Correlations Between Respiration and Direct Uptake of DDT in the Mosquito Fish *Gambusia affinis*

by PHILIP G. MURPHY and JOSETTE VIDRIL MURPHY
Hopkins Marine Station of Stanford University
Pacific Grove, Calif. 93950

Pesticide residues are widespread in natural waters and aquatic organisms. However, there are large interspecific and intraspecific variations in pesticide concentrations in fish, even from similarly contaminated waters (1,2). Although trophic levels and biological magnification undoubtedly account for much of this variation, it is becoming increasingly evident that there are other environmental and physiological factors which affect pesticide accumulation. Mayer et al (3) demonstrated that organochlorine insecticide interactions affect residue storage in rainbow trout. Murphy (4,5) reported effects of salinity and size on the uptake of DDT by mosquito fish. Macek et al (6) reported a generally increased susceptibility of trout and bluegills to many pesticides as temperature increased, and speculated that the mechanism involved was a higher rate of uptake at higher temperature.

In light of work by Holden (7) which indicates that gills are the main route of DDT uptake from water by fish, this study was undertaken to investigate the relationship between respiration rate and DDT uptake rate in the mosquito fish, Gambusia affinis.

MATERIALS AND METHODS

The fish, collected from a perennial pond on a golf course in Salinas, California, were of the same lot for which DDT residues have been previously reported (5). The fish were held in an outdoor pond for about one month before the experiments described here.

Respiration was measured by means of constant flow respirometers similar to those described by Fry (8). Erlenmeyer flasks of 500-ml capacity were used as chambers and contained from three to ten fish of the same size class. Oxygen analysis was performed by the Winkler method on 125-ml water samples (9). The fish were held at the experimental temperatures, 5°C and 20°C, for two days prior to the experiment. They were kept in the respirometers with a flow rate of about 10 ml of filtered pond water per min for 12 hr before samples were taken for analysis.

To ascertain the relationship of DDT uptake to body weight, 30 fish were exposed for 48 hr to 658±3

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nanograms (ng) of p,p' DDT-C^{14} (19.1 millicuries per millimole) in 16 l of aged tap water. Identical experiments were performed at 5°C and 20°C. The fish were kept at the experimental temperatures for two days prior to the experiment. Glass bottles of 22-l capacity served as aquaria. The fish were introduced and the aquaria stoppered 5 min after the DDT-C^{14} had been thoroughly mixed with the water. The DDT-C^{14} was added in 400 μl of ethanol.

To measure the effect of temperature on DDT uptake from water with a constant DDT-C^{14} concentration, continuous flow experiments were performed at 5°C and 20°C. For each of these experiments 743±8 ng of DDT-C^{14} in 520 μl of ethanol were mixed with 18 l of aged tap water, and 2 l were decanted into a 2-l Erlenmeyer flask; ten fish were placed in the Erlenmeyer flask and the remainder of the aqueous DDT-C^{14} solution was siphoned through this flask from a Mariotte bottle at a rate of 6.2 ml (5°C) or 6.1 ml (20°C) per min for 36 hr.

After exposure to the DDT-C^{14} the fish were each weighed to the nearest milligram, digested in 2 ml of an acid mixture consisting of equal parts of glacial acetic acid and 60% perchloric acid (10), and the DDT-C^{14} was extracted with three 2-ml washes of hexane.

Extracts were concentrated to 1 ml and the lipids removed by means of silica gel microcolumns (11); the eluent from the microcolumns was concentrated to one ml and mixed with 10 ml of toluene scintillation fluid. The radioactivity was measured by liquid scintillation counting using the channels ratio method of quench correction.

The effective gill area was measured by a modification of the method of Hughes (12). The gills were removed, and the gill filaments on each of the arches of the fish were counted. On each arch, the filament length and the interval of secondary lamellae of every eighth filament were measured with an ocular micrometer in order to determine the number of secondary lamellae per gill arch. The arches were fixed in Bouin's solution, embedded in paraffin, and sectioned at right angles to the gill filaments. The outlines of ten secondary lamellae from different filaments were projected onto paper by means of a camera lucida. Their areas were determined by cutting around their outlines and weighing the paper. For each arch the average area of a secondary lamella was multiplied by the number of lamellae on that arch and the areas of all the arches were summed.

We observed that a transverse section of a mosquito fish is approximately triangular. Therefore, we estimated the surface area of a fish to be twice the area of one side plus the area of the dorsal side. Measurements were made by laying the fish, with appropriate surface down, on paper, spraying it with paint from an aerosol can, cutting out and weighing the unpainted silhouette, and comparing these weights with the weight of a piece of the paper of known area.

RESULTS

Figure 1 shows the results of the respiration measurements. When plotted logarithmically a linear relationship exists between rate of oxygen consumption and body weight at both experimental temperatures.

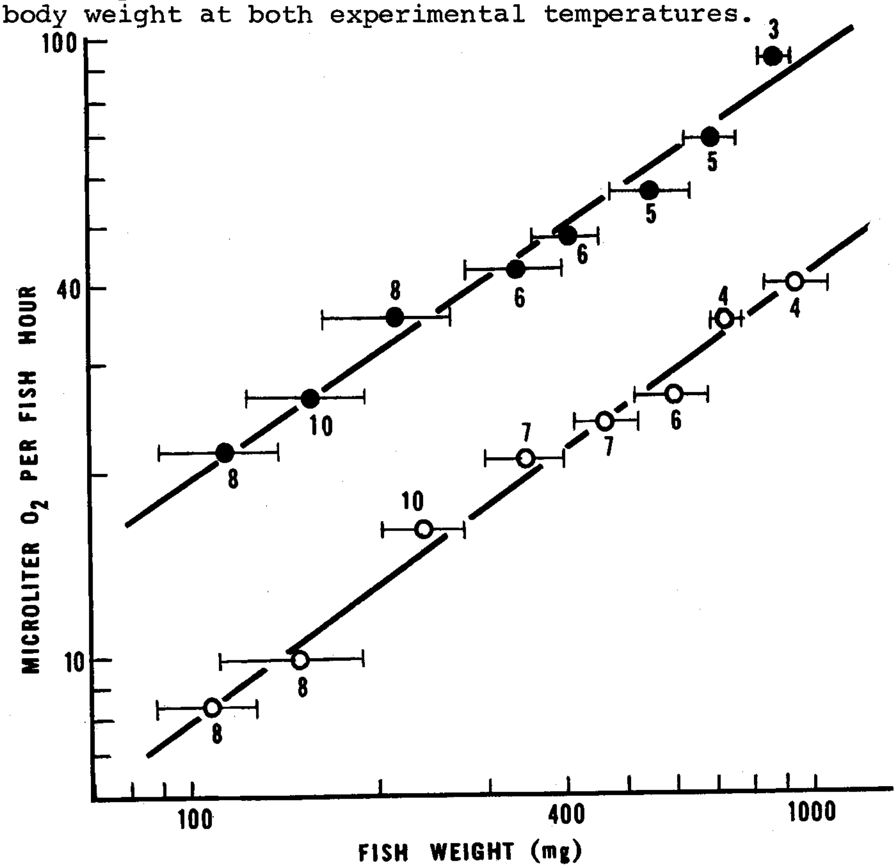

FIGURE 1. Log-log plots of respiration rate versus body weight at 5°C (open circles) and 20°C (solid circles). The lines were fitted by the method of least squares; their slopes and standard deviations, in log units, are 0.71±0.035 and 0.68±0.037, respectively.

The log-log relationship of DDT uptake to body weight is also linear as shown in figure 2.

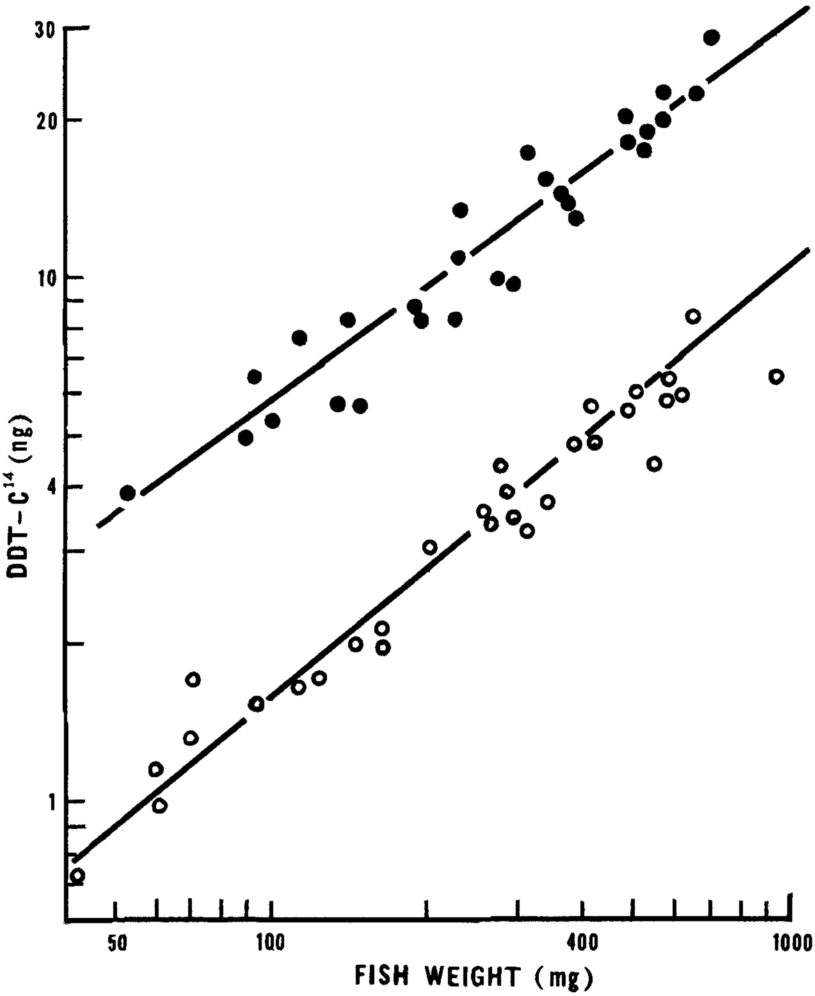

FIGURE 2. Log-log plots of DDT uptake from water with an initial concentration of 41 parts per trillion versus body weight at 5°C (open circles) and 20°C (solid circles). The lines were fitted by the method of least squares; their slopes and standard deviations, in log units, are 0.77 ± 0.029 and 0.75 ± 0.044, respectively.

Figure 3 shows the log-log relationships of gill area and body surface area to body weight.

The uptake of DDT-C^{14} from continuously flowing water is shown in table 1.

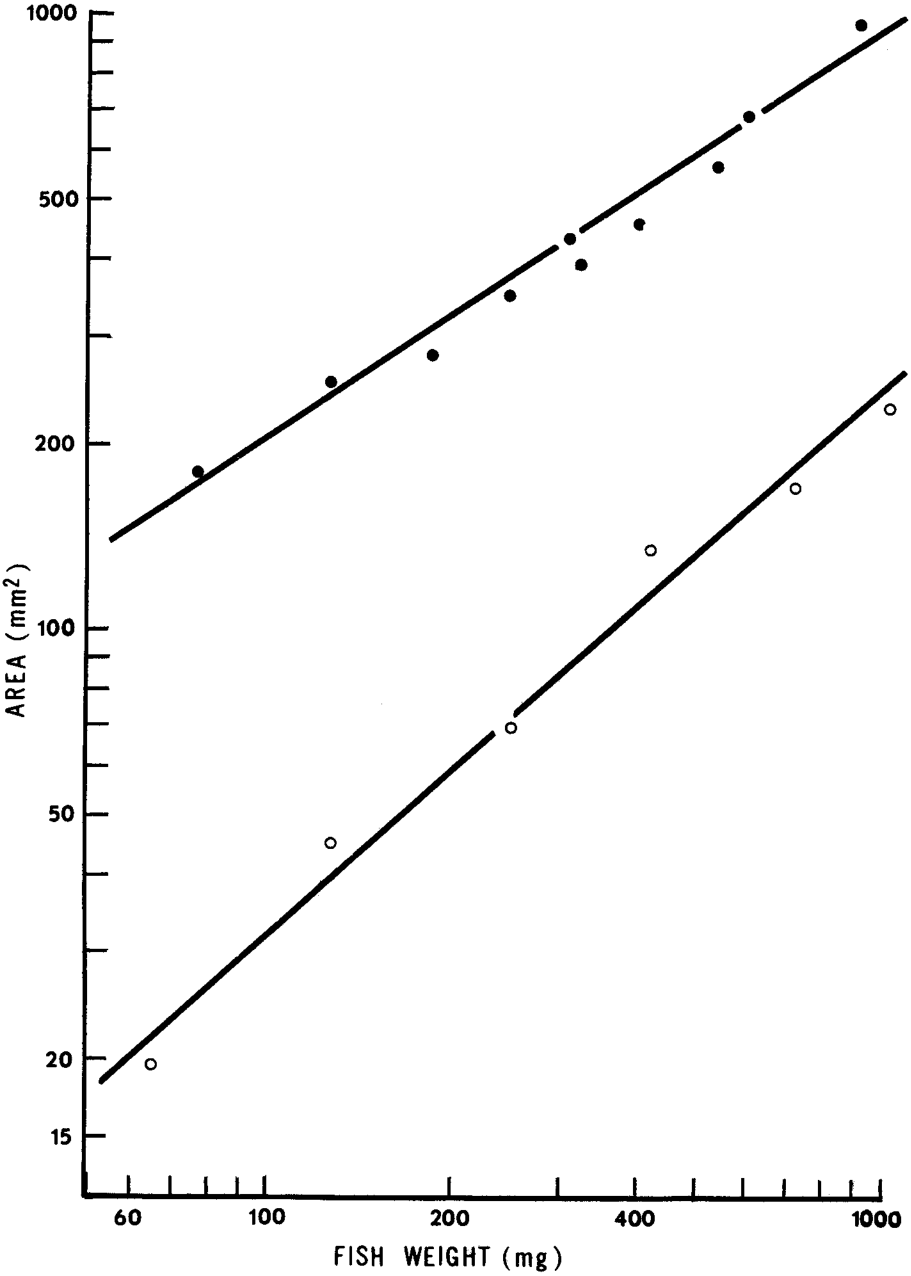

FIGURE 3. Log-log plots of body surface area versus body weight (solid circles) and gill area versus body weight (open circles). The lines were fitted by the method of least squares; the slopes and standard deviations, in log units, are 0.66±0.036 and 0.89±0.057 respectively.

TABLE 1. 36 HOUR DDT UPTAKE BY MOSQUITO FISH FROM FLOWING WATER WITH 40 PARTS PER TRILLION DDT-C^{14}

Temp.	No. Fish	Fish Weight (mg)[1]	ng DDT per Fish[2]	ml O_2 used per 36 hr[3]	ng DDT per ml O_2
5°C	10	280±36	3.0±0.6	0.60	5.0
20°C	10	250±22	9.6±1.3	1.32	7.3

1,2. Mean-standard deviation. 3. Oxygen consumption computed from equations graphed in figure 1.

DISCUSSION

The slope of the logarithmic relationship of oxygen uptake rate to body weight reported here for mosquito fish is approximately two-thirds (figure 1). This is similar to the log oxygen uptake-log weight relationships for two other top minnows, Fundulus parripinnis and Lebistes reticulatus, as well as for Cyprinis carpio (8). Although the slopes of the 5°C line and 20°C line are not statistically different, a slightly greater slope at 5°C than at 20°C, similar to our results, was reported by Job (13) for Salnelinus fontinalis which is the only other fish for which the 5°C and 20°C data are available.

The slopes of the 5°C and 20°C log DDT uptake rate-log weight relationships differ only slightly from the respective log oxygen uptake-log weight relationships (figures 1,2). The reduced oxygen consumption undoubtedly implies a smaller respiration volume at 5°C than at 20°C. It appears, at least for the DDT concentration used in our experiments, that the diffusion rates of DDT and oxygen through the gills are similarly affected by changes in respiration volume. Dead fish exposed to the same concentration of DDT-C^{14} at 20°C accumulated a maximum of 0.3 ng per fish in 48 hr, which confirms that only live fish take up DDT, and indicates that surface adsorbtion of DDT is not important.

The surface area to body weight ratio increases, as fish size decreases, faster than does the gill area to body weight ratio, which suggests that cutaneous respiration accounts for a larger proportion of the total respiration in smaller fish (figures 4,5). Since Holden (14) suggested that mucus is less permeable to DDT than to oxygen, and since the mucus layer on fish skin might be expected to be thicker than that on the gills, this might account for the steeper slopes for the log-log relations of DDT uptake to body weight than for the log-log relations of oxygen consumption to body weight.

From the equations for the lines in figures 1 and 2, as well as from the results of the continuous flow experiment (table 1), it is evident that, over the range measured, a nearly linear relation, with a slope of about 5 at 5°C and 6 or 7 at 20°C, exists between ng of DDT-C^{14} taken up and ml of oxygen consumed when water concentration of DDT is about 40 ppt. The uptake of DDT-C^{14} in the continuous flow experiment was predictably higher because the DDT-C^{14} concentration of the water did not diminish during the experiment. If cutaneous respiration, which does not depend on opercular movement, is less temperature dependent than gill respiration, a greater proportion of cutaneous respiration may account for the lower ratio of DDT uptake to oxygen consumption observed at 5°C.

If DDT uptake rates are similarly related to metabolism in other species, caution must be used when predicting the contribution of direct uptake to DDT contamination in fish on the basis of experiments with one size class of fish because the slope of the log oxygen uptake rate-log weight relationship is considerably less than unity for all species studied. That small fish are more efficient at removing DDT from water probably accounts for the inverse relationship between body weight and median tolerance limit to DDT for three salmonid species, as reported by Post and Schroeder (15), and may be especially important in light of evidence that fry are killed by body concentrations of DDT that are tolerated by larger fish (16).

Another implication of our results is that the temperature of natural waters will drastically affect the direct uptake of DDT residues, resulting in higher residues in fish from warm waters than in fish from colder waters given the same levels of water pollution.

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