TEMPERATURE PREFERENCE OF BAND-ED KILLIFISH, *FUNDULUS DIAPHANUS*, FROM SOUTHWESTERN PENNSYLVA-NIA.—Temperature is one of the most important single factors governing the distribution and behavior of aquatic poikilotherms (Stauffer et al., 1976; Gunter, 1957). Since the tissues of most fishes are in thermal equilibrium with the aquatic environment, fishes rely almost exclusively on behavior to regulate body temperatures. Hokanson (1977) noted that thermoregulatory behavior is a mechanism by which fish control their acclimatization temperature and optimize their physiological performance along a finite temperature gradient. Certain fishes can perceive temperature changes as small as 0.03 C (Bull, 1936), thereby avoiding areas of stress. Temperature acts as a directive factor by causing an organism to vary its behavior in the presence of a gradient (Stauffer et al., 1976; Fry, 1971). Gift (1977) noted that thermal gradients cannot be ignored when assessing the impact of power plant operation on aquatic organisms. Reynolds et al. (1977) showed that temperature was a more dominant factor than light intensity when California grunion (*Leuresthes tenuis*) were examined in gradients of these variables; however, Kutty and Sukumaran (1975) noted that rheotaxis overrode the influence of temperature in the swimming performance of *Tilapia mossambica*. Moss (1970) suggested that environmental factors (i.e., temperature) will cancel rheotaxis provided they are of sufficient magnitude. Numerous studies by Richards and Ibara (1978), Neumann et al. (1977), Cherry et al. (1976), Stauffer et al. (1976), Neill and Magnuson (1974) and Norris (1963) reported the manner in which fish respond to thermal gradients both in the field and laboratory.

Federal legislation mandates that preference tests be conducted to make effective decisions concerning the impact of power plant operation on populations of fishes in the vicinity of generating facilities (Dickson et al., 1976). Field and laboratory studies can predict which species are likely to be attracted or repelled by heated areas (Gift, 1977).

Much attention has been given to temperature preference of important game and commercial species, but temperature requirements of forage species have been largely ignored (Gift, 1977). Neill and Magnuson (1974), Fairbanks et al. (1971) and Rawson (1956) showed that predatory species spent significant amounts of time at water temperatures which were different than their preferred temperatures because of the presence of high concentrations of forage fish. Hatch (1973) showed that the movement of largemouth bass (*Micropterus salmoides*) in the vicinity of a heated discharge was governed by forage fish movements. The purpose of this paper is to report on temperature preference experiments conducted with banded killifish, an important prey for many game fish species (Scott and Crossman, 1973).

Materials and methods.—Banded killifish ranging in size from 39–79 mm SL (mean = 47.4; n = 48) were collected from High Point Lake using a 220-volt backpack shocker. High Point Lake is located in Somerset County, approximately 14.2 km west-southwest of Meyersdale, Pennsylvania. Specimens were placed in a 76 l plastic container partially filled with water from the collection site and transported to the laboratory within 1.5 hours of capture. Temperature in the transport container remained within 1 C of collection temperature (18 C).

A total of 12 to 15 fish was placed into 38 l glass aquaria. Aquaria were located in temperature control rooms to maintain desired temperatures and photoperiods. Water for all holding and experimental activities was supplied by the Frostburg City water supply. Prior to introduction into the apparatus and holding tanks, water passed through a filtration system which removed chlorine and iron. Illumination of the temperature control rooms was provided by Vita-lites (fluorescent lights emitting wavelengths equal to solar radiation on a clear day). Electric timers provided a L:D 12:12 photoperiod which was used in all experiments. Constant aeration was supplied by an electric air compressor.

Killifish were fed crushed trout chow between 1500 and 1600 daily except the day of testing. Fish were not fed on the day of the tests to insure consistent stomach contents. Aquaria were filtered constantly by air-filtration devices. Diatom filters were utilized to aid in maintaining water quality. Water in aquaria warmer than 18 C was changed weekly to prevent adverse effects caused by synergism of elevated temperatures and metabolic wastes. Dead or unhealthy fish were removed immediately upon observation.

TABLE 1. ACCLIMATION TEMPERATURES AND GRADI-
ENT RANGES IN THE TEST APPARATUS FOR TEMPERA-
TURE PREFERENCE TRIALS CONDUCTED WITH *Fundulus
diaphanus*.

Acclimation temperature (C)	Gradient range (C)
6	4.9–20.9
12	10.0–26.2
18	13.1–29.1
24	20.0–33.5
30	24.1–36.2
36	28.8–37.0

Fish were acclimated to 6, 12, 18, 24, 30 and 36 C at a rate of 1 C/day (Brett, 1944). Upon reaching the desired acclimation temperature, specimens were held five additional days at temperatures ±0.5 C of acclimation temperature.

The temperature preference apparatus was modeled after the one described by Meldrim and Gift (1971). Twenty-three thermistors were spaced evenly throughout the unit and monitored the temperature gradient. Water to the trough passed through a circulating temperature control bath which reduced the temperature 3–5 C below the acclimation temperature. These lower temperatures insured the presence of cooler water should the fish prefer temperatures lower than their acclimation temperature. Gradient ranges in the trough varied with acclimation temperature (Table 1). Overhead Vita-lites illuminated the test area. All tests were conducted during July and August, thereby eliminating seasonal effects.

Eight fish acclimated to each temperature were tested individually to eliminate problems due to social behavior. Each specimen was given a 40 minute habituation period to allow familiarization to test conditions. Tests were initiated upon completion of the habituation period. Fish were placed in the trough at the acclimation temperature. Movable doors shrouded the test area to minimize external stimuli. Upon completion of the orientation period, each fish was observed every minute for 20 minutes via overhead mirrors. The position of the fish with respect to the nearest thermistor was noted and the temperature recorded.

The relationship between acclimation and preferred temperatures was used to construct a model which best described the data. Raw data were used to calculate the r^2 terms, al-
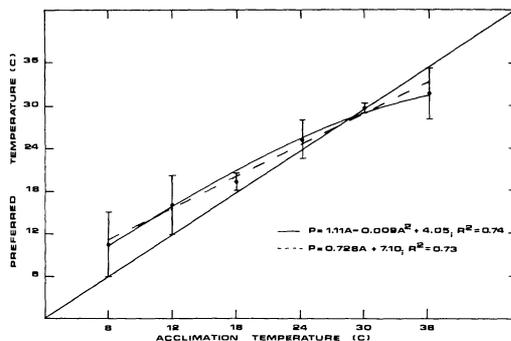

Fig. 1. Graphical representation of mean preferred temperatures at each acclimation temperature. The diagonal line represents the points where acclimation and preferred temperatures are equal. Linear (------) and quadratic (———) plots are presented. Mean preferred temperatures (●) and one standard deviation (I) at each acclimation temperature are plotted.

though only means and standard deviations were plotted (Fig. 1). Final temperature preferendum (FTP) was the point where the mathematical expression intersected a line having a slope of one (Fry, 1947). This point theoretically represented the highest temperature this species would prefer given an expanded temperature range for an indefinite time period.

Results and discussion.—A graphical representation of the mean preferred temperature is presented in Fig. 1. Stepwise regression analysis of 960 observations indicated that the quadratic equation $P = 1.11A - 0.009A^2 + 4.05$ ($r^2 = 0.739$) explained significantly ($P \leq 0.05$) more of the data than the linear expression, $P = 0.7284A + 7.1$ ($r^2 = 0.730$) where P = preferred temperature, and A = acclimation temperature. Coutant (*In*: Richards et al., 1977) felt that a linear expression of acclimation temperature's effect on preferred temperature is often inappropriate. However, the use of a linear model may be useful when comparing short sections of various response curves.

Fry (1947) reported that the final temperature preferendum can be considered a convenient, meaningful index of temperature as a directive factor, that is a factor eliciting a response due to the presence of a gradient. Laboratory tests, which minimize other stimuli (i.e., variation in current), have shown that temperature alone can dictate the distribution of fish; however, field studies have shown that many

species are often found in areas where temperatures are a few degrees below laboratory established FTP (Reynolds, 1977; Ferguson, 1958). This discrepancy in laboratory versus field preferenda may be explained by non-thermal stimuli such as bacterial infection (Reynolds et al., 1976), competition and niche segregation (Brett, 1970), light intensity (Sullivan and Fisher, 1954), social behavior (Pearson, 1952), current (Kutty and Sukumaran, 1975) and possibly other factors. However, Stauffer et al. (1976) showed that temperature was the most dominant parameter influencing the distribution of fishes in the vicinity of a power plant located on New River, Virginia.

Solving the quadratic equation for the point where P = A yielded a FTP of 28.6 C. The FTP for this population is considerably higher than that of a population from Nova Scotia which exhibited a FTP of 21.0 C (Garside and Morrison, 1977). Although different methods for determining these final preferenda were utilized, the results are not necessarily influenced by methlolology (Reynolds, 1977). The variation in preferenda between the two populations may be attributed either to physiological races adapted to geographic location or to differences in thermal history. Although McCauley (1958) and Hart (1952) found that ultimate incipient lethal temperature varied with subspecies, but not within physiological races, Hall et al. (1978) showed that the FTP of white perch (*Morone americana*) differed significantly according to geographic location with southern populations demonstrating a higher selected temperature than northern ones. Northern populations are expected to have lower selected temperatures because their thermal histories are dictated by lower thermal regimes. Our acclimation procedures probably eliminated the effects of short term thermal history; therefore, the higher FTP of the Pennsylvania population is probably indicative of a physiological race selected for by the influence of a warmer, more southern climate.

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PLASTICITY OF BURYING BEHAVIOR BY THE BANDED KILLIFISH, *FUNDULUS DIAPHANUS*.

—Earlier research (Colgan, 1974) described burying behavior in individuals of this species from Lake Opinicon, on the Rideau River system north of Kingston, Ontario, where the fish frequent shallows with fine gravel and sandy bottoms. Substrate preferences and the effects of group size on burying were also reported. Similarly, the burying behavior of Pacific gobies is influenced by the size of the group (Paulson, 1978). The present study focussed on population differences in burying and the behavioral plasticity underlying these differences.

Fish were collected from two populations. The first was the previously studied population in Lake Opinicon. The second was a small population in an approximately $160 \times 75 \times 4$ m deep quarry located about 40 km west of Kingston. The quarry had been abandoned 15 years earlier. No surface streams entered or exited the quarry. It contained, besides killifish, small numbers of bullheads (*Ictalurus* spp.) and several small cyprinoids, including exogenous goldfish (*Carassius auratus*). Inspection of the quarry by skin-diving revealed low densities of fish and a layer of light silt covering the rock bottom.

Killifish were seined from May to July 1978 and maintained, at 21 ± 2 C on a 12:12 L:D cycle, on a pasteurized liver-based food ("Gordon formula") in aerated aquaria ($31 \times 16 \times 21$ cm high) with bottoms covered with 3–4 cm of sand (particle size about 0.5 mm in cross section). The Opinicon fish averaged 53.5 ± 6.9 mm in total length, the Quarry fish 58.9 ± 9.7 mm. Pairs of fish from the same population were transferred to a similar aquarium and af-