea water with the appropriate oil solution. There were two replicates (beakers) per treatment in each run. Therefore, replication occurred at two levels - within days or families, and among days or families.

Preparation and Analysis of Oil WSFs

Hibernia crude oil was obtained from Mobil Canada Ltd. in St. John's, Nfld. It was provided in sealed drums lined with teflon. On receipt, the oil was transferred to 4L glass jars, which were then sealed and stored at 4C. Oil WSFs were prepared by adding 140ml of oil to 3360ml of sea water providing an initial 1:24 oil:sea water mix. This solution was then stirred at a constant speed for 10min with a paint stirrer powered by a mounted drill motor. The flask containing the solution was inverted and left to separate for 18h. The undissolved oil rose to the top; the WSF was drawn from the bottom and diluted as required.

Samples of the oil and its WSF were collected throughout the experiment. The samples were extracted with dichloromethane, dried over sodium sulphate, and analyzed by ultraviolet spectrometry to determine total

hydrocarbon concentrations. Three oil samples and 4 WSF samples were separated on a small silica gel column into aliphatic and aromatic fractions. The aliphatic fraction was eluted with hexane and the aromatic fraction was eluted with dichloromethane. Both fractions were analyzed by gas chromatography to determine their composition.

Fertilization and Rearing of Embryos

Spawning adult capelin were collected from the beach in Bryant's Cove, Nfld., and held in tanks provided with a continuous flow of unfiltered sea water pumped directly from the Cove. For each run, ova were stripped from 1 or 2 females into chilled plastic dishes and fertilized dry using sperm obtained by mashing the testes of 2-5 ripe males. Sea water was added after approx. 2 min. The dishes were then gently swirled to reduce adhesion and were placed in a 10°C water bath for 1-2 hours until the perivitelline space formed. Embryos were pipetted into 100ml glass beakers (50 per beaker) and 70ml of the appropriate test solution was added. The beakers were capped with aluminum foil to exclude debris. Four small holes were punched in each cap to permit oxygen exchange. The beakers were inserted into holes in a styrofoam pad, which was floated in a 10°C water bath.

The solutions in the beakers were changed daily to maintain hydrocarbon levels. Embryos exposed at age 5 days were placed in beakers containing sea water (changed daily) from age 0 to age 5, when the test solution was added instead of sea water.

Response Variables

Lethal Effects

Embryos were monitored at ages 1, 5, 9, 13, 17, and 21 days. Beakers were examined under a microscope and embryos scored as dead, developmentally retarded, and normal. All embryos were classified with the exception of those assessed at age 5 days. At this age, it was difficult to distinguish dead from developmentally retarded individuals. Therefore, a randomly selected subsample of >20 embryos was used to minimize time under the microscope. Unfertilized ova, and embryos that died during cleavage or epiboly, were easily recognized and classified as dead after age 5 days. Developmentally retarded embryos that had undergone some organogenesis were difficult to recognize because the only signs of life were infrequent movements. The dead category was therefore restricted to embryos that were obviously dead; even so, incorrect classification still occurred. After age 13 days, dead embryos began to yellow, decompose, or disintegrate, and became much more obvious. Survival was expressed as the percentage of eggs not scored as dead, and therefore included developmentally retarded individuals.

Hatching was monitored daily before the solutions were changed. Free embryos (live or dead) were counted and preserved in 10% formalin buffered with $CaCO_3$. Hatch was defined as the percentage of embryos hatching and remaining alive until collected. Therefore, survival was the most conservative measure of lethal effects, and hatch the most liberal. Only % survival and cumulative % hatch after 21 days (i.e., at the end of the experiment) were analyzed statistically.

Sublethal Effects

Mean age at hatch (live and dead free embryos) was calculated for each beaker from the daily records of the number hatched. Standard length (SL), head length (both as defined in Snyder et al. 1977), and body depth (at the pectoral fin insertion) were measured on the preserved free embryos. Yolk volume (V) was calculated by measuring the length (L) and height (H) of the yolk and using the formula for the volume of an ellipsoid:

$$V = LH^2/6.$$

The intensity of pigment in the eye was rated on a scale of 0 (pale) to 3 (dark) for free embryos from runs 2 and 4. Free embryos from run 3 were stained with alcian blue, to indicate cartilage, and cleared with trypsin, following Potthoff (1984) and Balon and Flegler-Balon (1985).

Statistical Analyses

The two variables measuring lethal effects, % survival and % hatch at age 21 days, were analyzed in a 2-way ANOVA, with one random factor (run) and one fixed factor (treatments; controls plus the combinations of age at exposure and oil concentration). Using orthogonal contrasts (Sokal and Rohlf 1981, p. 232-242), the variance among treatments was divided into variance due to differences between controls and all other treatments, and variance due to differences among the other (oil) treatments. The variance among oil treatments was further subdivided into variance due to age at exposure effects, oil concentration effects, and the interaction between the two. The significance of each contrast can be tested with

an F-test. Results from run 1, in which only one age at exposure was tested, were excluded to maintain a balanced design. Results for embryos exposed to 100% WSF were excluded because mortality approached or reached 100% in every beaker, producing variances much smaller than for other treatments. Both response variables were arcsin-transformed to equalize variances within the remaining treatments.

Because the interaction between age at exposure and oil concentration was significant for both response variables (see Appendix I), the effects of oil concentration were analyzed separately for each age at exposure. Again, 2-way ANOVAs with one random factor (run) and one fixed factor (oil concentration; 0, 10, 25, 50% WSF) were used; results from run 1 were included in the analysis of embryos exposed at age 0.

In any ANOVA, with replication, and with both random and fixed factors, the appropriate error term differs among effects (Table 1). The interaction between the fixed and random factors, used to test the fixed factor, has fewer degrees of freedom than the variance among replicates. Therefore, if the interaction is small, it is pooled with the variance among replicates to provide a more powerful test. (If the random term is small, it can also be pooled with the pooled interaction-among replicate term). In this report, we followed Weiner's (1971) rule - pool if $P>0.25$.

Williams' (1971) test was used to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The range NOEC to LOEC defines the maximum acceptable toxicant concentration (MATC). Normally, an F-test of the significance of differences among toxicant concentrations (i.e., an ANOVA) is not required for Williams' test. In this experiment, the 2-way ANOVA was required to remove the effects of the random factor and to provide the appropriate error term. Williams' test is based on testing the significance of the difference (D) between a concentration mean and controls:

$$t = D/(2s^2/r)^{1/2};$$

where s^2 = variance among replicates
r = number of replicates.

When MATCs were calculated over all runs, the best estimate of s^2 was the mean square for the run-concentration interaction, and r was the number of runs (i.e., the experiment was treated as a randomized block design). When MATCs were calculated for individual runs, the best estimate of s^2 was the mean square among replicates (beakers), and r was the number of replicates (=2) (i.e., each run was treated as a completely randomized experiment). When the run-concentration interaction was pooled with the variance among beakers, this pooled error was used as s^2 to calculate MATCs over all runs, and for each run.

Sublethal effects on mean age at hatch, size, and yolk volume were also analyzed in 2-way ANOVAs for each age at exposure. Concentrations ≥ LOEC for lethal effects were excluded because sublethal effects are irrelevant at concentrations producing lethal effects. MATCs for sublethal effects were calculated using Williams' test. Size measurement means for each beaker were used in analyses; all variables except yolk volume were untransformed. The cube root of yolk volume was used because it was normally distributed within beakers, had equal variances among beakers within treatments, and expressed volume on a linear scale so that it was comparable with other measures.

RESULTS

Composition and Concentration of Oil WSFs

Based on samples taken on 11 different days, the mean initial concentration of the WSF was 5.3ppm hydrocarbons (range=2.2-8.2, s.d.=1.5). When the extreme values at each end of the range were excluded, the mean remained 5.3 (range=4.0-6.5, s.d.=0.7). The mean concentration after 1 day (i.e., when solutions were changed) was 2.9ppm (n=7, range=2.3-3.4, s.d.=0.5) or 55% of mean initial concentration. A similar result was obtained by considering only those cases for which the same solution (including some dilutions) was measured when mixed, and after 1 day (mean=50%, n=5, range=42-56%, s.d.=6%). If the decline in hydrocarbon concentration is exponential, the geometric mean of initial and final concentrations ($=[2.9x5.3]^{1/2}=3.9$) gives the best estimate of the average concentration over the day. However, we use initial concentrations in this report to be consistent with other studies. Average initial concentrations for 50, 25, and 10% WSF would be 2.7, 1.3, and 0.5ppm.

Hibernia crude WSF was approximately 80% aromatics. Gas chromatography revealed that mono- and diaromatics accounted for most of the aromatic fraction. Because these are the most volatile hydrocarbons, it was not surprising that concentrations after 1 day were only 50% of initial concentrations.

Lethal Effects

Mean survival over all runs is shown in Fig. 1; patterns were similar in each individual run. Survival of embryos exposed to 100% WSF at age 0 declined rapidly, and most or all embryos were dead by age 13 days. Survival of embryos exposed to 100% WSF at age 5 was similar to that of embryos in other solutions through age 13 days, but declined rapidly thereafter. Survival of embryos exposed to 50% WSF at age 0 followed a similar pattern, except that the decline after age 13 days was not as great. Survival of embryos in other oil treatments did not differ greatly from survival in controls.

In runs 2-4 there was a sharp decline in survival from age 1 to age 5 in all treatments (controls are shown in Fig. 2). Perhaps gametes were of poorer quality in these runs than in run 1. The decline in survival occurred during the time when the paternal genome should begin contributing to embryonic development, suggesting that sperm may have been defective.

The interaction between oil concentration and age at exposure should be obvious from Figure 1, and is certainly obvious from % survival and % hatch at age 21 days (Table 2). For both response variables, age at exposure had little effect at 10, 25, and 100% WSF, but later exposure substantially increased survival and hatch at 50% WSF. Note also that age at exposure did have a strong effect on the timing of mortality in 100% WSF, as embryos exposed earlier died sooner (Fig. 1).

When the effects of concentration were analyzed separately for each age at exposure, differences among concentrations were significant for embryos exposed at age 0 but not at age 5 (Table 3). Results for both response variables were similar for embryos exposed at age 5 because values for % survival and % hatch had converged by age 21 days (Table 2). Concentration effects were much stronger on % hatch than on % survival for embryos exposed at age 0 (compare F-values in Table 3) because in each run there were a large number of apparently normal embryos in 50% WSF that failed to hatch. Differences between % survival and % hatch in Table 2 are almost entirely due to these normal unhatched embryos, as most developmentally retarded embryos were obviously dead by this time.

For both response variables at both ages at exposure, variation among runs was highly significant (Table 3). Survival and hatching success were considerably higher in most treatments in run 1, and to a lesser degree in run 4, than in other runs (Table 2). The interaction between run and concentration was either significant or large enough ($P \leq 0.25$) that it could not be combined with the variance among beakers for statistical tests. The presence of an interaction indicates that the dose-response curve differed among runs, although this did not seriously affect MATC values, which were similar for most runs (see below).

NOEC and LOEC values for each run, and overall, are given in Table 4; reductions relative to controls are also shown. Effects of 100% WSF were significant, regardless of how a common variance is calculated for Williams' test. Overall, and for most runs, the MATC for both response variables was 25-50% WSF (1.3-2.7ppm hydrocarbons) for embryos exposed at age 0, and 50-100% WSF (2.7-5.3ppm) for embryos exposed at age 5. The power of Williams' test was low, partly because there were few runs, and few beakers within runs, and because the error terms used in the tests were larger than expected if beakers represented random samples of 50 embryos. If each beaker represented a random sample of 50 embryos from the same population, and the number of replicates (r) was 2, the denominator for Williams' test ($[s^2/r]$) would be:

$$\{([p][100-p])/([50][2])\}^{1/2};$$

where p is % survival or hatch.

The largest value of this expression (5%) occurs when p=50%. Given a critical t value ≤2, any reduction in survival or hatch ≥10% would be significant. (The choice of number of replicates was in fact based on this calculation). However, there were many reductions of 10-20% which were not significant. The arcsin transformation should not reduce statistical power; variances of untransformed % survival or hatch among beakers treated the same were generally larger than expected if beakers were simply random samples of 50 embryos. The error term for Williams' test over all runs, the run-concentration interaction, was also larger than the variance among beakers. Thus each level of replication from individual fish to beaker to run adds variance. Therefore, replication should be at the highest level possible. However, the lack of statistical power is not a serious problem in this study; the MATCs effectively identify the concentrations at which lethal effects increased sharply. Although some of the effects observed at the NOEC in some runs may be biologically significant for that particular family, they may

not be biologically significant at the population level if they did not also occur in other runs.

Sublethal Effects

Mean Age at Hatch

At concentrations <LOEC for lethal effects, the relationship between mean age at hatch and oil concentration at both ages of exposure was an inverse parabola. Hatch was accelerated at intermediate concentrations, but was similar to controls at the NOEC for lethal effects (25% WSF for embryos exposed at age 0; 50% WSF for embryos exposed at age 5) (Table 5). Lethal concentrations delayed hatch; mean age at hatch for embryos exposed at age 0 to 50% WSF was approximately 18 days and mean age at hatch in 100% WSF could be considered infinity.

At both ages of exposure, age at hatch differed significantly among oil concentrations (Table 6). MATCs cannot be calculated because the parabolic relationship between age at hatch and concentration violates the assumption of a monotonic increase in effects necessary for Williams' test. Differences among runs, and the interaction between run and concentration, were relatively much smaller than for % survival and hatch (compare F-values in Table 4 and 6). Thus, mean age at hatch and its relationship with oil concentration varied much less among families than did survival and hatch, and their relationships with oil concentration.

Size and Yolk Reserves

At each age of exposure, free embryos from controls were longer, with longer heads, deeper bodies, and smaller yolks than free embryos from oil solutions (Table 7). Among oil solutions there was a mild tendency for body size measures to decrease, and yolk size to increase, with increasing oil concentration. For every size measure, differences among concentrations were significant at the P=0.05 level, and most were significant at the P=0.01 level (Table 8). These differences were not a function of differences in age at hatch; similar results were obtained when the analysis was restricted to embryos hatching 15 and 16 days after fertilization.

For all measures (except for body depth), differences among runs were significant for embryos exposed at age 0 but not for embryos exposed at age 5 (Table 8). Excluding run 1, and including 50% WSF, in the analysis of embryos exposed at age 5 was probably responsible for this result. Also, the interaction between run and concentration for embryos exposed at age 5 days for all measures except body depth was greater than the same interaction for embryos exposed earlier (Table 8). The same differences among control means from runs 2-4 obviously must exist at both ages of exposure. Similar differences among runs were observed at other concentrations for embryos exposed at age 0 days, but not necessarily for embryos exposed at age 5 days. The difference in the strength of the interaction term may also indicate that slight differences in growth response to oil developed among families between age 0 and 5 days.

All body size measures should be positively correlated; they should also be negatively correlated with yolk size. To determine if the measures used were largely redundant, two principal components (PCs) were computed by factoring the covariance matrix (see Leamy and Bradley 1982) of the log-transformed means from the 42 beakers for the three body size measures plus yolk height and yolk length. The first PC was an obvious size axis, positively correlated with the yolk dimensions, especially yolk height, and negatively correlated with the three body size measures. The second PC identified a trend for body depth to decrease with increasing yolk length once size effects (PC1) were removed.

Scores for these PCs for each beaker were then analyzed in 2-way ANOVAs for each age at exposure. This analysis was identical to the analysis of the original variables. There were significant differences in PC1 scores among oil solutions for both ages at exposure, but F-values were no higher than for yolk volume. Thus, adding the three body size measures did not increase our ability to detect oil effects. Given the high correlations among all measures, this was expected. The real test of redundancy was the analysis of PC2 scores, since this factor represented a measure (shape) independent of size. Differences in PC2 scores among solutions were not significant for embryos exposed at age 0, but were for embryos exposed at age 5. In the latter case, embryos in 50% WSF differed most from those in other solutions and PC scores were not correlated with oil concentration. Therefore, PC2 identified a possible effect on shape at one extreme concentration (and also identified a shape difference among embryos from different runs). This effect may be biologically interesting but its toxicological relevance is not clear. In conclusion, the variables measured were largely redundant, measuring the same effect on growth.

Which variables are the most sensitive to oil effects, and therefore the best for future studies? F-values for the solution term in Table 8 indicate that yolk volume and head length were the most sensitive. Williams' test confirmed this. LOECs for SL and body depth were occasionally >10% WSF; LOECs for yolk volume and head length were 10% WSF for every run, and over all runs, for both ages at exposure. Furthermore, the differences in head length and yolk volume at 10% WSF were significant at the P=0.01 level, with only one exception (head length for embryos from run 3 exposed at age 5 days). Yolk volume was a sensitive measure of oil effects because the effects were large (>20% difference from controls) relative to body size effects (5-10% difference). Head length was a sensitive measure because it had a lower coefficient of variation than did SL and was affected more by oil than was body depth. Head length is defined by a stiff rod of cartilage (the trabeculae and paranotochord) and is probably less subject to measurement error from shrinkage due to death or preservation than is SL, defined by the flexible notochord.

Eye Pigmentation

At both ages of exposure, the intensity of eye pigmentation decreased with increasing oil concentration (Fig. 3). Large differences in variance among beakers, and the small number of beakers made statistical analysis inadvisable. The trend is obvious, however.

Skeletal Development

There was no detectable variance in the presence/absence of specific skeletal elements within beakers or among solutions in run 3. Every properly stained and cleared specimen had the following cartilaginous elements:

(1) JAW - Meckel's cartilage, quadrate, symplectic, hyomandibular.

(2) SKULL (ventral) - trabeculae, fused anteriorly into the ethmoidal plate; basicapsular commissures with anterior and posterior dorsal extensions.

(3) PECTORAL GIRDLE - coracoscapula (occasionally with a faintly stained actinosal plate).

(4) BRANCHIOCRANIUM - ceratohyal with hypo- and interhyal; 3 ceratobranchials, each with a hypohyal; pharyngeal cartilage (4th ceratobranchial); basibranchials fused into anterior and posterior copulae.

Epibranchials were visible only on some specimens, but these small bones can be difficult to see except in a good dorsolateral view. Other embryos kept alive for a week or more after hatch were also examined. No new chondral bones were observed. Presumably, embryos attain the complement of chondral bones described some time before hatch, and new elements are not added until after the onset of exogenous feeding. Chondral bones were larger and more intensely stained in control, or more generally, larger, embryos; articulation surfaces were also more apparent. However, these are difficult effects to quantify.

DISCUSSION

This study provides the first quantitative results of the lethal and sublethal effects of Hibernia crude oil to capelin embryos. These effects are similar to those observed in other studies of oil toxicity to marine fish embryos. Lethal levels were within the range given for early life history stages of marine organisms by Connell and Miller (1984), although these ranges are based on 96h LC50s, usually conducted on larvae rather than embryos. There was certainly no indication that Hibernia crude oil had any unusual effects on capelin embryos. This may be partly because other oil WSF's are often also dominated by aromatics, particularly mono- and diaromatics (e.g., Anderson et al. 1974, Rice et al. 1977, Smith and Cameron 1979, Brodersen 1987, Carls 1987, Moles et al. 1987, Woodward et al. 1987).

In this study, the NOEC for embryos exposed at age 0 days (1.3ppm) was lower than that for embryos exposed at age 5 days (2.7ppm). Sharp et al. (1979) observed a similar age at exposure effect in studies on oil toxicity to the killifish Fundulus heteroclitus. In general, embryos exposed later should have lower mortality simply because they are exposed for a shorter interval. The lethality of compounds such as mono- and diaromatics, which are bioconcentrated, should be a relatively simple function of dose times exposure, with a critical level of contamination associated with death (Anderson 1979). Age at exposure effects will also occur when metabolic capabilities, and uptake of and permeabilty to toxicants, changes during ontogeny. Thus, Sharp et al. (1979) observed a discontinuous rather than smooth change in tolerance with increasing age at exposure. Presumably sharp increases in tolerance were associated with specific morphological or physiological developmental events. Assuming exposures in this study effectively lasted 15 days (median time to

hatch), NOECs expressed as ppm x days were 19.5 for embryos exposed at age 0, and 27 for embryos exposed at age 5. Because concentrations increased by a factor of 2 (>27/19.5), these values must be considered equal. Thus, there was no evidence of any age at exposure effect beyond a simple dose x exposure relationship.

Whipple et al. (1981) provide hypothesized modes of action and effects of monoaromatics, based on the assumption that these and other hydrocarbons behave similarly, acting as general stressors and affecting in particular enzymatic systems in metabolism. Their hypothesized modes account for most of the effects observed in this and other studies, although they do point out that specific hydrocarbons may also have other unique effects.

A complex relationship exists between oil concentration and age at hatch (Mazmanidi and Bazhashvili 1975, Ernst et al. 1977, Linden 1978, Leung and Bulkley 1979, Linden et al. 1979, Sharp et al. 1979, Neff and Anderson 1981, Hannah et al. 1982), with oil either accelerating or retarding hatch. This could occur if metabolism were inhibited at high concentrations but accelerated at low concentrations (Whipple et al. 1981). The premature hatch observed at low concentrations in our study is unlikely to be the result of accelerated metabolism, since yolk utilization was obviously retarded. Instead, oil may influence hatching by directly affecting hatching enzymes. We are not aware of any studies examining this possibility.

Reduced growth is another common sublethal effect of oil (see Anderson 1977, Capuzzo et al. 1984, Reish et al. 1985 for reviews) and is expected if oil inhibits metabolism and yolk utilization. Our measurements did not allow us to determine whether oil affected only the rate of growth and yolk conversion, or both the rate and efficiency. Preliminary results from the 1987 experiment in which embryos were exposed just prior to hatch indicate that exposed free embryos are smaller than control free embryos at the time of yolk exhaustion and death by starvation. These results suggest that reduced growth is a function of a metabolic cost as well as metabolic inhibition.

Effects on differentiation may be more difficult to detect at the whole-organism level because structure (e.g., skeletal elements in this study) is hard to quantify beyond presence/absence. Eye pigmentation was obviously affected (see e.g., Anderson et al. 1977). Some embryos were classified as 'developmentally retarded', suggesting effects on differentiation. However, these embryos were much more common in lethal solutions and died eventually, providing little information on sublethal effects. They were also distinguished primarily by their small size and immobility. The narcotic and behavioural effects of hydrocarbons have been described previously (Anderson 1979, Neff and Anderson 1981, Reish et al. 1984, 1985, Rand 1985).

Skeletal deformities, reported in other studies (reviewed by Spangenberg 1984; see also Mazmanidi and Bazhashvili 1975, Ernst et al. 1977, Smith and Cameron 1979, Winkler et al. 1983), were not observed. Hibernia crude oil WSF was composed primarily of mono- and diaromatics, rather than the more mutagenic and teratogenic polycyclic aromatics. Some exposed embryos appeared to have swollen pericardia, but certainly not as gross as observed by, e.g., Linden (1978).

How are these results likely to apply to field conditions (e.g., after an oil spill near capelin spawning grounds)? In extrapolating our results from the lab to the field, we face the usual problems involved in such an exercise (Kimball and Levin 1985) plus additional problems unique to oil toxicity studies (Anderson et al. 1977, Kunhold 1977, Anderson 1979). However, we do have the advantage of working with a test species and ecological system which have been extensively studied.

Oil and its soluble fraction are complex chemical mixtures whose toxicity depends on the properties of the component chemicals and their interactions. Our method of preparing oil WSFs was primarily a laboratory convenience designed to provide a standardized toxicant mixture. It was not designed to duplicate the mixture associated with an oil spill (see Neff and Anderson 1981, p. 17). Because oil spills are unique events, the toxicant mixtures they produce are likely to vary in composition, and the volatile mono- and diaromatics are unlikely to persist in sea water for as long as our exposure periods lasted (see Hardy et al. 1977, Butler and Levy 1978 for reviews of field concentrations). Pulse exposures to fresh WSFs or continuous expsoures to aged WSFs may provide more realistic results.

The conditions in our study may however duplicate the gradual leaching of hydrocarbons from oiled sediment into the water surrounding eggs. In future studies we plan to rear embryos in oiled sediments to examine this more directly.

Our results do show that hydrocarbon concentrations >2.5ppm, and certainly >5ppm, can be lethal if exposure is prolonged. More importantly, sublethal effects are expected at much lower concentrations. We also know what effects to look for in field experiments or after a spill. Anderson's (1979) dose times exposure relationship could be used to crudely predict critical concentrations for other exposure times. Information on factors affecting toxicity (e.g., age at exposure) can be used to evaluate the relative risks of different spill scenarios. Response surface analysis (e.g., Linden et al. 1979) is an example of extending this approach. This is probably a more important objective than estimating safe concentrations for a narrowly defined set of circumstances. Finally, this experiment provided baseline data on the appropriate effects to measure and levels of replication for future experiments.

What are the ecological implications of oil toxicity for capelin? Capelin have a number of life history characteristics which make them particularly susceptible to a localized event such as an oil spill. Lethal effects on embryos obviously affect the abundance of larvae, but the subsequent effects on year class strength are not so obvious. However, year class strength for age 2 capelin can be predicted from environmental conditions during the embryonic and larval periods (Leggett et al. 1984), suggesting that lethal effects on early life history stages would reduce the later abundance of that year class. The capelin fishery is based on the spawning population which consists almost entirely of one or two year classes (Templeman 1948, Bailey et al. 1977). Therefore, if an oil spill affected early life history stages, it could also affect the abundance of that year class as spawners 3-4 years later. Thus, a large portion of the harvested spawning population could be lost, if capelin stocks are discrete enough that most adults return to natal areas to spawn. (The extent of homing in capelin stocks is unknown.) Other species with many year classes in the spawning or harvested population, or without discrete stocks, or that are harvested in the open sea would not be as vulnerable. Because capelin have demersal adhesive eggs often spawned on beaches, a large number of eggs could be affected by a local spill. Pelagic eggs are unlikely to be as concentrated, and therefore as vulnerable.

The effects of oil on size and age at hatch may also have ecological implications for capelin populations. Embryos emerge from the beach sediment shortly after hatching, and coincident with onshore winds. This places larvae in a food-rich, predator-poor water mass (Frank and Leggett 1983). Unhatched embryos cannot emerge; thus, hatch is a more sensitive and meaningful endpoint than death is. The delayed hatch associated with lethal concentrations may add to the lethality of these concentrations. Premature hatch probably would not affect normal emergence, although it is possible that hatched embryos may be more vulnerable to displacement prior to the onset of onshore winds than unhatched embryos in adhesive eggs would be. In the future, we plan to directly examine the effects of oil on active emergence. Oil may have some additional behavioural effects which would impair emergence, and behavioural effects are expected at even lower concentrations than those producing growth effects (Connell and Miller 1984).

Survival of free embryos and larvae is thought to be positively correlated with size and ontogenetic stage (e.g., Ware 1975, McGurk 1986). If oil reduces growth and retards development, survival should decrease. However, time to starvation and therefore the probability of successful transition to exogenous feeding are positively correlated with yolk reserves (e.g., Blaxter and Hempel 1963). Thus, it is conceivable that exposed embryos with their larger yolks could enjoy enhanced survival. Obviously, a number of different selective forces act on size and age at hatch, and the transition to exogenous feeding. It is tempting to speculate that any departure from the norm (=controls) is suboptimal. However, in the absence of direct evidence, this argument rests on the Panglossian premise that all observed traits are optimal. In the past, phenotypic and genetic correlations between size and survival of capelin free embryos have been estimated (Chambers and Leggett, in prep.). Size selective predation on free embryos and larvae will be examined in the future. Thus, we do have a unique opportunity to estimate the effects of reduced growth on survival.

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FIGURE CAPTIONS

Figure 1. Survival of capelin embryos over the 21 day exposure period. Values are means over runs 1-4 for embryos exposed at age 0 days (a) and means over runs 2-4 for embryos exposed at age 5 days (b).

Figure 2. Survival of control embryos in each run. Note the mortality between days 1 and 5 that occurred in runs 2-4 but not in run 1. Also note the increase in survival on day 13 in run 3. This anomaly occurred because developmentally retarded individuals were not easily separated from dead individuals.

Figure 3. Intensity of eye pigmentation, on a scale of 0 (pale) to 3 (black), in free embryos from runs 2 and 4. Values are means for the two runs. • - exposed at age 0 days; o - exposed at age 5 days.

Table 1. Appropriate error terms for a 2-way ANOVA with one random factor and one fixed factor.

Source	Mean Square (MS)	F
Random factor (A)	MS(A)	MS(A)/MS(r)
Fixed factor (B)	MS(B)	MS(B)/MS(AxB)
Interaction (AxB)	MS(AxB)	MS(AxB)/MS(r)
Among replicates (r)	MS(r)	-

Table 2. Percentage survival and percentage hatch (in parentheses) at age 21 days for each treatment in each run and overall.

Run	Age at Exposure	Oil Concentration (%WSF) 0	10	25	50	100
1	0	87 (83)	87 (85)	95 (93)	71 (10)	0 (0)
2	0	59 (59)	50 (50)	53 (48)	14 (3)	0 (0)
	5	59 (59)	61 (61)	45 (44)	38 (35)	0 (0)
3	0	47 (43)	49 (47)	32 (25)	23 (6)	0 (0)
	5	47 (43)	40 (39)	34 (33)	46 (40)	4 (0)
4	0	69 (69)	71 (71)	53 (52)	41 (20)	0 (0)
	5	69 (69)	54 (54)	67 (67)	69 (66)	1 (0)
1-4	0	65 (64)	64 (63)	58 (54)	37 (10)	0 (0)
2-4	5	58 (57)	52 (52)	49 (48)	51 (47)	2 (0)

Table 3. Results of ANOVAs examining oil concentration effects on survival and hatch for each age at exposure.

Source	Error Term	DF	% Survival MS	% Survival F	% Hatch MS	% Hatch F
(a) Exposed at Age 0						
Run (R)	b	3	0.489	36.9**	0.308	19.3**
Concentration (C)	RxC	3	0.168	8.2**	0.800	16.6**
Interaction (RxC)	b	9	0.021	1.6	0.048	3.0*
Among beakers		16	0.013		0.016	
(b) Exposed at Age 5						
Run (R)	b	2	0.110	14.5**	0.134	15.6**
Concentration (C)	RxC	3	0.011	0.7	0.013	0.8
Interaction (RxC)	b	6	0.017	2.2	0.017	2.0
Among beakers		12	0.008		0.009	

* - $P \leq 0.05$
** - $P \leq 0.01$

Table 4. NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values for % survival and % hatch (in parentheses). Reductions from controls are also given; these reductions were calculated after arranging concentrations in an isotonic series (i.e., if the mean for a particular concentration was higher than the mean for any lower concentrations, a grand mean for those concentrations and any intervening ones was calculated, and that grand mean was taken as the mean for each concentration in the range).

Run	NOEC (% WSF)	Control minus NOEC	LOEC (% WSF)	Control minus LOEC
(a) Exposed at Age 0				
1	50 (25)	19 (0)	100 (50)	90 (77)
2	25 (25)	8 (11)	50 (50)	45 (56)
3	25 (25)	16 (20)	50 (50)	25 (29)
4	25 (25)	17 (18)	50 (50)	29 (50)
1-4	25 (25)	7 (10)	50 (50)	28 (54)
(b) Exposed at Age 5				
2	25 (25)	15 (16)	50 (50)	22 (25)
3	50 (50)	7 (6)	100 (100)	43 (43)
4	50 (50)	0 (7)	100 (100)	64 (69)
2-4	50 (50)	7 (10)	100 (100)	56 (57)

Table 5. Mean age at hatch (days) for each run and overall.

(a) Exposed at Age 0

Run	Oil Concentration (%WSF)		
	0	10	25
1	15.61	15.22	15.28
2	16.02	15.06	15.96
3	15.40	15.40	16.57
4	15.55	15.06	16.00
1-4	15.65	15.19	15.94

(b) Exposed at Age 5

Run	Oil Concentration (%WSF)			
	0	10	25	50
2	16.02	14.97	15.84	16.08
3	15.40	15.15	15.97	16.02
4	15.55	14.67	15.05	15.31
2-4	15.66	14.93	15.62	15.80

Table 6. Results of ANOVAs examining effects of oil concentration on mean age at hatch for each age at exposure.

Source	Error Term	DF	MS	F
(a) Exposed at Age 0				
Run (R)	Pooled error	3	0.380	1.7
Concentration (C)	Pooled error	2	1.502	6.7**
Pooled error		18	0.223	
Interaction (RxC)	b	(6)	(0.233)	1.1
Among beakers (b)		(12)	(0.218)	
(b) Exposed at Age 5				
Run (R)	Pooled error	2	0.815	5.1*
Concentration (C)	Pooled error	3	0.964	5.9**
Pooled error		18	0.162	
Interaction (RxC)	b	(6)	(0.134)	0.8
Among beakers (b)		(12)	(0.175)	

* - $P \leq 0.05$
** - $P \leq 0.01$

Table 7. Body size and yolk volume (cube root) means. All measurements are in mm, multiplied by the factors indicated.

(a) Exposed at Age 0

Run	Variable	Oil Concentration (% WSF) 0	10	25	Mean
1	SL (x 10^2)	559	515	524	532
	Head length (x 10^3)	674	627	627	643
	Body depth (x 10^3)	349	327	311	329
	Yolk volume (x 10^3)	204	270	287	254
2	SL (x 10^2)	568	533	505	535
	Head length (x 10^3)	695	654	624	658
	Body depth (x 10^3)	341	323	318	327
	Yolk volume (x 10^3)	214	257	282	251
3	SL (x 10^2)	519	502	470	497
	Head length (x 10^3)	660	623	600	628
	Body depth (x 10^3)	342	334	343	340
	Yolk volume (x 10^3)	233	295	312	280
4	SL (x 10^2)	526	487	483	498
	Head length (x 10^3)	677	615	598	630
	Body depth (x 10^3)	343	318	318	326
	Yolk volume (x 10^3)	234	295	294	274
1-4	SL (x 10^2)	543	509	495	
	Head length (x 10^3)	676	630	612	
	Body depth (x 10^3)	344	325	322	
	Yolk volume (x 10^3)	221	279	294	

Table 7. (Continued)

(b) Exposed at Age 5

Run	Variable	Oil Concentration (% WSF) 0	10	25	50	Mean
2	SL (x 10^2)	568	503	500	436	502
	Head length (x 10^3)	695	629	614	604	636
	Body depth (x 10^3)	341	310	314	297	315
	Yolk volume (x 10^3)	214	291	280	320	276
3	SL (x 10^2)	519	501	453	462	484
	Head length (x 10^3)	660	629	582	613	621
	Body depth (x 10^3)	342	330	322	312	327
	Yolk volume (x 10^3)	233	284	309	330	289
4	SL (x 10^2)	526	491	490	444	488
	Head length (x 10^3)	677	620	613	605	629
	Body depth (x 10^3)	343	318	324	298	321
	Yolk volume (x 10^3)	233	275	279	308	274
2-4	SL (x 10^2)	538	498	481	447	
	Head length (x 10^3)	677	626	603	607	
	Body depth (x 10^3)	342	321	320	303	
	Yolk volume (x 10^3)	227	284	289	319	

Table 8. Results of ANOVAs analyzing the effects of oil concentration on body size and yolk volume.

(a) Exposed at Age 0

Variable		Oil Concentration (C)	Run (R)	Inter-action (RxC)	Among Beakers (b)
SL	MS (x 10^4)	48.19	26.61	2.23	2.00
	F	23.2**	12.8**	1.1	
	Error term	RxC + b	RxC + b	b	
Head length	MS (x 10^6)	88.46	11.43	1.57	1.87
	F	50.0**	6.5**	0.8	
	Error term	RxC + b	RxC + b	b	
Body depth	MS (x 10^6)	10.70	2.23	1.35	0.60
	F	7.9*	3.7*	2.2	
	Error term	RxC	b	b	
Yolk volume	MS (x 10^6)	117.95	12.91	1.12	2.16
	F	65.1**	7.1*	0.5	
	Error term	RxC + b	RxC + b	b	
	DF	2	3	6	12

* - $P \leq 0.05$
** - $P \leq 0.01$

Table 8. (Continued)

(b) Exposed at Age 5

Variable		Oil Concentration (C)	Run (R)	Inter-action (RxC)	Among Beakers (b)
SL	MS (x 10^4)	84.92	7.05	7.69	2.60
	F	11.1**	2.7	3.0*	
	Error term	RxC	b	b	
Head length	MS (x 10^6)	69.73	4.43	3.17	1.75
	F	22.0**	2.5	1.8	
	Error term	RxC	b	b	
Body depth	MS (x 10^6)	15.76	2.93	0.61	0.85
	F	20.5**	3.8*	0.7	
	Error term	RxC + b	RxC + b	b	
Yolk volume	MS (x 10^6)	88.63	5.32	2.26	1.83
	F	44.9**	2.7	1.2	
	Error term	RxC + b	RxC + b	b	
	DF	3	2	6	12

* - $P \leq 0.05$
** - $P \leq 0.01$

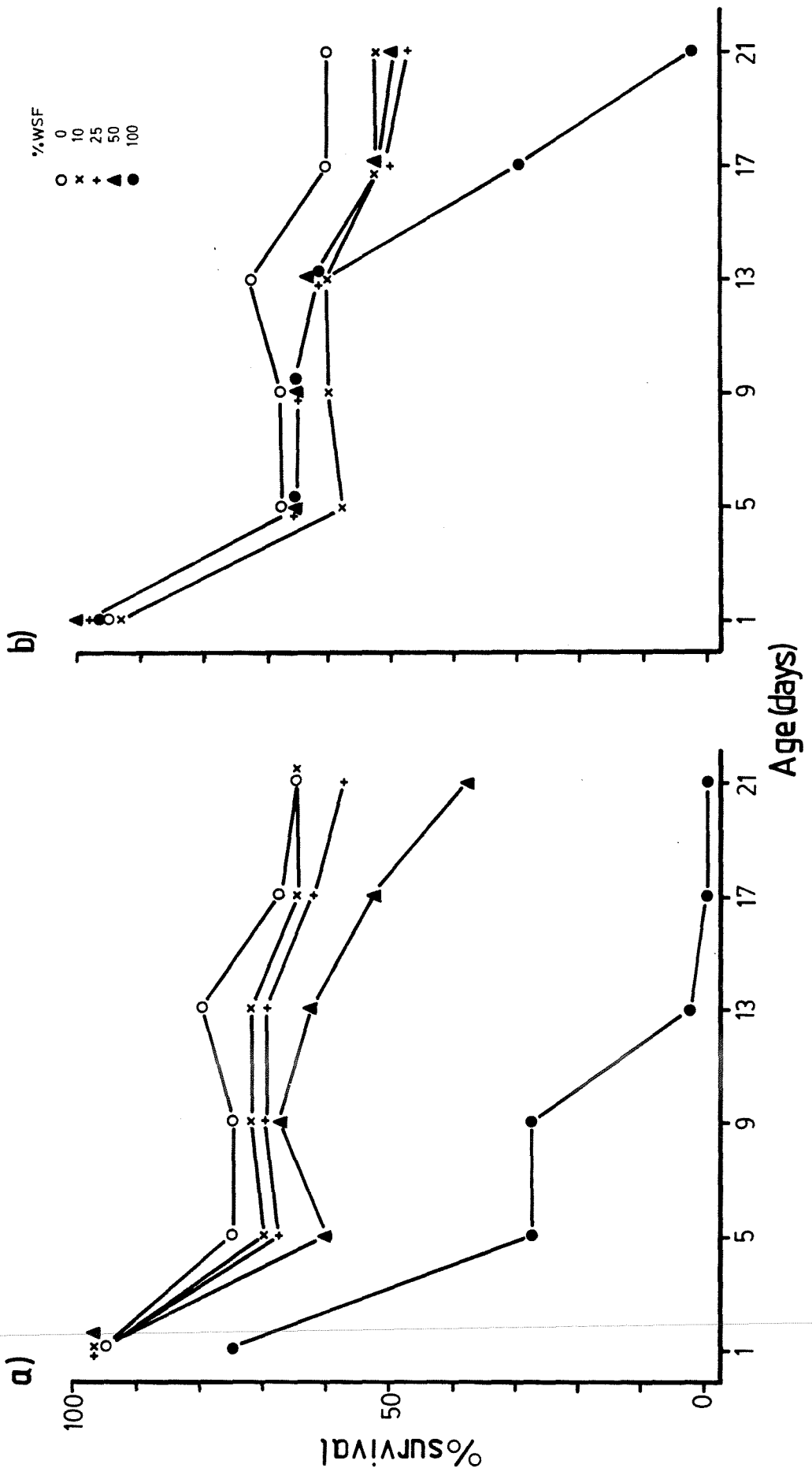

Figure 1.

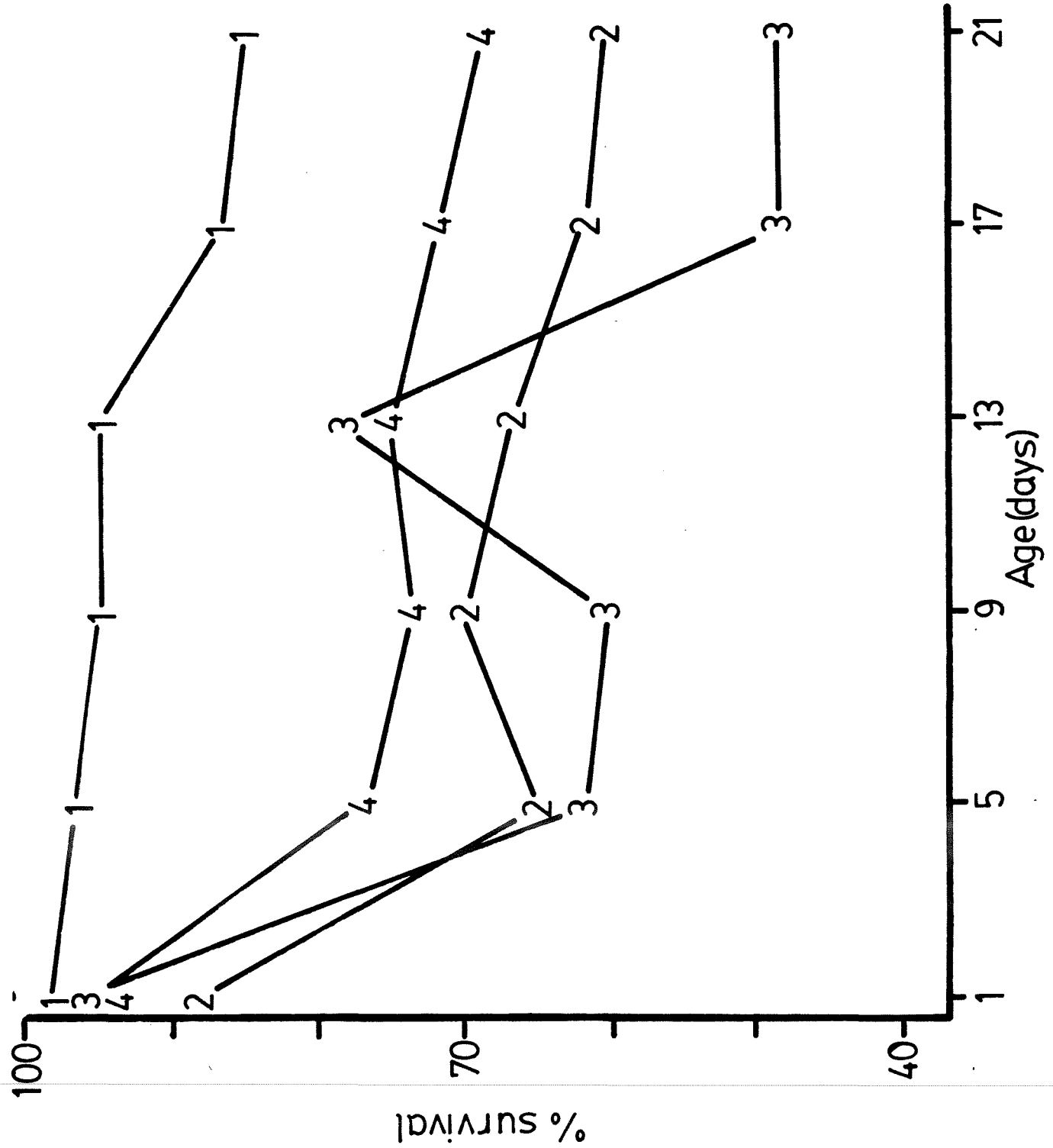

Figure 2.

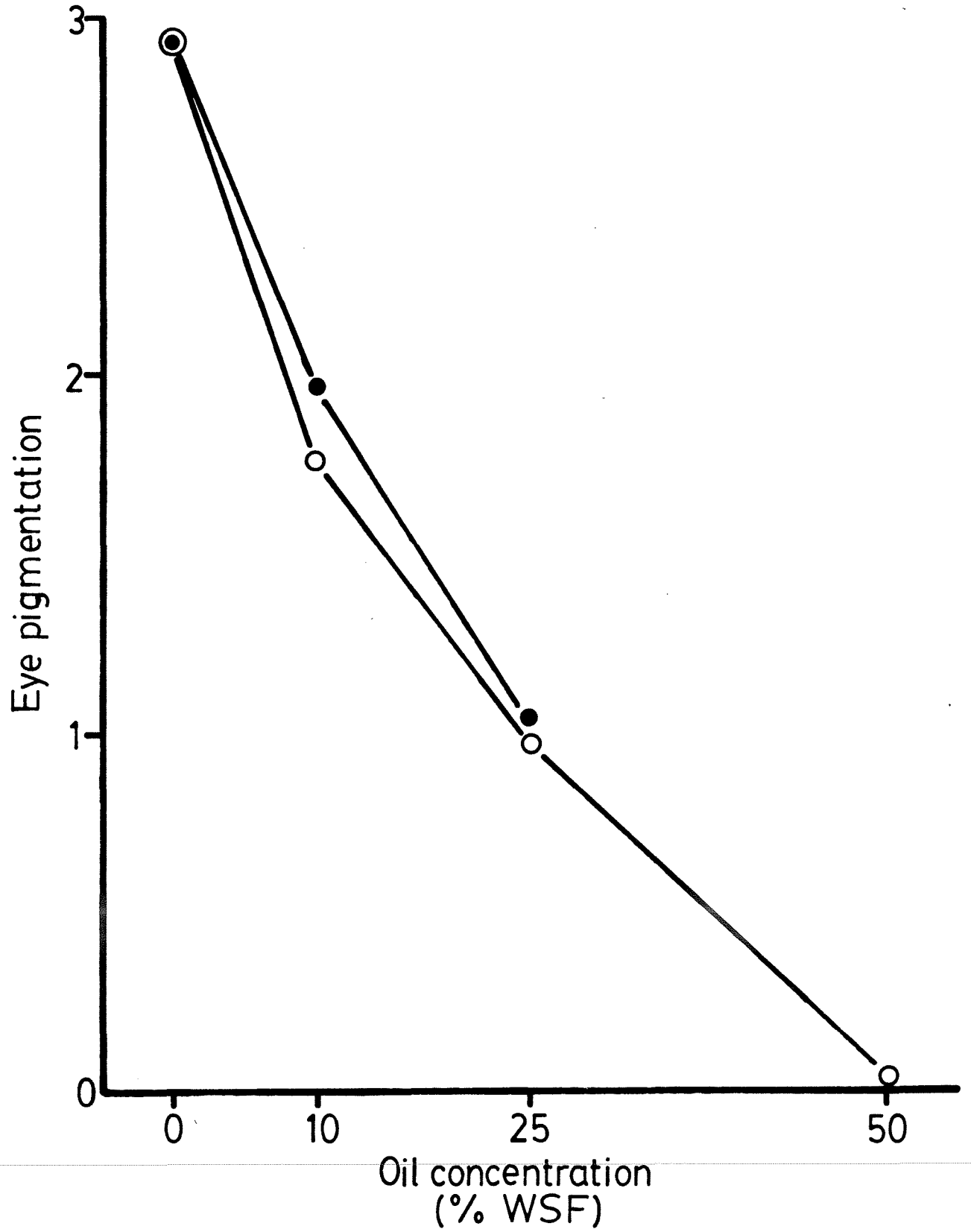

Figure 3.

APPENDIX I: INITIAL STATISTICAL ANALYSES

The variables measuring lethal effects (% survival, % hatch) were analyzed in 2-way ANOVAs, with one random factor (run) and one fixed factor (treatment) (Tables I.1, I.2). The levels of the treatment factor were controls plus the six combinations of three oil concentrations (10, 25, 50% WSF) and two ages at exposure (0, 5 days). Using orthogonal (independent) contrasts, the sum of squares (SS) among treatments can be subdivided into control versus all oil treatments, and among oil treatments. The among oil treatments SS can then be subdivided into the effects of oil concentration, the effects of age at expsoure, and the interaction between the two (e.g., Table I.1). Note that the SS and degrees of freedom (DF) for these contrasts add up to the total treatment SS and DF, respectively. Also, the concentration, age at exposure, and interaction SS are the same SS obtained from a 3-way ANOVA with run, concentration, and age at exposure as factors, and with controls excluded.

For both variables, the interaction between age at exposure and oil concentration was significant ($P \leq 0.05$). In fact, all contrasts were significant. The run-treatment interaction was not pooled with the residual term, because $P < 0.25$, even though it was not significant at the $P = 0.05$ level.

Table I.1. Results of ANOVA analyzing treatment effects on % survival for runs 2-4.

Source	Error Term	DF	SS	MS	F
Run (R)	b	2	0.374	0.187	25.4**
Treatment (T)	RxT	6	0.479	0.080	5.6**
Control vs others	"	(1)	(0.079)	(0.079)	5.5*
Age at exposure	"	(1)	(0.064)	(0.064)	4.5*
Oil concentration	"	(2)	(0.169)	(0.085)	5.9*
Interaction	"	(2)	(0.167)	(0.083)	5.8*
Interaction (RxT)	b	12	0.173	0.014	2.0
Among beakers (b)		21	0.154	0.007	

* - $P \leq 0.05$
** - $P \leq 0.01$

Table I.2. Results of ANOVA analyzing treatment effects on % hatch for runs 2-4.

Source	Error Term	DF	SS	MS	F
Run (R)	b	2	0.465	0.233	24.9**
Treatment (T)	RxT	6	1.438	0.239	15.5**
Control vs others	"	(1)	(0.144)	(0.144)	9.3**
Age at exposure	"	(1)	(0.248)	(0.248)	16.1**
Oil concentration	"	(2)	(0.589)	(0.295)	19.1**
Interaction	"	(2)	(0.457)	(0.229)	14.8**
Interaction (RxT)	b	12	0.186	0.015	1.7
Among beakers (b)		21	0.196	0.009	

* - $P \leq 0.05$
** - $P \leq 0.01$