

Abstract.—Continuous observations in a large research aquarium (121 kL) over a 14-week period were combined with field collection in the Navesink River estuary, New Jersey, to explore the behavior of winter flounder (*Pseudopleuronectes americanus*) during and after the spawning season. Ten males and ten females held in the aquarium spawned over a 60-day period, with an average of 40 spawns per female and 147 spawns per male. Males initiated all observed spawning events, which occurred throughout the night, but primarily between sunset and midnight. Spawning by one pair frequently elicited sudden convergence and spawning by secondary males (up to six individuals); consequently, strictly paired spawning was uncommon (22% of events). Males and females were almost entirely nocturnal during the reproductive season but became increasingly diurnal during the postspawning season.

Males and females arrived in the estuary in ripe spawning condition; fish with high gonadosomatic indices were collected during most of February and March 1997. All of the ripe females were >20 cm in total length and at least two years old, whereas ripe males measuring 10–15 cm were common. Ripe males were found throughout the system whereas ripe females were concentrated in the middle reach of the estuary. Field collections revealed that females began feeding, primarily on ampeliscid amphipods and on siphons of the clam *Mya arenaria* earlier in the season than males. Males in the laboratory also began feeding late in the season, after most spawning had ended. Field and laboratory results combine to indicate that male spawning strategy is adapted to maximize numbers of eggs fertilized. There is probably high genetic diversity in the offspring from any one female owing to frequent spawning and to multiple males participating in individual spawning events.

Behavior of winter flounder, *Pseudopleuronectes americanus*, during the reproductive season: laboratory and field observations on spawning, feeding, and locomotion

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Winter flounder, *Pseudopleuronectes americanus*, is an important commercial and recreational fishery species in estuarine and continental shelf habitats along the Atlantic coast of North America from Labrador to Georgia (Scott and Scott, 1988). A large number of investigations have been devoted to this species and its life history is relatively well known (Klein-MacPhee, 1978; Able and Fahay, 1998). Winter flounder populations include several substocks that mix during summer residence on the shelf, but adults return to natal estuaries to spawn (Saila, 1961; Poole, 1966; Howe and Coates, 1975; Pierce and Howe, 1977; Phelan, 1992). In the Mid-Atlantic Bight, adults make seasonal migrations into the estuaries of New York and New Jersey (Perlmutter, 1947; Phelan, 1992) where they spawn in shallow inshore waters during late winter (Scarlett and Allen, 1992).

Winter flounder in the mid-Atlantic region are believed to mature at 2–3 years, at 20–25 cm total length (Perlmutter, 1947, Danila, 1978; Witherell and Burnett, 1993). Con-

siderable attention has been given to seasonal movement (Perlmutter, 1947; Saila, 1961; Howe and Coates, 1975; Phelan, 1992) and to fecundity and reproductive state in wild populations (Kennedy and Steele, 1971; Danila, 1978; Burton and Idler, 1984; Nelson et al., 1991); however, information on spawning behavior is limited to observations made by Breder (1922) of fish in small laboratory tanks.

In this investigation we expanded Breder's (1922) description of spawning and courtship in winter flounder by making continuous observations on a population of winter flounder in a large research aquarium for a 14-week period during and after the reproductive period. We determined the total number of spawning events for males and females in the population and examined seasonal and diurnal variation in spawning, feeding, and locomotory activity. On the basis of field collection we described population structure, gonadal development, feeding, and localized movements by a wild population of winter flounder in an

estuarine spawning ground during winter and spring 1997.

Methods

Laboratory observations

Experimental animals Winter flounder were collected in nearshore waters between Rockaway Point and Coney Island, New York, on 27 January 1997 with a 9.2-m otter trawl fished from RV *Gloria Michelle*. Flounder were held in live cars with flow-through seawater and transported in large ice chests to the laboratory. Mature ripe males were identified by the extrusion of sperm under light pressure applied to the body. Females were identified by the presence of full ovaries distending the body, occupying a large portion of the body cavity, and extending almost to the caudal peduncle. Ten males and ten females, each greater than 200 mm in TL (total length), were marked with Peterson tags (13 mm diameter) before release into the research aquarium. To provide visual identification of gender on videotape, males and females were marked with orange and white tags, respectively. These colors were distinguishable with low-light video cameras during both day and night. At the beginning of the experiment the mean total length of male flounders was 29.8 cm (SD=3.2, range=24.3–34.4 cm). Mean length of females was 28.5 cm (SD=2.6, range=23.5–30.8 cm). Four fish (two males and two females) died near the end of the 14-week observational period; these were not replaced. The fish were remeasured for total length at the end of the experiment to estimate growth.

Research aquarium Observations of the winter flounder were made in the research aquarium at the James J. Howard Marine Sciences Laboratory at Sandy Hook, New Jersey. The aquarium (121 kL volume) is an oval 10.6 m long, 4.5 m wide, and 3 m deep, with eight rectangular windows (0.7 m wide and 1.2 m high), one in each end and three along each side. There is a recirculating system so that 10% of the water is replaced each week. Seawater upwells through coarse sand which covers the bottom of the aquarium (46 m²) to a depth of 40 cm, then exits through drains at the top of the tank.

For this experiment, photoperiod in the aquarium room, which was controlled by a computer-driven bank of fluorescent lamps (Duro-Test, Vita-Lite: model CRI91; 5500°K), was programmed to follow the natural sequence at the location of the laboratory (40°28'N, 74°00'W), with a 13.5-h dark period

at the end of January, when fish were first introduced into the system, and a 10-h dark period at the end of the study in mid-May. During nighttime hours, light intensity on the sediment surface was 0.17 lux (1.98×10^{-6} mEinsteins PAR [photosynthetic active radiation]). Light intensity increased at the time of twilight to 2.0 lux (2.92×10^{-5} mEinsteins PAR) for 20 min, followed by a natural rise in intensity to 206 lux (0.0027 mEinsteins PAR) at midday. Return to nighttime conditions followed an exact reverse pattern.

Water temperature was held at 4.0°C ($\pm 0.5^\circ\text{C}$) from the beginning of the observations until 27 March, when a cooling system malfunction allowed temperature to rise to 7.5°C. To follow conditions in the Navesink River, we held water temperature at 7.5°C until 10 April, then raised temperature $\sim 0.5^\circ\text{C}$ per day to 15°C on 14 April. This temperature was maintained until the end of observations on 13 May 1997.

Finely chopped pieces of frozen surf clam (*Spisula solidissima*) were made continuously available as food for the flounders. The food was renewed at least once per week during the early part of the period when feeding was light, and increased with ingestion rate to near-daily additions by 1 April 1997. The food was placed on the bottom in an area approximately 2 m in diameter within camera view so that all feeding bouts could be counted.

Recording observations Two low-light-sensitive video-tape cameras were set at the end windows of the aquarium to make continuous recordings of courtship and spawning events over a large portion of the tank from bottom to water surface. Each of these cameras viewed 53% (24.6 m²) of the tank bottom with minimal overlap in coverage. Recordings were made from about 1530 h through 0500 h between 29 January and 18 April 1997, after preliminary taping and routine visual observation indicated that virtually all activity was nocturnal. The validity of this approach was confirmed by cameras at the side windows set for 24-h recording throughout the study period. Twenty-four-hour recording from the end cameras was initiated 19 April 1997, concurrent with increasing daytime activity of the fish.

Spawning events were divided into two classes. An event was considered "definite" when characteristic behavior patterns were clearly observed in the recorded image. However, because most spawning occurred in darkness, some spawning events were not observed clearly. These likely but less certain spawning events were scored "probable." Two forms of courtship were recorded; these are described in the "Results" section. Records were kept of the numbers of males and females involved in each courtship and spawning encounter. A feeding event was scored once,

regardless of feeding duration, when a fish entered the food distribution area and made obvious feeding motions toward the clam pieces. Analysis of video tape from the two cameras for four dates during midspawning season revealed that there were no significant differences in daily counts of spawning, courtship, and feeding as recorded by the two cameras (paired-sample *t* tests, $P > 0.30$); therefore, the second camera was used only as a backup for recorder failure (e.g. on four dates).

Two other low-light cameras were set at side windows to provide data on locomotory activity and swimming speeds. Each viewed a 3.6-m wide section of the opposite tank wall and about 4.6 m² of bottom, and recordings were made for 5 min at the beginning of each hour. An index of locomotory activity was provided by the number of fish passing a central line in view of the camera. Swimming speeds (cm/sec) were determined by timing the passage of fish between two points 142 cm apart on the tank wall. Whenever possible, the gender of fish passing through this view was noted. The two cameras provided essentially identical information, and recordings from only one were analyzed for each day.

Observations were also made on the relative abundance of winter flounder larvae in the aquarium. Flounder larvae were almost constantly present in the aquarium after mid-February, and an index of their abundance was recorded each day. Because the larvae demonstrated diurnal vertical migration, observations were made at approximately 1200 h and general abundance was scored from zero to four. A score of one was recorded when only a few larvae were observed in any field of view and a score of four was given when thousands of larvae were present at all of the tank windows.

Field collections

To monitor the location, reproductive condition, and sex ratio of winter flounder in a wild population, weekly daytime otter-trawl surveys were conducted from 7 February to 24 April 1997 in the Navesink River estuary, New Jersey (Fig. 1), which is a known spawning area for the species (Scarlett¹). Eight fixed stations were established approximately 1 km apart

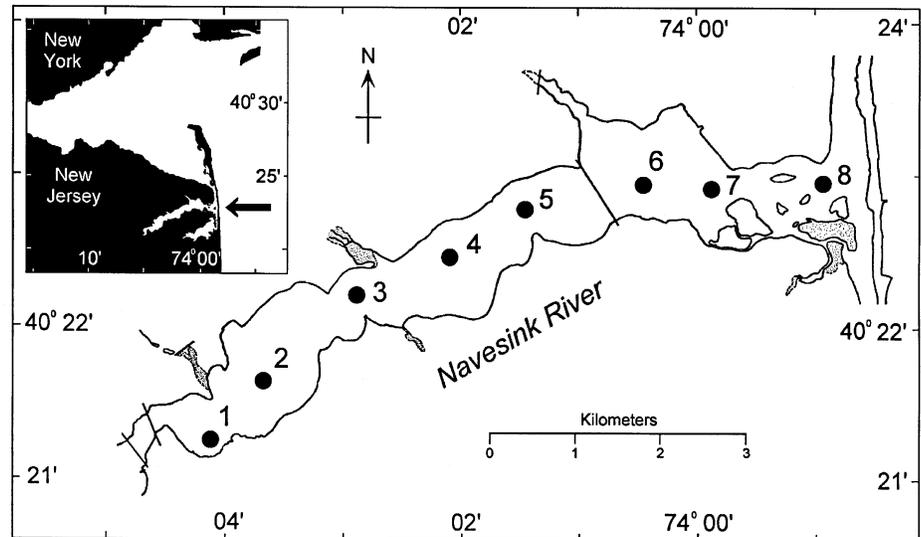


Figure 1

The Navesink River estuary, New Jersey, showing the stations where collections were made for winter flounder during winter and spring 1997.

throughout the system. Two 5-min tows (at 4.4 ± 0.9 km/h) were made at each station with a 5-m semi-balloon otter trawl with 3.5-cm wings and body and a 6-mm codend liner. Abundance of winter flounder was reported in catch per unit of effort standardized as number of individuals per 100 m of tow distance, which was estimated with a global positioning system (GPS).

Winter flounder collected in the trawl surveys were measured to the nearest millimeter of total length, blotted dry, and weighed to the nearest gram. Gender was determined by inspecting the gonads, and each fish was classified as immature, ripe, or spent. The gonads were weighed to the nearest gram, and gonadosomatic indices (GSI) were calculated according to LeCren (1951) as

$$GSI = 100 (\text{gonad weight} / \text{total body weight}).$$

The stomach of each winter flounder was removed, preserved in 10% formalin solution, and later transferred to 70% ethanol. Diet was analyzed as in Stehlik (1993), and fullness of the foregut was estimated visually on a scale from 0% to 100%. Prey items were identified to the lowest possible taxon and the proportion of the total volume of stomach contents con-

¹ Scarlett, P. G. 1991. Temporal and spatial distribution of winter flounder (*Pseudopleuronectes americanus*) spawning in the Navesink and Shrewsbury Rivers, New Jersey. Dep. of Environmental Protection, Div. Fish, Game and Wildlife, Bureau of Marine Fisheries, Nacote Creek Marine Fisheries Laboratory, Star Route, Absecon, New Jersey, 12 p. Unpubl. report.

tributed by each prey type was estimated visually (Williams, 1981). Fullness was averaged for fish grouped by date, gender, and stage of reproductive development. Fish examined were 63–460 mm TL.

Bottom water temperature, salinity, and dissolved oxygen were measured weekly at each station with YSI (Yellow Springs Instruments) oxygen and temperature-conductivity meters. HydroLab DataSonde II instruments were deployed near the bottom on piers extending from the south shore of the Navesink River near stations 1 and 6 to record temperature, salinity, and dissolved oxygen each hour.

Results

Laboratory observations

Behavior Two forms of simple courtship behavior were observed in the research aquarium: “following” and “avoidance.” “Following” was defined as oriented locomotion of one or more fish behind another, typically on the sediment surface. A following could be relatively slow or fast (approx. 20–50 cm/sec), continued for 5–20 sec, and sometimes resembled chasing. Turns by the lead fish were followed by the fish behind, and, in most cases, the distance between fish decreased but contact was never made. In all cases where genders of the fish could be determined the lead was female. One or more “followers” were always male. “Avoidance” was characterized by brief contact (<1 s) between a “follower” and the female being followed, then by rapid acceleration and evasive turning by the female. Typically, the female swam 1–2 m off the bottom. In at least 50% of such encounters additional males converged rapidly on the female, eliciting strenuous evasive swimming by the female.

Our observations of spawning in winter flounder were relatively similar to those of Breder (1922), except that females never initiated spawning. Spawning occurred after initial contact by a male as described above. Instead of fleeing, the female remained near the sediment surface (<1.0 m above it) and the pair immediately made several very rapid circles with diameters typically less than two body lengths. Spawning events could be detected even in the far reaches of the tank during darkness because the female nearly always rotated momentarily to the vertical position so that the white underside of her body was clearly visible on the outside of the circles. Participation by multiple males in the spawning event was common, and in approximately 10% of spawning events, one to several males converged on the spawning location immediately after the female had

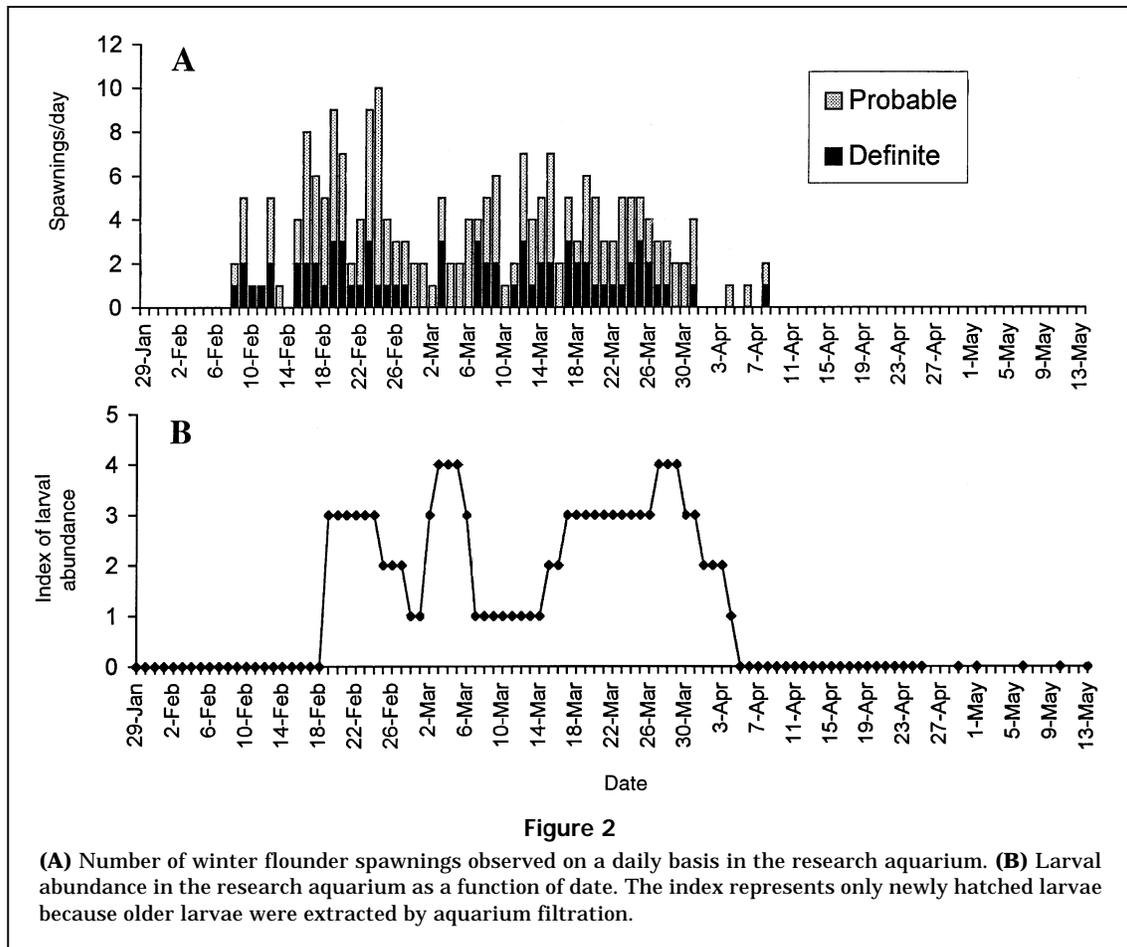
departed, making rapid undulating motions on the sediment surface. Despite convergence of males on spawning females and spawning locations, agonistic behavior among males was never observed. In less than 2% of observed spawning events, a second spawning by the same female occurred a short distance from the first encounter. In most cases the female avoided further contact.

Spawning Spawning began on 8 February 1997, about 2 weeks after the fish were introduced into the research aquarium. One or more spawnings occurred in camera view every day, except 14 February, until 1 April (Fig. 2A); maxima were evident in mid-February and mid-March. The last spawnings were observed on 8 April. In total, 211 spawning events were recorded, 34% of which were definite. In analyses that follow, we considered the “spawning season” in the aquarium to be the period between 8 February and 8 April, 1997. The “postspawning season” was the remainder of the observational period, 9 April through 13 May 1997.

Spawning began near or just before sunset (1700–1800 h) and reached maximum frequency around 2100 h (Fig. 3A). Seventy-five percent of all observed events occurred before midnight. Although some spawning occurred as late as 0430 h, only 10% occurred after 0200 h, when the fish became relatively inactive.

When the total number of spawning events during the observation period was extrapolated to the total dimensions of the aquarium and divided by the ten females in the system, we estimated that the average female flounder spawned 40 times during the reproductive season. It is unknown whether individual fish spawned over this extended observation period. However, given that the mean total number of spawns per night was just six, individuals probably spawned over a period of at least one week. Two facts suggest that a subset of the females may have been completely spent during the first three weeks of the spawning period: the nightly spawning frequency was bimodal, with a minimum on 2 March (Fig. 2A), and female feeding increased rapidly after approximately 3 March (Fig. 4).

None of the observed spawning events involved more than one female flounder, but multiple males were engaged in the majority of spawnings. In 151 events with records for the number of males, only 22.5% were pair spawnings, 11.3% had two males, 3.3% had three males, and the majority involved four to six males (62.9%). Given these numbers, a conservative estimate for the total number of male spawnings within camera view is 780. By extrapolating to total tank dimensions and dividing among the ten males



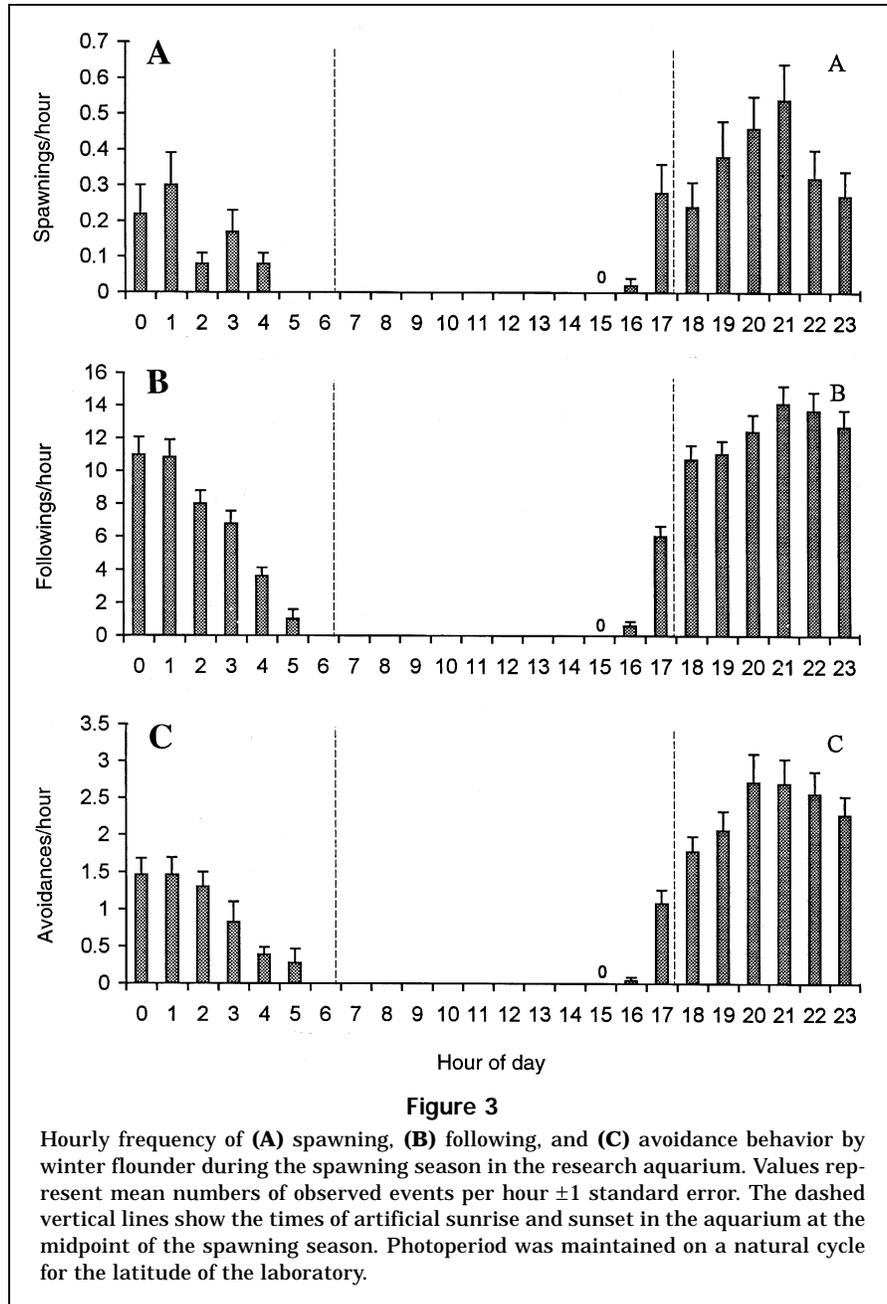
in the system, we estimated that each male (on average) spawned approximately 147 times during the season.

Larval abundance The first winter flounder larvae were observed in the aquarium on 19 February, 11 days after the first spawning was observed. A week of low larval abundance occurred in March, but larvae were constantly present until 5 April (Fig. 2B). Because there was no appreciable food for fish larvae in the aquarium, most of the larvae observed were at yolksac and early stages.

Courtship behavior The spawning season was characterized by a high frequency of following behavior (Fig. 5A). Only a small amount of following was observed in the days before and after the spawning season, but 50–200 following events were observed each night between 8 February and 8 April, decreasing steadily after mid-March. This pattern reflected spawning frequency (Fig. 2A), and there was a weak but positive correlation ($r=0.531$, $P<0.001$) between the two forms of behavior.

Avoidance events were approximately one order of magnitude less frequent than following events (Fig. 5), but the seasonal patterns were similar, and there was a positive correlation between these behaviors ($r=0.786$, $P<0.001$). The correlation between avoidance and spawning was also positive ($r=0.441$, $P<0.001$). Most courtship behavior occurred after sunset between 1700 and 0400 h, with a broad mode around 2100 h (Fig. 3, B and C). Very few interactions occurred in daylight.

Feeding and growth The seasonal pattern of feeding was entirely different from the patterns of spawning, following, and avoidance behaviors. First feeding occurred on 10 February, then increased slowly over time—some apparent oscillations, however, were embedded in the overall trend (Fig. 4). For example, an approximately 10-day periodicity occurred between 12 February and 20 March; this was independent of the dates when fresh food was added. All feeders that could be identified prior to 18 March were female. Females were responsible for 90% of all feeding events recorded ($n=3673$), and this percentage

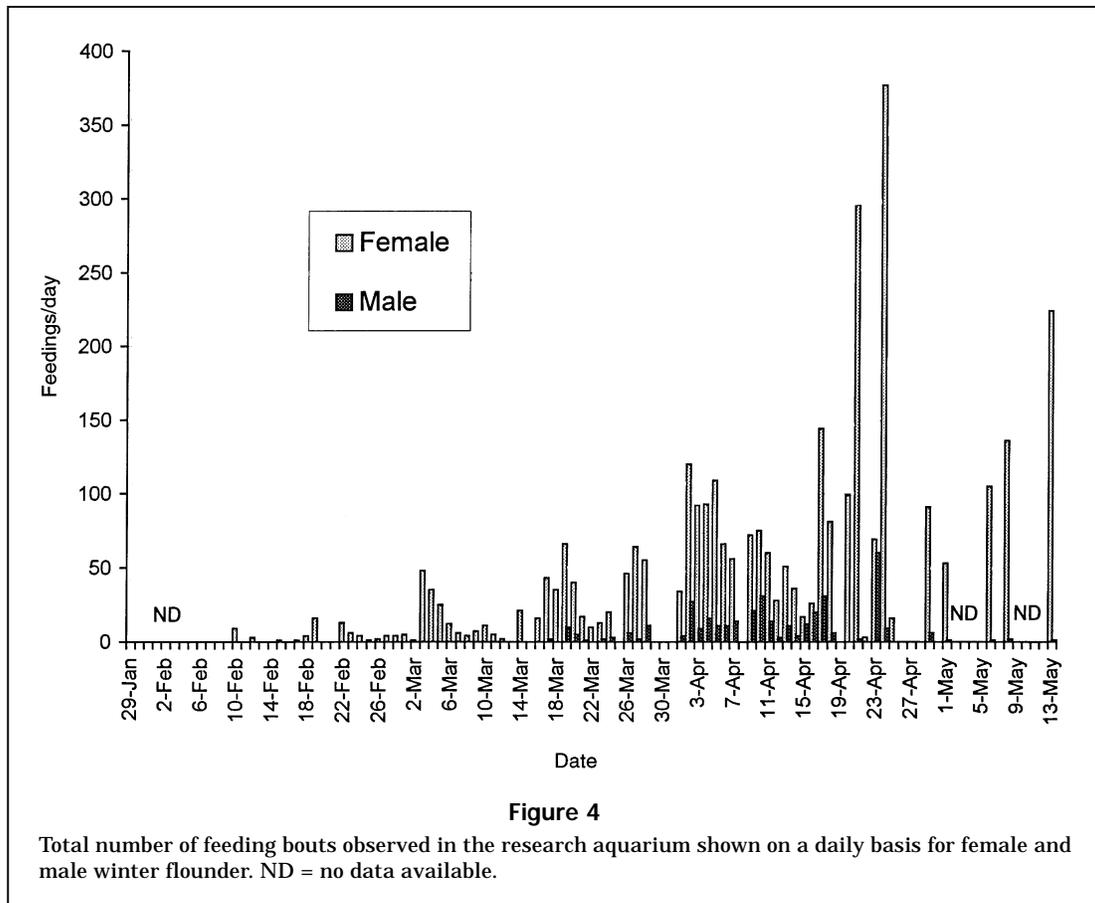


did not differ appreciably between spawning (90.3%) and postspawning (89.8%) periods.

It is possible that feeding frequency was related to water temperature. During the time when water temperature was 4°C (until 25 March), average feeding frequency was 14 bouts/day (SD=17, $n=45$). During the period when temperature was 9°C (26 March–8 April), feeding frequency increased to 94 bouts/day (SD=47, $n=11$), and when the temperature was 15°C (14 April–13 May) there were 159 bouts/day (SD=107, $n=17$).

Diurnal feeding periodicity was similar in males and females. During spawning season, most feeding

occurred in the hours immediately following sunset and ended before sunrise (Fig. 6, A and C). After spawning ended, feeding (particularly by females) was dispersed throughout day and night, and maximum feeding occurred from afternoon to early evening, primarily 1300–1900 h (Fig. 6B). The least feeding activity occurred from approximately midnight until 1100 h. Feeding by males during the postspawning season continued to be much less frequent than feeding by females; however, the diurnal pattern shifted similarly to an earlier period of maximum activity (Fig. 6D).



Most female winter flounder demonstrated growth in total length (mean=1.0 cm, SD=1.0) during the three-month experimental period, despite the cold temperature and high spawning activity. Change in length of males (mean=0.1 cm, SD=0.6) was less but the gender difference was not significant (Student's $t=-1.91$, $df=7$, $P<0.098$) because of large individual variation.

Locomotory pattern General activity in winter flounder during the spawning season began near sunset and remained high throughout the night until sunrise (Fig. 7, A and C). After spawning ended, approximately equal levels of activity were observed during day and night, with highest levels during afternoon and evening hours. Diurnal patterns of locomotion were somewhat different in males and females. During the spawning season, male fish began moving somewhat earlier in the evening than females, and the general level of activity was higher. Postspawning, female fish shifted more strongly to an afternoon activity pattern whereas males continued a high level of activity into the night and early morning hours (Fig. 7).

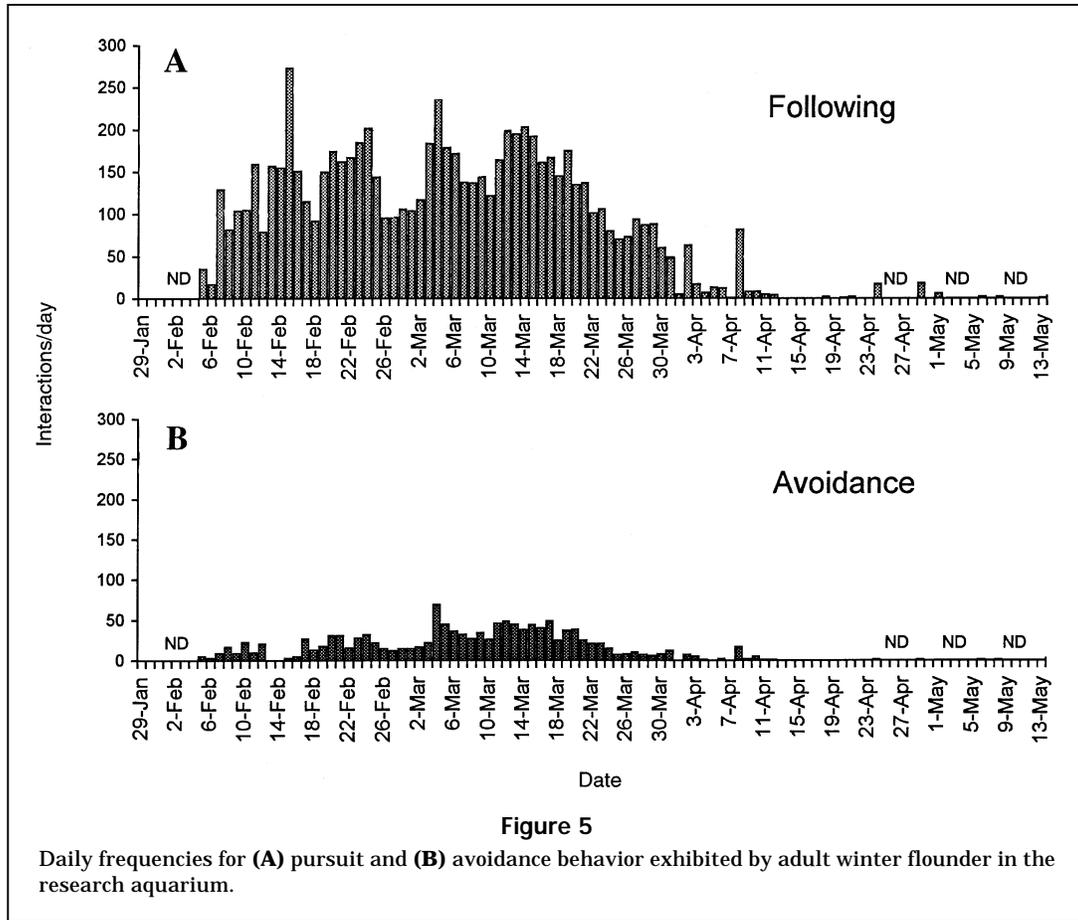
Swimming speeds of winter flounder were generally near 20–25 cm/sec, and there was no apparent

effect of date or time of day during active hours. However, male fish were significantly faster (overall mean=26.1 cm/sec, SD=6.6) than females (mean=21.2 cm/sec, SD=4.6; Student's $t=14.61$, $P<0.001$).

Field observations

Physical conditions Temperature in the Navesink River estuary ranged from 2.2°C in mid-February to 11°C at the end of the field study in late April, and there was relatively little temperature variation among the eight field stations (Fig. 8A). Salinity near station 6 in the eastern part of the study area ranged from 15 to 23 ppt over the study period. At station 1 in the western part of the study area, salinity was 10–21 ppt, except for a brief period of low salinity (7–10 ppt) on 1 April 1997. Therefore, the entire sampling area was polyhaline, and there was a slightly wider range of salinity in the west than in the east. Dissolved oxygen was always high (>8 mg/L) at the two sites where measurements were made continuously.

Population structure, reproduction, and migration Numbers of winter flounder in the Navesink River



estuary increased steadily between mid-February and early March, concurrent with the rise in water temperature (Fig. 8). Abundance remained relatively stable for the remainder of the collection period. Of the 389 winter flounder collected, 49% were female (mean length=28.9 cm, SD=8.9) and 32.5% were male (mean=25.1 cm, SD=6.9). Gender could not be determined for 18.5%, all of which were small individuals considered to be age-1 fish (mean=10.2 cm, SD=2.3). Females were significantly more abundant than males (Table 1; $F=4.62$, $P<0.05$), with a sex ratio of 1.5 to 1, and this pattern was consistent throughout the sampling period; analysis of variance (ANOVA) showed a nonsignificant *Date* × *Gender* interaction ($P=0.58$; Table 1).

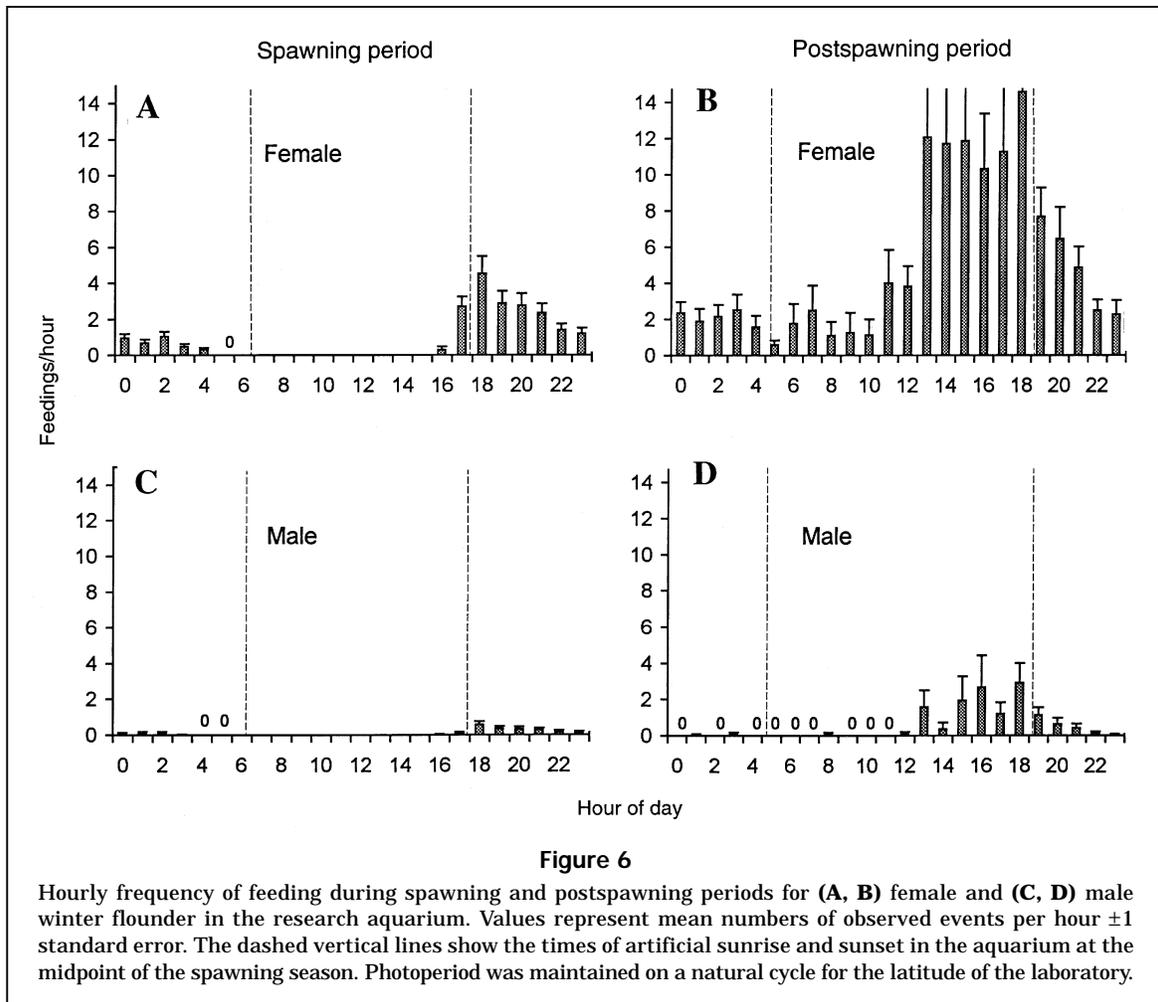
The majority of ripe female flounder were collected in the first half of the survey period (Fig. 9A); all of these fish were larger than 20 cm TL. Ripe females were divided into two obvious groups based on gonadosomatic index (GSI). Sixty-five percent of ripe females had GSIs greater than 20% (mean=30.9, SD=9.1). A second group of nearly spent females had GSIs of 3–8%. All female winter flounder collected after mid-March had GSIs less than 6%.

Table 1

Results of analysis of variance on catch data for winter flounder in the Navesink River during winter 1997. ANOVA was performed on natural log-transformed data on catch per unit effort. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = $P > 0.05$.

| Source of variation | df | Sum of squares | F ratio |
|-------------------------|-----|----------------|----------|
| Date | 9 | 0.002 | 3.27 ** |
| Station | 7 | 0.004 | 7.25 *** |
| Gender | 1 | 0.0003 | 4.62 * |
| Date × Station | 63 | 0.009 | 2.10 *** |
| Date × Gender | 9 | 0.0005 | 0.84 ns |
| Station × Gender | 7 | 0.004 | 7.22 *** |
| Date × Station × Gender | 63 | 0.003 | 0.59 ns |
| Error | 160 | 0.011 | |

Male winter flounder formed two size groups, small fish (<20 cm TL) assumed to be age-1 fish and larger individuals (20–35 cm TL). Through the end of February all males larger than 20 cm were reproduc-

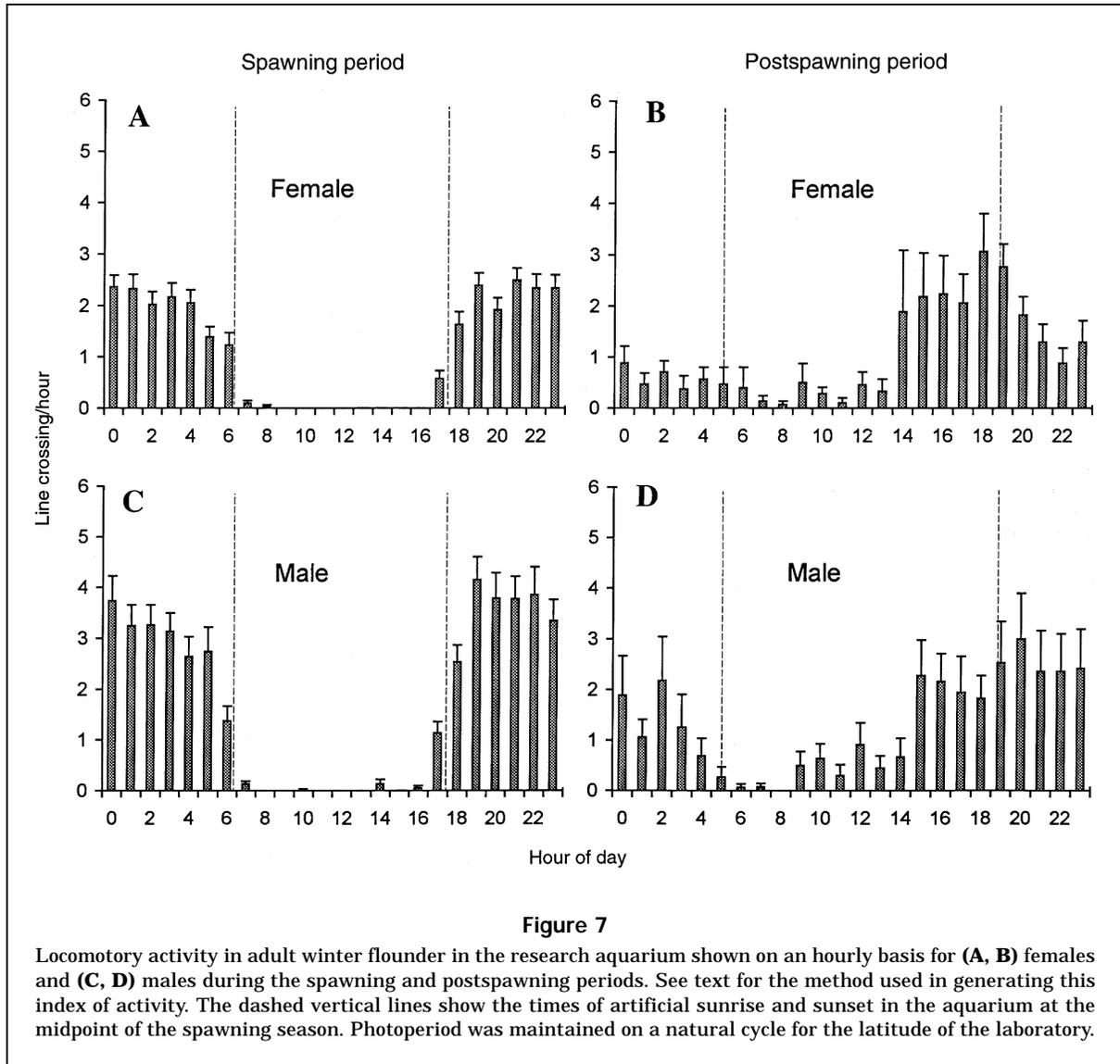


tively ripe, as were 40% of males between 10 and 15 cm. A steady decline in male GSI began in March (Fig. 9B), but ripe males (mean GSI=7.3, SD=4.4) were collected later than ripe females, through 11 April.

Although the number of fish collected at any one station was not large, the *Station* \times *Gender* interaction in the ANOVA indicated that male and female flounder had different distributional patterns in the estuary (Table 1). Males were more abundant than females at stations in the lower estuary (stations 7 and 8), whereas females were more abundant in the middle and upper reaches (stations 1–6) (Fig. 10). These gender-specific distribution patterns were generally consistent, as indicated by the nonsignificant *Date* \times *Station* \times *Gender* interaction term in the ANOVA (Table 1). Ripe males were collected throughout the estuary in proportions ranging from 14% of individuals at station 6, to 100% at stations 1 and 2. In contrast, all ripe females were collected at stations 1–4, with the exception of one partially spent female collected at station 7.

The length distribution of female winter flounder at most of the sampling stations showed two modes (Fig. 11). Large females (mean length=29.7 cm, SD=0.6) were collected at all eight stations but were most abundant in the middle reach (stations 3–6). Small females (<20 cm TL), all of which were reproductively immature, were most abundant in the upper estuary, with approximately equal numbers of small fish at stations 1–4. Size groups were less obvious in male winter flounder (Fig. 11), but it is clear that the mean size of male fish was larger at stations in the lower river (stations 5–8) than in the upper river, and small fish were most abundant at stations 1–3. From 28 March through the end of the sampling period, large numbers of flounders were collected only at station 6. Virtually all the fish in the lower river during this late part of the collecting season were spent.

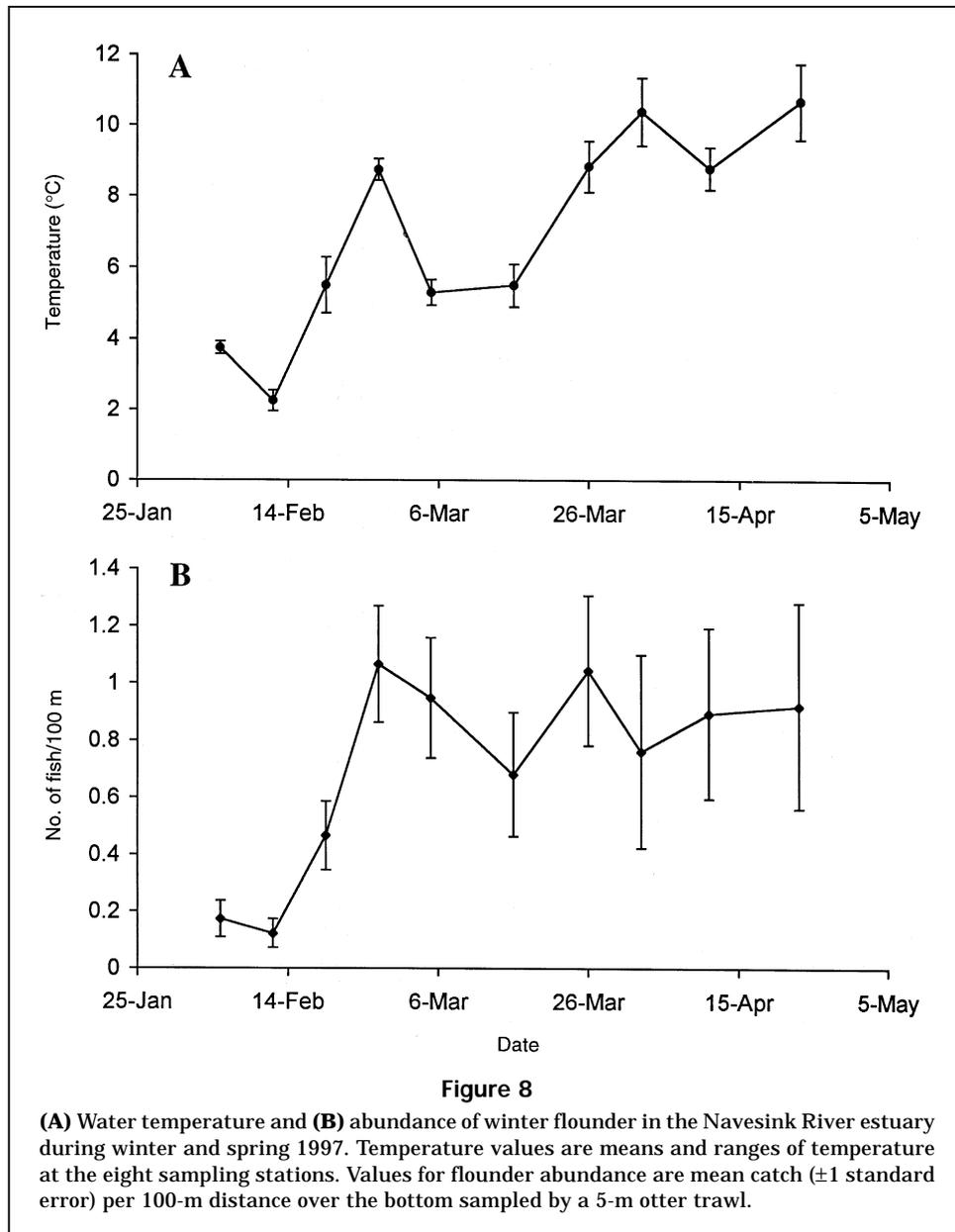
Feeding Stomach fullness in winter flounder varied with date, gender, and age (Fig. 12). Highest full-



ness indices (59%–84%) occurred in immature fish, most containing substantial amounts of food. Average fullness in females was also high throughout the survey period (60%–80%), but variation was large and associated with reproductive state. Ripe females had an average fullness of just 24%, whereas spent females had an average fullness of 72%. Variation among males was even higher, with an obvious increase in fullness from about 1% early in the survey to a range of 70%–90% during the last three collection dates (Fig. 12). Ripe males had average stomach fullness of only 17%, whereas average fullness of spent males was 69%. Therefore, the rapid increase in stomach fullness of males in late March was associated with rapidly declining numbers of ripe males. Stomach fullness was weakly correlated with GSI in

both females ($r=-0.439$) and males ($r=-0.582$) because fullness in fish with a low GSI spanned the entire range from 0%–100%.

Siphons of soft clam (*Mya arenaria*) were the most abundant prey items taken by winter flounder, on the basis of both percentage volume and frequency of occurrence. Of the 257 flounders that contained prey, 150 contained siphons with diameters of 3–13 mm. However, diet varied with collection site (Fig. 13). Siphons of *M. arenaria* were most abundant in fish collected in the middle reach of the estuary (stations 3–6), whereas another clam, *Macoma balthica*, was most abundant in fish from the upper reach (station 1). Ampeliscid amphipods, mysids, shrimps, and other crustaceans were the most important food items in the lower estuary.



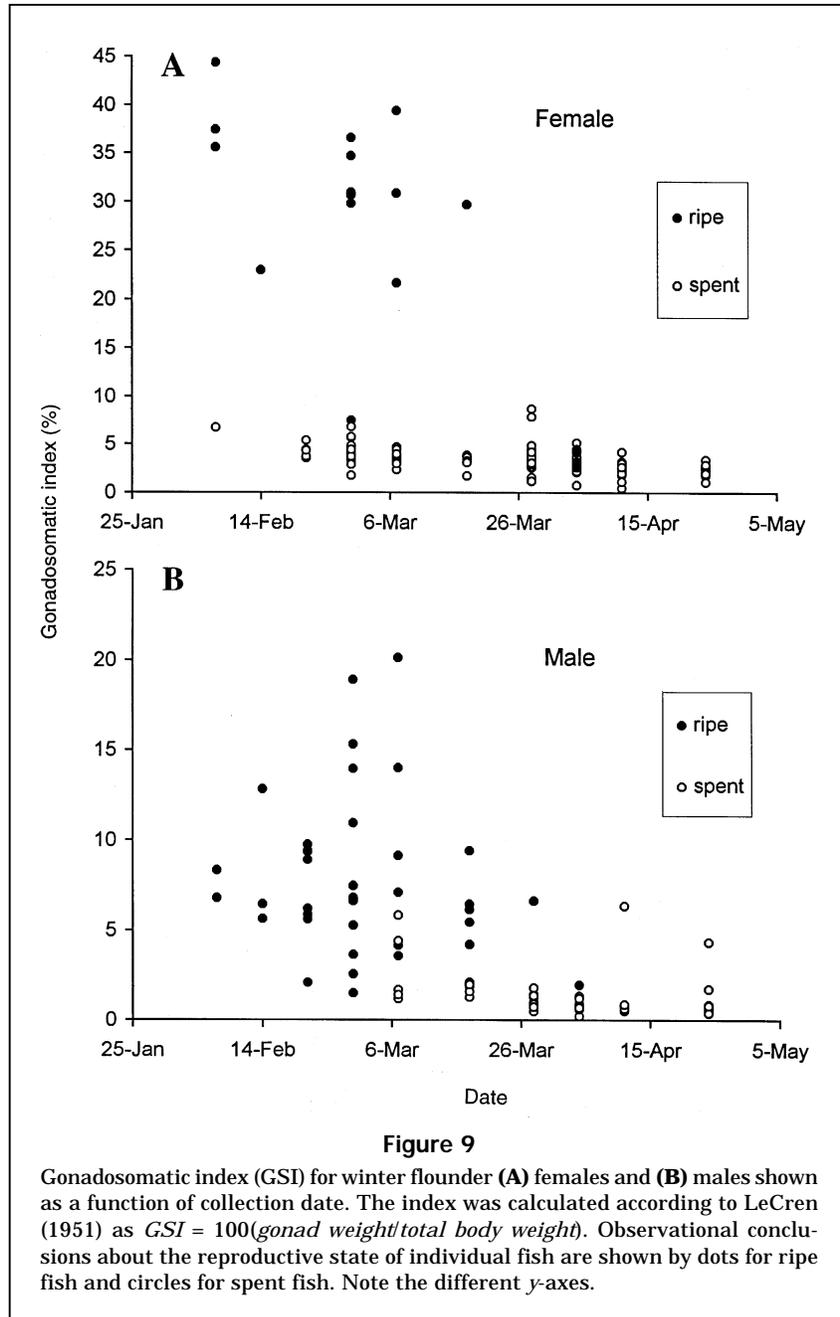
Discussion

Spawning behavior

Direct observation of spawning behavior has been documented for only a few flatfish, but spawning modes appear to vary substantially among species. Midwater pair-spawning has been observed in plaice (*P. platessus*; Forster, 1953), and in Dover sole (*Microstomus pacificus*; Baynes et al., 1994), but winter flounder spawned near the bottom and pairwise spawning (22%) was less common than spawnings involving multiple males (78%). Also, winter floun-

der spawning may be less elaborate or ritualized than in plaice or Dover sole or Caribbean bothids (Konstantinou and Shen, 1995). Although this could be an artifact of confinement, our research aquarium, with a bottom surface area of 46 m² and 3 m depth, was much larger than tanks where pair spawning has been observed in other species and where the density and size of fish were lower.

Our long-term observation of a single spawning population in the aquarium allowed us to record several different forms of reproductive behavior not reported earlier (Breder, 1922). Most notably, we were able to observe and record individual encounters



between males and females. Unlike Breder, and in thousands of observed encounters, we never observed females initiating spawning activities. Breder acknowledged that his relatively small tanks may have altered normal behavioral patterns and was correct in predicting that males would be more active than females in a more open environment. In fact, males attempted to initiate spawning in the laboratory long before and after females were receptive. A similar phenomenon most likely occurs in the field, where males are ripe and capable of spawning for a longer

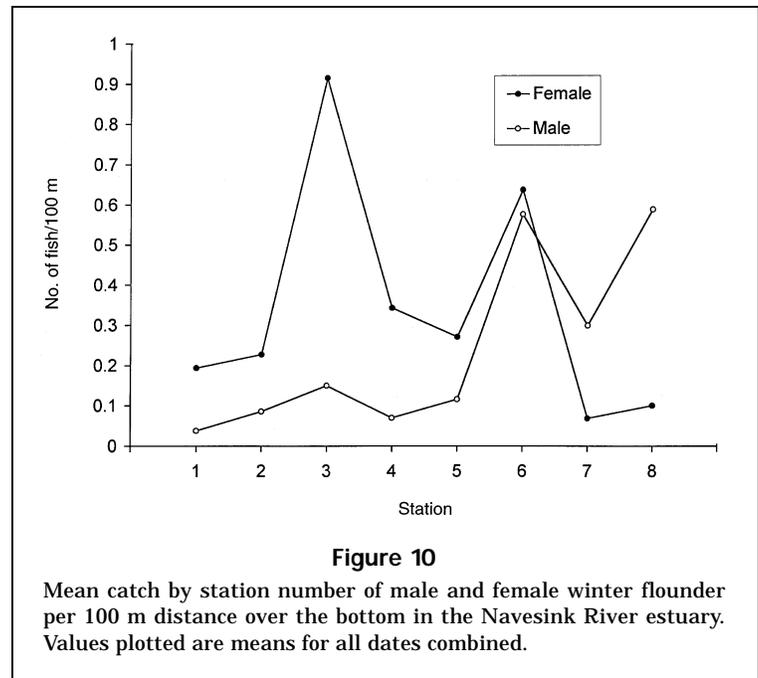
period of time than females. Similarly, Rijnsdorp (1989) has observed that male plaice are in spawning condition for a period twice as long as that for females. As in our observations with winter flounder, Baynes et al. (1994) observed that males of *Microstomus* were much more active than females, and male attempts to initiate spawning were often rejected.

Male winter flounder were able to detect the presence of a spawning female from distances of at least 5–10 m in the research aquarium and frequently con-

verged suddenly on a spawning pair or group. A variety of sensory mechanisms could play a role in detecting opportunities for egg fertilization. Male fish may respond to the obvious visual signal of light reflected from the white underside of spawning fish. It is also possible that hydroacoustic cues were emitted from vigorous bouts of spawning that occurred just above the bottom. It is less likely that spawning was detected by an olfactory cue because there was almost instantaneous response to spawning activity and there was little horizontal current in the aquarium. However, olfactory cues may be important in certain circumstances. Occasionally, males converged on locations where spawning had occurred although the spawners had departed, and these spawning locations were sometimes visited by additional males for several minutes after the initial spawning event. Future research will be needed to determine the precise mechanisms of spawning group formation and detection.

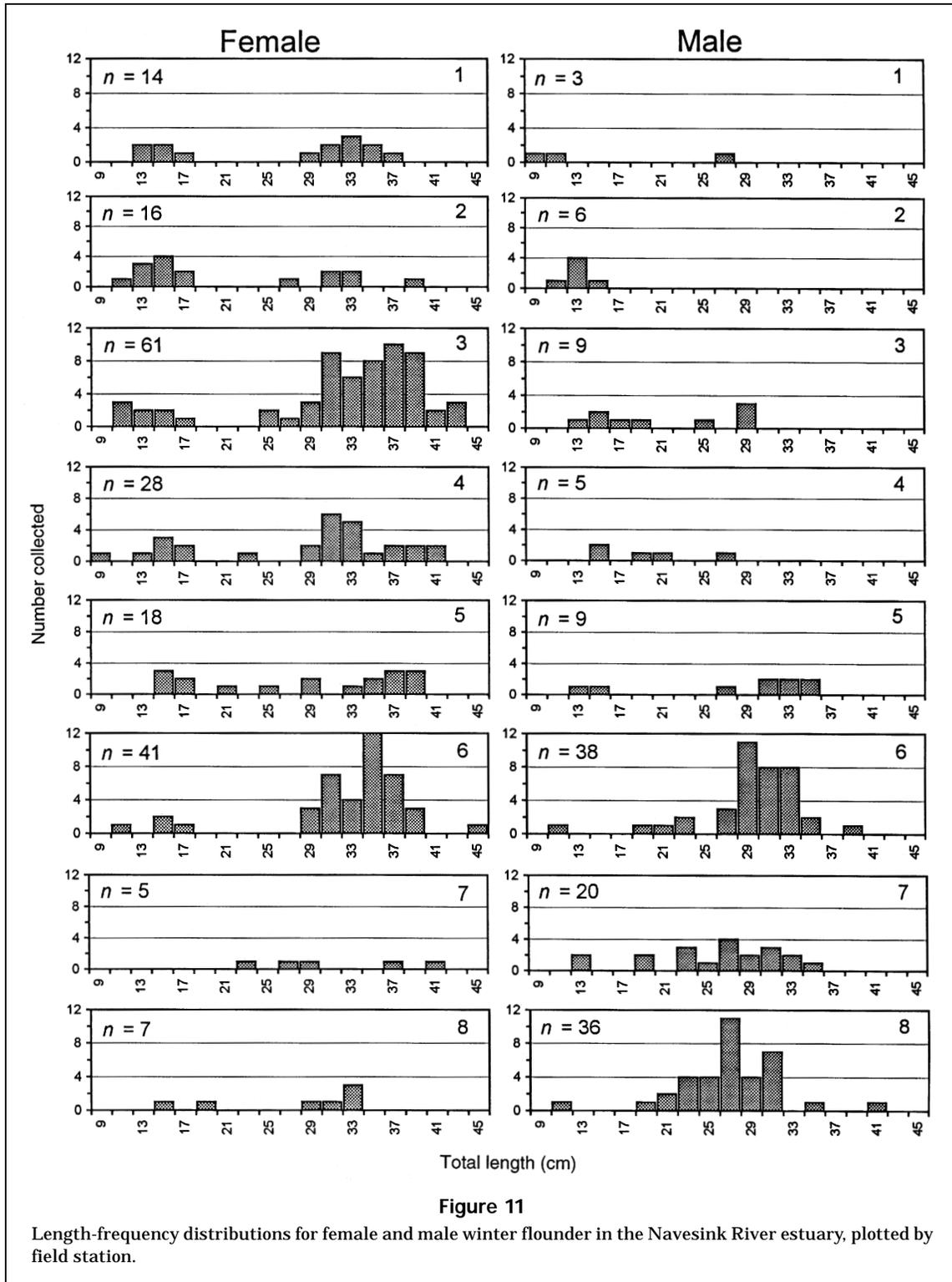
In female winter flounder just one batch of oocytes matures per year (Burton and Idler, 1984); nevertheless, spawning in the aquarium population of winter flounder had a duration of 60 days. It is clear that individual females spawn many times over a period of at least one week, and perhaps longer. Circumstantial evidence indicates that individual males were capable of spawning over most of the reproductive season in the research aquarium. Large numbers of males were often engaged in single spawning events; the total number of spawnings yielded a high average spawning frequency by males that would necessitate several weeks of spawning by each; and feeding by males did not begin until most females stopped spawning. It is unknown whether male winter flounder produce more than one batch of sperm or simply release one batch over time (Bert et al., 1988).

Spawning in the aquarium occurred over a broad range of times, but always during hours of darkness or just before. Nocturnal spawning in winter flounder is probably a function of endogenous diurnal rhythms rather than light level because Breder (1922) noted that spawning occurred between 2200 and 0330 h even under artificial light. Spawning times vary with flatfish species and are probably species-specific. For example, plaice (Nichols, 1989) and other pleuronectids (Woodhead, 1966) are also known to spawn primarily at night, but *Bothus ocellatus* spawns only around the time of sunset (Konstantinou and Shen, 1995).



The role of multiple releases of eggs and sperm is poorly understood but may be important in the reproductive biology and recruitment of winter flounder. Although release of gametes could not be recorded or verified beyond the fact that larvae were produced in the aquarium, it is evident that numerous males spawned in response to one female. It is likely, therefore, that eggs released in a single spawning event were often fertilized by more than one male. Given that the average female fish spawned 40 times during the reproductive season in the aquarium and that most of the males appeared to remain ripe throughout the observation period, it is likely that many, if not all, of the males could have contributed genetic material to the offspring of each female. Genetic diversity of embryos produced in the field could be high for similar reasons.

Although male winter flounder appeared to be in a constant state of readiness for spawning, future experiments will be needed to determine whether frequency of spawning and fertilization success is uniform among individuals and how this might be influenced by size structure, sex ratio, or density of fish on the spawning ground. For example, small (10–15 cm) but reproductively ripe males were abundant in the Navesink River estuary. It is possible that these small individuals have a lower frequency of success than large males in inducing females to spawn, given the order-of-magnitude difference in body weight between 10 and 22 cm fish. However, Baynes et al. (1994) observed female Dover sole spawning with males smaller than themselves, and



the same may occur with winter flounder. On the other hand, most fertilization by small males may occur during group spawning. "Sneaking," whereby subordinate males approach and fertilize some of the eggs when a pair is spawning, is a reproductive behav-

ior common in a variety of fishes (Chan and Ribbink, 1990; Oliveira and Almada, 1998). We observed no overt agonistic behavior in our long-term experiment, and there is no reason to believe that small males do not contribute to the reproductive population.

Spawning, migration, and habitat use

Previous studies have shown that winter flounder in the mid-Atlantic region migrate to shallow inshore waters to spawn during the late winter (Perlmutter, 1947; Poole, 1966; Scarlett and Allen, 1992), and it is known that the Navesink-Shrewsbury River estuarine system is an important spawning ground (Phelan, 1992; Scarlett¹). Phelan's study and our investigation provide direct evidence that winter flounder immigrate to the Navesink estuary between early February and March. Flounder abundance was lowest when water temperature was lowest (2.2°C in mid-February) and increased rapidly with subsequent temperature rise. In fact, high and relatively constant catches of winter flounder occurred in the Navesink estuary at temperatures of 6–11°C. This is consistent with earlier observations that adult winter flounder emigrated from bays in southern New Jersey when water temperature declined below 3.5°C and returned when temperature rose to 6°C (Danila, 1978). Most adult males and females had ripe gonads upon entry to the system in late winter, and most were spent by the end of April. This pattern of seasonality is consistent with previous findings (Percy, 1962; Scarlett and Allen, 1992).

It is likely that the most important spawning area for winter flounder in the Navesink River was in the middle reach. Reproductively ripe male winter flounder were found throughout the estuary; however, highest numbers of ripe females were found west of station 5, and only one nearly spent female was collected farther down the estuary. Scarlett and Allen (1992) found that the middle reach of the Manasquan River estuary, New Jersey, was a major spawning ground for winter flounder, as were the upper reaches of the Mystic River estuary, Connecticut (Percy, 1962), and Narragansett Bay, Rhode Island (Powell²).

² Powell, J. C. 1989. Winter flounder tagging study, 1986–1988, with comments on movements. Rhode Island Division of Fish, Wildlife, and Estuarine Research, P.O. Box 218, West Kingston, Rhode Island 02892. Unpubl. report.

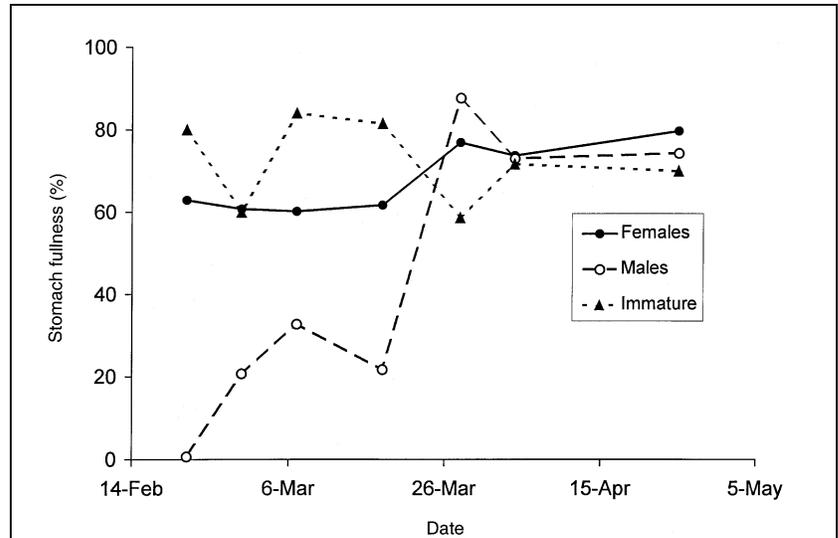


Figure 12
Temporal variation in percent stomach fullness of winter flounder in the Navesink River estuary. Fullness indices are shown by adult gender and for immature fish.

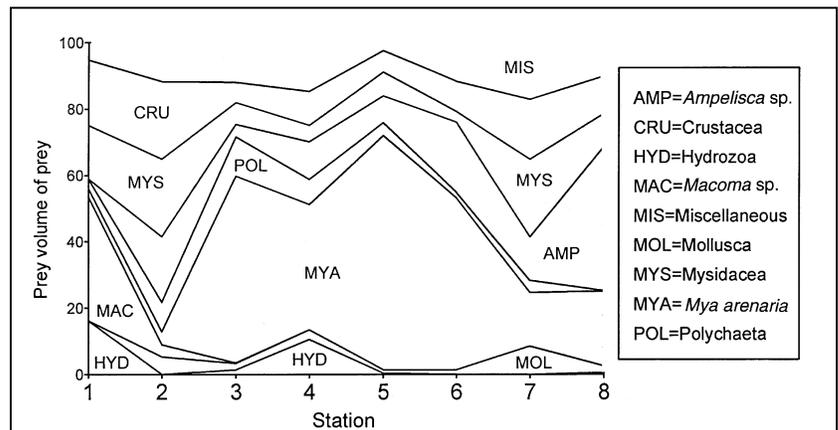


Figure 13
Composition of diet by percentage volume in winter flounder collected at eight stations along the length of the Navesink River estuary during winter and spring 1997.

Several different physical and chemical factors could affect the location of spawning habitats. Temperature may have influenced the seasonal migration to the Navesink estuary; however, there was little variation in temperature along the axis of the estuary, and other factors probably influenced exact spawning location. McCracken (1963) found that adult winter flounder died in 8 ppt salinity; therefore, upstream migration is probably limited by this variable. Neither males nor females were abundant in locations where salinity was frequently near or below 10 ppt.

The location of spawning in an estuary may also be related to temperature and salinity requirements of the demersal eggs. Scarlett and Allen (1992) collected eggs at salinities of 14–32 ppt and temperatures of 0.9–10°C in the Manasquan estuary, about 30 km south of our study site. Rogers (1976) found that the highest proportion of viable hatches occurred at 3°C over a salinity range of 15–35 ppt. At temperatures over 3°C, the optimal range was 15–25 ppt, which closely matches temperature and salinity ranges in the middle reach of the Navesink River.

Other factors that may influence the choice of spawning habitat by winter flounder are hydrodynamic properties and associated sediment characteristics. Strong flood tidal currents that characterize the lower Navesink River attenuate rapidly in the middle reach (Chant³). This attenuation is associated with deepening of the estuary and general reduction of sand bars, decrease in the mean grain size of sediments, and increase in sediment organic content (Stoner, unpubl. data). It is unknown whether spawning winter flounder respond to any of these habitat characteristics; however, the depositional qualities of the upper estuary and flood-dominated circulation may aid in the retention of winter flounder eggs and the evolution of habitat choice in spawners. Crawford and Carey (1985) noted that winter flounder spawned in two sites in an estuarine lagoon in Rhode Island where hydrodynamic features retained larvae. It is plausible that habitat choice, cued by either fine-grained sediments or low current velocities, could be associated with such retention features.

Seasonal variation in feeding activity

The general conclusion has been that winter flounder are opportunistic feeders, consuming polychaetes, small crustaceans, molluscs, and other prey according to their abundance (Pearcy, 1962; Richards, 1963; Klein-MacPhee, 1978; Franz and Tancredi, 1992; Martell and McClelland, 1994; Steimle et al., 1994; Carlson et al., 1997). Winter flounder collected in the Navesink River during this investigation consumed a mixture of invertebrate prey, but the vast majority of the diet was siphons of the soft clam *Mya arenaria*. The abundance of spent and feeding flounder in the lower estuary may, in fact, be associated with the observed abundance of this preferred prey in that location.

Cessation of feeding by adult winter flounder during the winter has been noted previously (Tyler, 1972;

Martell and McClelland, 1994), but we found that feeding was influenced strongly by gender and reproductive state. Both in the field and in the aquarium, female flounder appeared to resume feeding immediately after they completed spawning for the season. Early feeding probably provides an advantage for female fish because ovarian development for the next spawning season begins immediately after spawning (Burton and Idler, 1984). In the field, males did not begin feeding until the end of March, and feeding in the aquarium was light until after the last spawning events. We conclude that male behavior is dedicated to fertilizing the maximum number of eggs, at the expense of feeding and late winter growth.

There is now substantial evidence for seasonal variation in daily activity and feeding rhythms in winter flounder. During the summer, winter flounder are primarily diurnal in general activity (Olla et al., 1969) and feeding (MacDonald and Waiwood, 1987). Bharadwaj (1988) found that adults fed throughout the day and night in November, speculating that this was related to maturation of gonads for the winter spawning season. In our aquarium observations, adult winter flounder were almost entirely nocturnal throughout the spawning season, remaining buried during the day. Daytime activity began again when spawning was completed and increased throughout the remainder of the postspawning period. Primarily daytime activity during the nonspawning season could be associated with visual predation modes (Olla et al., 1969), but it remains to be determined how mates are located in darkness and why spawning occurs at night.

Conclusions

This study provides the first detailed observations on winter flounder spawning since Breder's (1922) original description over 75 years ago, and the combination of laboratory and field work provides new insight into the biology and ecology of spawners. The behavior of male winter flounder appears adapted to maximize encounters with reproductive females and numbers of eggs fertilized. From our field collections, it is apparent that one-year-old male winter flounder (10 cm TL) are capable of spawning, whereas female spawners all appeared to be at least two years old. Males also became reproductively ripe earlier in the season than did females and remained ripe to a later date than most females. Males were more active than females, had higher swimming speeds, and initiated all spawning events. The males fed very little during the reproductive season and appeared

³ Chant, R. J. 1998. Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 231, New Brunswick, New Jersey 08903. Unpubl. data.

to sacrifice winter growth to maintain a high level of reproductive behavior.

Spawning frequency was high in both males and females. Individual females were capable of spawning as many as 40 times over a period of at least one week, and males spawned throughout the season (at a rate at least three times the female rate) and perhaps several times per day. Clearly, individual records of spawning behavior would be useful in understanding the reproductive role of one-year-old males and in determining the duration of spawning in individual females. Given the frequency of spawning in both male and female winter flounder and the fact that several males may release sperm in response to egg-laying by one female, we conclude that the genetic diversity of offspring produced by a female in one year is probably very high.

Although our data provide new insights into the natural history and reproductive behavior of winter flounder, several questions important to sound management of spawning stocks remain to be answered. For example, we do not know if reproductive populations are scattered in estuarine and coastal waters or are concentrated in spawning aggregations on specific spawning grounds. We saw a concentration of ripe fish in the middle reach of the Navesink River estuary, but ripe fish were also present in a variety of habitats in adjacent Raritan Bay during the winters of 1997 and 1998 (Stoner and coauthors, personal obs.), and it is possible that winter flounder spawn in more than one location over the spawning season. It is also important to learn whether or not spawning behavior and reproductive output from a spawning ground are density-dependent.

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