

**MOVEMENT OF SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*) IN
RELATION TO HYPOXIA IN AN ESTUARINE TRIBUTARY**

by

Margaret H. Miller

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fulfillment of the requirements for the degree of Master of Science in Marine Studies

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RELATION TO HYPOXIA IN AN ESTUARINE TRIBUTARY**

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Margaret H. Miller

Approved: _____
Timothy E. Targett, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Charles E. Epifanio, Ph.D.
Director of the School of Marine Science and Policy

Approved: _____
Nancy M. Targett, Ph.D.
Dean of the College of Earth, Ocean, and Environment

Approved: _____
Debra Hess Norris, M.S.
Vice Provost for Graduate and Professional Education

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ABSTRACT

Estuarine waters generally provide favorable physiochemical and biological conditions for early growth and survival of ecologically and economically important fishes. However, given their close proximity to expanding urban and rural coastal populations, estuaries are especially susceptible to the threat of increased nutrient loading and may contain areas of low dissolved oxygen (DO), or hypoxia. The present study looked at the effects of hypoxia on movement of estuary-dependent juvenile and adult summer flounder (*Paralichthys dentatus*). Summer flounder were captured from Indian River Bay, Delaware, surgically implanted with Vemco V7-4L acoustic tags, and released into Pepper Creek, a tributary of Indian River Bay that experiences diel-cycling hypoxia. Overall, 17 juveniles and 8 adult summer flounder were released into Pepper Creek during September in 2007 and 2008, and July in 2009. Vemco VR2 acoustic receivers and YSI 600XLM multi-parameter sondes were deployed along the longitudinal axis of Pepper Creek to capture the spatial and temporal dynamics of summer flounder movement in relation to hypoxia. Individual fish tracks were plotted on spatiotemporal DO contour plots using MATLAB software and analyzed with respect to the following environmental variables: DO, temperature, spatial and temporal DO and temperature gradients, insolation, and tide. Fish detections were labeled as either “movement” or “stay put” detections, with “movement” detections further coded as “upstream” or “downstream.” The results show that summer flounder remained fairly sedentary in Pepper Creek during healthy

DO concentrations, but responded actively to growth reducing and lethal DO concentrations. Some flounder avoided unhealthy DO (≤ 4.8 mg O₂/l) while others initiated movement only when DO fell into the severely hypoxic range (≤ 2.3 mg O₂/l), with activity driven by the specific nature of the DO environment. Rapid drops in DO to unhealthy levels triggered active movement responses, as did absolute DO values ≤ 2.3 mg O₂/l, whereas slowly declining and/or spatially extensive DO > 2.3 mg O₂/l triggered a passive behavior (no movement). Binary logistic regression was used to determine which environmental variables predict overall activity and specifically directional movement. With respect to the general summer flounder population, the temperature variables significantly discriminated between movement and stay put detections. For fish that experienced severe hypoxia, DO and the spatial temperature gradient predicted overall movement activity. Summer flounder also relied primarily on the spatial DO gradient and tidal flow for directional guidance within Pepper Creek. Upstream movements were generally done when the spatial DO gradient was negative and tide was flooding, while downstream movements were made during positive spatial DO gradients and ebbing tides. Additionally, tidal flow may have occasionally inhibited escape from hypoxic areas for smaller summer flounder. Residency time in the creek was shorter for fish released into unhealthy DO concentrations as opposed to fish released in DO > 4.8 mg O₂/l, as was the exposure duration to unhealthy DO levels, with diel-cycling hypoxia compressing optimal summer flounder habitats. Offshore emigration from the bay also appeared to be delayed for a few summer flounder. Given the relatively low DO concentrations that elicit movements, summer flounder in Pepper Creek are experiencing DO levels that may stunt growth or result in mortality. Overall, these findings suggest that many

flounder will tolerate progressively declining or steady unhealthy DO concentrations (≤ 4.8 mg O₂/l), perhaps reaping the benefits of the productive nursery grounds to counteract growth-reducing DO conditions, but once exposed to severe hypoxia (≤ 2.3 mg O₂/l), will abandon this potentially risky passive behavior and move permanently out of the creek.

INTRODUCTION

Many ecologically and economically important fishes inhabit estuaries at some point during their lives (NOAA 2008). Due to favorable physiochemical and biological characteristics, fishes often use shallow estuarine waters as nursery habitats during juvenile, and sometimes larval, stages (Weinstein 1979, Able 1999, Minello 1999). Estuaries are highly productive environments, and their value as nursery habitats for young fishes is attributed to prey resources, predation refuge, and favorable conditions for fast growth, such as warm temperatures, throughout the spring and summer season (Joseph 1973, Miller et al. 1985, Ruiz et al. 1993). The quality and quantity of suitable nursery habitats are believed to disproportionately contribute to year class strength and recruitment to the adult population (Fogarty et al. 1991, Gibson 1994, Beck et al. 2001). Adult fishes, such as weakfish (*Cynoscion regalis*) and summer flounder (*Paralichthys dentatus*) have been shown to return from offshore to their estuarine nursery grounds, spending the summer and fall in these dynamic environments (Thorrold et al. 1998, 2001, Sackett et al. 2007)

Given their proximity to expanding urban and rural coastal populations, estuaries are especially susceptible to increased nutrient loading (Diaz 2001). Excess nutrients (eutrophication) are commonly associated with areas of low dissolved oxygen (DO), or hypoxia (Diaz 2001, Gray et al. 2002).

Hypoxia refers to dissolved oxygen concentration that is less than saturation but the term is frequently used to refer to waters with $< 2 \text{ mg O}_2/\text{l}$ (US EPA 2000, Diaz 2001, Tyler et al. 2009). Anoxic waters are generally considered to be those with $< 0.2 \text{ mg O}_2/\text{l}$. The US EPA (2000) has set dissolved oxygen criteria for coastal and estuarine waters from Cape Cod, MA to Cape Hatteras, NC, with DO levels $> 4.8 \text{ mg O}_2/\text{l}$ required for growth of aquatic organisms and $> 2.3 \text{ mg O}_2/\text{l}$ for survival. There are three types of hypoxia that commonly occur in coastal waters: 1) seasonal, 2) episodic, and 3) diel-cycling. Seasonal hypoxia, as found in Chesapeake Bay (US EPA 2000), can last from weeks to months and occurs in deeper water when temperature and salinity gradients prevent the more oxygenated surface water from mixing with the deeper water. Episodic, multiple-day, hypoxia occurs in unstable systems with relatively weak pycnoclines, where low DO levels are mainly influenced by water temperatures, freshwater and tidal mixing, and wind (Pihl et al. 1991, Tyler et al. 2009). Diel-cycling hypoxia occurs on a 24-hour time scale in shallow water and above the pycnocline in deeper water (Tyler et al. 2009). Diel-cycling hypoxia is synchronized with the daily cycle of photosynthesis of algae and respiration of biota and is highly influenced by nutrient loading (D'Avanzo and Kremer 1994, Boynton et al. 1996, Tyler et al. 2009). In these systems, DO is highest just before sunset due to daytime photosynthesis, and drops to a low just after sunrise during the time respiration dominates. In Delaware's Coastal Bays, the amplitude and duration of this diel DO cycle is primarily driven by the abiotic factors of water temperature and insolation (Tyler et al. 2009).

Hypoxia has become a frequent problem in coastal and estuarine environments (Diaz and Rosenberg 2008). Since the 1960s, the number of marine ecosystems reporting hypoxia has drastically increased, doubling each decade, showing a highly probable link between anthropogenic effects and declining oxygen levels (Diaz 2001, Diaz and Rosenberg 2008). In fact, most of the reported hypoxic zones are located close to anthropogenically modified watersheds and dense coastal populations, major sources of nitrogen influx to large riverine and estuarine systems (Howarth et al. 1996, Diaz 2001, Diaz and Rosenberg 2008). For example, the extent of hypoxia in the northern Gulf of Mexico has been closely linked with freshwater discharge from the Mississippi River, with periods of increased river flow resulting in larger spatial areas of hypoxia (Rabalais et al. 2002, Diaz and Rosenberg 2008, LUMCON 2008).

Nutrient loading in Delaware's coastal bays has increased due to urbanization and input from agricultural practices, and hypoxia has become a frequent problem, with the potential to intensify with the growing coastal population, increased development, and associated rise in nitrogen input (Martin et al. 1996, C. Bason pers. comm.). More than 60,000 people live in the Delaware Coastal Bays watershed, and the bays themselves are three shallow (average 0.9 – 2.4 m) coastal bays (Rehoboth Bay, Indian River Bay, and Little Assawoman Bay) covering around 83 square kilometers (CIB 2007) and located in Sussex County. Most of the recent growth in Delaware has been along the coastal towns, especially in Sussex County, where population has increased 59% between 1990 and 2006 (US Census Bureau 1990, 2007).

Shallow estuarine waters, such as in Delaware's Coastal Bays, are particularly susceptible to diel-cycling hypoxia, and this poses a problem for fishes that use these areas as nursery grounds and adult habitats. Exposure to low DO levels has been shown to elicit a variety of responses, ranging from primary physiological responses, such as an adjustments in ventilation frequency (Breitburg 1992, Wannamaker and Rice 2000, Taylor and Miller 2001, Robb and Abrahams 2003) and metabolic activity (Fischer et al. 1992), to altered behavioral responses in the form of decreases in physical activity and feeding rates (Bejda et al. 1987, Heath 1995, Chabot and Dutil 1999, Domenici et al. 2000, Shoji et al. 2005, Stierhoff et al. 2006, Brady et al. 2009, Brady and Targett in press). Although these strategies help species subsist during short-term low DO events, the negative longer-term effects on growth have implications for survival (Steffensen et al. 1982, Coiro et al. 2000, Gray et al. 2002, Herbert and Steffensen 2006).

In the field, fish may actively avoid areas of low DO concentrations (generally ≤ 2 mg O₂/l) (Howell and Simpson 1994, Eby and Crowder 2002, Bell and Eggleston 2005, Craig and Crowder 2005, Tyler and Targett 2007, Brady 2008). Depending on the duration of a particular hypoxia event, this habitat shift may strongly alter trophic dynamics (Diaz and Breitburg 2009). For example, in estuaries, fishes and crustaceans occupy more habitat space when sufficient oxygen is available (Eby and Crowder 2002, Tyler and Targett 2007) but when DO drops, their normal habitat range is compressed and they are forced into less favorable conditions. During habitat compression, a variety of negative effects can occur, including: more inter- and intra- specific competition, less available food, density-dependent growth, and sub-

optimal abiotic conditions (Eby and Crowder 2002, Craig and Crowder 2005, Eby et al. 2005). For example, Craig and Crowder (2005) found that during lethal hypoxia (0.5 – 1.4 mg O₂/l) on the Gulf of Mexico continental shelf, Atlantic croaker (*Micropogonias undulatus*), which reside in warm (26-30°C) waters, were displaced into cooler temperatures (< 22°C) while brown shrimp (*Farfantepenaeus aztecus*), which prefer offshore waters, were found in highest densities in the warmest inshore waters (28-31°C). Both species also avoided the lowest DO levels on the shelf but remained close to the hypoxic edge, choosing to endure relatively moderate hypoxia (Craig and Crowder 2005). This behavior has been frequently documented in the field, whereby during short-term (episodic and diel-cycling) hypoxia events, fish and crustaceans remain in suboptimal low DO regions until risk of mortality necessitates their exit, but will reinvade these zones once DO rises above ~2 mg O₂/l (Pihl et al. 1991, Nestlerode and Diaz 1998, Meng et al. 2001, Eby and Crowder 2002, Tyler and Targett 2007, Brady 2008).

This migration behavior has been suggested as a means for fishes to at least partially compensate for reductions in growth by taking advantage of potential increases in prey abundance following severe hypoxia events. Previous work has shown that low DO may stress infaunal organisms, forcing them to the sediment surface and making them more vulnerable to predation by demersal fishes (Diaz et al. 1992, Pihl et al. 1992, Diaz and Rosenberg 1995). Young weakfish have been shown to exploit stressed infaunal organisms during and after hypoxic events. In Pepper Creek, a tributary of the Delaware Coastal Bays that experiences frequent diel-cycling hypoxia during the summer, weakfish have been documented leaving the estuary

during severe hypoxic events but returning later in the day when the DO rises above 2.0 mg O₂/l, primarily to feed on the stressed benthos at the creek headwaters (Tyler and Targett 2007, Tuzzolino 2008). Therefore, although fish experience decreased growth rates in hypoxic waters (Meng et al. 2001, Taylor and Miller 2001, Stierhoff et al. 2006, Stierhoff et al. 2009a, 2009b) they may counter this growth detriment through increased feeding opportunities, resulting in a higher trophic energy transfer.

Most research on fish behavior during hypoxia has been conducted on fusiform fishes, which are efficient swimmers and can perhaps more easily escape low DO events either through lateral or vertical migrations (Bell and Eggleston 2005, Brady 2008, Brady et al. 2009). Flatfish, on the other hand, are sedentary by nature and are assumed to initiate avoidance responses to hypoxia at higher levels of DO and with a greater energy expense than roundfish (Deubler and Posner 1963, McEnroe and Krozlowitz 1997, Bell and Eggleston 2005, Brady and Targett in press). Because flatfish are unable to easily escape bottom water hypoxia through vertical migrations, their active behavioral response to hypoxia is commonly displayed as an increase in horizontal swimming speed as DO decreases from normoxic conditions (Deubler and Posner 1963, McEnroe and Krozlowitz 1997, Bell and Eggleston 2005, Brady and Targett in press). In the lab, Brady and Targett (in press) documented similar active responses of increased swimming speed in declining DO for both the flatfish summer flounder and the fusiform weakfish; however the summer flounder increased speed by 248% compared to 42% for the weakfish. In the field, Bell and Eggleston (2005) found that summer and southern flounder (*Paralichthys lethostigma*) strongly avoided waters with DO < 3 mg O₂/l and were rarely found in trawls conducted in hypoxic

waters. On the other hand, two roundfish, the spot (*Leiostomus xanthurus*) and Atlantic croaker, both exhibited weak avoidance responses to hypoxia, with spot frequently caught in hypoxic waters (found in 82% of trawls in hypoxia) and croaker always present in waters with DO of 1-2 mg O₂/l. It seems the early and energetic response to hypoxia exhibited by flatfish may provide the fish with more swimming time to escape areas where DO is decreasing to potentially lethal levels. However, little information exists on hypoxia-induced movements of juvenile and adult flatfish in the natural environment.

The present study was the first to examine both juvenile and adult summer flounder behavior using real-time tracking data and synoptically measured environmental conditions in a hypoxia-impacted estuary. The specific objectives of this study were to a) determine the effects of diel-cycling hypoxia on movement patterns of juvenile and adult summer flounder, b) characterize the impacts of diel-cycling hypoxia on habitat quality by examining hypoxia-exposure duration and potential mortality of adult and juvenile summer flounder, and c) compare juvenile versus adult summer flounder residency time, low DO exposure duration, and movement patterns to examine life stage effects.

MATERIALS AND METHODS

Study Area

Pepper Creek is a shallow mesohaline estuarine tributary of Indian River Bay, one of Delaware's three Coastal Bays (Figure 1). It has an average depth of about 1 meter and salinity ranges from 15-25 (Tyler et al. 2009). It experiences limited tidal flushing because the creek mouth is located about 10 km from Indian River Inlet (~80 m wide), the only source of ocean circulation for the Indian River and Rehoboth Bays (Figure 1). Limited flushing contributes to the frequency of hypoxia occurrences in Pepper Creek (Brady 2008, Tyler et al. 2009).

Pepper Creek typically experiences diel-cycling DO during summer months, with lowest levels of DO occurring around 2 hours after sunrise and then increasing throughout the day due to net algal photosynthesis (Tyler et al. 2009). DO concentrations reach a peak around 2 hours before sunset and then decrease throughout the night due to respiration of the biota (Brady 2008, Tuzzolino 2008, Tyler et al. 2009). Diel-cycling hypoxia in Pepper Creek becomes more frequent and severe as the summer season progresses because of the increase in water temperatures and high primary production.

Based on previous work conducted in our laboratory (Tyler 2004, Tyler and Targett 2007, Brady 2008, Tuzzolino 2008, Tyler et al. 2009, Stierhoff et al. 2009a), six locations were chosen along the axis of the creek for placement of water

quality monitoring equipment and acoustic telemetry receivers (Figure 2). These sites encompass the longitudinal variation of diel-cycling hypoxia in Pepper Creek and were sufficient to pick up fish locations along the entire width and length of the tributary (based on receiver range data from Brady 2008; Figure 3). Each year minor adjustments were made to the six sites to increase tracking efficiency and diminish equipment loss or damage.

Water Quality Monitoring

For the continuous spatiotemporal coverage of water quality in Pepper Creek, three YSI Model 600XLM multi-parameter sondes were deployed on steel pyramid stanchions and suspended about 15 cm above the creek bottom at sites PC2, PC4, and PC5 (Figure 2). These three sondes were left in the creek in order to collect data for the entire summer season (May-October in 2007, and May-September in 2008, 2009). During the fish tracking experiments in 2008 and 2009, three more data sondes were added at sites PC1, PC3, and PC6 for finer spatial coverage of the water quality in Pepper Creek. Sondes measured salinity, temperature, and dissolved oxygen every 15 minutes. Each sonde was cleaned once a week in the field to remove biofouling accumulation. Every two weeks, sondes were brought back to the laboratory, water quality data were downloaded, sondes were cleaned, recalibrated, and placed back in Pepper Creek.

Fish Surgeries

Juvenile (≤ 320 mm TL) and adult (> 320 mm TL) summer flounder (age classification based on Smith and Daiber (1977), Able et al. (1989) and Szedlmayer et

al. (1992)) were collected from Pepper Creek using a 3-meter otter trawl and from Indian River Bay using rod-and-reel. Flounder were placed in a re-circulating holding tank in the laboratory at ~22°C, ~25 salinity, and 14hL:10hD photoperiod for at least 24 hours before surgery. Flounder were fed frozen mysid shrimp (*Mysis relicta*) and live mummichogs (*Fundulus heteroclitus*).

Surgeries to implant tags began in early September (2007), late August (2008), and early July (2009) to ensure that tagged fish were released into Pepper Creek during summertime diel-cycling hypoxia. Surgeries followed the protocol of Brady (2008) and Fabrizio and Pessutti (2007) as follows: instruments were autoclaved and soaked in a strong disinfectant Cidex solution for 12-24h before being rinsed in saline solution prior to implantation. Summer flounder were immersed in a container of anesthetic MS222 solution (170 ppm). Once anesthetized, fish were weighed and measured and immediately placed ocular side down on a padded surgical cradle of foam, saturated with a solution of seawater and MS222 (100 ppm). This MS222 solution was continuously circulated through the fish's mouth and over the gills. A 10 mm incision was made on the non-ocular side, about halfway between the pectoral and pelvic fins, and a V7-4L tag (22.5 mm length, 7 mm diameter, 1.8 g in air, 1.0 g in water) was inserted into the peritoneal cavity perpendicular to the long axis of the body. The implanted transmitters weighed less than 1.7% of the body weight for 22 of the 25 summer flounder (three fish had transmitters that weighed 2.3, 2.6, and 3.0% of their body weight). The incision was closed with 4-0 MAXON™ monofilament sutures and swabbed with betadine ointment and an antibiotic cream. After surgery, fish were placed into a container of seawater and gently moved to

increase ventilation until consciousness was regained and swimming initiated. Fish were given at least 3 days to recover before release into Pepper Creek.

Fish Releases

Fish were released in September of 2007 and 2008 and July of 2009. An array of six Vemco VR2 receivers at locations PC1-PC6 (Figure 2) recorded the date, time, and tag ID of a fish every time it swam within the range of the receiver (Figure 3). Receiver data were downloaded and the receivers were cleaned in the field on a weekly basis to remove biofouling.

Fish were released in batches, with approximately four fish per batch. Seven batches were released from 2007-2009 (Table 1). In 2007 and 2008 fish were transported to Pepper Creek in an aerated cooler of seawater and released at site PC2, near the head of Pepper Creek (Figure 2). Fish were released between 1000 and 1300, before the time of the daily peak in DO, so that initial fish movement was tracked before the start of the diel decline in DO.

In 2009, fish were held in cages and acclimated in Pepper Creek for 10-11h before release. An acclimation period of at least 6-8 hours has been suggested by previous work on spot (*Leiostomus xanthurus*) to result in normal movement following release (K. Craig pers. comm.). Based on this information, a review of the summer flounder tracks from 2007 and 2008, and logistical considerations, an acclimation period of 10-11h was deemed sufficient.

Each cage (0.9 m length x 0.5 m width x 0.3 m height) was separated into two compartments by netting. Two cages were used per batch release, with a flounder

in each compartment. A preliminary 10 h trial showed no negative effects (i.e. no body lesions/cuts/sores). Cages were placed about mid-way between sites PC3 and PC4 (Figure 2) during the morning, to increase the likelihood of missing the minimum DO concentrations that occur just after sunrise. After acclimation, fish were released (between 1600 and 1800); fish in one cage were released at site PC2 and fish in the other cage at site PC4 (with the exception of batch 7 which was released at sites PC2 and PC3). This release approach differed from that in 2007 and 2008 due to concern that fish released at PC2 would likely only move downstream, whereas fish released at sites of PC3 and PC4 could move in either direction (Figure 2).

To assess the effect of acclimation in 2009 versus no acclimation in 2007 and 2008, two Kruskal-Wallis (K-W) tests were run on residency time (in minutes; Table 1). The first K-W test examined residency times from different batch groups and the second test examined residency times from the different years. Neither test showed a significant difference ($p=0.101$, $p=0.207$), so that the null hypothesis that the residency times could be drawn from identical populations was not rejected. Thus, the change in release approach in 2009 did not appear to have a significant effect on residency time.

It was harder to determine whether or not the additional release sites in 2009 had significant impacts on the behavior of the fish. In batches 5 and 6, when fish were released at sites PC2 and PC4, upstream movements were only made by fish released at site PC2. On the other hand, when the batch 7 fish were released at sites PC2 and PC3, the only upstream movements were made by a summer flounder released at site PC3. This difference in behavior may be partly explained by creek

geomorphology. Moving from site PC4 to sites further upstream, Pepper Creek narrows and bends, while it widens and deepens from PC4 to sites further downstream (Figure 2). This is in contrast to site PC3, where the creek maintains its geomorphology to sites further upstream, remaining wide and open. Therefore, access to upstream as oppose to downstream habitats would have potentially been easier for summer flounder from a PC3 as oppose to PC4 release site, while the opposite is true for a PC4 release site.

Meteorological variables that may also influence summer flounder movement, such as precipitation, insolation, and barometric pressure, were recorded during the tracking periods at the Delaware Environmental Observing System (DEOS) stations at Bethany Beach (BB) and Indian River Inlet (IR), and at Weather Underground (wunderground.com) stations at Lewes (KDELEWES2) and Georgetown airport (GTA) (Figure 1). Tidal height data were collected from the USGS Rosedale Beach Tidal Gauge (RB) (Figure 1).

Data Analysis

Tracking data were analyzed using MatLab software and fish position was plotted against DO concentration, location, and time (using altered MatLab scripts from Brady (2008)). Fish tracks were randomly jittered so that each detection could be seen on the MatLab plot. This approach provided an examination of the behavioral responses of summer flounder to DO dynamics, allowing assessment of detailed fish movements along the length of Pepper Creek during multiple days and diel DO cycles. To obtain a spatiotemporal depiction of DO in Pepper Creek, DO conditions were

interpolated using a piecewise cubic Hermite interpolating polynomial (PCHIP). The PCHIP method interpolates between two data points using less oscillation than spline interpolation, and does not create false minimums or maximums (i.e. minimums and maximums are based on the actual data). Dissolved oxygen plot coloring and classification was based on the EPA criterion for the growth ($\text{DO} > 4.8 \text{ mg O}_2/\text{l}$) and survival ($\text{DO} > 2.3 \text{ mg O}_2/\text{l}$) of aquatic organisms (US EPA 2000). Therefore, DO levels $\leq 4.8 \text{ mg O}_2/\text{l}$ are referred to here as “unhealthy” with a sub- classification of DO levels $\leq 2.3 \text{ mg O}_2/\text{l}$ considered severely hypoxic. Other abiotic conditions, such as temperature and salinity, were also assigned to each fish track using PCHIP when the detection time fell between the 15-minute intervals of the data sonde collection period. Insolation, precipitation, pressure, and tide height were assigned to each fish track using the abiotic value that corresponded to the nearest point in time to the fish detection. Also, if detections of the same fish were picked up by two different acoustic receivers within a 5 minute interval, the location of the fish was assumed to be mid-way between those two receivers.

Spatial and temporal gradients of DO, temperature, and salinity were solved for by determining the derivative of the PCHIP value at every fish detection. Thus, a negative spatial gradient meant that the target variable was higher upstream of the fish’s current location, and a negative temporal gradient meant that the target variable was currently decreasing with respect to time. The rate of change in tide height and barometric pressure was also calculated by solving for the derivative of the PCHIP interpolated tidal height and pressure, respectively. Thus, during ebb tides, the rate of tide height change was always negative while the opposite was true for flood

tides, and a negative rate of pressure meant that the barometric pressure was decreasing with respect to time and vice versa.

Binary logistic regression (BLR) was used to identify environmental variables that were significant predictors of summer flounder movement in Pepper Creek (Hosmer and Lemeshow 2000). Using two consecutive time points (t_1 and t_2), fish track movement was coded into binary form as follows: When a flounder at t_2 did not move from its previous location (into another receiver range) at t_1 , a value of 0 was assigned for that detection and it was referred to as a “stay put” detection. Alternatively, when the location of the flounder at t_2 differed from its location at t_1 , a value of 1 was assigned and it was referred to as either an “upstream movement” or “downstream movement”, depending on which direction the fish moved. Because one of the assumptions of BLR is that observations are independent, a time interval between detections had to be determined that would satisfy this assumption. To accomplish this, a detrended correlogram showing the autocorrelation between fish locations at a specified time (Y_t) and lag $Y_{(t+k)}$ was created for each individual PCHIP interpolated fish track. Correlograms are commonly used in time-series analyses to determine correlations among specific time lags (Zuur et al. 2007, Cowpertwait and Metcalfe 2009). Independence of detections was determined using the first lag number that fell within the 95% confidence interval range, giving evidence to support the null hypothesis that autocorrelation did not exist. The lag unit, or sampling interval, was 5 minutes. The median time lag for independence of flounder detections was 55 minutes. Using this result, a time lag of 1 hour was deemed sufficient and biologically reasonable for satisfying the assumption of independence of detections.

Thus individual fish tracks were filtered, starting with the initial detection and proceeding in hourly increments, removing only those detections that fell within the hourly interval from the dataset. Although this process drastically truncates the data (by 92%), it provides a statistically manageable dataset. The filtered dataset from this study included all movement detections from 15 summer flounder but censored one movement detection from 2 separate fish, 2 movement detections from 1 fish, and 12 movement detections from another fish (which also happened to be the most active flounder in the dataset). Therefore, it appears, that this data mining procedure is better suited for fish tracking studies involving less active species, such as flatfish, as opposed to more mobile species, like weakfish, where frequent movements may occur within a time lag and be lost during statistical analysis.

For model testing, these individual summer flounder tracks were placed into pre-determined groups based on exposure to specific DO concentrations and also fish age class. The following explains the 6 groups into which individual fish were placed for inclusion in each BLR model: 1) All fish, 2) Fish that were *released* into unhealthy DO conditions (≤ 4.8 mg O₂/l), 3) Fish that *encountered* unhealthy DO conditions after release (≤ 4.8 mg O₂/l), 4) Fish that experienced severe hypoxia (≤ 2.3 mg O₂/l), 5) Juvenile fish, 6) Adult fish.

Covariates in the BLR models that were used to predict movement included DO, temperature, insolation, the spatial gradients in DO (ndxdo) and temperature (ndxtemp), the temporal gradients in DO (ndtdo) and temperature (ndttemp), and the rate of tide height change (ntide). Barometric pressure and the rate of pressure change were excluded from the model because they did not show

significant change throughout the course of tracks. Salinity and the spatial gradient of salinity (ndxsal) were excluded from the model as all upstream movements would result in decreased salinity and vice versa. The rate of salinity change (ndtsal) was also excluded from the model as the variable would be correlated with the tidal covariate. Because the environmental variables were of different units and distributions, the covariates were standardized to their z-scores, a dimensionless unit, in order for easier comparison in the model output.

Two BLR models were run for each of the six groupings of fish. The first BLR model was used to identify environmental predictors of movement (regardless of direction). The logit transformation of the first BLR model was:

$$\text{logit}(Y_{\text{movement}}) = \ln [\pi/(1-\pi)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n \quad (1)$$

where $\text{logit}(Y_{\text{movement}})$ predicts the log odds of a “movement”, π is the probability of the “movement”, $1-\pi$ is the probability of “stay put”, α is the Y intercept, β s are the regression coefficients, and X s are the environmental predictors (or covariates). The second BLR model examined directionality, identifying environmental predictors of downstream and upstream movements. The logit transformation of the second BLR model was:

$$\text{logit}(Y_{\text{downstream}}) = \ln [\pi/(1-\pi)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n \quad (2)$$

where $\text{logit}(Y_{\text{downstream}})$ predicts the log odds of a “downstream movement”, π is the probability of a “downstream movement”, $1-\pi$ is the probability of an “upstream movement”, α is the Y intercept, β s are the regression coefficients, and X s are the environmental predictors (or covariates). In total, 12 BLR models were tested (with 6 examining overall movement and 6 examining directionality).

The maximum likelihood estimation method was used to calculate logit coefficients. For each model output, the coefficients used to predict the log odds (logit) of “movement” or “downstream movement” are represented as “B”s while the odds ratios are referred to as “OR”s. For the spatial and temporal covariates, a negative “B” means the probability of “movement” or “downstream movement” increases when the covariate value is negative and decreases when the covariate value is positive. Converting the probability to an odds ratio provides for easier comparison of covariate effects. Briefly, the odds ratio is e^B and is the factor by which the odds (of “movement” or “downstream movement”) changes for a one standard deviation (SD) change in the environmental predictor variable. Therefore, for each predictor variable, if the:

OR > 1, A one SD increase in the predictor variable increases the odds of a “movement” for BLR model 1, or “downstream movement” for BLR model 2.

OR = 1, predictor variable has no effect on movement.

OR < 1, A one SD increase in the predictor variable increases the odds of a “stay put” detection in BLR model 1, and increases

the odds of an “upstream movement” in BLR model 2. (The exact change in odds is calculated by taking the OR inverse).

To determine the effectiveness of the overall model, the likelihood ratio, Rao’s efficient score, and Wald chi-square tests were calculated. If all three tests showed significance ($p < 0.05$) then the model was considered an improvement over the intercept-only model, or null model, meaning at least one of the environmental covariates was significantly related to the movement variable. The Hosmer and Lemeshow chi-square test (H-L test) of goodness-of-fit was used to determine the significance of the overall fit of the covariates in the model against the actual outcomes. This test is recommended for data that consist of continuous covariates and small sample sizes (Garson 2009). A finding of non-significance ($p > 0.05$) means that the model adequately fits the data. For individual regression coefficients (β s), the Wald chi-square statistic was used with ($p < 0.05$) as an indicator of significance for a predictor of movement.

To determine usefulness of the logit, the degree to which the full model accurately predicts the response variable was compared to the intercept-only model using classification tables. For each logit, two classification tables were produced: one for the intercept-only model and one using the full model. The classification table using the intercept-only model classified all cases by assigning a level of the dependent variable (0 or 1) at random and weighting according to the number of cases in the levels. The sum of the squared marginal percentages was calculated and used as the “proportional by chance hit rate.” The classification table produced using the full model calculated the “observed hit rate” by classifying cases based on a predefined

probability of 0.50. (If the model predicted the probability of an event to be greater than 0.50, it classified the case as a “movement” in BLR model 1 or “downstream movement” in BLR model 2 and assigned it a “1”). A model that was deemed “good” gave an observed hit rate that was 20-25% better than the proportional by chance hit rate. (This percentage was arbitrarily chosen; 25% is the most common criteria for a “good” model (Garson 2009)).

Due to the reliance on a predefined probability cutpoint, and the subjectivity of how to qualify a “good” model, an additional test of model accuracy was employed. Hosmer and Lemeshow (2000) suggest looking at the area under the Receiver Operating Characteristic curve (ROC). The ROC curve plots the probability of detecting correct classifications of “movement” or “downstream movement” (referred to as “sensitivity”) and false classifications of “stay put” or “upstream movement” (referred to as “1-specificity”) over a range of possible probability cutpoints. The area under the curve can range from 0.5 (no discrimination, equivalent to chance classification rate) to 1 (ideal model with perfect classification) with the following guideline for determining the model’s discriminate ability:

ROC area = 0.5: no discrimination

$0.6 \leq \text{ROC area} < 0.7$ = fair discrimination

$0.7 \leq \text{ROC area} < 0.8$ = acceptable discrimination

$0.8 \leq \text{ROC area} < 0.9$ = excellent discrimination

ROC area ≥ 0.9 = outstanding discrimination

(Hosmer and Lemeshow 2000, Simon 2008)

Model building was accomplished in two ways since sample sizes sometimes limited the number of covariates allowed in the model. Hosmer and Lemeshow (2000) suggest 10 as the minimum number of cases (e.g. detections) per independent variable (e.g. environmental covariate). In other words, including two environmental covariates in a model would require a dataset with at least 20 detections. When applicable, all covariates were used in the model unless they showed multicollinearity. When there were < 10 cases per independent variable, another model was created with covariates chosen using the method of stepwise logistic regression.

Stepwise logistic regressions are only suggested for exploratory purposes since they tend to overfit the model to noise in the data (Garson 2009). The method of stepwise logistic regression automatically determines which environmental covariates to add or drop based on the maximum likelihood estimation (MLE). It computes the $-2 \log$ likelihood for a model and then re-estimates the $-2 \log$ likelihood for a new model after an environmental covariate has been added or removed. Stepwise logistic regression re-evaluates each altered model using the likelihood ratio statistic until deleting or adding another environmental covariate does not significantly improve the model. In this way, the variables that appeared to most significantly affect the model were included as covariates.

Fish that did not move during the course of the tracking period ($N=4$) were excluded from the analysis. In addition, the two fish released in 2007 were also excluded after a non-parametric multiple comparisons test (Behrens-Fisher and Steel-type as described in Munzel and Hothorn (2001)) revealed that the DO concentrations

experienced were significantly different ($p < 0.05$) than those experienced by fish in 2008 and 2009. The fish in 2007 were released late in the season, when diel-cycling hypoxia had relaxed, and therefore did not encounter the same low DO concentrations as the fish in 2008 and 2009. In addition, only 3 data sondes located at sites PC2, PC4, and PC5 were in use during the tracking period. Because of this, and the fact that the purpose of this study was to examine fine spatial movements in relation to diel-cycling hypoxia, the 2007 fish were not included as replicates for any of the BLR models but were included in non-BLR movement, residency time, and exposure analyses.

Residual analysis was conducted to determine outliers in the regression models. Standardized residuals > 2.58 were considered outliers at the $p = 0.01$ significance level. Outliers were excluded from the model only if exclusion increased the significance of the model and observed classification accuracy rate by 2% or more (Schwab 2005).

The amount of time that passed between flounder release and its first movement detection into another receiver range was quantified using the entire fish track dataset (i.e. not reduced to only periods of independent consecutive detections, see above). Differences between this time in first movement for fish released into unhealthy DO (≤ 4.8 mg O₂/l) and fish released into healthy DO (> 4.8 mg O₂/l) but later encounter unhealthy concentrations were explored using a *t*-test for independent samples. In addition, average residency time was calculated for these two groups of fish.

Duration of exposure to severe hypoxia and unhealthy DO conditions was quantified using the entire fish track dataset (i.e. not reduced to only periods of independent consecutive detections, see above). Duration of exposure for each flounder was calculated using segments of consecutive detections separated by a ≤ 30 -minute time interval. Most detections included in the analysis were separated by less than 5 minutes, but when the time interval increased to the maximum of 30 minutes, continued exposure duration was considered only if the location of the flounder did not change from the previous detection. (This method made the assumption that the fish had not moved during those 30 minutes and experienced the low DO during the entire time interval. The 30 minute maximum seemed a reasonable amount of time to allow for flounder movement into another receiver range). These duration segments were summed to calculate the total duration exposure for each individual flounder. This technique underestimates exposure duration since individual detections separated by more than 30 minutes were not considered as a continuous exposure to low DO (even if the fish did not move from its location) but as separate duration exposure segments. Also, individual detections that occurred during low DO but did not have any detection immediately before or after it were excluded from the analysis because it was not possible to determine exposure duration for that specific detection. The percent of residency time spent in unhealthy DO and severe hypoxia was calculated for each fish. A *t*-test for independent samples was conducted to determine differences in duration exposure for fish released into unhealthy DO and fish that encountered unhealthy DO concentrations, and also fish that experienced $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$ but $> 2.3 \text{ mg O}_2/\text{l}$ and fish that experienced severe hypoxia.

Although mortality could not be verified, analysis of fish tracks allowed speculation on survival. For example, if a fish was continuously detected at a receiver over multiple days, with detections separated by a time interval close to that of the acoustic receiver transmitter rate (i.e. 40-120s), the fish was considered stationary and likely dead. Also, exposure to severe hypoxia and subsequent lack of movement also suggested mortality.

Differences between adult and juvenile summer flounder residency time and low DO exposure were explored using a *t*-test for independent samples. Adult and juvenile activity patterns were compared using binary logistic regression models (as explained above) to determine if differences existed among the predictor variables for movement.

All statistical analyses were performed using SPSS software except the non-parametric multiple comparison test, which was performed using the R package.

RESULTS

Water Quality

Temperature, salinity, and DO conditions were monitored at sites PC2, PC4 and PC5 throughout the summers (May-September) of 2007, 2008, and 2009 (Figures 4-6). Temperature was generally highest at the upper tributary site (PC2) and decreased downstream toward the mouth. Mean temperatures at PC2 over the summer 2007, 2008, and 2009 seasons were 25.7°C, 26.9°C, and 26.1°C, with maximum temperatures reaching 32.5°C, 33.2°C, and 32.3°C, respectively. Mean temperatures at PC4 over the summer 2007, 2008, and 2009 seasons were 24.9°C, 26.8°C, and 25.9°C, with maximum temperatures reaching 32.1°C, 32.4°C, and 32.0°C, respectively. Mean temperatures at PC5 over the summer 2007, 2008, and 2009 seasons were 24.5°C, 26.0°C, and 25.8°C, with maximum temperatures reaching 31.2°C, 31.7°C, and 36.6°C, respectively. Average temperature at PC2, PC4, and PC5 over the three-year summer period was 26.2°C, 25.8°C, and 25.4°C, respectively.

Salinity ranged from 15-30, with drops in salinity evident following storm events (Figures 4-6). Salinity increased along the axis of the tributary, with PC2 having the lowest mean salinity (21.7) and PC5 having the highest (23.5) over the three-year summer period. From 2007-2009, salinity averaged 22.5 over all three sites.

DO concentrations showed the most daily variability among the environmental variables, ranging from 0 mg O₂/l (anoxia) to 23.5 mg O₂/l (super-saturated conditions). Severe diel-cycling hypoxia did not occur frequently until late May in 2007 and 2009, and early June in 2008 (Figures 4-6). DO dropped to anoxic conditions during these diel-cycling events at all three sites and in all three years. During the months when diel-cycling hypoxia was most severe (June-September), 2009 had the greatest number of occurrences of severe hypoxia, followed by 2008 and then 2007. In these months, DO fell below 2.3 mg O₂/l over 90% of the days in 2009, 68% in 2008, and 64% in 2007. The daily running average of DO at all three sites over the three-year summer period ranged from 0 mg O₂/l to 14.04 mg O₂/l (Figures 4-6) and frequently fell below the EPA DO criterion of 4.8 mg O₂/l for the safe growth of aquatic organisms. In fact, the daily running DO average also dropped below the EPA criterion of 2.3 mg O₂/l for the survival of aquatic organisms during all three summers. Compared to the other sites and years, PC2 in 2009 had the most occurrences of severe average DO concentrations (Figure 6d).

During the fish tracking experiment, temperature, salinity, and DO conditions were monitored at sites PC2, PC4, and PC5 in 2007 and at sites PC1-PC6 in 2008 and 2009. Analysis of temperature during the tracking period showed that flounder in 2009 experienced the greatest mean temperature (27.4°C), with a maximum reaching 29.0°C. The mean temperature experienced by the 2008 flounder was 25.1°C with a maximum of 27.7°C while in 2007 the mean temperature was 22.3°C with a maximum reaching 26.7°C. The lower temperatures late in the summer tracking season of 2007 may be the reason why those flounder rarely experienced

hypoxic concentrations. Examination of salinity revealed that flounder in 2009 experienced the lowest average salinity (21.1) and largest range (16.2-27.3). Fish in 2008 experienced an average salinity of 24.9 with range 21.8-28.2, while in 2007 salinity averaged 25.4 with range 21.4-28.6. With respect to dissolved oxygen levels, the fish released in 2009 experienced the widest range of DO concentrations, from 0 mg O₂/l to 14.4 mg O₂/l, and a mean of 6.53 mg O₂/l. The 2008 flounder endured the lowest average DO concentration (5.57 mg O₂/l) with DO ranging from 1.34 mg O₂/l to 11.87 mg O₂/l. In 2007, DO from detections ranged from 1.4 mg O₂/l to 15.8 mg O₂/l with an average of 7.68 mg O₂/l. Even though DO dropped to anoxic conditions during all three years, only those fish released in 2009 experienced anoxic conditions during the experimental tracking period.

Fish Tracking

A total of 4,385¹ fish detections were collected (see Table 1) with the majority of detections logged during daylight hours (0500 to 1900). Only 124 of the detections were movements, with summer flounder generally displaying their primarily sedentary nature during the experimental tracking period. Flounder movement occurred mostly during the dark hours (1900 to 0500), with 60% of movements detected during this time period. In 2007 and 2008, when fish were released in late morning and early afternoon (1000-1300), 35% of flounder movements occurred at night, with 42% of these movements during the early morning

¹ The fish “Cutty” was excluded from the detection count due to the uncertainty surrounding the exact date/time of the flounder’s mortality. Including this fish would have greatly overestimated the detection count (see Table 1).

hours (0100 to 0500). In 2009, when flounder were released in the late afternoon (1600 to 1800), 83% of movements occurred at night, with 29% of these movements during the early morning hours. On average, 36% of the flounder dark hour movements in Pepper Creek were made in the early morning, when DO was decreasing to its lowest concentration.

BLR Models Groups 1-4

Group 1 - All fish: Nineteen fish (206 detections) were included in this group (Tables 1 and 2; Table 2 excludes fish with no movement detections and the 2007 fish, see Methods). Of these detections, 133 were stay put and 73 were movement detections. The movement logit showed only fair discrimination (area under ROC curve = 0.69), meaning the model did not fit the data well. This is not surprising, as all fish were used in this model, including those that did not experience unhealthy DO or severely hypoxic conditions. Important predictors ($p < 0.05$) in the movement logit, in order of Wald chi-square significance, included: the temporal and spatial gradients in temperature, and temperature (Table 3). Fish were more likely to stay put than move at warmer and warming temperatures. A one standard deviation (SD) increase in the rate of temperature change nearly quadrupled the odds that the fish would remain in its location (inverse of 0.266; see OR column in Table 3). In addition, an increase of 1.4°C (one SD unit) of water temperature resulted in a 1.4-fold increase in the odds of a stay put detection (inverse of 0.723; see OR column in Table 3). Although increasing temperature lowers the solubility of DO, it also increases metabolic rate and aids in growth for juvenile summer flounder. Warm temperatures are one of the

reasons provided for why summer flounder migrate to estuaries during the spring and summer months. However, an SD increase in the spatial temperature gradient also increased the odds of remaining stationary by a factor of 1.4 (inverse of 0.696; see OR column in Table 3). Although flounders tolerated increases in water temperatures in Pepper Creek, they did not actively swim to warmer areas.

The BLR model that examined directionality of movement fit the data much better than did the movement model. The directional logit showed excellent discrimination (area under ROC curve = 0.85). Of the 73 movement detections, 60 were downstream and 13 were upstream (Table 2). Important covariates ($p < 0.05$) in the model, in order of Wald chi-square significance, were: the spatial gradient in DO, temperature, and the rate of tide height change (Table 4; some covariates were excluded during model-building due to multicollinearity or sample size restrictions, see Methods). Specifically, downstream movements followed increases in the spatial DO gradient with flounder 9 times more likely to move downstream than upstream along a positive spatial DO gradient (DO higher downstream of the flounder's location). The average DO gradient for downstream movements was 9.72×10^{-4} mg O_2 /l/m, compared to -2.18×10^{-3} mg O_2 /l/m for upstream movements. In addition, the probability of a downstream movement increased when the tide was ebbing, as evidenced by the sign of the ntide coefficient (Table 4). Upstream movements often occurred during the flooding tide (average rate of tidal height change = 2.9×10^{-3} m/min) compared to downstream movements, which coincided with the ebbing tide (average = -1.1×10^{-3} m/min). Temperature also played a role in predicting upstream versus downstream movement, with a 1.45°C (one SD unit) increase in water

temperature more than tripling the odds of an upstream over a downstream movement (Table 4).

One fish that was not included in this group was Aurora² (Table 1), since no movements were detected by the Pepper Creek receiver array. Aurora was released at PC3 on 7/20/09 into saturated (~8 mg O₂/l) DO conditions. The flounder was detected for an hour and a half before the detections ceased. Five days later Aurora was detected just outside Indian River Inlet (Figure 1) on an acoustic array deployed by Dr. Dewayne Fox from Delaware State University. Thus, this fish exhibited behavior consistent with fall emigration from the estuary, moving through the inlet en route offshore to winter on the continental shelf.

Groups 2 & 3 - Fish that were released in unhealthy DO conditions ($\leq 4.8\text{mg O}_2/\text{l}$) & fish that encountered unhealthy DO conditions after release: To investigate the effects of low DO on flounder movement, fish that never experienced unhealthy DO concentrations ($\leq 4.8\text{ mg O}_2/\text{l}$) were removed from the dataset. This new group included 16 fish (Table 2), however a review of the MatLab-produced movement plots indicated dramatic differences in behavior based on the timing of exposure to unhealthy DO. Analysis of tracks (using the entire (non-filtered) dataset, see Methods) revealed that flounder released into $\text{DO} \leq 4.8\text{ mg O}_2/\text{l}$ initiated movements significantly sooner ($p=0.015$) than the flounder released into higher DO conditions. Fish released into $\text{DO} \leq 4.8\text{ mg O}_2/\text{l}$ ($N = 6$) had an average time before first

² Sexes of the flounder were not determined; names used in this thesis were chosen purely for the author's and readers' entertainment.

movement of 2.5 hours (median = 2.5 h; range = 1.8 – 3.6 h) whereas fish released into higher DO conditions (N = 12; includes 2007 fish but excludes fish that never moved) did not initiate movement until an average of 9.4 hours after release (median = 4.8 h; range = 2.1 – 27.8 h). Average residency time also differed between these two fish groups, with those flounder released in unhealthy DO concentrations emigrating from Pepper Creek after an average of 1.2 days (median = 0.7 d; range = 6 h – 3.6 d, Table 1), while flounder released in DO concentrations > 4.8 mg O₂/l remained in Pepper Creek for an average of 4.3 days (median = 3.1 d; range = 8.9 h – 11.2 d, Table 1).

Flounder released into DO > 4.8 mg O₂/l remained relatively sedentary until confronted by encroaching unhealthy DO conditions (≤ 4.8 mg O₂/l) where DO was decreasing to severely hypoxic concentrations, a level that generally appeared to trigger movement (Figure 7). Because of this delayed response to DO > 2.3 mg O₂/l, initial movement of these fish from unhealthy DO conditions was not as immediate as it was for flounder released in waters of the same DO range (Figure 8). In other words, the fish that encountered DO ≤ 4.8 mg O₂/l (Figure 7) appeared to tolerate and remain in those conditions longer than those that were released into the same low DO concentrations (Figure 8). For example, Donkey (Figure 7a) was released into healthy DO conditions at site PC2 on 7/27/09 and ~40 minutes later was exposed to DO concentrations ≤ 4.8 mg O₂/l. This fish remained in the unhealthy DO waters, even experiencing some pockets of severe hypoxia (DO ≤ 2.3 mg O₂/l), staying at the release location for > 8.5 hours.

Two other flounder that were released into $\text{DO} > 4.8 \text{ mg O}_2/\text{l}$ also seemed to tolerate lower DO concentrations. Paula and Simon (Figures 7b and 7c) were released at site PC2 in the early afternoon on 9/08/08 and remained there for > 6 hours in healthy DO concentrations. Because DO had peaked about an hour after their release, DO was steadily declining, with the upper tributary the first to fall below $4.8 \text{ mg O}_2/\text{l}$. Paula spent a documented 1 hour 17 minutes in $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$ and Simon spent a documented 1 hour 19 minutes under these conditions, although this time may be underestimated. Both of these flounder were detected only at upper tributary sites from 9/08/08 – 9/09/08, and although there were no detections for over a 14 hour period for Paula and 9 hour period for Simon, there was a spatially extensive zone of $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$ which both flounder likely experienced during that whole time period. Instead of traveling downstream as soon as DO dropped below $4.8 \text{ mg O}_2/\text{l}$, both fish remained at the upper sites, exhibiting a passive strategy of little movement and potentially experiencing prolonged durations of unhealthy DO levels.

Summer flounder that encountered $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$ spent significantly more time ($p=0.016$) in these DO conditions compared to those fish that were released into them. Fish released into unhealthy DO conditions spent an average of 24 minutes (range 2 – 54 min) in $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$, while fish that encountered unhealthy DO concentrations spent an average ~ 43 minutes (range 1 – 222 min) in these levels.

Because of these marked differences in behavior, BLR models were run separately for these two groups of fish:

Group 2 - Fish that were released into unhealthy DO conditions ($\leq 4.8 \text{ mg O}_2/\text{l}$):

Six fish (from release batches 2 and 4; 44 detections) were included in this group (Tables 1 and 2). Of these detections, half were stay put and half were movement detections. A BLR model examining movement could not be fitted to the data, as the overall movement logit was not significant (i.e. the model was not an improvement over the intercept-only model, or null model, see Methods). However, because of their more immediate downstream movements, these flounder experienced significantly higher DO levels, lower temperatures, and higher salinities than did Group 3 fish (see Figure 9).

Comparison of upstream and downstream movements could not be evaluated as there was only one upstream movement detected. Being released in unhealthy DO conditions ($\leq 4.8 \text{ mg O}_2/\text{l}$) appeared to trigger a response in summer flounder to swim only downstream, toward deeper, more oxygenated waters. Flounder released in batch 2 were tracked moving downstream and down the spatial DO gradient, thus escaping the unhealthy DO area that spanned the upper portions of the creek (Figure 10). Fish released in batch 4 all made downstream movements on 9/11/08, on ebbing tides and during zero to positive spatial DO gradients (average = $1.4 \times 10^{-3} \text{ mg O}_2/\text{l/m}$) (Figure 11). Moving down the spatial DO gradient using ebb tidal flow toward deeper water allowed flounder fast and directed escape from the unhealthy DO into which these batches had been released.

Group 3 - Fish that encountered unhealthy DO conditions ($\leq 4.8 \text{ mg O}_2/\text{l}$) after release: Ten fish (154 detections) were included in this group (Tables 1 and 2). Of

these detections, 108 were stay put and 46 were movement detections. A BLR model predicting movement could not be fitted to the data, as the overall movement logit was not significant. However, the BLR model that examined directional movement fit the data extremely well (area under the ROC curve = 0.84). The environmental covariates of spatial DO gradient and rate of tide height change were significant discriminants between downstream and upstream movements (Table 5). A one SD increase in the spatial DO gradient resulted in a 3.6-fold increase in the probability of a downstream movement. Summer flounder commonly swam downstream along a positive DO gradient (DO higher downstream; average = 1.4×10^{-3} mg O₂/l/m) and upstream when the DO gradient was negative (average = -2.4×10^{-3} mg O₂/l/m), perhaps using spatial differences in DO to find waters of higher oxygen content. In addition to the spatial DO gradient, flounders used tide to influence their movements, as evidenced by the sign of the ntide coefficient (Table 5). On average, tide height decreased by -1.3×10^{-3} m/min during downstream movements while it increased by 2.6×10^{-3} m/min during upstream movements. In other words, flounder exhibited the use of selective tidal stream transport to assist during migrations in the tributary.

Use of the spatial DO gradient and tide helped flounder escape areas in which they encountered low DO. These two predictors of directional movement are illustrated in many of the Group 3 fish tracks. For example, flounder Paula appeared to use the spatial DO gradient to guide movement in the tributary (Figure 12). This was especially evident in the early morning on 9/09/08, when Paula, experiencing unhealthy DO at PC2, swam down the spatial DO gradient to PC1, making upstream

movements during a steep negative spatial DO gradient (DO higher upstream; average = -7.4×10^{-3} mg O₂/l/m) (Figure 12).

Another example of the above directional movement predictors is evident in the fish tracks of Flora and Fauna (Figure 13). These fish were released into super-saturated DO conditions (~14 mg O₂/l) at site PC2, and remained there while DO decreased to saturated conditions (~8 mg O₂/l). Although DO was decreasing steadily at their location (Figure 13b), the spatial DO gradient was negative (DO lower downstream), and so the flounder remained in an area of higher DO in the upper tributary. By early morning on 7/21/09, a spatially extensive unhealthy DO zone (≤ 4.8 mg O₂/l) had developed, with an area of severe hypoxia in the upper reaches of Pepper Creek (Figure 13). Flora and Fauna moved downstream from this area following the increase in the spatial DO gradient (as it transitioned from negative to positive), and exited the tributary on the early morning ebbing tide. Fauna was ultimately detected on Dr. Dewayne Fox's acoustic array just outside Indian River Inlet (Figure 1) on 11/28/09, indicating late fall emigration from the estuary.

In addition to Flora and Fauna, the flounder Fiona also demonstrated use of the directional movement environmental covariates. Following Fiona's release at site PC3, DO dropped to unhealthy and severely hypoxic levels along the entire length of the tributary (Figure 14). Fiona got caught in this spatially extensive low DO and approximately 2 days later was detected leaving Pepper Creek. As predicted by the BLR results for this group of fish, Fiona appeared to use the ebbing tide (rate of tide height change = -5.0×10^{-3} m/min) and positive spatial DO gradient (Figure 14b) to influence directional movement downstream. Fiona was also detected on Dr.

Dewayne Fox's acoustic array just outside Indian River Inlet (Figure 1) on 11/28/09, the same day as Fauna's departure, again indicating late fall emigration from the estuary.

Group 4 - Fish that experienced severely hypoxic DO conditions (≤ 2.3 mg O₂/l): To determine if severe hypoxia, in particular, increased the probability of movement, the dataset was filtered to include only those fish that experienced $\text{DO} \leq 2.3$ mg O₂/l. This group is a subset of the Group 3 fish. Four fish (100 detections) were included in this group (Tables 1 and 2). Of these detections, 75 were stay put and 25 were movement detections. The movement logit showed acceptable discrimination (area under ROC curve = 0.73), fitting the data reasonably well. There were two significant environmental predictors of movement: dissolved oxygen and the spatial temperature gradient (Table 6). By examining only fish that experienced severe hypoxia, results from the BLR model showed that increasing temperature at the flounder's location was no longer a significant predictor of a stay put detection, as it was for the general population of summer flounder (Group 1). Instead, the emphasis shifted to those environmental variables that assisted flounder with escape from and avoidance of unhealthy and severely hypoxic areas.

For flounder that experienced severe hypoxia conditions, a 2.40 mg O₂/l (one SD unit) increase in DO doubled the odds that the fish would move, as oppose to stay in its location, whereas an increase in the spatial temperature gradient (temperature increasing downstream) decreased the odds of movement (Table 6). The four fish that experienced severe hypoxia also spent a great deal of time in unhealthy

DO areas (see Figure 15), with an average of 3.98 hours in $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$ but $> 2.3 \text{ mg O}_2/\text{l}$ (range 1.7 hrs – 7.4 hrs) and 1.1 hours in severe hypoxia (range 9 min – 5.45 hrs). Therefore, as these fish endured severe hypoxia, they may have waited to elicit movements until DO increased, providing the fish with a chance to search the creek for higher DO. On the other hand, an increase in the spatial temperature gradient indicated higher temperatures downstream (and potentially less saturated oxygen available), and thus perhaps elicited the stay put response. In general, fish exposed to severe hypoxia were more likely to become active with increases in DO, expending energy for escape swimming while avoiding areas of higher temperatures, perhaps ultimately aiding the fish with locating waters with more favorable DO concentrations.

The BLR model that analyzed directional movement showed outstanding discrimination (area under the ROC curve = 0.91). Significant environmental predictors of directional movement were the spatial DO gradient and the rate of tide height change (Table 7). Not surprisingly, these are the same covariates as those from the directionality BLR model for fish that encountered unhealthy DO (Group 3). However, the probability of a directional movement based on increases in the environmental covariates was much more pronounced for fish that experienced severe hypoxia. Specifically, a one SD increase in the spatial DO gradient resulted in an incredible nearly 8-fold increase in the probability of a downstream movement versus an upstream movement (Table 7), as oppose to a 3.6-fold increase in Group 3 (Table 5). Enhanced use of the spatial DO gradient can greatly improve the chances of escape from severely hypoxic areas that are not spatially extensive.

In addition to using the spatial DO gradient to influence directional movement, summer flounder also appeared to employ selective tidal stream transport in the sense that when the fish chose to move, it followed the tidal flow. Specifically, the probability of a downstream movement increased with an increasingly negative rate of tide height change (Table 7). The flounder tracks illustrate that upstream movements generally occurred during flooding tides and downstream movements during ebbing tides (Figures 16, 17, and 18). However, movements influenced by tidal flow occasionally inhibited escape from lethal DO zones. This tidal entrapment is shown in the flounder Kevin's track (Figure 16). Kevin was the smallest juvenile tagged in 2009. Figure (16) shows the progression of Kevin's movements in relation to hypoxia, the spatial DO gradient, and the tidal flow. After release at site PC2 on 7/14/09, Kevin moved upstream on a flooding tide into severely hypoxic conditions. The fish experienced 26 minutes of severe hypoxia at PC1 before DO conditions relaxed and it swam, against the tide, downstream to PC2. As the tide transitioned to ebb-flow, Kevin moved further down the tributary, thereby escaping the severely hypoxic zone that developed at the upper sites on the morning of 7/15/09. Kevin was detected back at the upper site the following morning where the fish tolerated unhealthy DO concentrations (≤ 4.8 mg O₂/l). When the waters became severely hypoxic (≤ 2.3 mg O₂/l) Kevin moved downstream against the flow of tide during a positive spatial DO gradient, in an apparent attempt to escape low DO. The flounder was successful in reaching higher DO concentrations, but only for a couple hours before exhibiting a tidally-assisted movement upstream into dangerously low DO (Figure 16). In the early morning of 7/17/09, Kevin tolerated another unhealthy DO

zone and, as before, once the DO dropped to severely hypoxic concentrations Kevin moved downstream, swimming down the DO gradient and against the tide.

Unfortunately, just like the previous escape attempt, Kevin did not remain in these healthy DO conditions for long, moving back upstream, all the way to the severely hypoxic PC1 site, during a flooding tide. For Kevin, tide appeared to be an obstacle that prevented escape from severe hypoxia.

Brittany and Simon, two other flounders that experienced severe hypoxia, were able to detect the local spatial DO gradient and use the flow of tide to escape low DO waters. Brittany's track (Figure 17) illustrates the flounder in an area of severe hypoxia on the morning of 7/15/09. After enduring over an hour and half of unhealthy DO concentrations, with 40 minutes of that time spent in severe hypoxia, Brittany finally moved upstream with the flooding tide, remaining at site PC1 during a negative spatial DO gradient (DO higher upstream). The flounder stayed at PC1 until the tide changed to ebb (rate of tide height change = -3.3×10^{-3} m/min) and subsequently moved downstream to PC2 into a severe hypoxia zone (Figure 17). Brittany remained at PC2 over the tidal phase, enduring pockets of $\text{DO} \leq 2.3 \text{ mg O}_2/\text{l}$, instead of escaping downstream against tidal flow. On the following ebb tide, Brittany swam with the flow of tide and moved quickly downstream at a speed that peaked at 1 m/s (or ~ 4 Body Lengths (BL)/s), avoiding further exposure to severely hypoxic waters in the upper tributary. Thus, similarly to Kevin, Brittany followed an ebb tide into severely hypoxic waters, but was able to use the flow on the next tidal phase to escape the low DO area.

The flounder Simon (Figure 18) also used tidal flow to escape severe hypoxia. Simon was not very active (as evident in the lack of movement detections), even in unhealthy DO conditions, until DO dropped to severely hypoxic levels at his location. When this occurred on the morning of 9/09/08, and the spatial DO gradient transitioned from being predominantly negative to positive, Simon made multiple consecutive downstream movements on ebb tides, and escaped the severely hypoxic area (Figure 18). From these results, it appears that the spatial DO gradient and, for the most part, tidal flow assists summer flounder escape from areas of severe hypoxia. But for smaller flounder (e.g. Kevin), tide may also inhibit escape, especially when the flow is toward areas of lower DO.

Low DO Exposure

The amount of time a summer flounder spent in waters of unhealthy DO (≤ 4.8 mg O₂/l) and severe hypoxia conditions (≤ 2.3 mg O₂/l) was quantified (using the entire (non-filtered) dataset, see Methods) in terms of the length of individual duration segments (segments of time where the fish only experienced these DO conditions) and additive exposure (the sum of all duration segments) (Table 8). Eighteen of the 25 fish tracked (Table 1) were exposed to unhealthy DO conditions at some point during the tracking period. Duration segments where fish were only exposed to DO ≤ 4.8 mg O₂/l ranged from 1 minute to 222 minutes (3 hrs 42 min) with an average duration segment of 40 minutes and median of 29 minutes. Additive exposure duration to unhealthy DO conditions ranged from 3 minutes to 772 minutes

(12 hrs 52 min). With respect to residency time, summer flounder spent 0.1% to 20.9% of their time in Pepper Creek inhabiting waters with ≤ 4.8 mg O₂/l (Table 8).

Of the 18 fish that were exposed to unhealthy DO concentrations, 8 of them also experienced severe hypoxia (≤ 2.3 mg O₂/l). Severe hypoxia duration segments ranged from 3 minutes to 83 minutes (average duration segment = 22 min; median = 16 min). Additive exposure duration to severe hypoxia ranged from 9 minutes to 327 minutes (5 hrs 27 min), with an average of 1.1 hours. With respect to residency time, summer flounder spent 0.1% to 7.0% of their time in Pepper Creek inhabiting waters with ≤ 2.3 mg O₂/l (Table 8).

A comparison of the total duration exposure to unhealthy DO ≤ 4.8 mg O₂/l but > 2.3 mg O₂/l of the 10 fish that never experienced severe hypoxia and the 8 fish that did revealed that the severe hypoxia-exposed fish spent significantly more time in these unhealthy DO conditions ($p=0.001$). Specifically, the 8 severe hypoxia-exposed fish spent an average total of ~ 4 hours in DO ≤ 4.8 mg O₂/l but > 2.3 mg O₂/l, while the 10 fish that never experienced severe hypoxia spent an average of only 53 minutes in these DO conditions.

Mortality

There were 3 potential summer flounder mortalities (Figure 19). Two of these may have been the result of exposure to severe hypoxia whereas the third (Cutty) was probably surgery-induced. The two potential hypoxia-related deaths (Brittany and Puss) were classified as such because these fish failed to move after encountering severe hypoxia and were never detected exiting the tributary. Brittany (Figure 19a)

initially demonstrated active avoidance of severe hypoxia, but on 7/17/09 stopped moving and remained at site PC3, close to the edge of severe hypoxia for over 4 days. Before 7/17/09, this fish had experienced > 40 minutes of severe hypoxia, with DO concentrations falling to 0.5 mg O₂/l. The other flounder, Puss, was released on 7/27/09, at a site where DO was quickly dropping at rate of -0.12 mg O₂ l⁻¹ s⁻¹ (Figure 19b). This fish never exhibited movement, even when DO dropped to a potentially lethal level of 1.4 mg O₂/l on 7/28/09. Although it is unclear whether or not these were in fact hypoxia-related deaths, it seems improbable that either flounder, after enduring such low DO concentrations, would otherwise not eventually attempt to escape the tributary.

One summer flounder mortality was more definite (Cutty). This fish was released on 9/4/08 (Figure 19c). Immediately after release, Cutty was detected at site PC2 about every 2 minutes until the second ebbing tide when the fish was detected at PC3 about every 2 minutes. Whereas longer delays between detections imply the fish is moving around the creek, Cutty's detections occurred frequently, indicating that this fish was stationary and within receiver range. Cutty was detected at site PC3 continuously for 11 days, even during severely hypoxic DO conditions, a pretty clear indication of mortality. However, unlike Brittany and Puss, where low DO potentially caused mortality, Cutty's detections were coinciding with the receiver transmitter rate (every 40-120s) even before DO dropped to severe hypoxia. Instead, Cutty's mortality likely resulted from surgical implantation of the transmitter, or subsequent tag extrusion. All transmitters were the same weight, but Cutty was the smallest flounder tagged (Table 1), and the transmitter-to-body-weight ratio (3.04% body

weight) was the highest of the 25 tagged fish. The transmitter may have been too large for Cutty's body cavity, possibly explaining why some of the sutures ripped during the recovery period and had to be replaced before release. It appears that this flounder's small size and post-operation complications resulted in its mortality.

Adult Versus Juvenile Behavior

Difference in residency time between adult and juvenile summer flounder was determined using a *t*-test for independent samples. Mean residency time for adult summer flounder was 3.15 days while for juveniles it averaged 2.6 days. The *t*-test was not significant ($p=0.722$); therefore, there was no statistical difference in mean residency time between juvenile and adult summer flounder in Pepper Creek.

Duration of low DO exposure was compared for adult and juvenile summer flounder, also using a *t*-test for independent samples. The test compared duration of exposure to unhealthy DO conditions (≤ 4.8 mg O₂/l) between juvenile and adult flounder. The *t*-test was not significant ($p=0.979$); therefore, mean total exposure duration did not differ between the age groups. Average duration of exposure to DO levels ≤ 4.8 mg O₂/l was 40.1 min and 40.5 min for juvenile and adult summer flounder, respectively. A *t*-test for comparison of exposure duration to severe hypoxia (≤ 2.3 mg O₂/l) could not be used since there was only one adult that experienced severe hypoxia. This is in contrast to the juvenile group, where seven of the 17 flounder swam in severely hypoxic waters after release (Table 8). These results suggest that adult summer flounder prefer to avoid severe hypoxia concentrations more so than juveniles.

To compare activity patterns of juvenile and adult summer flounder, binary logistic regression models were run to examine potential differences in environmental predictors of movement:

BLR Models Groups 5-6

Group 5 - Juvenile fish: Twelve fish (153 detections) were included in this group (Tables 1 and 2). Of these 153 detections, 106 were stay put and 47 were movement detections. A BLR model predicting movement could not be fitted to the data, as the overall movement logit was not significant.

The BLR model predicting directionality showed excellent discrimination between upstream and downstream detections (area under ROC curve = 0.84). Of the 47 movement detections, 10 were upstream while 37 were downstream detections. The environmental covariates of rate of tide height change and the spatial DO gradient were significant predictors of directional movement (Table 9). On average, juvenile summer flounder made upstream movements during the flood tide (mean rate of tide height change = 4.2×10^{-3} m/min) whereas downstream movements occurred during ebbing tides (mean rate of tide height change = -2.0×10^{-3}). These results show that juvenile flounder use tidal flow to influence their directional swimming in the tributary, thus likely maximizing energetic efficiency during movement. Regarding the spatial DO gradient, a one SD increase in the spatial DO gradient tripled the odds of a downstream versus an upstream movement (Table 9). Thus, juvenile summer flounder may be able to sense the spatial DO gradient and, if tidal flow does not inhibit escape

from upstream hypoxic areas, use the DO gradient to influence directional movement toward higher oxygenated waters.

Group 6 - Adult fish: Seven fish (54 detections) were included in this group (Tables 1 and 2). Of these 54 detections, 29 were stay put and 25 were movement detections. A BLR model predicting movement could not be fitted to the data, as the overall movement logit was not significant. In addition, a comparison between upstream and downstream movements could not be evaluated as there were only two upstream movements detected.

DISCUSSION

The results from this study suggest that summer flounder movement in relation to diel-cycling hypoxia is mainly driven by the specific nature of the DO environment. In Pepper Creek, the type of behavioral strategy that a summer flounder chose depended on the spatiotemporal DO situation and the absolute DO concentration. Specifically, summer flounder actively responded (exhibited movement) to rapid drops in DO to unhealthy concentrations (≤ 4.8 mg O₂/l) but also to absolute DO levels of severe hypoxia (≤ 2.3 mg O₂/l), while they passively responded (remained sedentary) to gradually decreasing or spatially extensive DO concentrations > 2.3 mg O₂/l. Subsequently, exposure to unhealthy and severe hypoxia varied according to the strategy taken. Flounder that passively responded to decreasing DO spent significantly more amount of time in unhealthy and occasionally experienced severely hypoxic DO concentrations. Residency time also varied with respect to strategy, with flounder released in unhealthy DO concentrations initiating movement sooner (2.5 hours vs 9.4 hours) and exiting Pepper Creek faster (1.2 days vs 4.3 days) than flounder released in DO > 4.8 mg O₂/l.

A more detailed examination of summer flounder activity revealed temperature to be an important environmental predictor of overall movement while the spatial DO gradient and rate of tide height change were significant discriminants of directional movement. Specifically, the temperature variables helped distinguish

between movement and stay put detections, with increases in temperature correlating with inactivity. Summer flounder endured temporally warming waters in Pepper Creek but spatially did not actively seek higher water temperatures in the area. In a New Jersey estuary, summer flounder have also been documented remaining in rather narrow temperature ranges during their spring/summer residencies (Szedlmayer and Able 1993, Sackett et al. 2007, 2008). Optimal temperatures for growth in small juvenile summer flounder have been found to range between 21-24°C, with decreases in growth rate and efficiency at temperatures above this range (Packer et al. 1999, Howson 2000, Gaylord et al. 2004). Therefore, by remaining stationary during increases in the spatial temperature gradient, summer flounder perhaps avoided moving to sites where their growth may have been negatively impacted from high water temperatures.

However, since DO becomes less soluble in higher temperatures (WOW 2007), flounder may have also been avoiding areas in the creek with lower oxygen concentrations through detection of the DO gradient. For those fish that experienced unhealthy and severely hypoxic DO concentrations in Pepper Creek, an increase in the spatial DO gradient greatly favored downstream movement. The fish from batch 2 and batch 4 (Table 1, Figures 10 and 11), for example, all swam downstream along a zero to positive spatial DO gradient (DO higher downstream). Tyler (2004) speculated that juvenile summer flounder may have the ability to detect small spatial differences in DO, and use a DO gradient to find waters of higher oxygen content. In this study, juveniles but also adult summer flounder frequently swam down the DO

gradient into higher DO waters, using the gradient to influence the directionality and timing of their escape from lower DO waters.

In addition to the spatial DO gradient, summer flounder also exhibited the use of selective tidal stream transport. Summer flounder made the majority of upstream movements during flood tides and downstream movements during ebb tides, with 60% of these movements occurring at night. Similar patterns of increased summer flounder nighttime activity have been documented in the Wachapreague lagoon system in Virginia (Capossela 2010) and also in the Chesapeake Bay (Fabrizio et al. 2007). Since flounder are known to be visual feeders (Olla et al. 1972, Packer et al. 1999, Howson 2000), the reason behind the increased nighttime activity, at least in Pepper Creek, may be attributed to the early morning hypoxia occurrences and an active avoidance of these low DO areas through tidal movement. Selective tidal stream transport has been previously documented in juvenile summer flounder movement within other shallow estuaries (Wirjoatmodjo and Pitcher 1984, Rountree and Able 1992, Szedlmayer and Able 1993, Sackett et al. 2007). For example, in Schooner Creek, NJ, an estuary with physical characteristics similar to Pepper Creek, Szedlmayer and Able (1993) observed that 57% of juvenile summer flounder movements were in the same direction as the tidal flow, with fish detected further upstream during high tides as opposed to low tides.

By using both the spatial DO gradient and selective tidal stream transport to influence their movements, summer flounder were able to swim efficiently toward waters of higher DO within the estuary. In Pepper Creek, where environmental parameters change on an hourly basis, moving with the tide toward higher DO

conditions minimized the need for metabolic adjustments and oxygen-consuming swimming activity against tidal flow in order to escape hypoxia. However the use of selective tidal stream transport occasionally trapped smaller summer flounder in lethal DO conditions. This was especially true during spatially extensive hypoxia, when the flooding tide failed to advect higher DO water upstream. Kevin and Brittany were two flounder that illustrated movement into low DO conditions by following tidal flow into severely hypoxic areas. However, Kevin and Brittany were not the only fish to endure severe hypoxia.

In addition to tidal flow, another factor that may explain the presence of fish in severe hypoxia is their previous exposure to unhealthy DO concentrations > 2.3 mg O₂/l. The severe hypoxia-exposed fish were documented spending significantly more amount of time in DO ≤ 4.8 mg O₂/l but > 2.3 mg O₂/l compared to the fish from Group 3 that were able to completely avoid severely hypoxic concentrations (4 hours versus 53 minutes). For the Group 4 fish, failure to escape from severely hypoxic areas after prolonged exposure to unhealthy DO concentrations may be, to some degree, a result of hypoxia-induced exhaustion and disorientation. In laboratory experiments, recovery from low DO exposure has been shown to occur slowly for summer flounder acclimated to diel-cycling hypoxia (Brady and Targett in press). After > 4.5 hours of exposure to unhealthy DO (≤ 4.8 mg O₂/l), with about half of that time spent in severely hypoxic conditions (≤ 2.3 mg O₂/l), juvenile summer flounder were shown to be disoriented, exhibit extremely uncorrelated (random) movements, and slow to resume normal swimming activity until DO climbed above 4.2 mg O₂/l (Brady and Targett in press).

As flounder experience stressful DO levels, they allocate available energy resources toward primary respiratory metabolism, consuming energy that could have been used for other physical activity, such as escape swimming. For example, Tallqvist et al. (1999) observed that juvenile European flounder (*Platichthys flesus*) increased ventilation rate as DO dropped below 40% saturation. Below 30%, their predation efficiency significantly decreased (Tallqvist et al. 1999), implying a reduction in routine activities to conserve energy and oxygen. In the present study, Brittany appeared to exhibit a similar hypoxia-induced reduction in physical activity. After exposure to DO concentrations ≤ 4.8 mg O₂/l for ~2.5 hours, with about half of that time spent in severe hypoxia, this summer flounder appeared to lack the required energy to escape from low DO by swimming against the tidal flow.

Prolonged exposure to insufficient DO can also lead to disorientation and a lack of responsiveness. The golden grey mullet (*Liza aurata*) and European sea bass (*Dicentrarchus labrax*) show a significant decrease in responsiveness (startle response) in water with only 10% oxygen saturation compared to normoxic conditions (Lefrancois et al. 2005, Lefrancois and Domenici 2006), and they lose their left/right orientation under hypoxia (at $\leq 20\%$ O₂ saturation for golden grey mullet, and $\leq 50\%$ O₂ saturation for European sea bass). In the present study, summer flounder Kevin appeared to exhibit hypoxia-induced disorientation after > 3 hours exposure to unhealthy and severely hypoxic DO. This fish attempted to move from an area of severe hypoxia with a burst of swimming that peaked at 2 m/s (or 8 BL/s) against the flow of tide. This energy and oxygen-consuming escape response may have proven

too metabolically costly, as Kevin, after reaching DO-saturated waters, proceeded to move back upstream into severe hypoxia during the following flood tide.

The overall reaction of aquatic organisms to hypoxia is often described in terms of whether it is a primarily active or passive response (Chapman and McKenzie 2009). When an animal is exposed to decreasing DO, does it exhibit an active response, such as increased swimming, or a passive response, such as reduced metabolic rate? In a review of spontaneous swimming activity in hypoxia, Chapman and McKenzie (2009) concluded that active responses occur more frequently in pelagic, schooling, fishes whereas slower moving, benthic, fishes tend to take a primarily passive approach. However, these authors reported that many species employ both types of responses, and suggested the approach depends on the extent and duration of hypoxia. An active response would allow fish to escape from pockets of hypoxia encountered during routine activity, whereas a passive response would allow fish to conserve energy for respiratory metabolism if escape from low DO was impossible. Herbert and Steffenson (2005) found that Atlantic cod (*Gadus morhua*) exhibited this dual behavior when exposed to a stepwise decrease (lasting 30 min) in dissolved oxygen with intervals of steady (unchanging) DO levels after each step. In the steady low DO state, indicative of spatially extensive or long-term hypoxia, cod favored the passive approach, significantly reducing their swimming speed by up to 41%. However during the initial 30 minute progressive decline in DO, indicative of diel-cycling hypoxia or quickly changing DO conditions, cod exhibited an active response, increasing swimming speed by 18%.

Results from the present study suggest that summer flounder also use a combination of active and passive strategies in response to different spatiotemporal DO conditions and at different absolute DO concentrations. All summer flounder actively responded to absolute DO levels ≤ 2.3 mg O₂/l, but when DO concentrations were above 2.3 mg O₂/l, the strategy a flounder employed was driven by the rate of DO change and the spatial extent of unhealthy DO concentrations. In particular, summer flounder exhibited an active response to DO concentrations as high as 4.8 mg O₂/l but generally only when released in these conditions (Group 2, Table 2), otherwise flounder exhibited a more passive strategy when encountering DO levels above 2.3 mg O₂/l (Group 3, Table 2).

All summer flounder that were released in DO ≤ 4.8 mg O₂/l (batches 2 and 4 in 2008, Group 2) had been previously held for over a week in air-saturated DO conditions. Thus, upon release, they experienced a rapid drop in DO into unhealthy levels and subsequently avoided these DO conditions, moving quickly away from the release site and spending significantly less amount of time in DO ≤ 4.8 mg O₂/l compared to other flounder (e.g. Group 3, Table 2). While the active avoidance response may have been influenced by the lack of an acclimation period in 2008, summer flounder in batch 3 that were also released in 2008 but into higher DO concentrations (> 4.8 mg O₂/l) remained stationary for a significantly longer period of time before initiating their first movements in Pepper Creek. Therefore, it is more likely that the immediate drop in dissolved oxygen, specifically to DO levels that impact growth, triggered the early active response to increase movement toward higher DO concentrations.

In a review of hypoxia impacts, Breitburg (2002) concluded that fish commonly avoid DO concentrations that are 2-3 times higher than those levels that cause 50% mortality in 24 – 96 hour exposure durations, and that these avoided DO levels are also about equal to the levels that inhibit growth for the fish. Interestingly, summer flounder in the present study avoided DO concentrations of ~ 4.8 mg O₂/l, which is approximately 3 times higher than the 24 hr LC50 for juvenile summer flounder (estimated at 1.1-1.6 mg O₂/l (Poucher and Coiro 1997)). Additionally, in the laboratory, a significant reduction in juvenile summer flounder growth rate has been shown to occur at DO levels of 3.5 mg O₂/l in temperatures of 20°C, 25°C, and 30°C, and at 5 mg O₂/l in temperatures of 30°C over a 2-week period (Stierhoff et al. 2006). These temperatures and growth-reducing DO levels are commonly found in Pepper Creek and other estuarine tributaries during spring and summer months.

Attempting to steer clear of $DO \leq 4.8$ mg O₂/l can help summer flounder avoid DO concentrations that constrain growth. In laboratory experiments, summer flounder have been shown to initiate this active response; increasing swimming activity as DO levels drop from 5.6 to 4.2 mg O₂/l (Brady and Targett in press). In New Jersey estuaries, summer flounder have been tracked remaining in rather narrow DO limits, with averages greater than 4.8 mg O₂/l (Szedlmayer and Able 1993, Sackett et al. 2008). Although collectively these findings indicate that DO levels as high as 4.8 mg O₂/l can elicit an active avoidance, as seen in this study, this strategy is not completely effective for avoiding all growth-reducing DO levels. Summer flounder that used this strategy still occasionally experienced unhealthy DO concentrations, especially when the DO was spatially extensive, or when tidal flow was in the

opposite direction of higher DO. Consequently, Stierhoff et al. (2009) found that wild-caught juvenile summer flounder showed lower than expected (compared to laboratory-calculated) growth rates based on RNA:DNA in Pepper Creek.

However, another reasonable explanation for the lower growth rates of free-ranging flounder may be attributed to the increased duration of exposure to DO levels $> 2.3 \text{ mg O}_2/\text{l}$ for those flounder that choose a passive strategy in Pepper Creek. The summer flounder that took the primarily passive approach in unhealthy DO concentrations did so after experiencing a gradual, as opposed to sudden, decrease in DO from conditions $> 4.8 \text{ mg O}_2/\text{l}$, and additionally remained sedentary when the unhealthy DO areas were spatially extensive. Donkey, Paula, and Simon are examples of summer flounder that demonstrated this passive strategy. Tyler (2004) observed sedentary behavior of juvenile summer flounder in the laboratory in unhealthy DO concentrations. In DO choice trials, Tyler (2004) found that steady unhealthy DO concentrations as low as $2 \text{ mg O}_2/\text{l}$, indicative of spatially extensive hypoxia, failed to elicit an avoidance response in sedentary summer flounder.

Other benthic flatfishes that have also been shown to exhibit a passive response to gradually decreasing DO and spatially extensive low DO concentrations, include the common sole (*Solea solea*), winter flounder (*Pseudopleuronectes americanus*), and southern flounder (*Paralichthys lethostigma*). In a study by Dalla Via et al. (1998), sole reduced routine activity as DO decreased from 80 to 20% air-saturation. McEnroe and Krozlowitz (1997) found no significant increase in activity levels of juvenile winter flounder as DO concentrations were decreased at a rate of $1 \text{ mg O}_2/\text{l/hr}$ from normoxia to $3 \text{ mg O}_2/\text{l}$. The number of horizontal movements was

also lower for the period of DO decline from 8 to 4 mg O₂/l versus those in 4 to 2 mg O₂/l, suggesting that winter flounder also choose a passive strategy to conserve energy during slowly decreasing DO concentrations from saturation to unhealthy levels. In diel-cycling DO conditions (2.8 – 6.2 mg O₂/l), the southern flounder, a species closely related to summer flounder, was shown to significantly reduce routine oxygen consumption as DO decreased from the day time oxygen levels of 6.2 mg O₂/l to the night time levels of 2.8 mg O₂/l conditions (Taylor and Miller 2001). By decreasing physical and routine respiratory activity as DO decreases from healthy concentrations, benthic fish can reserve energy for primary metabolic processes in order to survive DO concentrations > 2.3 mg O₂/l.

However, when unhealthy DO concentrations decline into the severely hypoxic range (≤ 2.3 mg O₂/l), summer flounder exhibit an active escape response. The outcome of such a response is commonly observed in the field, with the marked decrease and absence of summer flounder, and many other fish species, in regions where the absolute DO concentration is below ~ 2 mg O₂/l (Howell and Simpson 1994, Bell and Eggleston 2005, Tyler and Target 2007). In the present study, Paula and Simon, who employed a passive strategy during tracking at DO concentrations > 2.3 mg O₂/l, exhibited this active escape response to severe hypoxia. Paula, who likely experienced > 14 hours of unhealthy DO concentrations, spent only 9 minutes in severe hypoxia (where DO dropped to a minimum of 2.1 mg O₂/l) before moving out of the tributary. Simon, who likely experienced > 11 hours in unhealthy DO waters, spent just a little over 1 hour in spatially severe hypoxia (where DO dropped to a

minimum of 1.68 mg O₂/l) before following an ebb tide downstream to DO levels higher than 2.3 mg O₂/l.

An active escape response in summer flounder has been documented at similar absolute levels of hypoxia in the laboratory. Brady and Targett (in press) measured the swimming speed of juvenile summer flounder exposed to decreasing dissolved oxygen after initially acclimating the fish to either air-saturation or diel-cycling hypoxia conditions. Results showed that although summer flounder increased swimming speed with decreasing DO, speed did not reach a maximum until DO fell to 2.8 mg O₂/l for diel-cycling hypoxia acclimated flounder and 1.4 mg O₂/l for air-saturation acclimated fish. Furthermore, fish also demonstrated three distinct bouts of acceleration at DO levels of 2.8, 0.8, and 0.4 mg O₂/l. From the tracking data in the present study, those flounder that passively responded to unhealthy DO levels initiated an active response at absolute DO levels similar to this general range of values. Flounder in the field swam downstream when DO levels fell between 2.1 and 0.44 mg O₂/l; however, it appears that the fish did not gradually increase swimming speed as DO decreased from normoxia. Instead, the fish remained at location during the diel decline in DO, and increased swimming speed only when the absolute DO concentration fell to severely hypoxic levels (≤ 2.3 mg O₂/l). This hypoxia-induced activity is similar to the behavior exhibited by the air-saturation acclimated summer flounder that did not survive low DO exposure in the Brady and Targett (in press) experiments. Of the 13 air-saturation acclimated flounder, 23% died during the low DO treatment. The authors attribute this mortality to the fishes delayed active response whereby the fish coupled peak swimming speed with highly stressful

hypoxic conditions. Instead of increasing swimming speed with decreasing DO, these non-survivors became active only when DO fell from 2.8 to 1.4 mg O₂/l, potentially increasing their susceptibility to hypoxia-induced exhaustion. Of the 8 summer flounder that experienced severe hypoxia in the current study, 25% were concluded to have died due to severe hypoxia exposure during the tracking period. These laboratory findings and tracking results show that the passive response strategy to progressively declining DO > 2.3 mg O₂/l may be risky and can lead to mortality. By decreasing physical activity, summer flounder may conserve energy to partially compensate for the growth-reducing effects of unhealthy DO, however if levels continue to decrease, flounder may not be able to initiate sufficient high-energy swimming to escape severe hypoxia.

A comparison of adult and juvenile summer flounder tracks showed that life stage did not affect residency time in Pepper Creek, nor did it distinguish between environmental predictors of movement or directionality. However, the comparison of low DO exposure duration for juveniles and adults provided insight into potential life stage specific hypoxia-avoidance strategies. With the exception of one (Simon), none of the adult summer flounder endured severely hypoxic concentrations whereas 41% of juveniles released spent time in DO ≤ 2.3 mg O₂/l, suggesting adult flounder may take a more active approach to decreases in DO levels, or avoid a higher absolute DO concentration compared to juvenile flounder. This age-based preference of strategy seems counter-intuitive given the growth-reducing effects of low DO. For juvenile summer flounder, exposure to diel-cycling hypoxia can severely stunt growth. Stierhoff et al. (2006) found that standard growth rate for juvenile summer flounder

was significantly reduced (~35%) when flounder were exposed to 14 days of diel-cycling hypoxia (2-11 mg O₂/l). One juvenile even died during the diel-cycling hypoxia treatment at 25°C. However, smaller juvenile fish, compared to larger fish, may better withstand decreases in DO in high water temperatures because of physiological differences in oxygen demands (Voyer and Morrison 1971). Generally, larger fish species have higher lethal DO levels than smaller fish species (Robb and Abrahams 2002, Robb and Abrahams 2003, Shoji et al. 2005), but very few studies have examined DO tolerance of juveniles versus adults within a species. Further study on the oxygen demand requirements of adult summer flounder in decreasing DO is needed to support this concept.

Because the summer flounder tracking in the present study was conducted on a very fine temporal and spatial scale, it is difficult to compare the results to other field tracking studies, where the focus has been on larger-scale migration triggers with environmental data averaged over periods of time (Szedlmayer and Able 1993, Fabrizio et al. 1997, Sackett et al. 2007, 2008, Caposella 2010). Generally, from these tracking studies, it appears that with respect to DO, summer flounder consistently avoid absolute concentrations < ~ 2.3 mg O₂/l. For example, summer flounder in New Jersey were tracked remaining in relatively narrow DO values, specifically concentrating in waters of DO around 7 mg O₂/l (Sackett et al. 2008) and never found in DO < 2.4 mg O₂/l (Szedlmayer and Able 1993). Also, although this study was not investigating emigration patterns, two of the summer flounder (Fiona and Fauna) were detected moving offshore on November 28th, an unusually late emigration for flounder in the Delaware Coastal Bays. Most studies examining summer flounder behavior in

New Jersey and Delaware estuaries found that emigration occurs mainly during the late summer and early fall (Smith and Daiber 1977, Szedlmayer et al. 1992, Able and Kaiser 1994, Sackett et al. 2007). Even in water bodies further south of Delaware, where timing of emigration occurs later, Fabrizio et al. (1997) and Caposella (2010) documented 50% of emigrating flounder leaving the Chesapeake Bay, MD and Wachapreague lagoon, VA by August 26th and November 11th, respectively.

Many environmental variables have been linked to summer flounder emigration including temperature, dissolved oxygen and changes in barometric pressure (Sackett et al. 2007). In a New Jersey estuary, Sackett et al. (2007) found a strong negative correlation between the change in barometric pressure and the number of summer flounder emigrating offshore from the estuary. A change in barometric pressure might explain Fiona and Fauna's emigration, as the day before the flounder were detected on Dr. Dewayne Fox's acoustic array, the barometric pressure dropped to a monthly low of 999 mbars, signaling an upcoming storm. Regardless of the trigger, this late emigration is evidence that juvenile summer flounder may inhabit the Delaware Coastal Bays for longer periods of time than previously thought.

From 2006-2008, movements of juvenile weakfish (a sciaenid) in Pepper Creek were documented, using similar protocols as in the present study (Brady 2008), allowing for a behavioral comparison of another estuary-dependent fish species to summer flounder. Results from both tracking studies (Brady 2008 and the present study) reveal that weakfish and summer flounder share many similarities in their response to diel-cycling DO, however they differ in the absolute DO concentrations that trigger an active movement response. Summer flounder released into unhealthy

DO conditions (≤ 4.8 mg O₂/l) generally avoided DO levels as high as 4.8 mg O₂/l, whereas weakfish released in these concentrations commonly remained in them. It was not until DO levels fell to ≤ 2.8 mg O₂/l that weakfish began swimming downstream, with some laboratory-acclimated weakfish to diel-cycling hypoxia becoming active only when DO fell below 1.4 mg O₂/l (Brady 2008). None of the weakfish tracked experienced DO below 1.4 mg O₂/l. On the other hand, summer flounder that initially took a passive approach to decreasing DO actively responded to DO when it fell ≤ 2.3 mg O₂/l, an absolute DO level about 1 mg O₂/l higher than the threshold for the hypoxia acclimated weakfish. However, given the slower swimming speeds of flounder compared to weakfish (Brady et al. 2009, Brady and Targett in press), some of the summer flounder that actively responded to severe hypoxia still endured concentrations below 2.3 mg O₂/l, even as low as 0.44 mg O₂/l, before escaping the tributary. So it appears that by delaying activity until DO drops into the range of severe hypoxia, summer flounder will occasionally experience lower DO levels (< 1.4 mg O₂/l) than weakfish.

Ultimately, the DO levels that were generally avoided (≤ 4.8 mg O₂/l for summer flounder vs ≤ 2.8 mg O₂/l for weakfish) or sparked an active escape response (≤ 2.3 mg O₂/l for summer flounder vs ≤ 1.4 mg O₂/l for hypoxia-acclimated weakfish) were about twice as high for summer flounder as those levels for weakfish. This finding is not surprising given the nature of these two species and the difference in the levels of hypoxia that negatively affect their growth rate. In Pepper Creek, summer flounder were mainly sedentary, with an average of 5 movements/fish done mostly as a means of escaping unhealthy and hypoxic DO concentrations. Summer

flounder rarely moved back into areas where they previously experienced DO levels detrimental to growth and survival. Instead, diel-cycling hypoxia events appeared to trigger summer flounder movement permanently out of Pepper Creek. Weakfish, on the other hand, exhibited many up and downstream movements during and after hypoxic events, with an average of 16 movements/fish (Brady 2008). Under laboratory conditions, Stierhoff et al. (2009b) discovered that juvenile weakfish growth was not affected by hypoxia (down to levels of 2 mg O₂/l), which may explain why weakfish commonly swam along the hypoxic edge in Pepper Creek (Brady 2008), but in the wild, weakfish have been found with lower than expected growth rates (Stierhoff et al. 2009a). To perhaps compensate for the reduced growth, weakfish frequently return to forage in areas where they previously experienced hypoxic conditions (Tuzzolino 2008). In other words, diel-cycling hypoxia only temporarily compresses weakfish optimal habitats, whereas it permanently influences summer flounder movement out of productive estuarine nursery grounds.

With respect to predictors of movement and direction, results from the tracking studies in Pepper Creek revealed that both species appear to have the ability to detect the local spatial DO gradient. Summer flounder, especially those confronted by unhealthy and severely hypoxic DO conditions, relied primarily on the spatial DO gradient for directed escape from the tributary to avoid low DO conditions. Weakfish exhibited the same reliance on the spatial DO gradient to relocate to areas of higher oxygen content, commonly swimming to the upper sites during negative spatial DO gradients and vice versa (Brady 2008). Furthermore, in laboratory experiments weakfish and summer flounder have both shown an ability to distinguish between

small (separated by 1 mg O₂/l) differences in DO concentrations (Tyler 2004, Stierhoff et al. 2009b).

In addition, both species, and including spot (which were also tagged and released with weakfish in the Brady 2008 study), were documented using the tide to move directionally in the tributary, avoiding low DO areas. The flounders Simon and Brittany are two examples of fish that escaped spatially extensive severe hypoxia by swimming downstream along ebb tides during positive spatial DO gradients. In the Brady (2008) study, the active escape response by weakfish and spot was commonly exhibited during ebbing tides in decreasing DO, allowing fish to move tidally downstream into higher oxygenated waters. However, use of selective tidal stream transport was also shown to sometimes trap smaller fish in severely hypoxic areas. For example, the flounder Kevin in the present study made multiple tidally-induced movements into severely hypoxic areas after failing to escape these areas by swimming against tidal flow. Likewise, in the Brady (2008) study, a smaller spot also became trapped in hypoxic DO, remaining at the mouth of the tributary during a flood tide and thus enduring DO concentrations ≤ 2.8 mg O₂/l.

Findings from the present study suggest that an increase in the occurrence and severity of diel-cycling hypoxia may result in an increase in hypoxia-induced mortalities and/or significantly reduced growth rates for summer flounder in Pepper Creek. For those summer flounder that actively avoid growth-limiting DO concentrations (≤ 4.8 mg O₂/l), an increase in diel-cycling hypoxia events may lead to a general decline in usage of such nursery habitats. On the other hand, for those flounder that initiate active responses only when absolute DO concentrations fall ≤ 2.3

mg O₂/l, an increase in severe hypoxia events would have significant negative effects on growth and survival. Ultimately these fish will be forced to utilize more open bay habitats, less impacted by diel-cycling hypoxia, for longer seasonal durations. This trend is already evident in the Delaware Coastal Bays (as documented in this study but see also Tyler 2004, Tuzzolino 2008). In these deeper estuarine waters, where there is an increased abundance of large piscivorous fish (Paterson and Whitfield 2000), smaller summer flounder would likely become more susceptible to predation. Therefore, studies on predation and growth rates in open bay habitats relative to tidal tributaries are needed to further assess the potential impacts of increased occurrence and severity of diel-cycling hypoxia on summer flounder at the population level.

Table 1 Fish, batch number, age, wet weight, standard and total length, tag ID, and detection information for fish released from 2007-2009 in Pepper Creek.

Fish	Batch	Age	Weight (g)	TL (mm)	Tag ID	Release Date (1st Detected)	# Detections	Last Detected	Residency Time
Phil	1	Juvenile	N/A	215	6638	9/19/2007 (10:57)	568	9/22/2007 (13:02)	3d 2:05
Stan	1	Juvenile	N/A	210	6639	9/19/2007 (11:01)	809	9/30/2007 (16:40)	11d 5:39
Carl	2	Juvenile	79.1	218	2398	9/4/2008 (10:25)	52	9/4/2008 (16:19)	5:54
Cappie	2	Juvenile	68.4	208	2397	9/4/2008 (10:24)	86	9/6/2008 (5:11)	1d 18:47
Cutty	2	Juvenile	59.2	197	2396	9/4/2008 (10:26)	9803	9/15/2008 (9:47)	10d 23:21
Cougar	2	Adult	224.5	348	317	9/4/2008 (10:25)	118	9/5/2008 (7:40)	21:15
Paula	3	Juvenile	107.9	236	310	9/8/2008 (13:27)	216	9/17/2008 (1:11)	8d 11:44
Simon	3	Adult	299.8	385	318	9/8/2008 (13:25)	241	9/15/2008 (20:16)	7d 6:51
Porky	3	Adult	489.1	447	2399	9/8/2008 (13:27)	56	9/8/2008 (15:42)	2:15
PeeWee	3	Adult	234.7	366	6483	9/8/2008 (13:26)	47	9/17/2008 (12:21)	8d 22:55
Flipper	4	Adult	331.2	385	6485	9/11/2008 (10:07)	90	9/14/2008 (23:38)	3d 13:31
Frank	4	Adult	293.4	367	6484	9/11/2008 (10:03)	119	9/11/2008 (17:21)	7:18
Flappy	4	Adult	355.0	410	6486	9/11/2008 (10:03)	49	9/12/2008 (0:08)	14:05
Brittany	5	Juvenile	145.3	270	52876	7/14/2009 (17:08)	484	7/22/2009 (3:56)	7d 10:47
Kevin	5	Juvenile	114.8	279	58855	7/14/2009 (17:12)	675	7/17/2009 (23:28)	3d 6:15
Minnie	5	Juvenile	133.8	290	58856	7/14/2009 (17:13)	88	7/15/2009 (20:58)	1d 3:44
Mickey	5	Juvenile	124.9	257	58850	7/14/2009 (17:14)	38	7/15/2009 (3:54)	10:39
Flora	6	Juvenile	132.5	291	58852	7/20/2009 (17:07)	77	7/21/2009 (2:01)	8:54
Fauna	6	Juvenile	158.7	298	58857	7/20/2009 (17:06)	56	7/21/2009 (2:52)	9:45
Merryweather	6	Juvenile	135.2	295	58851	7/20/2009 (17:02)	16	7/20/2009 (17:50)	0:47
Aurora	6	Juvenile	138.6	290	58858	7/20/2009 (16:56)	62	7/20/2009 (18:26)	1:30
Shrek	7	Juvenile	222.1	308	58853	7/27/2009 (16:36)	106	7/29/2009 (4:22)	1d 11:46
Fiona	7	Juvenile	218.2	312	58854	7/27/2009 (16:35)	88	7/30/2009 (19:51)	3d 3:15
Puss	7	Juvenile	233.1	353	58859	7/27/2009 (16:33)	73	7/28/2009 (5:27)	12:53
Donkey	7	Juvenile	163.3	288	58860	7/27/2009 (16:35)	171	7/28/2009 (16:17)	23:41

Table 2 **Number of detections (after dataset was filtered for independent observations) and binary logistic regression (BLR) model group inclusion for each summer flounder.**

Fish	Movement Logit # Detections		Directionality Logit # Detections		BLR Model Group Inclusion					
	Stay Put	Movement	Upstream	Downstream	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6
Carl	1	3	0	3	X	X			X	
Cappie	4	3	0	3	X	X			X	
Cougar	6	4	0	4	X	X				X
Paula	13	7	2	5	X		X		X	
Simon	9	6	2	4	X		X	X		X
Porky	1	1	0	1	X					X
PeeWee	1	3	0	3	X					X
Flipper	7	5	1	4	X	X				X
Frank	2	3	0	3	X	X				X
Flappy	2	4	0	4	X	X				X
Brittany	34	5	2	3	X		X	X	X	
Kevin	26	14	4	10	X		X	X	X	
Minnie	9	1	0	1	X		X		X	
Mickey	1	1	0	1	X				X	
Flora	3	2	0	2	X		X		X	
Fauna	2	3	0	3	X		X		X	
Shrek	3	5	2	3	X		X		X	
Fiona	3	2	0	2	X		X		X	
Donkey	6	1	0	1	X		X	X	X	
TOTAL	133	73	13	60						

Table 3 **Group 1 – All fish. Binary logistic regression results for the movement logit, including regression coefficients (B), Wald chi-squares, significance values (p), and odds ratios (OR). For spatial and temporal predictors, a negative B indicates an increase in movement probability when the predictor value is negative (e.g. -ndxdo and -ndxtemp when DO and temperature are decreasing downstream, -ndtdo and -ndttemp when DO and temperature are decreasing with time, -ntide when tide is ebbing) and a decrease in movement probability when the predictor value is positive (e.g ndxdo and ndxtemp when DO and temperature are increasing downstream, ndtdo and ndttemp when DO and temperature are increasing with time, ntide when tide is flooding). For all other variables (DO, Temp, Insol), a negative B indicates a decrease in movement probability (an increase in stay put) with increases in the predictor value. Opposite movement trends occur when B is positive. ORs >1 indicate the specific increase in the odds of movement and ORs < 1 indicate the specific increase in odds of stay put, per SD change in the predictor variable.**

							95% CI for OR	
Predictor	B	S.E.	Wald χ^2	df	p	OR ($\frac{\text{movement}}{\text{stay put}}$)	Lower	Upper
ndttemp	-1.323	0.519	6.496	1	0.011	0.266	0.096	0.737
ndxtemp	-0.363	0.177	4.206	1	0.040	0.696	0.492	0.984
Temp	-0.325	0.159	4.156	1	0.041	0.723	0.529	0.988
Non-significant covariates								
DO	0.165	0.169	0.947	1	0.331	1.179	0.846	1.642
Insol	0.230	0.159	2.082	1	0.149	1.259	0.921	1.721
ntide	0.115	0.158	0.533	1	0.466	1.122	0.824	1.528
ndxdo	0.131	0.167	0.614	1	0.433	1.140	0.822	1.581
ndtdo	-0.754	0.543	1.933	1	0.164	0.47	0.162	1.362
Constant	-0.613	0.157	15.224	1	0	0.542		
Model Tests			χ^2	df	p			
Overall Model Evaluation:								
Likelihood ratio test			20.201	8	0.010			
Score test			16.409	8	0.037			
Wald test			17.592	1	0			
Goodness-of-fit test:								
Hosmer & Lemeshow			7.734	8	0.46			

Table 4 **Group 1 – All fish. Binary logistic regression results for the directionality logit, including regression coefficients (B), Wald chi-squares, significance values (p), and odds ratios (OR). For spatial and temporal predictors, a negative B indicates an increase in downstream movement probability when the predictor value is negative (e.g. -ndxdo and -ndxtemp when DO and temperature are decreasing downstream, -ndttemp when temperature is decreasing with time, -ntide when tide is ebbing) and a decrease in downstream movement probability when the predictor value is positive (e.g ndxdo and ndxtemp when DO and temperature are increasing downstream, ndttemp when temperature is increasing with time, ntide when tide is flooding). For all other variables (DO, Temp, Insol), a negative B indicates a decrease in downstream movement probability (an increase in upstream) with increases in the predictor value. Opposite movement trends occur when B is positive. ORs > 1 indicate the specific increase in the odds of downstream movements and ORs < 1 indicate the specific increase in the odds of upstream movements, per SD change in the predictor variable.**

							95% CI for OR	
Predictor	B	S.E.	Wald χ^2	df	p	OR ($\frac{\text{downstream}}{\text{upstream}}$)	Lower	Upper
ndxdo	2.177	0.759	8.226	1	0.004	8.822	1.992	39.057
Temp	-1.226	0.551	4.952	1	0.026	0.293	0.1	0.864
ntide	-1.006	0.461	4.76	1	0.029	0.366	0.148	0.903
Non-significant covariates								
DO	0.709	0.457	2.402	1	0.121	2.032	0.829	4.981
Insol	-0.119	0.421	0.08	1	0.778	0.888	0.389	2.025
ndxtemp	-2.204	1.363	2.617	1	0.106	0.11	0.008	1.594
ndttemp	-0.329	0.84	0.153	1	0.695	0.72	0.139	3.736
Constant	2.573	0.646	15.856	1	0	13.102		
Model Tests			χ^2	df	p			
Overall Model Evaluation:								
Likelihood ratio test			25.75	7	0.001			
Score test			21.907	7	0.003			
Wald test			24.993	1	0			
Goodness-of-fit test:								
Hosmer & Lemeshow			12.358	8	0.136			

Table 5 **Group 3 – Fish that encountered unhealthy DO conditions (≤ 4.8 mg O₂/l) after release. Binary logistic regression results for the directionality logit, including regression coefficients (B), Wald chi-squares, significance values (p), and odds ratios (OR). For spatial and temporal predictors, a negative B indicates an increase in downstream movement probability when the predictor value is negative (e.g. -ndxdo when DO is decreasing downstream, -ntide when tide is ebbing) and a decrease in downstream movement probability when the predictor value is positive (e.g ndxdo when DO is increasing downstream, ntide when tide is flooding). For all other variables (DO, Temp, Insol), a negative B indicates a decrease in downstream movement probability (an increase in upstream) with increases in the predictor value. Opposite movement trends occur when B is positive. ORs > 1 indicate the specific increase in the odds of downstream movements and ORs < 1 indicate the specific increase in the odds of upstream movements, per SD change in the predictor variable.**

							95% CI for OR	
Predictor	B	S.E.	Wald χ^2	df	p	OR ($\frac{\text{downstream}}{\text{upstream}}$)	Lower	Upper
ndxdo	1.284	0.539	5.672	1	0.017	3.61	1.255	10.384
ntide	-0.982	0.429	5.247	1	0.022	0.374	0.162	0.868
Non-significant covariates								
DO	0.528	0.409	1.669	1	0.196	1.696	0.761	3.781
Temp	-0.798	0.453	3.107	1	0.078	0.45	0.185	1.093
Insol	-0.465	0.37	1.579	1	0.209	0.628	0.304	1.297
Constant	1.49	0.473	9.94	1	0.002	4.438		
Model Tests			χ^2	df	p			
Overall Model Evaluation:								
Likelihood ratio test			14.126	5	0.015			
Score test			12.61	5	0.027			
Wald test			9.62	1	0.002			
Goodness-of-fit test:								
Hosmer & Lemeshow			6.94	7	0.435			

Table 6 **Group 4 – Fish that experienced severely hypoxic DO conditions (≤ 2.3 mg O₂/l). Binary logistic regression results for the movement logit, including regression coefficients (B), Wald chi-squares, significance values (p), and odds ratios (OR). For spatial and temporal predictors, a negative B indicates an increase in movement probability when the predictor value is negative (e.g. -ndxdo and -ndxtemp when DO and temperature are decreasing downstream, -ndtdo and -ndttemp when DO and temperature are decreasing with time, -ntide when tide is ebbing) and a decrease in movement probability when the predictor value is positive (e.g ndxdo and ndxtemp when DO and temperature are increasing downstream, ndtdo and ndttemp when DO and temperature are increasing with time, ntide when tide is flooding). For all other variables (DO, Temp, Insol), a negative B indicates a decrease in movement probability (an increase in stay put) with increases in the predictor value. Opposite movement trends occur when B is positive. ORs >1 indicate the specific increase in the odds of movement and ORs < 1 indicate the specific increase in odds of stay put, per SD change in the predictor variable.**

							95% CI for OR	
Predictor	B	S.E.	Wald χ^2	df	p	OR ($\frac{\text{movement}}{\text{stay put}}$)	Lower	Upper
DO	0.716	0.324	4.869	1	0.027	2.046	1.083	3.863
ndxtemp	-0.741	0.361	4.211	1	0.04	0.477	0.235	0.967
Non-significant covariates								
Temp	-0.37	0.256	2.077	1	0.15	0.691	0.418	1.142
Insol	-0.013	0.263	0.002	1	0.962	0.988	0.589	1.655
ntide	-0.128	0.304	0.179	1	0.673	0.88	0.485	1.595
ndxdo	0.52	0.315	2.72	1	0.099	1.682	0.907	3.12
ndtdo	-0.992	1.043	0.904	1	0.342	0.371	0.048	2.864
ndttemp	-1.718	1.054	2.658	1	0.103	0.179	0.023	1.416
Constant	-1.177	0.284	17.139	1	0	0.308		
Model Tests			χ^2	df	p			
Overall Model Evaluation:								
Likelihood ratio test			16.931	8	0.031			
Score test			16.145	8	0.04			
Wald test			22.63	1	0			
Goodness-of-fit test:								
Hosmer & Lemeshow			1.923	8	0.983			

Table 7 **Group 4 – Fish that experienced severely hypoxic DO conditions (≤ 2.3 mg O₂/l). Binary logistic regression results for the directional logit, including regression coefficients (B), Wald chi-squares, significance values (p), and odds ratios (OR). For spatial and temporal predictors, a negative B indicates an increase in downstream movement probability when the predictor value is negative (e.g. -ndxdo and -ndxtemp when DO and temperature are decreasing downstream, -ntide when tide is ebbing) and a decrease in downstream movement probability when the predictor value is positive (e.g ndxdo and ndxtemp when DO and temperature are increasing downstream, ntide when tide is flooding). Opposite movement trends occur when B is positive. ORs > 1 indicate the specific increase in the odds of downstream movements and ORs < 1 indicate the specific increase in the odds of upstream movements, per SD change in the predictor variable.**

							95% CI for OR	
Predictor	B	S.E.	Wald χ^2	df	p	OR (downstream upstream)	Lower	Upper
ndxdo	2.053	1	4.219	1	0.04	7.793	1.098	55.282
ntide	-1.419	0.717	3.92	1	0.048	0.242	0.059	0.986
Non-significant covariates								
ndxtemp	-1.866	1.966	0.901	1	0.343	0.155	0.003	7.295
Constant	1.684	0.779	4.678	1	0.031	5.387		
Model Tests			χ^2	df	p			
Overall Model Evaluation:								
Likelihood ratio test			12.379	3	0.006			
Score test			10.919	3	0.012			
Wald test			4.496	1	0.034			
Goodness-of-fit test:								
Hosmer & Lemeshow			5.714	6	0.456			

Table 8 Duration of exposure to unhealthy DO (≤ 4.8 mg O₂/l) and severe hypoxia (≤ 2.3 mg O₂/l) for each summer flounder. Duration is expressed in terms of the length of individual segments of continuous exposure, additive exposure (total duration), and as a percentage of residency time in Pepper Creek.

	Duration of Exposure					
Flounder	Unhealthy DO segments (min)	Total Duration: Unhealthy DO (min)	Severe Hypoxia segments (min)	Total Duration: Severe Hypoxia (min)	% Residency Time in Unhealthy DO	% Residency Time in Severe Hypoxia
Juveniles:						
Carl	19,21,34	74			20.9%	
Cappie	2,3,29	34			1.3%	
Paula	7,16,31, 33,40,85	212	9	9	1.7%	0.07%
Phil	40,56,63, 86	245			5.5%	
Stan	3,3,16,28, 39,198	287	9	9	1.8%	0.06%
Brittany	8,13,13,18 ,21,24,41, 42,73,78, 100	431	3,5,40	48	4.0%	0.45%
Kevin	4,4,8,9,13, 15,16,21,2 3,26,27,29 ,32,36,44, 61,67,115, 222	772	7,9,15,17, 18,18,21,2 3,26,27,31 ,32,83	327	16.4%	7.0%
Flora	1,2	3			0.56%	
Fauna	1,34	35			6.0%	
Shrek	121	121	9,9	18	5.6%	0.84%
Fiona	6	6			0.13%	
Puss	15,47,81	143	15	15	18.5%	1.9%
Donkey	2,43,43, 53,141	282	10,15	25	19.9%	1.8%
Adults:						
Cougar	20	20			1.6%	
Simon	3,20,56, 112	191	71	71	1.8%	0.68%
Flipper	30	30			0.53%	
Frank	54	54			12.3%	
Flappy	29	29			3.43%	

Table 9 **Group 5 – Juvenile summer flounder. Binary logistic regression results for the directionality logit, including regression coefficients (B), Wald chi-squares, significance values (p), and odds ratios (OR). For spatial and temporal predictors, a negative B indicates an increase in downstream movement probability when the predictor value is negative (e.g. -ndxdo when is decreasing downstream, -ntide when tide is ebbing) and a decrease in downstream movement probability when the predictor value is positive (e.g ndxdo when DO is increasing downstream, ntide when tide is flooding). For all other variables (DO, Temp), a negative B indicates a decrease in downstream movement probability (an increase in upstream) with increases in the predictor value. Opposite movement trends occur when B is positive. ORs > 1 indicate the specific increase in the odds of downstream movements and ORs < 1 indicate the specific increase in the odds of upstream movements, per SD change in the predictor variable.**

							95% CI for OR	
Predictor	B	S.E.	Wald χ^2	df	p	OR (downstream upstream)	Lower	Upper
ntide	-1.318	0.48	7.547	1	0.006	0.268	0.104	0.685
ndxdo	1.076	0.544	3.907	1	0.048	2.932	1.009	8.518
Non-significant covariates								
DO	0.58	0.437	1.763	1	0.184	1.786	0.759	4.203
Temp	-0.827	0.482	2.941	1	0.086	0.437	0.17	1.125
Constant	1.882	0.537	12.276	1	0	6.568		
Model Tests			χ^2	df	p			
Overall Model Evaluation:								
Likelihood ratio test			14.421	4	0.006			
Score test			13.678	4	0.008			
Wald test			13.475	1	0			
Goodness-of-fit test:								
Hosmer & Lemeshow			8.346	7	0.303			

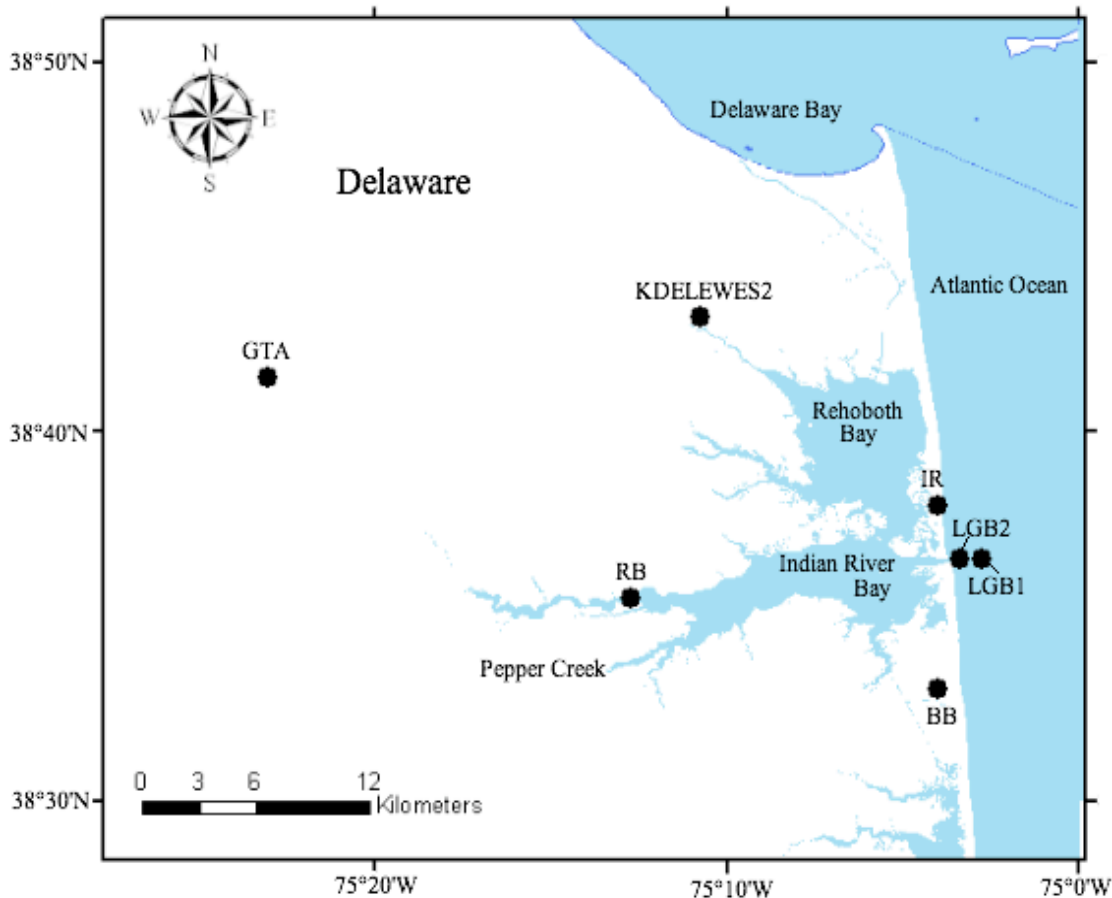


Figure 1 Delaware's Coastal Bays (USA) with locations of the environmental recording stations of meteorological variables and tidal height with respect to Pepper Creek. Precipitation, insolation, and barometric pressure data were collected from the Delaware Environmental Observing System (DEOS) stations at Bethany Beach (BB) and Indian River Inlet (IR), and from the Weather Underground stations at Lewes (KDELEWES2) and the Georgetown airport (GTA). Tidal height data was collected from the USGS Rosedale Beach Tidal Gauge (RB). Also displayed is the location of the Indian River Inlet receiver array at Lighted Gong Buoys (LGB) 1 and 2, deployed by Dr. Dewayne Fox of Delaware State University.

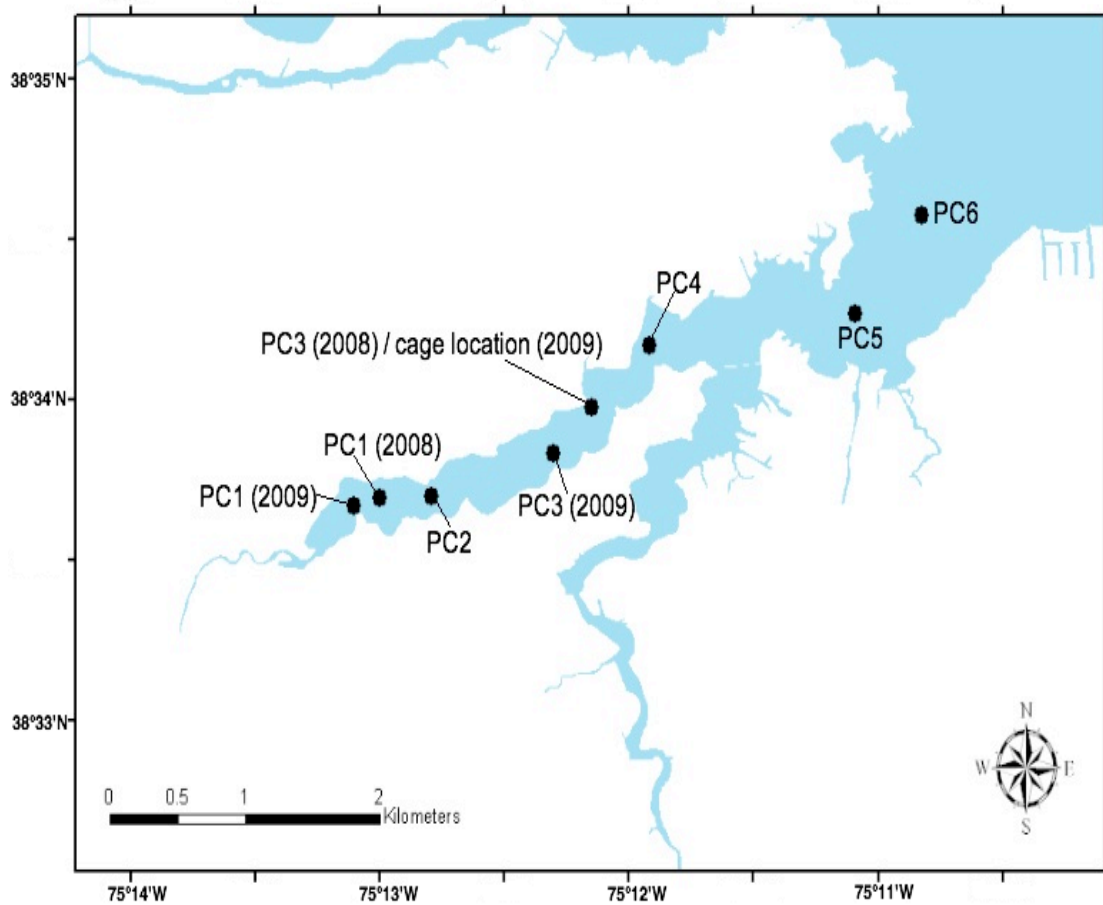


Figure 2 Location of the six sampling stations (PC1 – PC6) within Pepper Creek displaying yearly adjustments to the six sampling sites, in addition to the acclimation cage location in 2009.

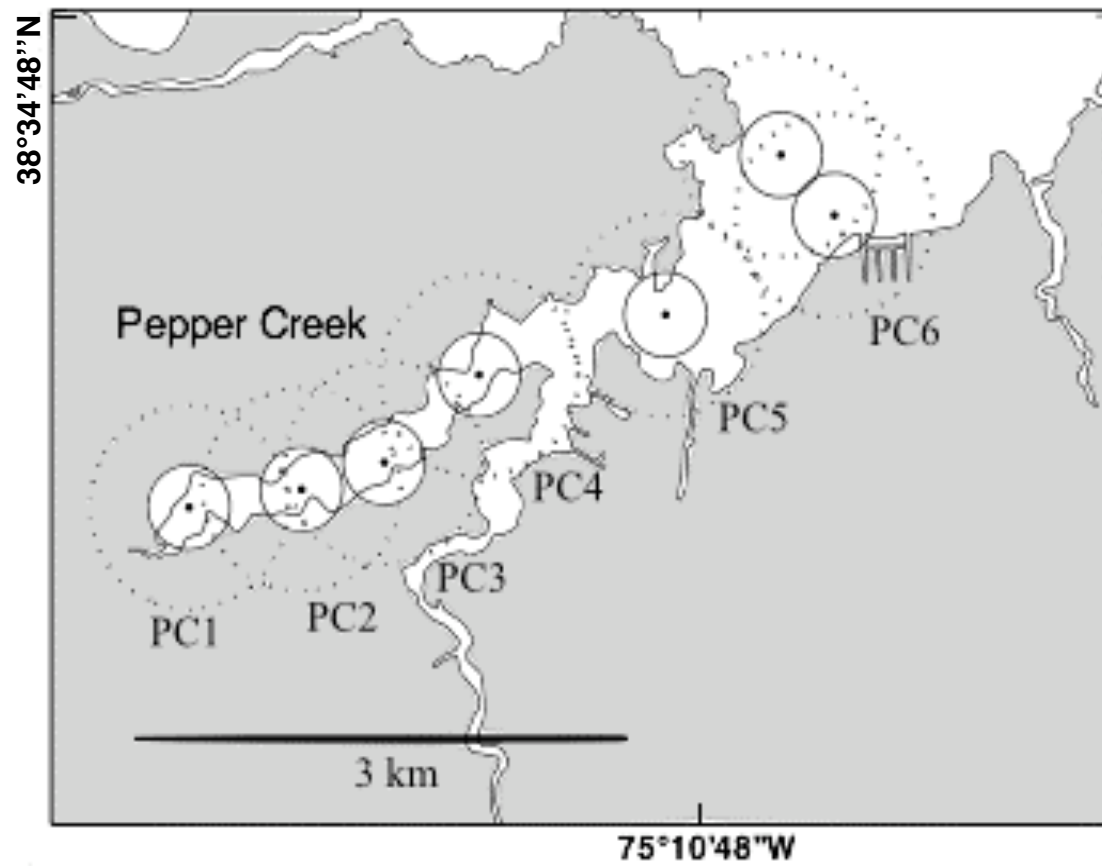


Figure 3 Pepper Creek showing the range testing on a number of receivers (from Brady and Targett in prep).

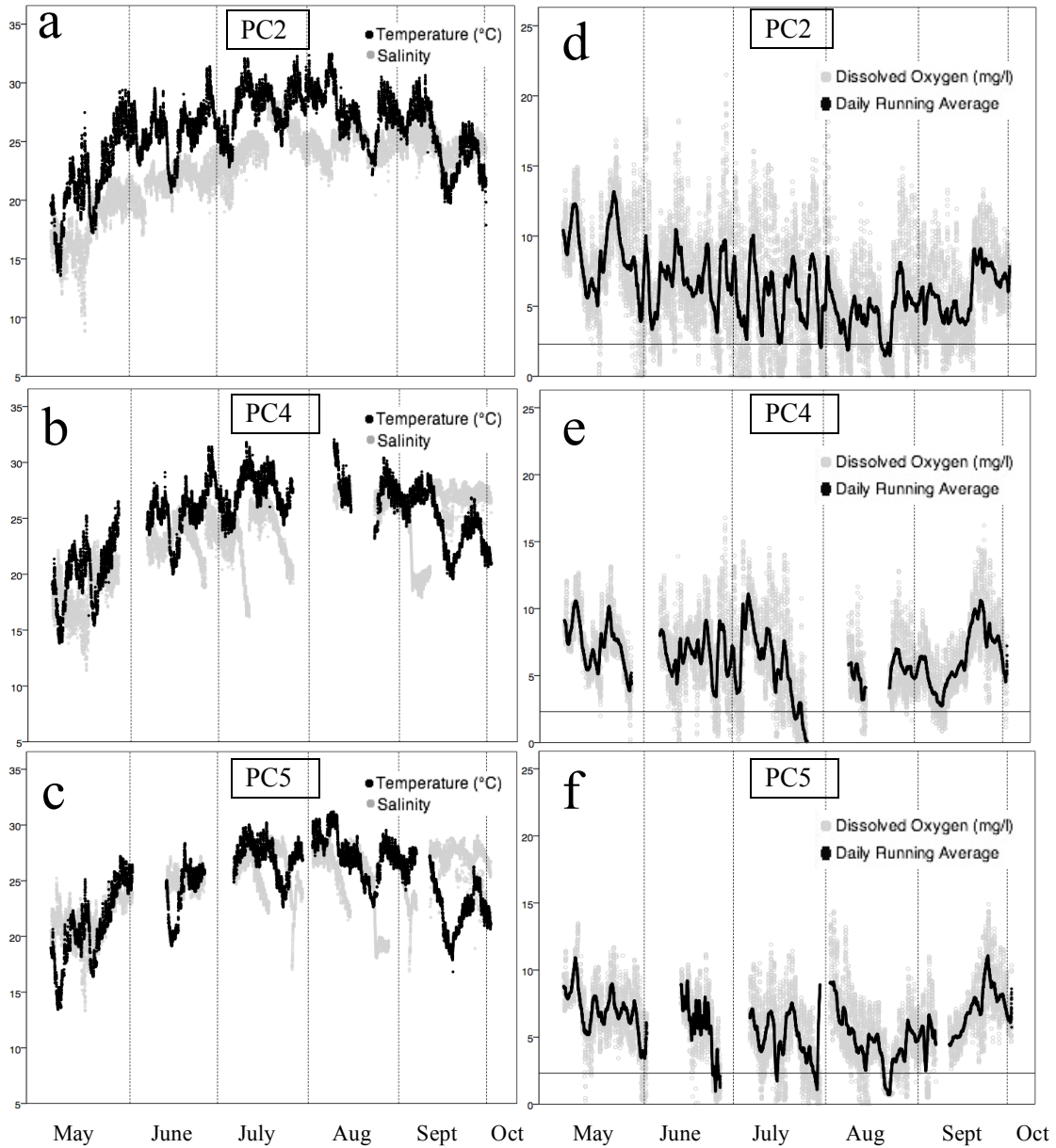


Figure 4 Water quality data from the Pepper Creek sites PC2, PC4, and PC5 in 2007. (a) – (c) Temperature (°C) and Salinity, collected every 15 min from May – October. (d) – (f) Dissolved oxygen (mg O₂/l) collected every 15 min from May – October and the daily running average of dissolved oxygen, with a horizontal line denoting severe hypoxia (2.3 mg O₂/l).

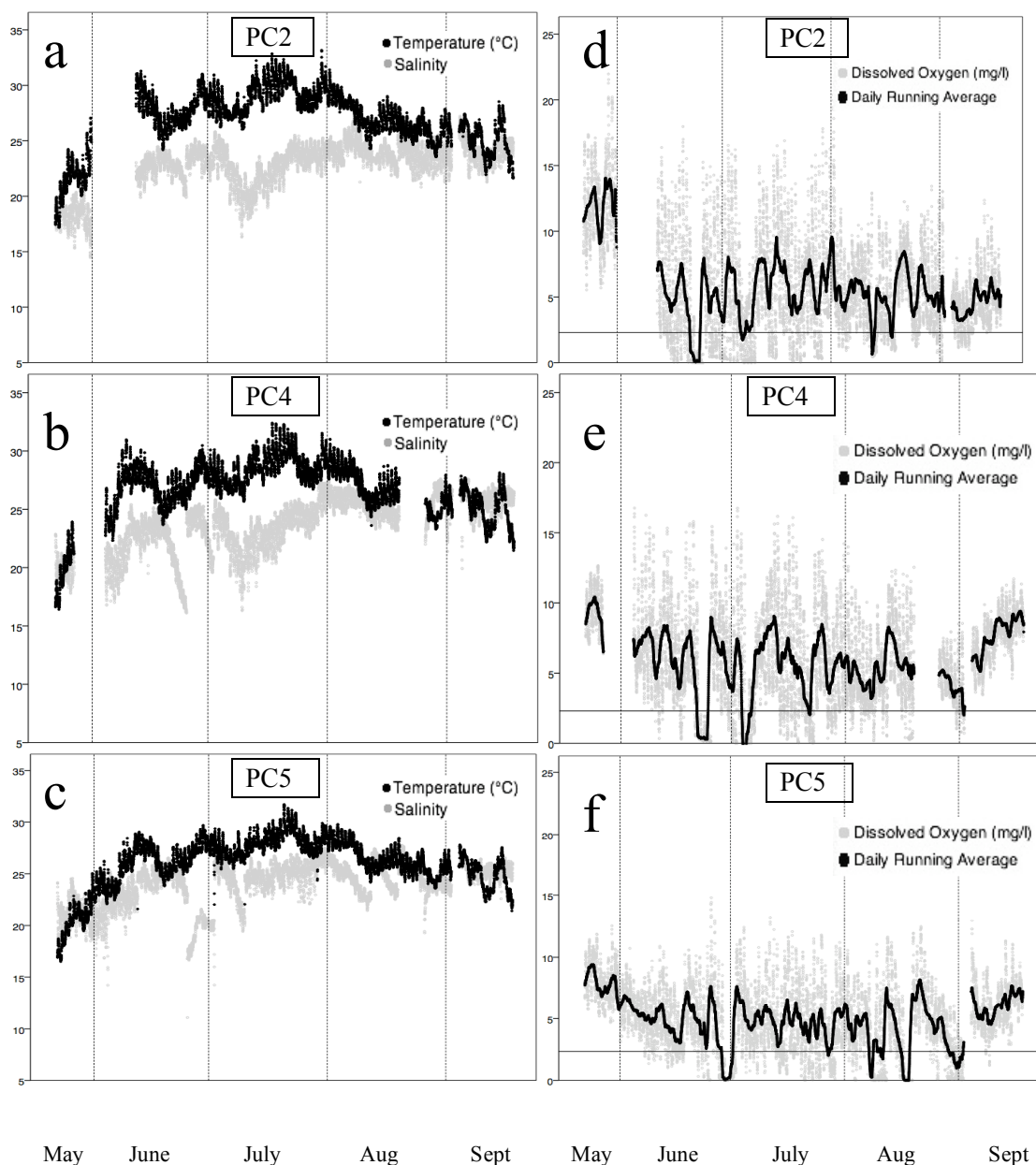


Figure 5 Water quality data from the Pepper Creek sites PC2, PC4, and PC5 in 2008. (a) – (c) Temperature (°C) and Salinity, collected every 15 min from May – September. (d) – (f) Dissolved oxygen (mg O₂/l) collected every 15 min from May – September and the daily running average of dissolved oxygen, with a horizontal line denoting severe hypoxia (2.3 mg O₂/l).

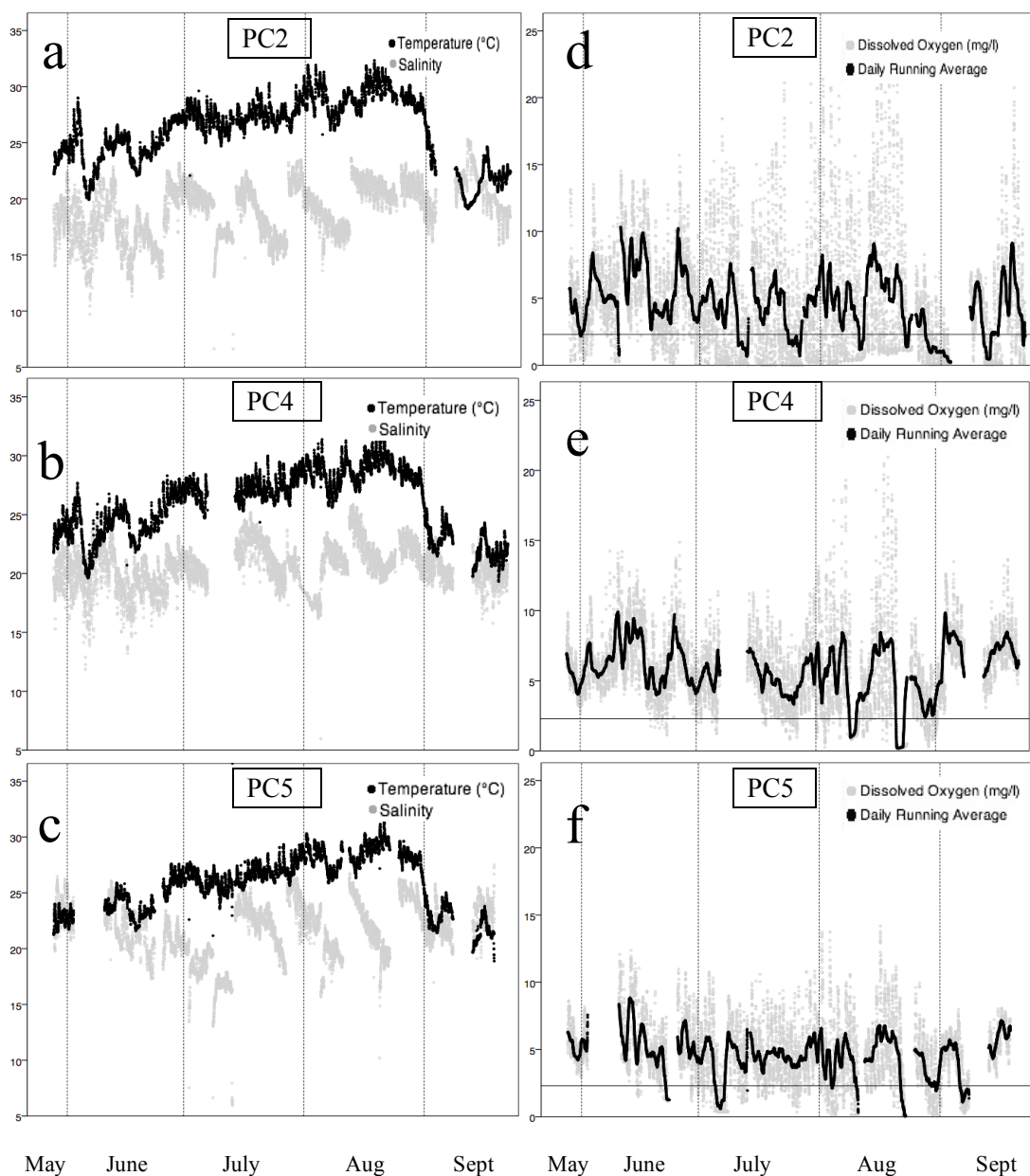


Figure 6 Water quality data from the Pepper Creek sites PC2, PC4, and PC5 in 2009. (a) – (c) Temperature (°C) and Salinity, collected every 15 min from May – September. (d) – (f) Dissolved oxygen (mg O₂/l) collected every 15 min from May – September and the daily running average of dissolved oxygen, with a horizontal line denoting severe hypoxia (2.3 mg O₂/l).

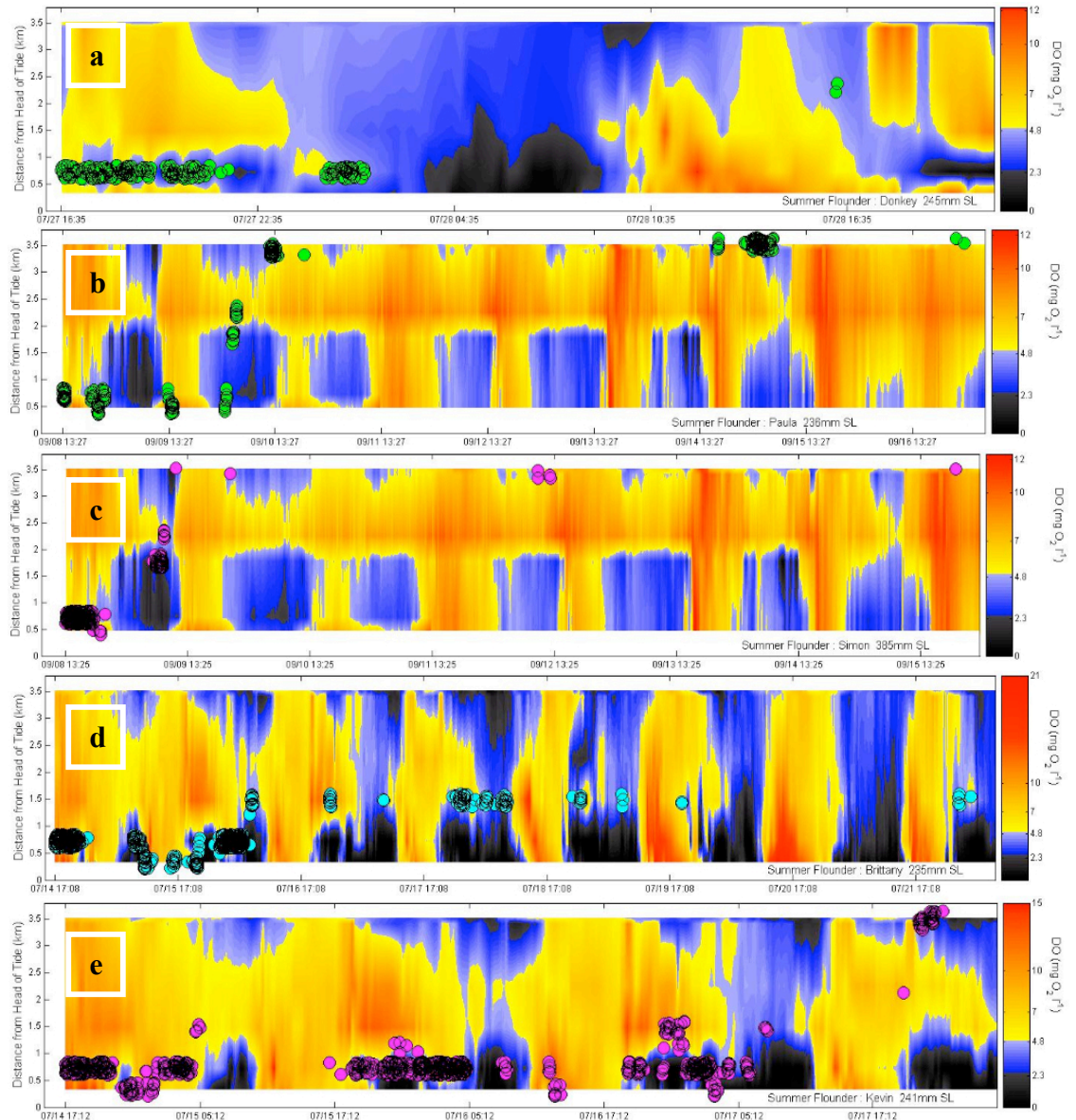


Figure 7 (a) – (e) Plots of dissolved oxygen and fish tracks for summer flounder released into DO concentrations $> 4.8 \text{ mg O}_2/\text{l}$ that subsequently encountered DO concentrations $\leq 4.8 \text{ mg O}_2/\text{l}$. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms ($\leq 4.8 \text{ mg O}_2/\text{l}$) and black representing the survival criterion (severe hypoxia) ($\leq 2.3 \text{ mg O}_2/\text{l}$). Note individual fish identifications (see Table 1) in the lower right of each panel.

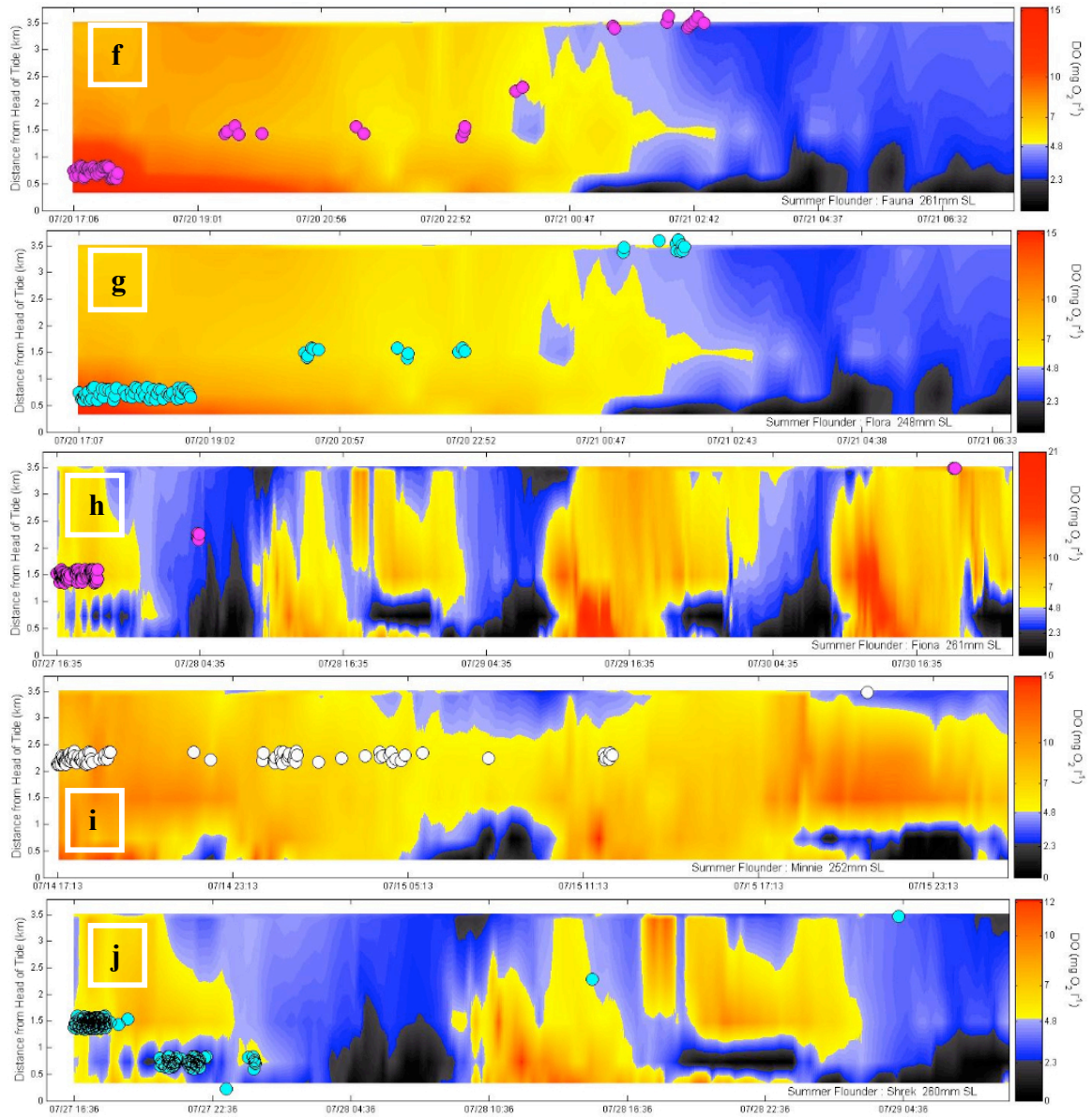


Figure 7(cont.)(f) – (j) Plots of dissolved oxygen and fish tracks for summer flounder released into DO concentrations > 4.8 mg O_2 /l that subsequently encountered DO concentrations ≤ 4.8 mg O_2 /l. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O_2 /l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O_2 /l). Note individual fish identifications (see Table 1) in the lower right of each panel.

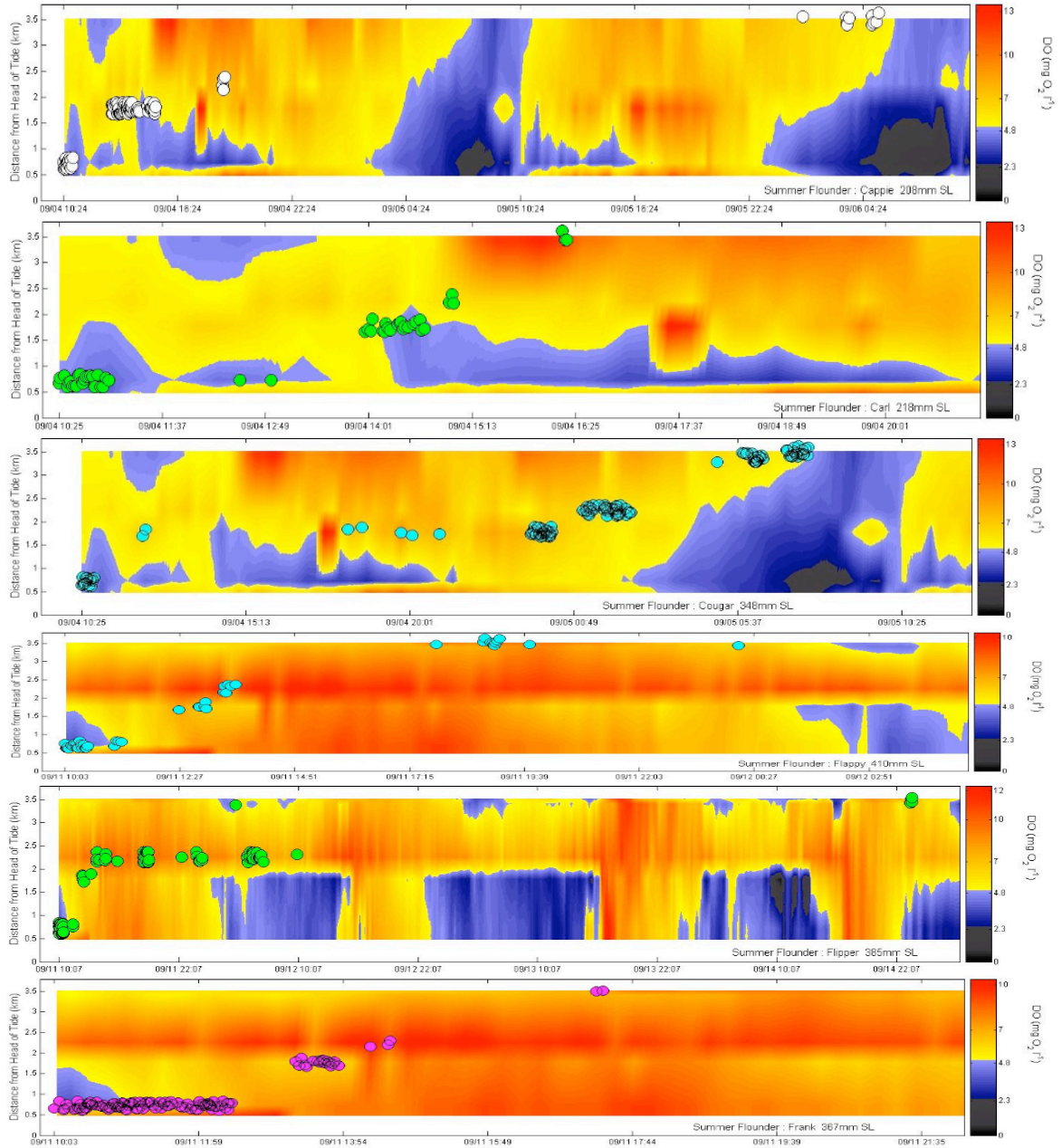


Figure 8 Plots of dissolved oxygen and fish tracks for summer flounder released into $\text{DO} \leq 4.8 \text{ mg O}_2/\text{l}$. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms ($\leq 4.8 \text{ mg O}_2/\text{l}$) and black representing the survival criterion (severe hypoxia) ($\leq 2.3 \text{ mg O}_2/\text{l}$). Note individual fish identifications (see Table 1) in the lower right of each panel.

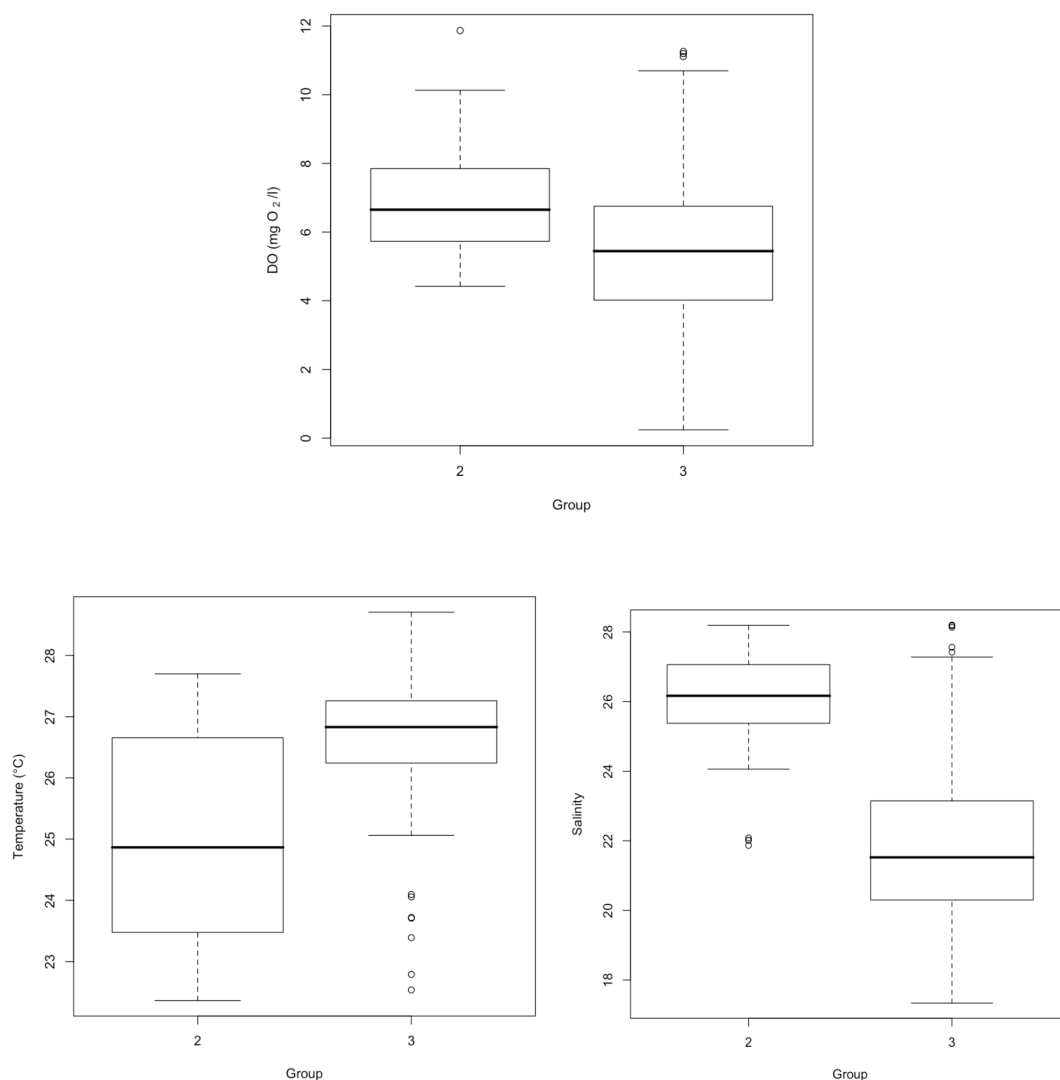


Figure 9 (a) Dissolved oxygen (DO; mg O₂/l), (b) temperature (°C), and (c) salinity values experienced by the summer flounder in Group 2 and Group 3. The bold horizontal line is the median value of the data. The box extends from the first quartile to the third quartile. The dashed lines (whiskers) extend from the quartiles to the smallest non-outlier and largest non-outlier in the dataset. Unfilled circles on the plot signify values that are suspected outliers.

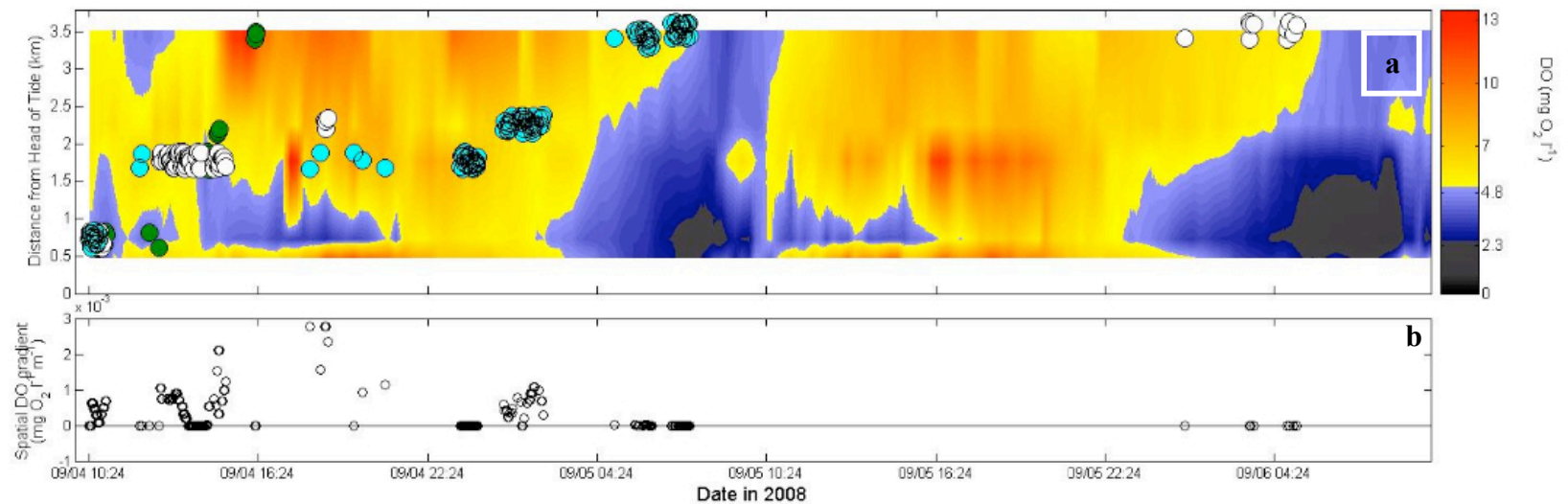


Figure 10 (a) Plot of dissolved oxygen and fish tracks for the summer flounder released in batch #2 and (b) spatial DO gradient ($\text{mg O}_2/\text{l}/\text{m}$) associated with each detection. (● Cougar (348mm SL), ○ Cappie (208mm SL), ● Carl (218mm SL)). The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms ($\leq 4.8 \text{ mg O}_2/\text{l}$) and black representing the survival criterion (severe hypoxia) ($\leq 2.3 \text{ mg O}_2/\text{l}$).

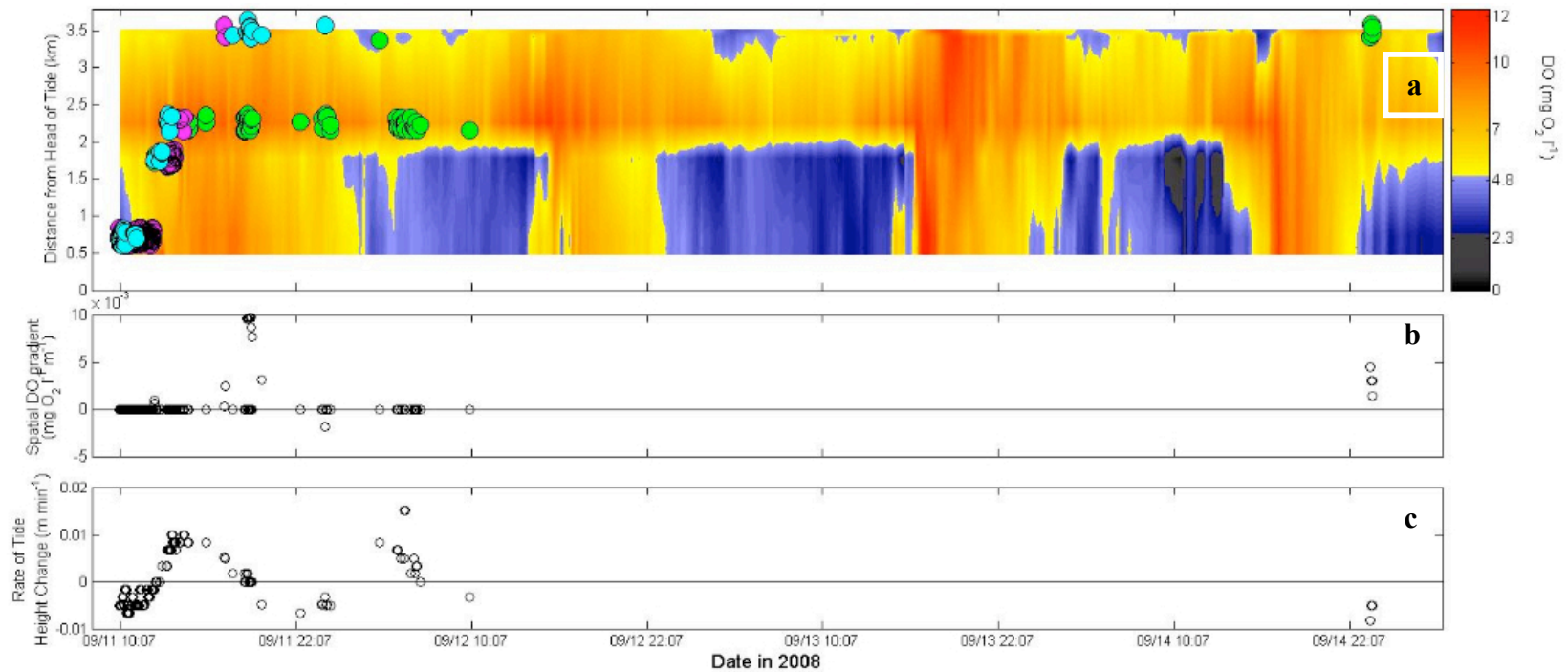


Figure 11 (a) Plot of dissolved oxygen and fish tracks for the summer flounder released in batch #4, (b) spatial DO gradient ($\text{mg O}_2/\text{l}/\text{m}$), and (c) rate of tide height change (m/min) associated with each detection (● Flappy (410mm SL), ● Frank (367mm SL), ● Flipper (385mm SL)). The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms ($\leq 4.8 \text{ mg O}_2/\text{l}$) and black representing the survival criterion (severe hypoxia) ($\leq 2.3 \text{ mg O}_2/\text{l}$).

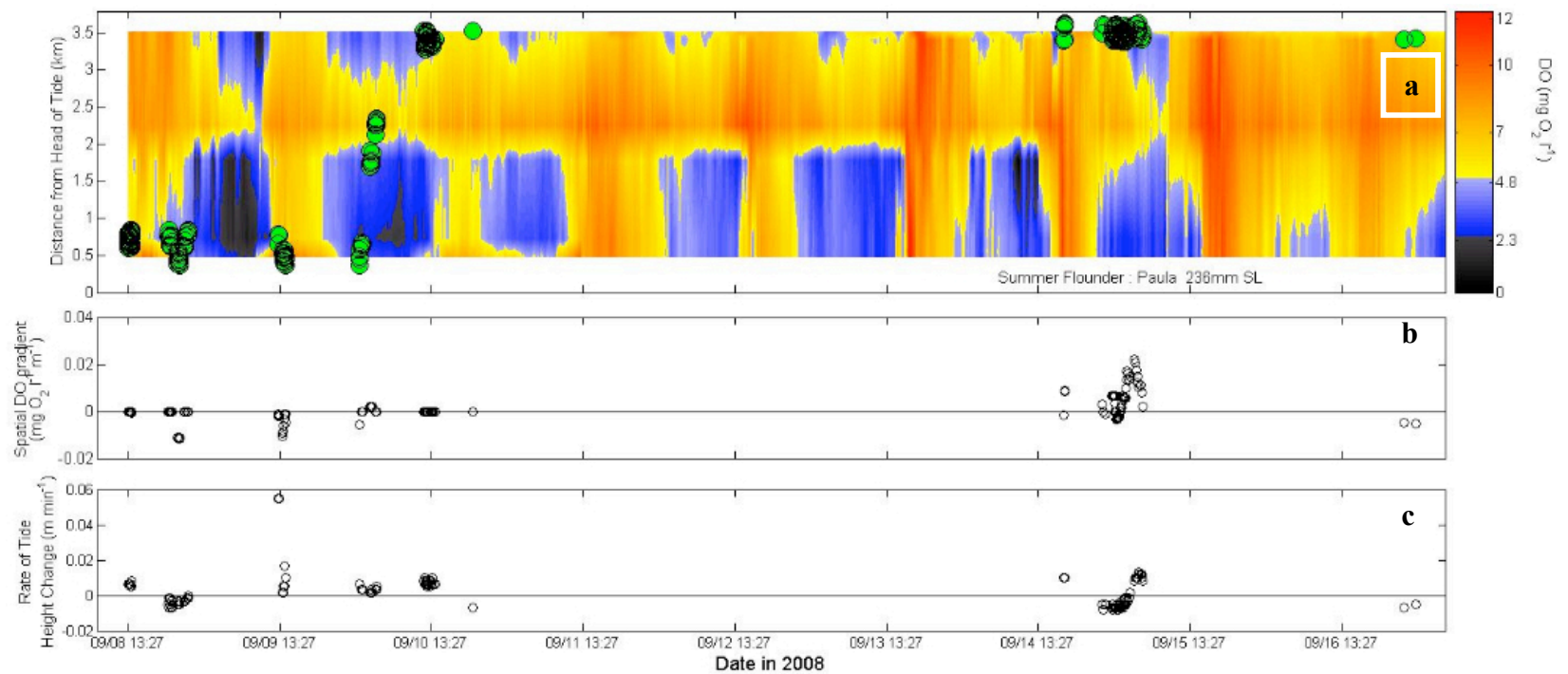


Figure 12 (a) Plot of dissolved oxygen and fish tracks for the summer flounder Paula, released 09/08/08, (b) spatial DO gradient (mg O₂/l/m) and (c) rate of tide height change (m/min) associated with each detection. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l). Note individual fish identifications (see Table 1) in the lower right of each panel.

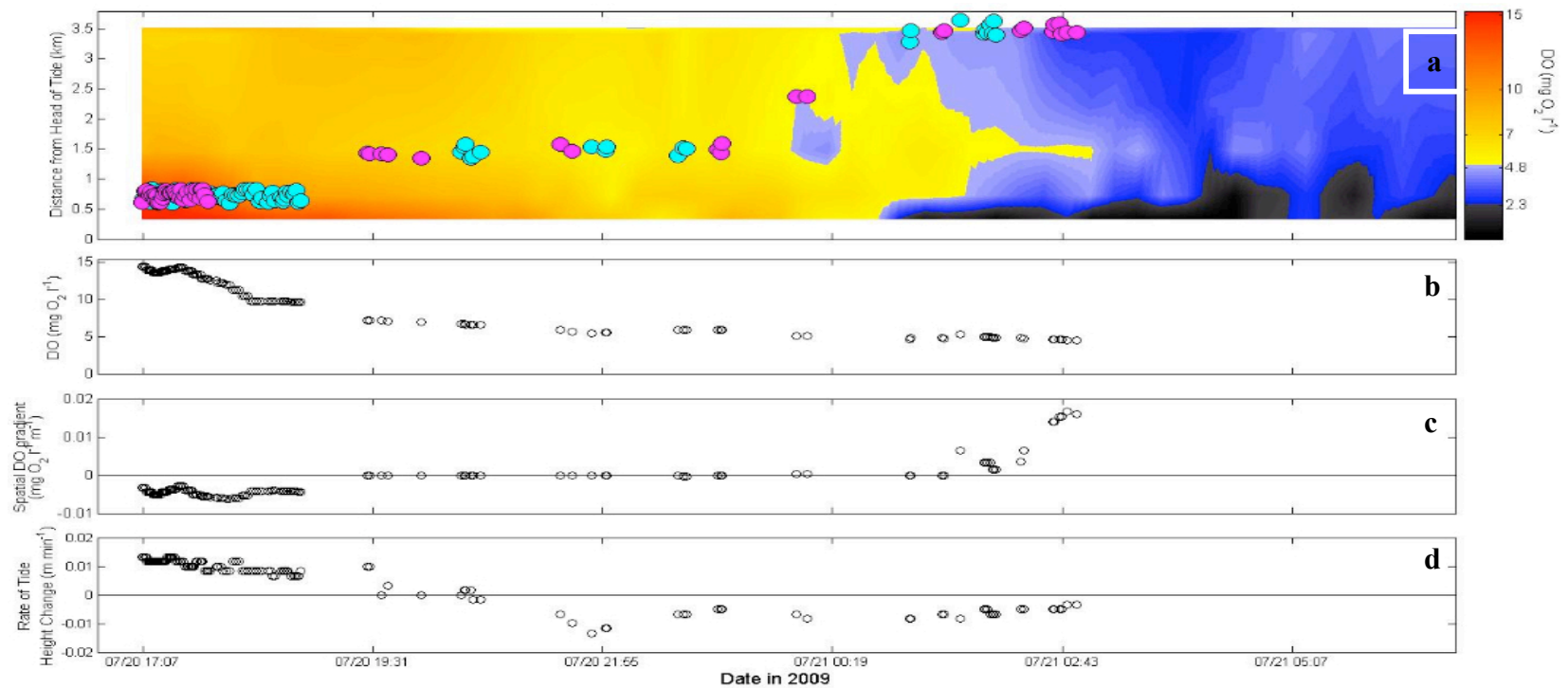


Figure 13 (a) Plot of dissolved oxygen and fish tracks for the summer flounder Flora and Fauna, released 07/20/09, (b) DO (mg O₂/l), (c) spatial DO gradient (mg O₂/l/m), and (d) rate of tide height change (m/min) associated with each detection (● Flora (248mm SL), ● Fauna (261mm SL)). The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l).

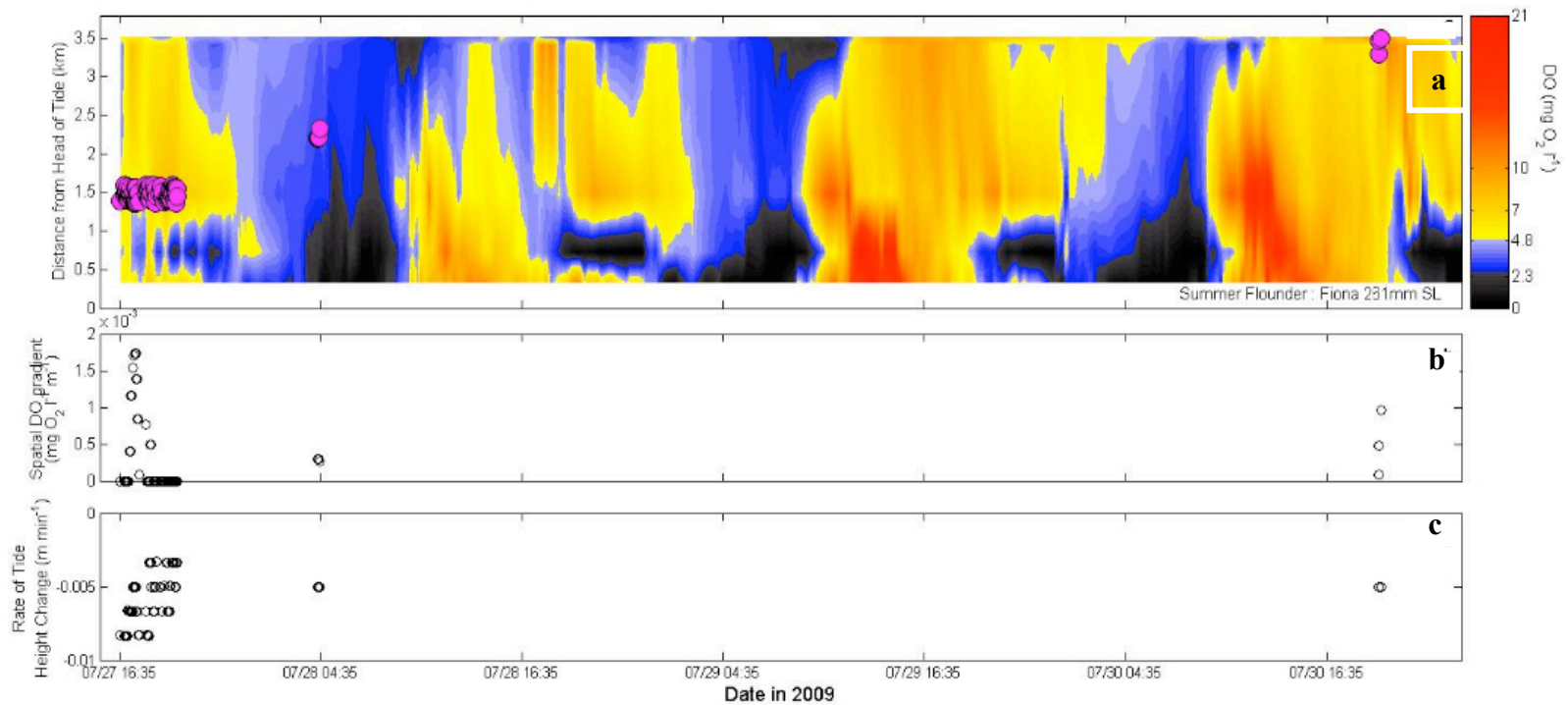


Figure 14 (a) Plot of dissolved oxygen and fish tracks for the summer flounder Fiona, released 07/27/09, (b) spatial DO gradient ($\text{mg O}_2/\text{l}/\text{m}$) and (c) rate of tide height change (m/min) associated with each detection. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms ($\leq 4.8 \text{ mg O}_2/\text{l}$) and black representing the survival criterion (severe hypoxia) ($\leq 2.3 \text{ mg O}_2/\text{l}$).

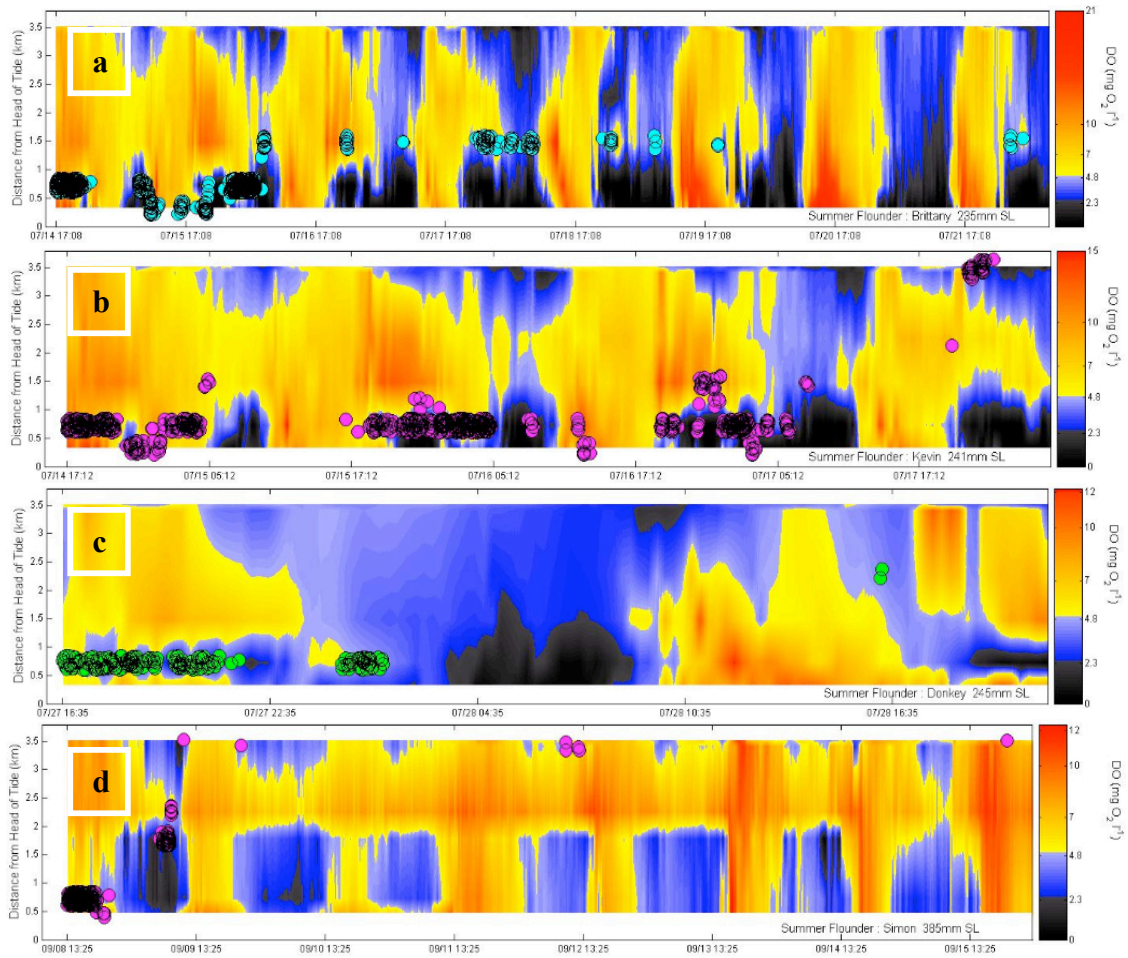


Figure 15 Plots of dissolved oxygen and fish tracks for summer flounder that experienced hypoxic DO concentrations ≤ 2.3 mg O₂/l. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l). Note individual fish identifications (see Table 1) in the lower right of each panel.

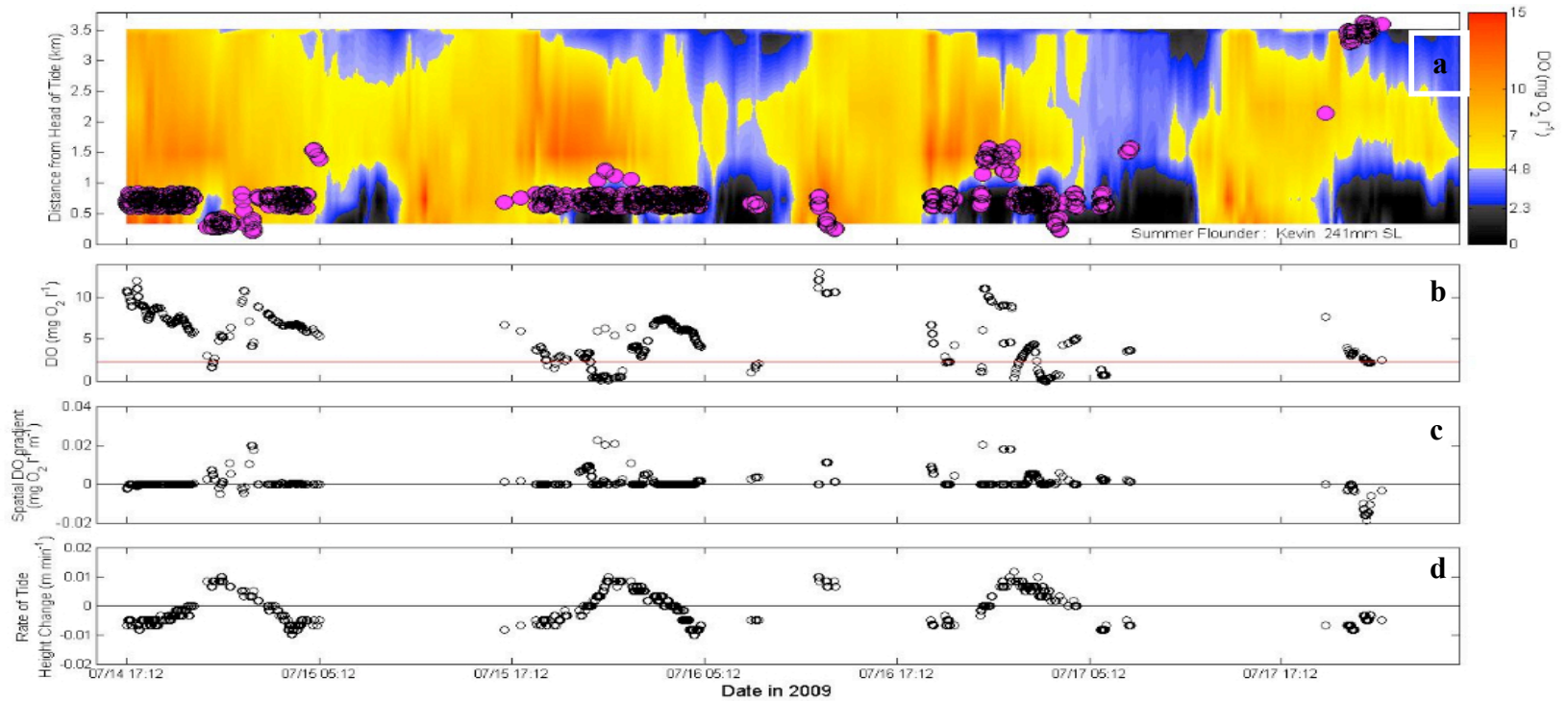


Figure 16 (a) Plot of dissolved oxygen and fish tracks for the summer flounder Kevin, released 07/14/09, (b) DO (mg O₂/l) with a red horizontal line denoting hypoxia (DO ≤ 2.3 mg O₂/l), (c) spatial DO gradient (mg O₂/l/m), and (d) rate of tide height change (m/min) associated with each detection. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l).

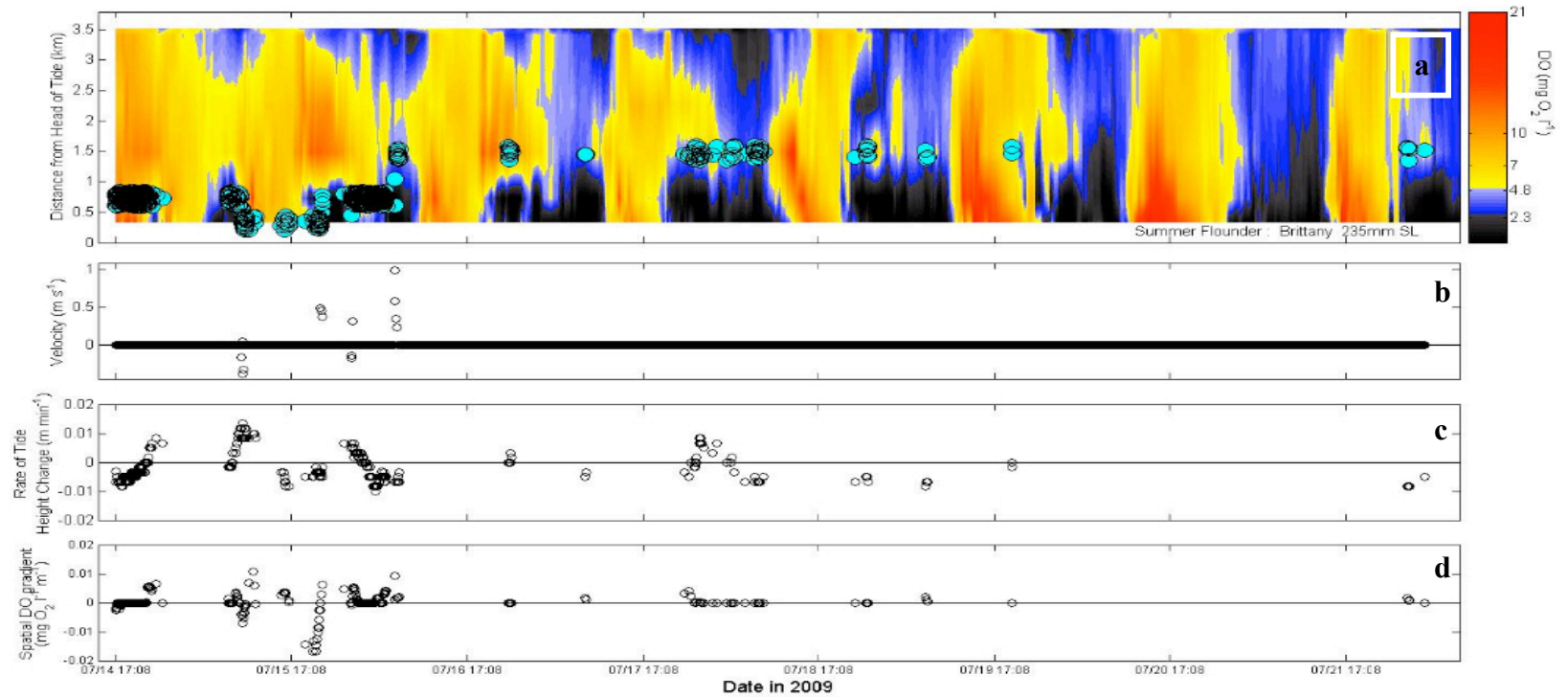


Figure 17 (a) Plot of dissolved oxygen and fish tracks for the summer flounder Brittany, released 07/14/09, (b) velocity (m/s), (c) rate of tide height change (m/min), and (d) spatial DO gradient (mg O₂/l/m) associated with each detection. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l).

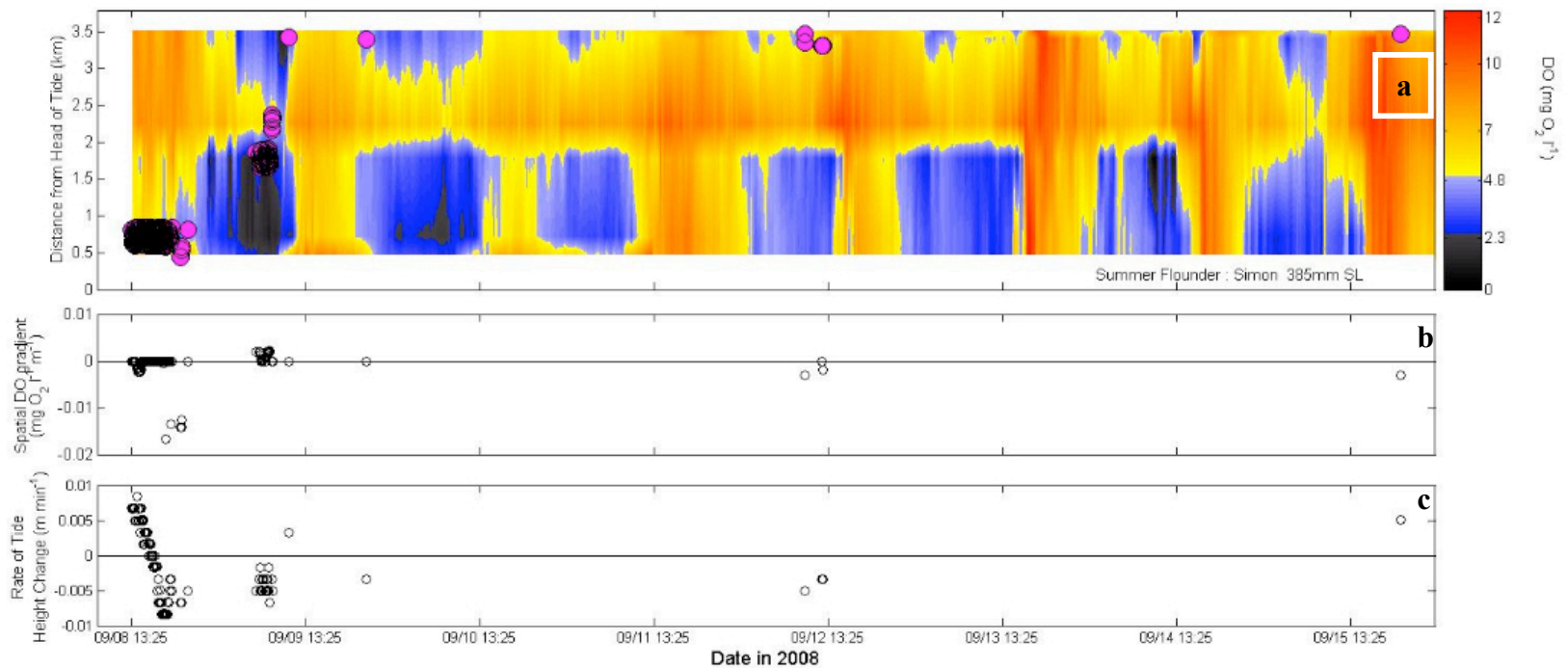


Figure 18 (a) Plot of dissolved oxygen and fish tracks for the summer flounder Simon, released 09/08/08, (b) spatial DO gradient (mg O₂/l/m), and (c) rate of tide height change (m/min) associated with each detection. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l).

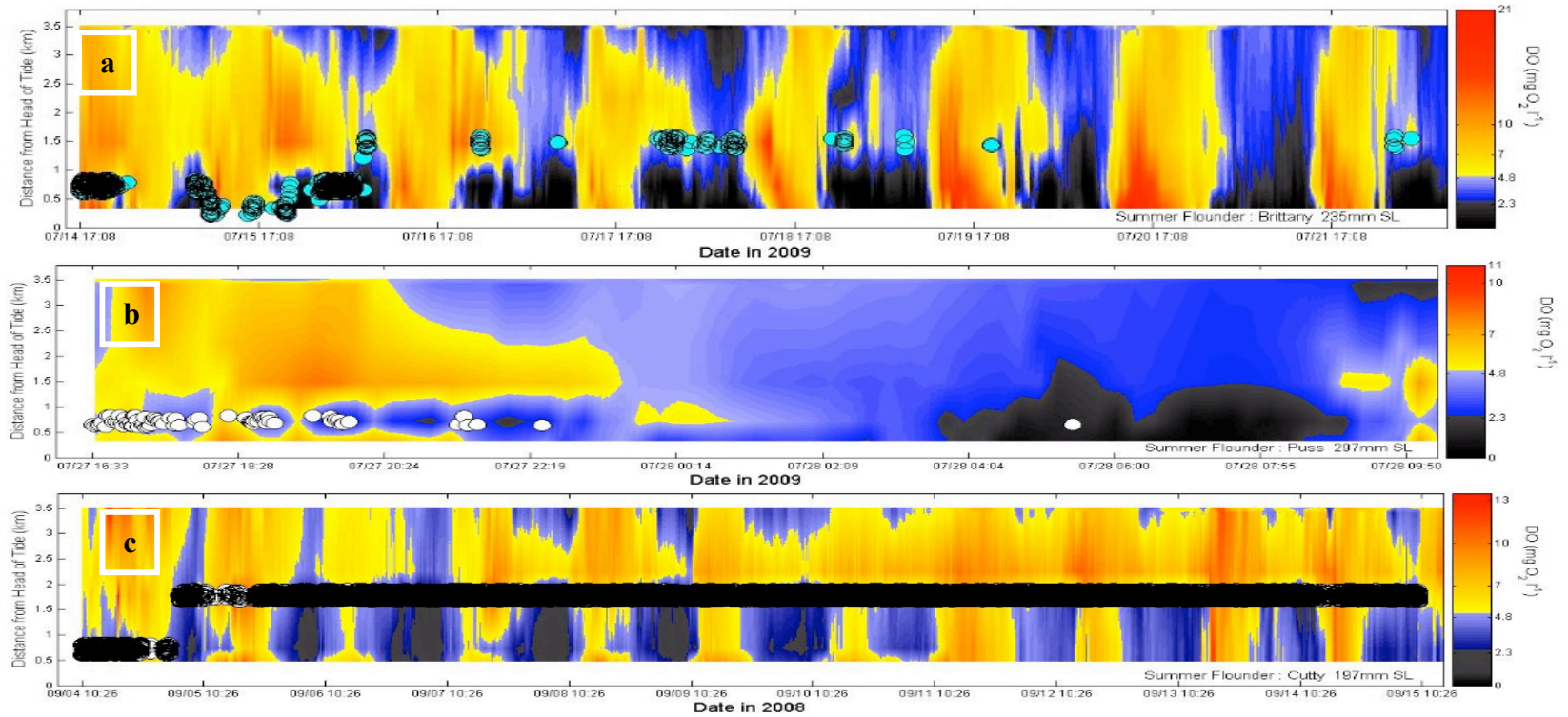


Figure 19 (a) – (c) Plots of dissolved oxygen and fish tracks for the summer flounder assumed to have died during the tracking period. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O_2/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O_2/l). Note individual fish identifications (see Table 1) in the lower right of each panel.

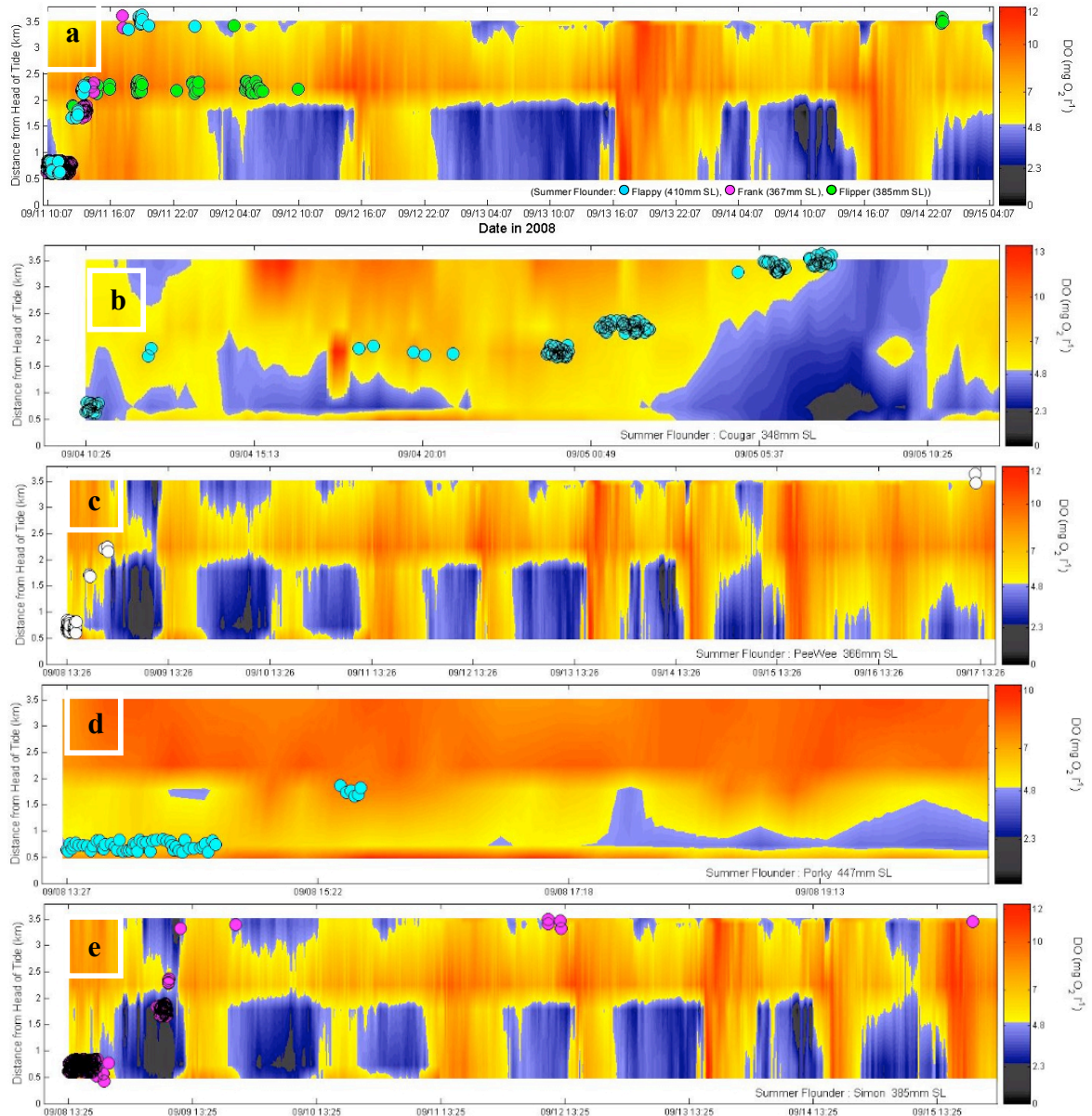


Figure 20 (a) – (e) Plots of dissolved oxygen and fish tracks for all seven adult summer flounder, released in 2008. The DO color scale is based on the US EPA (2000) DO criteria, with blue representing DO concentrations detrimental to growth of aquatic organisms (≤ 4.8 mg O₂/l) and black representing the survival criterion (severe hypoxia) (≤ 2.3 mg O₂/l). Note individual fish identifications (see Table 1) in the lower right of each panel.

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