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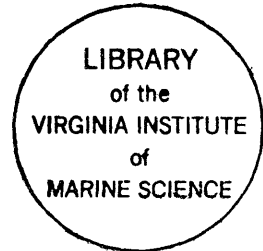
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COMPARISON OF DAILY GROWTH RATES OF THE MUMMICHOG,
FUNDULUS HETEROCLITUS IN HABITATS OF DIFFERENT
SALINITY REGIMES

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia



In Partial Fulfillment
of the requirements for the Degree of
Masters of Arts

by

James G. Hoff

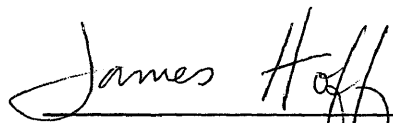
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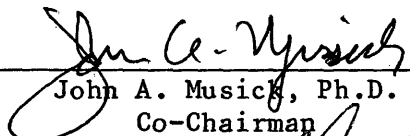
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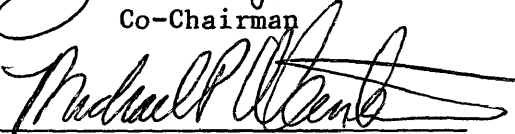


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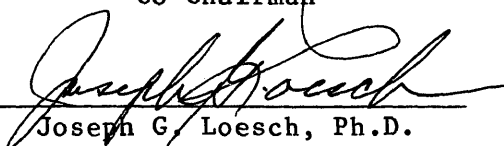
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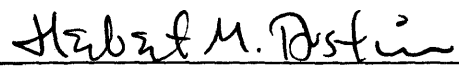
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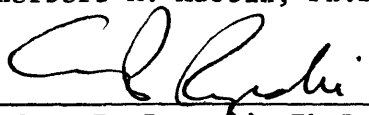
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ABSTRACT

Daily growth increments from otoliths of the mummichog, Fundulus heteroclitus were compared from habitats of different salinity regimes. Biweekly collections were made at four stations in both Blevins (polyhaline) and Goalders (oligohaline) creeks within the York River estuary, Virginia. This sampling design enabled the comparison of growth rates within and between creeks. Linear regressions of length vs. adjusted age were computed for samples from each of the eight stations. Analysis of covariance indicated that there were no significant differences in the regression lines when data were grouped to compare among creek growth rates. Pooled data from each creek were then compared, and no significant differences in lines were detected.

A mark and recapture study was undertaken to determine the home range of Fundulus heteroclitus within Blevins creek. The majority of individuals in the population exhibited a home range of less than 30 meters along the creek bank.

A COMPARISON OF DAILY GROWTH RATES OF THE MUMMICHOG,
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INTRODUCTION

The mummichog, Fundulus heteroclitus, is a common cyprinidontid fish found along the Atlantic coast of the United States, where it is one of the most abundant species of fish in shallow estuarine waters. Its life history has been documented by Bigelow and Schroeder (1953), Schmeltz (1964) and Fritz and Garside (1975).

Mummichogs are abundant at virtually all salinities, occurring over a broad range of shallow habitats in the estuary (Tagatz and Dudley, 1961; deSilva et al., 1962; Keup and Bayliss, 1964; Tagatz, 1968; Samaritan and Schmidt, 1982), and are one of the few fishes that reside in the marsh year-round (Nixon and Oviatt, 1973; Cain and Dean, 1976; Hoff and Ibara, 1977; Kneib and Stiven, 1978; and Weinstein, 1979). The adaptability of the species is highlighted by its euryhaline nature.

Fundulus heteroclitus is considered to be one of the most stationary species of marine fishes (Bigelow and Schroeder, 1953). Lotrich (1974) reported a home range of less than 36 meters for F. heteroclitus from a tidal creek in Delaware and documents this range as being restricted to one side of the creek. He further suggested that variation in the length of home range might exist over the fish's wide geographic distribution. If fish are confined to a limited home range, each creek might harbor a resident, distinct population which may reside in a different physiochemical regime from other populations.

The hypothesis of this study is that the growth rates of mummichogs will differ between creeks with different salinity regimes. Fishes regulate their plasma ions such that internal osmotic pressure of their body fluids is equivalent to approximately 10 ‰ salinity (± 2 ‰). A fish that lives with a small osmotic differential might require less energy for osmoregulation than those that live in areas that are highly hypo- or hyperosmotic. Studies by Brocksen and Cole (1972) and Wohlschlag and Wakeman (1978) support this hypothesis by reporting maximum metabolic efficiency of certain fishes at specific salinities. Gibson and Hirst (1955) reported that an isotonic solution (physiologically saline solution) produced better growth than freshwater in guppies and Canagavatnam (1959) documented that juvenile coho, sockeye and chum salmon also grew more rapidly in saltwater.

The discovery of daily growth increments in the otoliths of some teleost fishes by Panella (1971, 1974) provided an accurate means for estimating the ages of individual fish from which growth rates were obtained. Otoliths are composed of calcium carbonate crystals projecting outwards from a nucleus through an organic matrix. Three pairs (sagitta, lapillus, and astericus) are found on each side of the skull in the membranous labyrinths in many fishes. The sagitta are the largest of these and are among the calcified structures in fish known to have annual growth rings. The daily nature of otolith growth increments has been verified recently for larvae and juveniles (<40mm) of several species (Brothers et al., 1976; Radtke, 1978, 1980; Radtke and Waiwood, 1982; Uchiyama and Struhsaker, 1981; Miller and Stork, 1982; Taubert and Tranquill, 1982; and Siegfried, in prep.).

Radtke (1978) verified that daily growth increments were present in the sagittal otoliths of laboratory-reared Fundulus heteroclitus, and concluded that age structure and growth rates of F. heteroclitus could be determined in wild populations with daily increments in otoliths.

This study examines the effects of salinity on growth rates of Fundulus heteroclitus in its natural environment. Age estimates were obtained for individuals by analysis of daily growth increments in their sagittal otoliths. These age estimates provided a more accurate means of obtaining accurate age-growth information than traditional methods.

The objectives of the present study were: 1) To compare the home range of Fundulus heteroclitus in Virginia to that of previously studied areas (in Delaware) and, 2) To compare growth rates of F. heteroclitus that are resident in two different salinity regimes within the York River estuarine system.

METHODS & MATERIALS

DESCRIPTION OF STUDY AREA

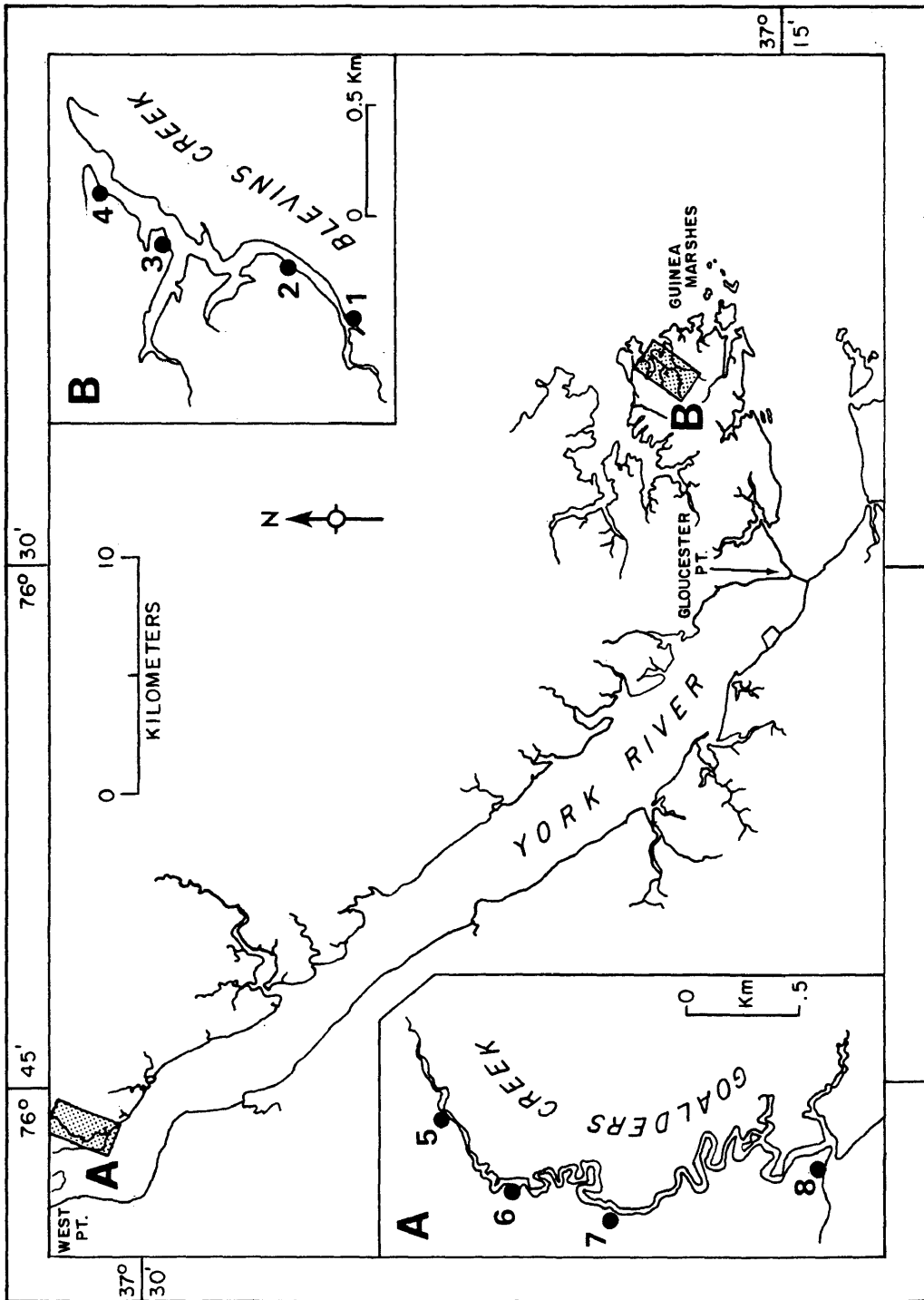
The York River is one of Virginia's three major rivers which empty into the Chesapeake Bay along its Virginian border. The York estuary covers about 200 km² and extends 46 km from the Tue Marsh light to West Point, Va.. The general trend of morphology, hydrography, and ecology were described by McHugh (1967) and Boesch (1971).

Two localities with different salinity regimes were selected for sampling (Fig. 1): 1) Goalders Creek- an oligohaline tributary at the head of the York River estuary, located in King and Queen County, Va. and, 2) Blevins Creek- a polyhaline tributary just outside the mouth of the York River, located in the Guinea Marshes of Gloucester County, Va.. Both creeks are natural systems, similar in length and depth, and typical of the ecosystems in the area.

FIELD METHODS

Biweekly collections were made at four stations in both Blevins and Goalders creeks from 13 May to 15 Sept. 1982. In Blevins creek station 1 was located at the mouth of the creek, stations 2, 3, and 4 were located progressively upstream as far as possible. In Goalders Creek, stations 5, 6, 7 and 8 were established in a similar order. All stations were located with numbered stakes along the shoreline.

FIGURE 1. Location of Goalders and Blevins Creeks within the
York River estuary



This design enabled the comparison of growth rates within and between each creek.

The substrate at all stations was soft and silty precluding the use of any collecting device other than commercially available minnow traps (5mm mesh). Juvenile fish (<25mm) escaped entrapment, and were collected with a dip net.

Stations were sampled in the same order, from 1 to 8, and as close to low tide as possible. Water temperature and salinity were recorded at each station with an immersion thermometer and a temperature compensated refractometer, respectively.

MARK AND RECAPTURE STUDY

Six sites, approximately 30 meters apart in Blevins creek, were selected for a mark and recapture study. Fish were trapped, injected subcutaneously at the base of the caudal peduncle with acrylic dye (Lotrich and Meredith, 1974), and released at each of the six stations on 17-22 August. Stations of original marking were subsequently sampled monthly for recaptures over all tidal stages. Fish captured at each station were distinctly marked by dye color and/or placement of the mark; if recaptured, fish were again distinctively marked. Different body locations and dye colors enabled a large number of specific marking combinations. For example, red was used as a mark for recaptured fish, and when placed on the right side of the caudal peduncle, this indicated a once-recaptured fish. This fish would now have two marks (color and location of original marking and recapture mark). If this fish was again recaptured, a red mark was placed on the left side of the

caudle region. Because of this process, Fundulus recaptured at any station were distinctly double or triple marked. All recapture data were log transformed and subjected to a Model II analysis of variance (ANOVA) on the total number of fish recaptured at 0 meters (point of original capture), 30m, 60m, 90m, 120m and 150m. When ANOVA indicated significance a Student-Newman-Keuls (SNK) test of multiple comparison was used to separate means which were significantly different.

LABORATORY METHODS

Fish were preserved in 95% ethanol and measured at a later date with Vernier calipers to the nearest 0.1 mm standard length (SL). Five or more individuals were randomly selected from each sample, giving a minimum of 20 fish per creek for each collecting day.

The sagittal otoliths were extracted from each fish and embedded in Spurr (1969) electron microscopy embedding medium. Spurr blocks were rough cut to expose the transverse plane, and ground to the primordium with 400 and then 600 grit wet-or-dry sandpaper. This side was polished with 0.3 micron alumina polishing compound (Fisher Scientific Co.) and attached to a piece of a glass microscope slide with commercially available cyanoacrylate adhesive. The opposite side was then ground and polished to obtain a thin transverse section [this procedure is only a slight modification of that described by Haake et al.(1982)].

Growth increments were counted with a light microscope at 1000X magnification. The mean of four successive increment counts on each otolith was used for all age determinations. Verification of

counting precision was made by comparisons of light microscopy counts with scanning electron microscopy (SEM) counts of the same otolith preparation. Only otoliths in which the difference among successive counts was 5% or less were used. This included approximately 75% of all samples prepared. Counts were made by the same reader and in the same region of the otolith. The counts were hand tallied on a mechanical counter and were made without the knowledge of previous counts.

Otoliths used for SEM comparisons were prepared as described above and then attached to viewing stubs. The exposed side of the otolith preparation was then etched for one to five minutes with 5 percent disodium ethylenediaminetetraacetate [EDTA, (pH 7-8)], coated with gold-palladium, and observed under SEM operated at 20 to 40 kV.

In order to gain further confidence in reading otoliths a known age series was obtained. These fish were prepared as described above, and the "art" of reading daily growth rings for this species was developed by the author.

Length vs. age regressions of age on length were computed for each of the eight sampling stations by Model II analysis of variance. Analysis of covariance was used to determine if differences existed in the age-length regressions. Based on this analysis, growth rates were compared among sites over the entire sampling period. The results of statistical analysis are reported as the probability (P) of observing a deviation as large or larger, solely due to chance.

RESULTS

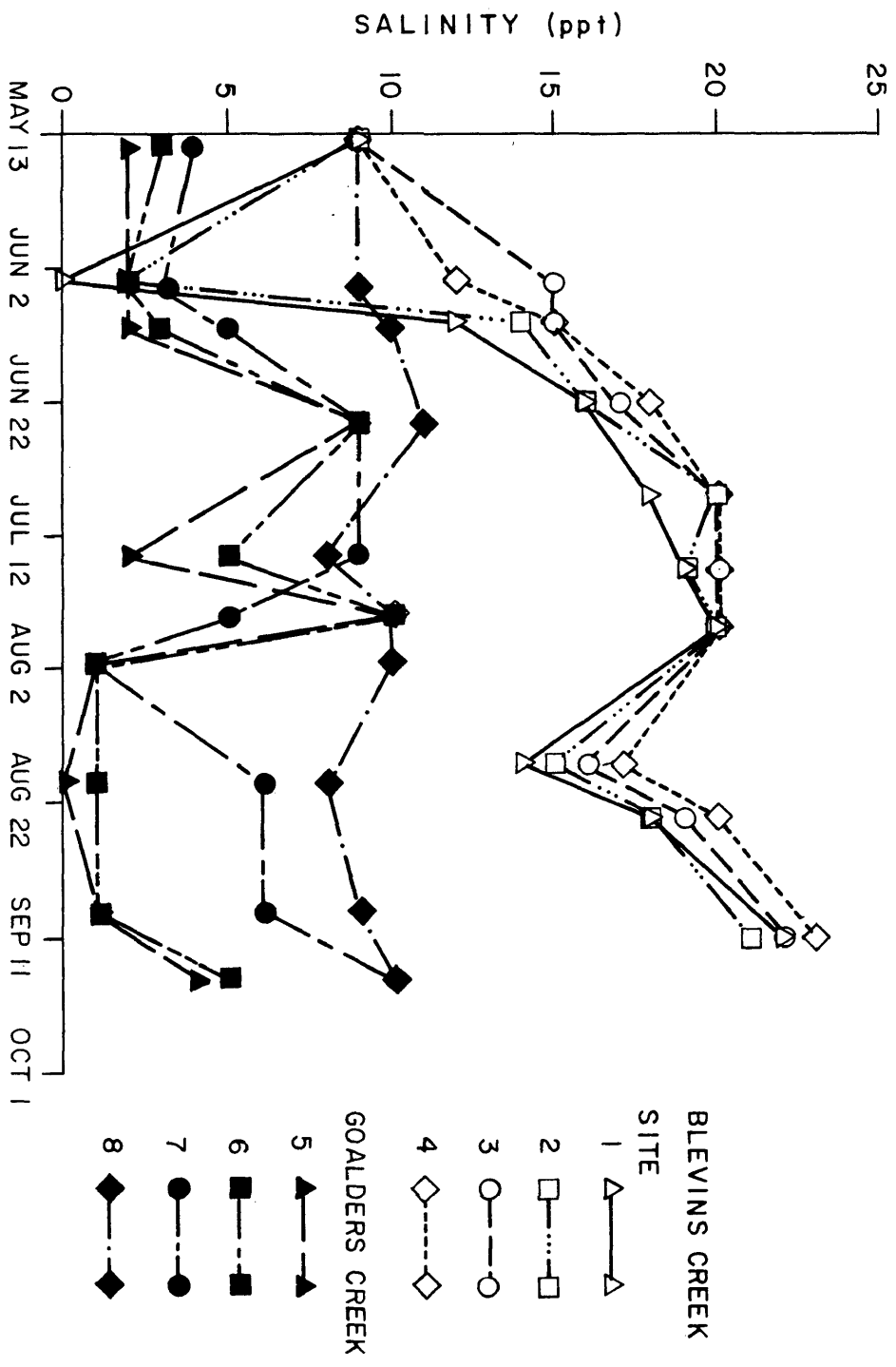
PHYSICAL DATA

Stations 1-8 were situated at depths of less than 1 meter. Temperature and salinity data were subject to strong influences by environmental conditions, which accounted for much of the variability of these data.

With the exception of the two upstream stations in Blevins Creek on 2 June, salinity values were higher at Blevins creek than at Goalders creek (Fig. 2). An ANOVA detected a significant difference between stations at the $P < .001$ level. The results of SNK indicates a significant salinity difference at low tide between Blevins and Goalders Creeks (Table 1). No differences were detected among the Blevins Creek salinity data. In Goalders Creek a difference was shown between the two most downstream stations (stations 5 and 6) and the two most upstream stations (stations 7 and 8). Although the salinity values within each creek showed little or no significant differences among stations, (ANOVA, $P < .001$) within Goalders Creek a more variable salinity regime was detected.

The trend was similar at all stations for temperature data (Fig. 3.), ranging from 20-25°C in late May and June, 28-34 °C in August and 22-26°C in September. Analysis of variance indicated no two

FIGURE 2. Salinity data for Blevins and Goalders Creeks



MEAN SALINITY VALUES

	Goalders Creek					Blevins Creek			
Mean	3.89	4.11	5.89	9.33		14.80	15.40	17.30	17.70
Station	<u>8</u>	<u>7</u>	<u>6</u>	5		<u>4</u>	<u>3</u>	<u>2</u>	<u>1</u>
			—						

TABLE 1. Results of SNK test for multiple comparisons of salinity data. Lines connect samples in which no significant differences in means were detected at $P < .001$.

FIGURE 3. Temperature data for Blevins and Goalders Creeks



stations to be significantly different at the $P < .001$ level.

Stations were sampled in the same order and at the same tidal stage whenever possible in an attempt to standardize the temperature and salinity data.

HOME RANGE

The prediction for the mark and recapture study was that fish at any station would be recaptured primarily at that station, with some captured at 30 meters and almost no recaptures greater than 30 meters away.

A total of 2500 fish were marked and subsequently released. Of the 15.7% (394) recaptured, 82.7% were recaptured at the same site (Table 2). An ANOVA detected significant differences among sites at the $P < .001$ level. The results of the SNK test show significant difference between 0m (site of original marking) and 30m (next closest point of recapture). The conclusion from this analysis is that Fundulus heteroclitus are usually restricted to a home range of less than 30 meters in Blevins creek, although some individuals may move greater distances.

AGE AND GROWTH

Examination of known age Fundulus heteroclitus have shown that 2-3 increments were present at the time of hatching (Radtke, 1978),

TOTAL RECAPTURED

<u>SITE</u>	<u>MARKED</u>	<u>RECAPTURED</u>	<u>0M</u>	<u>30M</u>	<u>60M</u>	<u>90M</u>	<u>120M</u>	<u>150M</u>
1	329	100	84	0	4	4	8	0
2	231	73	51	18	0	2	2	0
3	368	59	51	2	4	0	0	2
4	294	68	53	15	0	0	0	0
5	438	55	50	1	4	0	0	0
6	510	39	37	0	0	2	0	0
<hr/>								
TOTAL	2500	394	326	36	12	8	10	2

TABLE 2. Blevins Creek mark and recapture data.

therefore, age estimates in this study were equal to the mean of four successive counts less three for each individual.

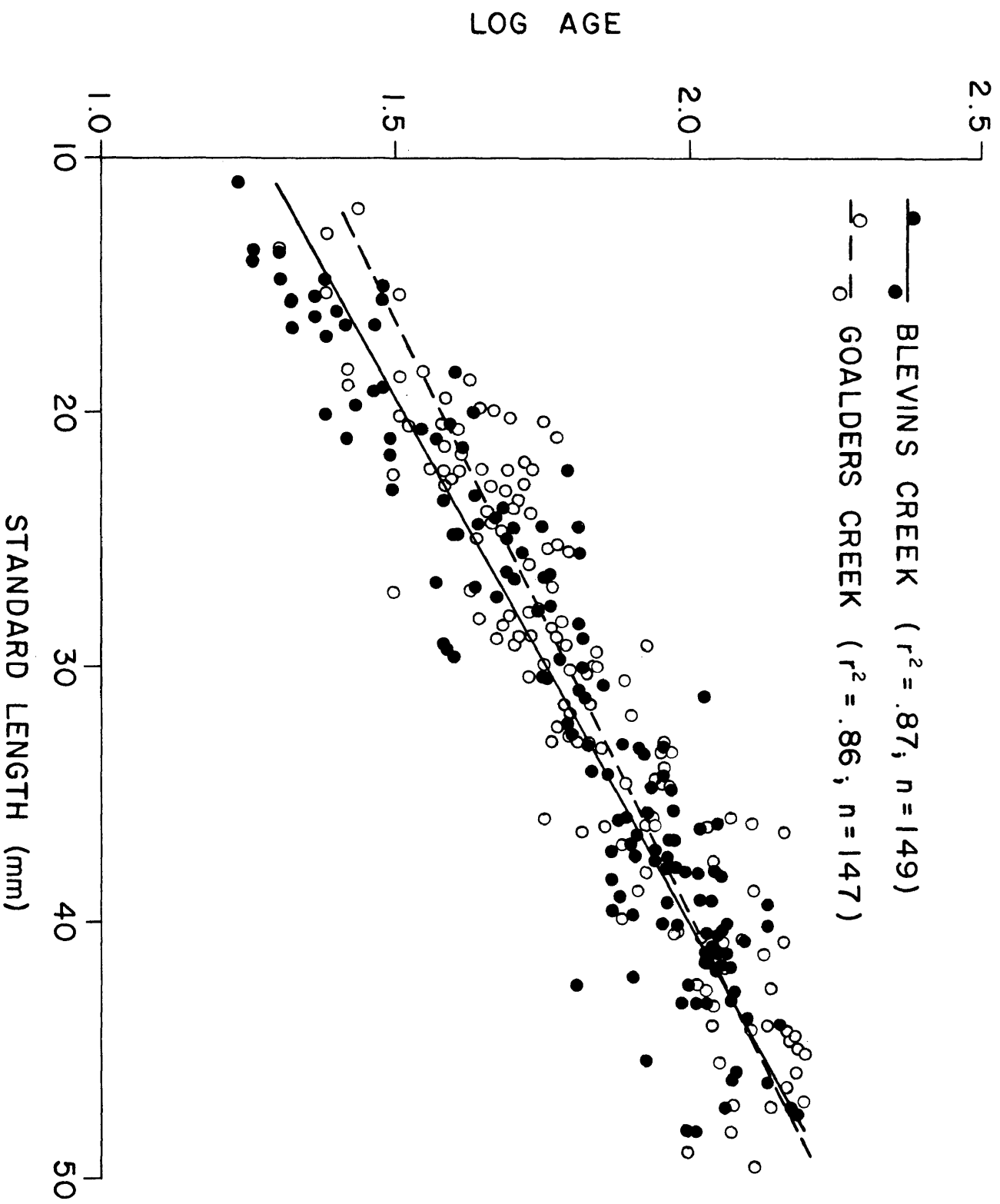
A total of 396 otoliths were counted. Otolith based age estimates ranged from 30 days for a 12 mm (SL) fish, to 160 days for a 47 mm (SL) fish.

Linear regression of length on adjusted age were computed for samples from each of the eight stations. Length was the independent variable and rings the dependent variable. In this way the number of rings depended on the size of the fish, which was assumed to be fixed and measured without error.

Analysis of covariance indicated that there were no significant differences in the regression lines when data were grouped to compare within creek growth rates ($P > .05$). Therefore, values from each creek were pooled and the regression of age on length of Blevins Creek data was compared with the regression of age on length from Goalders Creek (Fig. 4).

The model which best described age with growth for Goalders Creek was a linear regression of $\log(\text{age})$ vs. standard length: $SL = A(\text{age}) + B$, where B is the y-intercept and A is the slope of the line. For Blevins Creek the equation was: $SL = .024 - 1.03(\text{age})$; while Goalders Creek was: $SL = .022 - 1.14(\text{age})$. Analysis of covariance indicated that there was no significant difference between the two regression coefficients or adjusted means at the $P > .05$ level.

FIGURE 4. Regression lines of the Otolith Data of Blevins and
Goalders Creeks



DISCUSSION

The relationship between salinity and growth rates was examined in this study. Redeke (1932), Segerstrale (1951,1953), Kilby (1955), Gunter (1961), and Weinstein et al. (1980) all supplied evidence that the salinity of water was an important factor governing the distribution of estuarine species.

Mummichogs are abundant at virtually all salinities within the estuarine system. Weinstein et al. (1980) reported that Fundulus heteroclitus displayed equal abundance over a salinity range of 0-31 ‰, in the Cape Fear River estuary, North Carolina. Richards and Castagna (1970) collected F. heteroclitus over a range of 0.4 to 28.7 ‰ in the seaside waters of Virginia's eastern shore. Under laboratory conditions a tolerance range for the species was determined as 0 to 113 ‰ (Griffith, 1974). In the present study, F. heteroclitus were collected at all stations in salinities from 0 to 22 ‰. Although mummichogs were found at all stations, their abundance was considerably lower in the three most upstream stations of Goalders Creek (Smith et al. 1985). Despite this lower observed abundance, fish were consistently trapped there with increased effort. The salinity data indicate that these stations were significantly fresher than the downstream station and indeed the least saline stations in this study. This may be an indication that there is a preference for low salinity habitats by F. heteroclitus during summer months.

Fundulus heteroclitus's ability to osmoregulate in varying salinities is shared by other euryhaline teleosts. Many physiological functions first described by Smith (1930) are involved. In the marine environment the fish must balance the net efflux of water and influx of sodium and chloride by drinking the medium, excreting small quantities of urine that contain less sodium and chloride than the blood and excreting the excess sodium and chloride across the gills. In freshwater a fish must balance the net influx of water and efflux of sodium and chloride by excreting large quantities of dilute urine that contain much less sodium and chloride than the blood by actively pumping in sodium and chloride from the medium across the gills.

The distinct physiological strategies described above lead to an attractive hypothesis. The environment which is isotonic to the fish's bloods should be an optimal habitat. It does not seem unreasonable to assume that in general more metabolic work is required to sustain a large osmotic gradient than to sustain a small osmotic gradient. This hypothesis is supported by the data of Gibson and Hirst (1955), Canagavatnam (1959), Brocksen and Cole (1972), and Wohlschlag and Wakeman (1978).

The data presented in this study supports an alternate hypothesis. The growth of the euryhaline Fundulus heteroclitus does not appear to be affected by residing in different salinity regimes. This would suggest that the fish has adapted physiologically to a wide salinity spectrum without sacrificing energy that could otherwise be utilized in growth. This adaptation may be in the

development of specific enzyme systems, impermeability, and/or specialized ion transport mechanisms.

Euryhaline species adapt to a wide range of salinity by utilizing a variety of mechanisms. The gills are the principle site of monovalent ion excretion. Movement of these ions catalyzed by the enzyme adenosine triphosphatase (ATPase) results in active transport via "sodium pumps". Epstein et al. (1967) reported that the gills of killifish acclimated to seawater possessed Na^+ and K^+ -ATPase activity two to seven times that of killifish acclimated to freshwater. Towle et al. (1977) have documented similar evidence and further reported intermediate enzyme activity associated with acclimation, suggesting that the same enzyme system may function to absorb ions at low salinities and excrete ions at high salinities. This increase in Na^+ and K^+ activated adenosine triphosphatase activity coupled with an increase in active sodium outflux suggests a specific enzyme system important to Fundulus heteroclitus osmoregulation. This system may explain how mummichogs are able to maintain similar growth rates in both hypertonic and hypotonic media.

LOCAL MOVEMENTS

Past studies on the local movements and activity of Fundulus heteroclitus have been reported by Chidester (1920), Butner and Brattstrom (1960), Nixon and Oviatt (1973), Lotrich (1974) and Fritz et al. (1975). While Chidester (1920) observed that F. heteroclitus appeared to remain in the tidal creek throughout the summer, and Lotrich (1974) suggested a limited home range of 36 meters, Buttner

and Brattstrom (1960) found little evidence of F. heteroclitus maintaining a home range in salt marshes.

The mark and recapture data from this study suggests a home range limited to include 30 meters but not more than 60 meters for most individuals. Although 82.7% of the marked fish were recaptured at the site of marking, 16.3% were recaptured at a distance greater than 30 meters away from site of original marking. It is clear that while Fundulus heteroclitus maintains a limited home range, some individuals drift out of the 30 meter range. With the wide geographic distribution and diversity of local habitats occupied by F. heteroclitus, it is not surprising that variation in local movements occurs. Genetic composition of local populations, physical conditions, resources as related to fish density and interspecific interactions are a few factors that may influence the home range for any species. These data support Lotrich's (1974) idea of an overlapping continuum of fish along the creek bank as being the principle mechanism for genetic exchange for this species.

The use of baited minnow traps may introduce a bias if certain individuals are more or less susceptible to trapping (Lotrich, 1974). If a group of individuals exists which has zero probability of entering a trap, their behavior is not testable by methods employed in this study. Some species and/or individuals may become "trap happy", introducing a bias in the other direction.

GROWTH

The hypothesis of an isotonic medium influencing growth rates is not supported by this study. The data presented indicate no

difference in growth rates of mummichogs from at least two different natural salinity regimes.

The relatively sedentary mummichog, with its high densities along the marsh bank, may be prone to heavy predation. In the evolutionary history of the species some strategies should have evolved to counter the disadvantage of such limited movements. Weisberg (1982) reported that Fundulus heteroclitus must have access to the high marsh in order to obtain food of maximum caloric value. This would be of significance during periods of high water where marshes are regularly flooded. Additional advantages gained by high marsh utilization include a refuge from subtidal predatory type fishes such as, Paralichthys spp., Cynoscion spp., Pomatomus spp., and Anguilla spp., in an area of restricted current flow. In habitats where the high marsh is inaccessible other strategies must have developed. Lotrich (1974) suggested that as the fish maintains position, food is carried past the organism and little energy is expended in food search and capture. Development of these strategies allows the energetic costs of osmoregulation, physiological stress, and maintaining a position against currents to be offset.

There are many factors or combinations of factors that may play an important role in daily ring formation. Exactly which factors affect this rhythmic deposition of rings have not yet been elucidated. Radtke (1978) worked with Fundulus heteroclitus in controlled laboratory experiments where salinity was held constant. He concluded that otolith increment formation appeared to be exogenous. Length of daylight, temperature, and food availability seemed to have the major effects in his study. In the present study

food availability is the only of these factors that might have differed between creeks. If differential growth rates were observed, the availability of food and its particular caloric value would have to be investigated. The abundance and distribution of particular food items with potentially higher caloric values might easily be a factor contributing to an optimal environment.

In addition to food availability, many other biotic and abiotic conditions should be considered when growth rates are being studied. Unstable physical conditions, increased energy expenditure in the search and capture of prey, interspecific relationships contributing to size-selective mortality, and the numerous interactions of these important variables influence growth in natural habitats. The data presented in this study should not be interpreted as unequivocal evidence that salinity has little or no effect on the growth of Fundulus heteroclitus. To draw this conclusion, further research is needed from controlled laboratory experiments to isolate the primary effects of salinity on growth rates.

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