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Thermoregulatory behavior and diel activity of yearling winter flounder, *Pseudopleuronectes americanus* (Walbaum)

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Synopsis

Sixteen yearling winter flounder, tested individually for 3-day periods in electronic shuttleboxes, voluntarily occupied an 8–27° C range of temperature, with a modal final thermal preferendum of 18.5° C (mean 18.7° C, median 19° C, midpoint 17.5° C, s.d. 1.9° C, S_k +0.33). The locomotor activity pattern of the yearling fish in the laboratory was markedly nocturnal, with mean hourly nocturnal activity exceeding mean hourly diurnal activity by a factor of 3.4. Maximum activity occurred at 0300 EST, minimum activity at 1400 EST. While activity generally increased with temperature, a local activity minimum occurred at 18.7° C, coinciding with the 24-hour mean final thermal preferendum. Comparison of these laboratory data with previously published field data suggest that behavioral responses to temperature and light play significant roles in determining age- and size-specific differences in seasonal depth stratification and onshore-offshore distributions in this species.

Introduction

The winter flounder, *Pseudopleuronectes americanus* (Walbaum), is an important commercial species zoogeographically distributed along the Atlantic coast of North America from Labrador to Georgia (Bigelow & Schroeder 1953). Because of its commercial importance, much attention has been paid to the environmental behavior and physiology of this species, in an attempt to better understand its distribution in space and time with respect to environmental factors. On the basis of field distributional analyses, it has been inferred (for literature review, see Klein-McPhee 1978) that temperature and light are among the most important abiotic factors influencing seasonal and shorter-term movements of winter flounder, and that there may be differences among the responses of different size classes that result in age segregation by depth. However, there have been very few of the critical laboratory experiments needed to substantiate the inferred age-specific behavioral responses to temperature and light. McCracken (1963) found that underyearling juveniles (60–90 mm TL) are photophilic, while yearling juveniles (120–180 mm TL) flounder are photophobic, and mature adults (280–330 mm TL) are indifferent to light intensity. There are no published data on laboratory thermal preference or avoidance behavior of this species (Richards et al. 1977), nor on its diel activity patterns. There are data indicating that the upper thermal tolerance limit (near 29° C) is greater

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in immature than in mature individuals (Klein-McPhee 1978). We have sought to fill important remaining gaps in the knowledge of environmental biology of winter flounder, by measuring the thermoregulatory behavior (preferred and avoided temperatures), diel patterns of locomotor activity and preferred temperature, and the relationship of locomotor activity to temperature in yearling (120–130 mm TL, age 1+) winter flounder, which we here report for the first time.

Materials and methods

Sixteen yearling *P. americanus*, 120–130 mm TL, were captured by otter trawl in Saco Bay, Maine, during September and October, 1979. These were held in the laboratory at 15–17°C for at least 2 weeks prior to testing, in natural sea water of 25–30‰ salinity (corresponding to salinities measured in the bay). The fish were fed various small, live crustaceans and worms ad libitum. Diffuse, indirect natural daylight was provided through windows, with no artificial lighting.

The fish were tested individually, for 3-day periods, in two-chambered versions of Ichthyotron-type electronic shuttleboxes described by Reynolds (1977). These shuttleboxes allow the fish to control water temperatures by means of normal, unconditioned swimming movements, which are monitored by paired light beams and photocells as the fish move along the bottom between chambers. Locomotor activity is quantified as the number of light-beam interruptions per hour, and recorded automatically along with water temperatures. Thus, during the tests the fish are not disturbed by human interference or observation. Pooled data for all the fish were used to construct a relative frequency distribution (Fig. 1) of voluntarily occupied (self-controlled) or preferred temperatures. Mean hourly water temperatures and spontaneous locomotor activity levels were also plotted against time of day to determine the presence of any diel rhythms (Fig. 2), and mean hourly activities were plotted against mean hourly temperatures to determine the interrelationship of activity and temperature (Fig. 3).

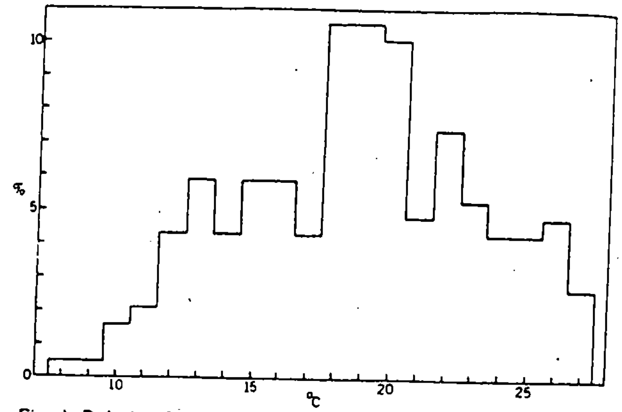

Fig. 1. Relative frequency distribution of temperatures selected by 16 yearling (age 1+, 120–130 mm TL) winter flounder in electronic shuttleboxes. The fish were tested individually for 3 days each.

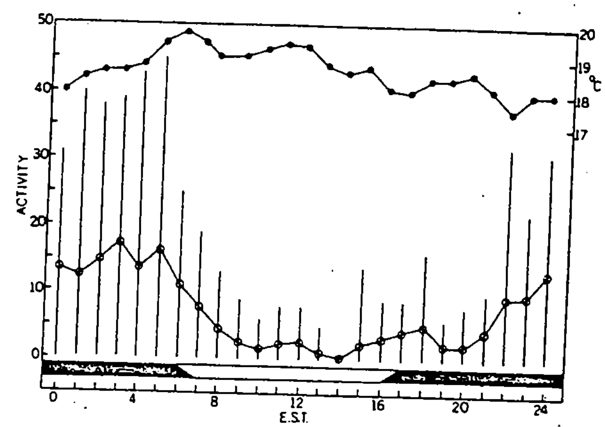

Fig. 2. Diel patterns of locomotor activity (○) and of preferred temperature (●). Hourly means for 16 yearling winter flounder are plotted against time (hours EST, Eastern Standard Time). Vertical lines are ranges of activity quantified as photocell-monitored light-beam interruptions per hour. Horizontal bar shows natural photoperiod (diffuse window light); shaded portion is night or scotophase, unshaded portion is day or photophase, with tapered crepuscular transitions at dawn and dusk.

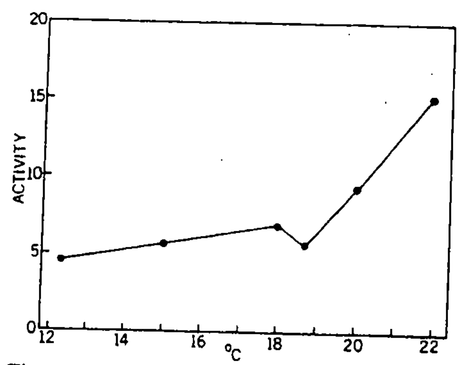

Fig. 3. The relationship of locomotor activity to temperature for 16 yearling winter flounder tested individually for 3 days each in electronic shuttleboxes.

Results

The range of voluntarily occupied temperatures spanned 8–27° C, with a modal final preferendum of 18.5° C (Fig. 1). The 24-hour mean preferendum was 18.7° C ± 1.9 S.D.; the median was 19° C, and the midpoint of the range 17.5° C. Failure of the various measures of central tendency to coincide resulted from skewness ($S_k = +0.33$). Mean diurnal (19.0° C) and nocturnal (18.5° C) temperatures differed little; the highest hourly preferred temperature occurred just before dawn (19.8° C at 0600 E.S.T.), while the lowest (17.5° C) occurred at 2200 hours (Fig. 2).

Locomotor activity was markedly nocturnal (Fig. 2), with mean hourly nocturnal activity (9.7 units · $hour^{-1}$) exceeding mean diurnal activity (2.8 · h^{-1}) by a factor of 3.4. Minimum activity (0.1 units · h^{-1}, range 0–1) occurred at 1400 E.S.T., while the maximum ($\bar{x}$ = 17.3 units · h^{-1}) occurred at 0300 E.S.T. The maximum range of activity (0–45 units) occurred before dawn, at 0500 E.S.T. Variability in activity was greatest at night (Fig. 2). While activity generally increased with temperature (Fig. 3), a local minimum occurred at 18.7° C, coinciding with the 24-hour mean final thermal preferendum.

Discussion

Our data indicate that winter flounder thermoregulate somewhat less precisely than do many other fish species (Reynolds & Casterlin 1979, 1980). Temperatures preferred by yearling winter flounder correspond to those typically experienced in shallow nursery areas frequented by immature fish during the summer (Klein-MacPhee 1978). The upper avoidance temperature of 27° C evident in our data (Fig. 1) is consistent with the temperature at which Radle (1971) observed cessation of feeding, and is near the ultimate upper incipient lethal temperature reported by Hoff & Westman (1966). The lower avoidance temperature of 8° C (Fig. 1) is consistent with the lower incipient lethal temperature of fish acclimated to the final preferendum of 18–19 C, although the species readily tolerates near-freezing temperatures at lower acclimation temperatures.

The nocturnal activity of the immature flounder in our tests is consistent with McCracken's (1963) report of photophobic behavior in fish of this size and age class, although younger and older fish may behave differently, leading to intraspecific size and age segregation. The relationship of activity to temperature (Fig. 3) exhibits a pattern which has been observed in a number of other species: a local activity minimum in the final preferendum region which appears as an anomaly in an overall pattern of increasing activity with increasing temperature (Reynolds & Casterlin 1980). Activity increases threefold ($Q_{10} = 3$) over the range 12–22° C, but changes little between 16–19° C, resulting in increased homeostasis in the preferred temperature range. A thermokinetic mechanism of temperature preference behavior might also be inferred from this relationship.

Based upon our own experimental data presented herein, and other data summarized by Klein-MacPhee (1978) from the work of other investigators and field observations, we have reached several tentative conclusions regarding the behavioral ecology of *Pseudopleuronectes americanus*. The species spawns inshore at cold winter temperatures, below 6° C. Embryonic and larval development similarly occur at cold temperatures, below 10° C, in late winter and early spring. The larvae metamorphose into juveniles during the spring, as inshore waters begin to warm up, and the juveniles enter the shallow estuarine and cove nursery areas which during the summer may reach temperatures exceeding 20° C or even 25° C. Between 20 and 29° C, sublethal effects such as inhibition of feeding occur, and avoidance responses are initiated at or below 27° C. If the young fish do not succeed in escaping to deeper, cooler water (or by burying themselves in cooler soft bottom sediments), they will succumb to heat death at about 29° C. Barring such excessive inshore temperatures, the young-of-the-year (YOY or 0+) fish remain in the shallow nursery areas throughout the summer and early autumn, then move out of the shallows into deeper waters in late autumn, as the shallows become cooler (below 8°) than the deeper water. In contrast, spawning adults move into the cold shallows in winter for reproduction.

The following spring, yearling (1+) fish again move into the shallower water as inshore temperatures rise, but because they are more photophobic, they tend to remain in deeper water than the YOY fish, or perhaps move into the shallowest waters only at night. Thus, during the summer the YOY fish will remain in the shallowest water because they are photopositive and not aversive to relatively warm temperatures, while yearling fish will remain in somewhat deeper water at least during daylight because of the photophobic response, and mature fish will tend to avoid the shallower waters because of a slightly lower preferred temperature range that has been inferred to be about 12–15°C for these older fish on the basis of their field distributions (Klein-McPhee 1978). Thus, flounder of different ages and sizes would tend to have different depth distributions because of their different responses to temperature and light, resulting in intraspecific niche differentiation which may tend to minimize intraspecific competition. Temporal niche partitioning may also occur on the basis of different diel activity patterns of the different size and age classes. The yearling flounder in our tests were nocturnally active, in contrast to the diurnal activity reported for older fish (Olla et al. 1969). A similar ontogenetic change in preferred temperature and diel activity has been reported for a freshwater fish, the yellow bullhead *Ictalurus natalis* (Reynolds & Casterlin 1978).

References cited

Bigelow, H.B. & W.C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Government Fishery Bulletin No. 74. 577 pp.

Hoff, J.G. & J.R. Westman. 1966. The temperature tolerances of three species of marine fishes. J. Marine Res. 24: 131–140.

Klein-MacPhee, G. 1978. Synopsis of biological data for the winter flounder, *Pseudopleuronectes americanus* (Walbaum). NOAA Techn. Rept. NMFS Circular No. 414; FAO Fisheries Synopsis No. 117. 43 pp.

McCracken, F.D. 1963. Seasonal movements of the winter flounder, *Pseudopleuronectes americanus* (Walbaum), on the Atlantic coast. J. Fish. Res. Board Can. 34: 749–752.

Olla, L.B., R. Wicklund & S. Wilk. 1969. Behavior of winter flounder in a natural habitat. Trans. Amer. Fish. Soc. 98: 717–720.

Radle, E.W. 1971. A partial life history of the winter flounder (*Pseudopleuronectes americanus*) exposed to thermal addition in an estuary, Indian River Bay, Delaware. M.S. Thesis, Univ. Delaware, Lewes. 74 pp.

Reynolds, W.W. 1977. Fish orientation behavior: an electronic device for studying simultaneous responses to two variables. J. Fish. Res. Board Can. 34: 300–304.

Reynolds, W.W. & M.E. Casterlin. 1978. Ontogenetic change in preferred temperature and diel activity of the yellow bullhead, *Ictalurus natalis*. Comp. Biochem. Physiol. 59A: 409–411.

Reynolds, W.W. & M.E. Casterlin. 1979. Behavioral thermoregulation and the 'final preferendum' paradigm. Amer. Zool. 19: 211–224.

Reynolds, W.W. & M.E. Casterlin. 1980. The role of temperature in the environmental physiology of fishes. pp. 497–518. *In*: M.A. Ali (ed.) Environmental Physiology of Fishes. Plenum Press, New York.

Richards, F.P., W.W. Reynolds, R.W. McCauley, L.I. Crawshaw, C.C. Coutant & J.J. Gift. 1977. Temperature preference studies in environmental impact assessments: an overview with procedural recommendations. J. Fish. Res. Board Can. 34: 728–761.