

**Abstract.**—The sublethal effects induced by a model carcinogen and environmental contaminant on salmonid emergence behaviors have been studied. Rainbow trout embryos were exposed for 24 hours to 25 µg/mL of benzo[a]pyrene 1 week prior to hatching. Exposures occurred during the late organogenesis period of development and allowed assessment of how a single embryonic exposure might affect emergence behaviors nearly 6 weeks later. Though no differences in numbers of alevins successfully emerging were observed, a significant decrease was noted in performance of the upstream orientation behaviors characteristic of emergence among wild individuals. These findings are discussed in terms of a model describing the role of upstream swimming behavior after emergence.

## Decreased Performance of Rainbow Trout *Oncorhynchus mykiss* Emergence Behaviors Following Embryonic Exposure to Benzo[a]pyrene

**Gary K. Ostrander**

College of Ocean and Fishery Science, University of Washington WH-10  
Seattle, Washington 98195

Present address: Department of Zoology, Oklahoma State University  
Stillwater, Oklahoma 74078

**James J. Anderson**

**Jeffrey P. Fisher**

**Marsha L. Landolt**

**Richard M. Kocan**

College of Ocean and Fishery Science, University of Washington WH-10  
Seattle, Washington 98195

As aquatic ecosystems worldwide continue to function as ever-increasing reservoirs of environmental pollutants, the presence of many diverse classes of compounds is being documented (Champ and Park 1982). One class of compounds, the aromatic hydrocarbons, has been implicated in carcinogenesis in feral fish populations (Baumann 1989). Previous studies (Ostrander et al. 1988, 1989) and work presented here suggest that aromatic hydrocarbons may also exert subtle, yet profound, behavioral effects during early development.

Benzo[a]pyrene (B[a]P) is an aromatic hydrocarbon that is combustion product of organic materials (e.g., forest fires). Furthermore, anthropogenic activities have introduced significant amounts of B[a]P into the aquatic environment both directly (industrial emissions and oil spills) and indirectly (automobile exhaust and coal burning). This compound has been well studied in mammalian systems (reviewed in Gelboin 1980), and much is known of its mode of action. Recently we have verified the sen-

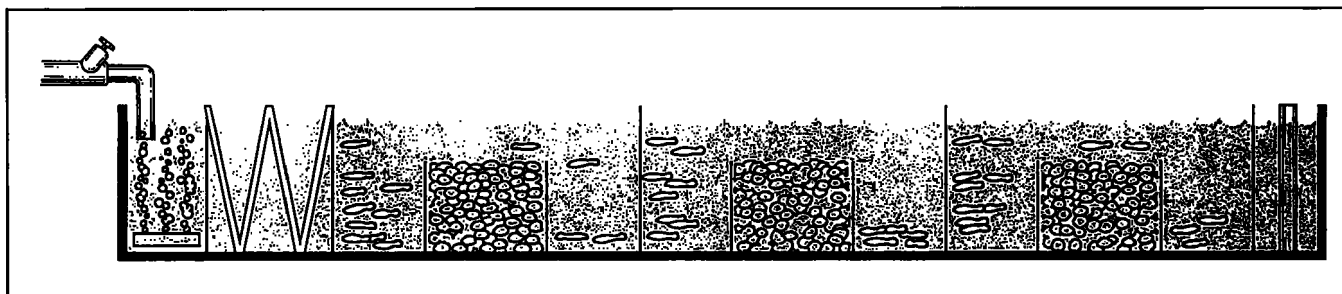
sitivity of coho salmon early-life-history stages to a single embryonic exposure to benzo[a]pyrene (Ostrander et al. 1988). Embryonic exposure to B[a]P resulted in temporal differences in hatching, reduced and altered emergence success, and decreased foraging efficiency.

We next studied emergence behaviors following embryonic B[a]P exposure of the rainbow trout *Oncorhynchus mykiss*. We describe the effect of B[a]P exposure on the emergence success and upstream swimming behavior. A qualitative model, utilizing data from our rainbow trout and coho salmon studies, is proposed to explain the effects of toxicants on these early life behaviors.

### Materials and methods

#### Experimental animals and exposures

Two replicates of a single experiment with rainbow trout were conducted with eggs obtained from the McCleary Trout Lodge, Tacoma, Washington.



**Figure 1**

A single raceway containing three artificial gravel-filled redds and respective upstream and downstream compartments. Entering water was aerated and passed through a series of four baffles to reduce turbulence over the redds. Water exited through a standpipe at the opposite end of each raceway. Emerging fish, after entering either the upstream or downstream compartment, were removed and scored 3–5 times daily.

Eggs of a single female were fertilized with the pooled sperm of 12 males. Following fertilization, eggs were suspended in a continuous flow of lake water regulated with dechlorinated city water to a constant temperature of  $12^{\circ} \pm 1^{\circ}\text{C}$ . One week prior to hatching (late organogenesis), eggs were divided into three groups of 150 eggs and each group was transferred to glass petri dishes for a 24-hour exposure in a volume of 0.4 mL water per egg. B[a]P (Aldrich Chemical Co, Product #B1,008-0) was suspended in spectrophotometric grade DMSO (Schwarz/Mann Biotech, Product #820636) to make a stock solution of 10 mg/mL. This solution was diluted in lake water to make the final exposure concentrations of 25  $\mu\text{g}/\text{mL}$ . The control groups were exposed to filtered lake water, the solvent control group was treated with 0.5% solution of dimethylsulfoxide (DMSO) in filtered lake water, and the exposure group was treated with 25  $\mu\text{g}/\text{mL}$  of B[a]P in 0.5% DMSO in lake water.

### Experimental apparatus

Artificial redds were constructed in replicate raceways by subdividing each raceway into ten compartments (Fig. 1). The upstream compartment contained baffles to reduce water turbulence in the redds. The remaining nine compartments included three redds (30 cm wide  $\times$  40 cm long) each occupying a single compartment and centered between an open upstream and downstream compartment (30 cm wide  $\times$  20 cm long). The redds consisted of smooth rocks (2–4 cm diameter) contained within perforated stainless steel plates. Adjacent open compartments were separated by plastic netting. The redd surface was within 8 cm of the water surface and the open compartments were 20 cm deep.

The water, filtered to remove large particulates, flowed through each raceway at a velocity of 45 cm/second.

Following exposure, the eggs were rinsed with lake water and buried 8–10 cm deep in artificial redds. The position of the various groups in each raceway was randomized. The redds were maintained in darkness throughout embryonic development at a temperature of  $12.0^{\circ} \pm 1^{\circ}\text{C}$ . Beginning 60 days postspawning, the raceways were checked daily for emerged fry, and, following emergence of the first fry, the raceways were checked three to five times daily. Alevins emerged and held station above the redds while orienting against the water flow. After several minutes they would (1) swim into the upstream compartment, (2) swim into the downstream compartment, or (3) drift backward into the downstream compartment.

### Data collection

The presence of an alevin in either the upstream or downstream compartment provided a quantitative measure of the ability of the fish to perform its typical upstream swimming behavior. Fish in the upstream and downstream compartments were counted and removed. Furthermore, 20 fry from the upstream and downstream compartments of each exposure group were randomly selected for measurements of total length, dorsal fin height, caudal fin height, pectoral fin height, anal fin height, and dry weight. An analysis of variance followed by a Student-Newman-Kuels multiple range test was used to test for significant differences among the means of the various exposure groups.

### Results

Rainbow trout were exposed to a sublethal 24-hour pulse (25  $\mu\text{g}/\text{mL}$ ) of B[a]P during late organogenesis. The emergence success of rainbow trout treated with

Mention of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

**Table 1**

Emergence and upstream swimming behavior of rainbow trout and coho salmon, showing number of fish performing each behavior. In each experiment 150 apparently healthy salmon or trout eggs (embryos) were buried 1 week prior to hatching.

Replicate number	Fish species	Exposure group	Number emerged	Number upstream (% of emerged)	Number downstream
1	Rainbow trout	Control	136	108 (76)	33
		DMSO	138	104 (75)	34
		B[a]P	147	68* (46)	79
2	Rainbow trout	Control	139	115 (83)	24
		DMSO	145	115 (79)	30
		B[a]P	143	60* (42)	83
3 <sup>a</sup>	Coho salmon	Control	148	132 (89)	16
		DMSO	142	115* (81)	27
		B[a]P	118**	76** (64)	42
4 <sup>a</sup>	Coho salmon	Control	147	137 (93)	10
		DMSO	150	105* (72)	45
		B[a]P	103**	70** (68)	33

<sup>a</sup>Data from Ostrander et al. 1988.

\* Significantly different from control group at  $P < 0.05$ , chi-square analysis.

\*\* Significantly different from control group at  $P < 0.001$ , chi-square analysis.

**Table 2**

Morphological development of rainbow trout alevins at emergence. Values are means and ranges for groups of 20 fish.

Group	Total length (mm)	Dry body weight (mg)	Caudal ----- fin height (mm)	Dorsal ----- fin height (mm)	Pectoral ----- fin height (mm)	Anal ----- fin height (mm)
Upstream control	24.29 (22.6-25.6)	44.09 (43.9-44.4)	4.53 (3.8-5.0)	1.86 (1.5-2.2)	2.52 (2.2-3.2)	1.67 (1.4-2.0)
Downstream control	24.13 (21.5-25.1)	44.61 (44.4-44.9)	4.85 (4.0-5.6)	1.86 (0.8-2.3)	2.85 (1.9-3.3)	1.80 (0.8-2.1)
Upstream DMSO	24.41 (23.1-25.2)	44.47 (44.2-45.1)	4.69 (3.9-5.2)	1.80 (1.5-2.3)	2.96 (2.6-3.3)	1.80 (1.5-2.1)
Downstream DMSO	24.02 (21.4-24.9)	44.52 (44.3-44.8)	4.91 (4.3-5.4)	1.90 (1.4-2.3)	2.87 (1.8-3.3)	1.79 (1.4-2.1)
Upstream B[a]P	24.19 (21.4-25.5)	44.17 (43.9-44.5)	4.53 (3.8-5.4)	1.75 (0.8-2.1)	2.59 (1.9-3.3)	1.63 (1.4-2.3)
Downstream B[a]P	24.17 (23.3-24.9)	44.33 (44.1-44.6)	4.56 (4.0-5.2)	1.96 (1.6-2.4)	2.63 (2.3-3.0)	1.77 (1.4-2.1)

B[a]P was not significantly different ( $\chi^2$ ,  $P < 0.05$ ) from embryos treated with DMSO (solvent controls) or filtered lake water controls (Table 1). In both replicates more than 95% of B[a]P-exposed fish successfully emerged.

The two B[a]P-exposed groups, however, exhibited a decreased tendency to orient upstream when compared with DMSO or untreated controls ( $\chi^2$ ,  $P < 0.001$ ). Normal upstream orientation observed among emerged untreated and DMSO solvent controls was 75-83% of emerging alevins, while among B[a]P-

treated groups less than 46% of the emerged alevins reached the upstream compartment (Table 1).

Measurements of total body length, dry weight, and caudal, dorsal, pectoral, and anal fin heights are summarized in Table 2. There were no significant differences in the morphology of upstream and downstream fish or between respective groups when tested with a one-way analysis of variance.

## Discussion

The salient result of these studies is that both rainbow trout and coho salmon exposed as embryos to a sublethal dose of B[a]P exhibit decreased ability to swim upstream after emergence from artificial redds.

Emergence and orientation are activities that terminate a sequence of early-life behaviors critical for salmonid survival. Following hatching, developing salmonid eleutheroembryos remain in gravel redds for 2–4 months until yolk sac absorption and fin development are nearly complete (Fast 1987). As yolk sac reserves are exhausted the eleutheroembryos migrate, primarily at night, to the upper layers of the redd. Emergence is complete when eleutheroembryos, now called fry, leave the redd and swim to the water surface to inflate the air bladder and begin feeding. The direction in which emergent fry migrate is species-specific. Coho salmon and rainbow trout fry typically orient and swim upstream to counteract downstream transport and to colonize natal and nearby streams unavailable to, or not used by, spawners (Mason 1976). Soon after emergence, fry establish and defend territories as feeding begins (Chapman 1962, Mason and Chapman 1965).

Our previous work (Ostrander et al. 1989) demonstrated that the lipophilic nature of B[a]P coupled with the expansive yolk sac reserves of the embryo resulted in significant uptake of the compound and, furthermore, the B[a]P was only gradually lost from the egg throughout development. We found that over 60% of the B[a]P is retained in the eleutheroembryo at hatching when this exposure regimen is utilized. Consequently, rather than exposing our B[a]P-treated fish to an acute 24-hour pulse of B[a]P, they faced continuous exposure to B[a]P and its harmful metabolites (Gelboin 1980) as they exhausted their yolk reserves throughout early development.

There were no significant differences in numbers of fish emerging among the various control and exposure groups. Yet, fewer rainbow trout exposed to B[a]P as embryos exhibited typical upstream swimming behavior following emergence. These results are similar to previous findings for coho salmon (Ostrander et al. 1988). In that study, however, significantly fewer coho salmon, successfully emerged (Table 1). Perhaps the larger size of the coho alevin coupled with compromised abilities due to B[a]P exposure resulted in reduced success in negotiating the interstitial spaces of the redd during emergence. Fish in the downstream compartment did generally appear less active than fish in the upstream compartment. In neither study, however, were significant differences in weight or fin development among control and treatment groups observed,

nor were any morphometric differences detected between fish going upstream or downstream in the experiments.

In these experiments, fish emerged from the gravel and oriented into the water flow. After about 1 minute, the majority of fish moved into the upstream compartment of the test apparatus. A smaller number of fish, generally less active, either swam or drifted into the downstream compartment. Successful upstream movement requires that the time to travel the distance across the redds is less than the time to become fatigued, assuming that fish in the downstream compartment became fatigued before they reached the upstream compartment. This is expressed,  $T_f > T_t$ , where  $T_t$  is the travel time to the upstream compartment and  $T_f$  is the fatigue time of the fish. Travel time to the upstream compartment depends on the average velocity of the current across the redds,  $U$ , the average fish swimming velocity,  $V$ , and the distance,  $L$ , it travels across the redd, and is expressed:

$$T_t = L/(V - U).$$

Fatigue time also depends on swimming velocity. Laboratory experiments indicate that among adult rainbow trout, the time to fatigue decreases exponentially with swimming velocity for velocities above 3 body lengths per second (bl/s). However, at 2 bl/s adult rainbow trout can swim indefinitely (Beamish 1978). Alevins probably have a similar relationship, although (due to smaller size) they can maintain considerably larger swimming velocities on a body length basis, exceeding 30 bl/s (100 cm/s) (Ostrander et al. 1989). The model assumes time to fatigue is infinite when swimming velocity is zero and decreases as swimming velocity increases. A simple power function has these properties:

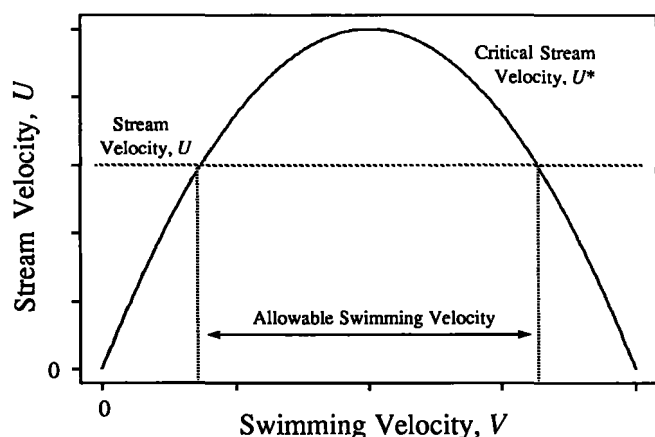
$$T_f = k V^{-a}$$

where  $k$  and  $a$  are parameters that depend on condition of the environment and the fish. Equating the two time scales, a critical stream velocity is defined:

$$U^* = V - L/kV^a$$

where successful upstream migration requires  $U < U^*$ . The intersection of  $U$  and  $U^*$  defines a swimming velocity range required for successful movement into the upstream compartment (Fig. 2). For velocities outside the allowable range fish become fatigued before they reach the upstream compartment.

The experiments indicated that fish exposed to B[a]P had a greater chance of ending up in the downstream compartment. This result could be achieved if fish



**Figure 2**

Critical stream velocity curve. Successful upstream movement requires that the critical stream velocity is greater than the stream velocity. Intersection of  $U$  and  $U^*$  defines the allowable swimming velocity,  $V$ , required for successful movement into the upstream compartment.

exposed to B[a]P became fatigued faster or swam upstream slower. In the first case, the critical stream-velocity curve of exposed fish would fall below the curve for unexposed fish. In the second case, the lower swimming velocity of the exposed fish would fall outside the allowable velocity range for successful upstream migration. The experiments were not designed to resolve such differences.

The model provides a qualitative basis for interpreting the experiments. It differs from standard dose-response models that describe the degree of change as a continuous function of the level of duration of toxicant exposure (Krewski and Brown 1981). The model allows a possible interplay of behavior and stamina for successful upstream movement, although such a separation was not pursued in the study.

Our results indicate that low levels of toxicants may affect important early-life-history behaviors critical to survival. Though the concentration of B[a]P used in this study is not frequently encountered in nature, the value of this study in predicting what could happen following some type of major accidental spill or discharge should not be overlooked. Given that a single 24-hour pulse of B[a]P elicited significant behavioral changes, future studies could be directed at understanding the potential effects of low-level chronic exposures which may more accurately mimic natural conditions.

## Acknowledgments

Data presented in this manuscript are included in a dissertation submitted to the College of Ocean and Fishery Science, University of Washington, in partial fulfillment of the requirements for the Ph.D. degree (GKO). The authors thank Kathleen Sabo for excellent technical assistance. This study was supported by a grant (R-811348) from the U.S. Environmental Protection Agency.

## Citations

- Baumann, P.C.**  
1989 PAH, metabolites, and neoplasia in feral fish populations. In Varanasi, U. (ed.), *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment*, p. 269-289. CRC Press, Boca Raton, FL.
- Beamish, F.W.H.**  
1978 Swimming capacity. In Hoar, W.S., and D.J. Randall (eds.), *Fish physiology*, vol VII, p. 101-187. Academic Press, NY.
- Champ, M.A., and P.K. Park**  
1982 *Global marine pollution bibliography*. Plenum Publ., NY, 399 p.
- Chapman, D.W.**  
1962 Aggressive behavior of juvenile coho salmon as a cause of emigration. *J. Fish. Res. Board Can.* 19:1047-1080.
- Fast, D.E.**  
1987 The behavior of salmonid alevins in response to changes in dissolved oxygen, velocity, and light during incubation. Ph.D. diss., Univ. Wash., Seattle, 150 p.
- Gelboin, H.V.**  
1980 Benzo(a)pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev.* 60(4):1107-1116.
- Krewski, D., and C. Brown**  
1981 *Carcinogenic risk assessment: A guide to the literature*. Biometrics 37:353-366.
- Mason, J.C.**  
1976 Some features of coho salmon, *Oncorhynchus kisutch*, fry emerging from simulated redds and concurrent changes in photobehavior. *Fish. Bull., U.S.* 74:167-175.
- Mason, J.C., and D.W. Chapman**  
1965 Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. *J. Fish. Res. Board Can.* 22:173-189.
- Ostrander, G.K., M.L. Landolt, and R.M. Kocan**  
1988 The ontogeny of coho salmon (*Oncorhynchus kisutch*) behavior following embryonic exposure to benzo(a)pyrene. *Aquat. Toxicol. (Amst.)* 13:325-346.  
1989 Whole life history studies of coho salmon (*Oncorhynchus kisutch*) following embryonic exposure to benzo(a)pyrene. *Aquat. Toxicol. (Amst.)* 15(2):109-126.