

these tests were conducted during March–April when juvenile mackerels are rare in the coastal waters of South Carolina.

In 1986 we collected juvenile mackerels during July through October. Because of incomplete temporal sampling, we do not know if they were present earlier and later in the year in this region. Based on the occurrence of early larval stages, spawning of both mackerels in the South Atlantic Bight extends from May through at least September (Collins and Stender 1987). If growth rate estimates of ca. 3 mm/day for juveniles are correct (M. R. Collins, unpubl. data), king mackerel spawned in early May could be recruited into the bycatch of the shrimp fishery in June. Late-spawned fish from the previous year may also be present at this time. In South Carolina, the open season for commercial trawling for penaeid shrimps in state waters usually extends from June through December, which coincides with the presence of juvenile mackerels in the heavily fished nearshore waters. In addition, mackerels were much more abundant in tows made in depths <9 m, which includes the preferred shrimping areas, than in deeper waters. This may be due either to greater abundance in these depths or to greater catchability in response to the fact that the trawl nets fish a larger portion of the water column in shallower areas.

It is difficult to accurately estimate the bycatch of mackerels in the commercial shrimp fishery owing to lack of current, detailed information from throughout the region on number of vessels, effort expended, gears used, and areas fished. However, our catch rates suggest that the impact of tongue nets on mackerel stocks may be significant. As the current status of these stocks is such that strong restrictions have been imposed on both the recreational and commercial fisheries, it is unfortunate that the situation may be exacerbated by a potentially large bycatch of juvenile mackerels in the shrimp fishery. More information is needed on the ecology and behavior of young mackerels, and their vulnerability to various gears, in order to resolve this conflict.

#### Acknowledgments

We wish to express our appreciation to the many people who assisted in the tedious tasks of collecting and processing trawl samples over the

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#### STOMACH CONTENTS OF COMMERCIALY CAUGHT HUDSON RIVER STRIPED BASS, *MORONE SAXATILIS*, 1973–75

The Hudson River estuary is a detritus-driven ecosystem. Only a few of the 100 or more reported fish species function as tertiary piscivores more typical of a grazing food chain. Of these few species, which include the American eel, *Anguilla rostrata*, and the summer-transient juvenile

bluefish, *Pomatomus saltatrix*, striped bass, *Morone saxatilis*, are probably the most important in terms of biomass and commercial value. However, commercial fishing for the American eel and striped bass has been banned in the Hudson since 1976 because both species are contaminated with higher levels of toxic polychlorinated biphenyls (PCB's) than most other Hudson River fish species.

To date, only one published paper has touched upon the diet of prespawning Hudson River striped bass larger than 400 mm TL (Gardinier and Hoff 1982), and their results for fish of that size are based on less than 10 samples for which fish remains were identified to species or family level. The present paper describes the findings of stomach content analysis for 510 striped bass, most of which were prespawning adults larger than 400 mm TL (Fig. 1) collected during the spring months of 1973–75 by commercial gill nets in the Tappan Zee region of the Hudson River.

#### Description of Sampling Area and Methods

The Tappan Zee gill-net fishery is located approximately 90 m south of the Tappan Zee Bridge at river km 43.5 (measured from the Manhattan Battery) in a relatively shallow section that is 4.5 km wide, a location through which many of the spawners move on the way to upstream spawning grounds. The staked gill nets were set perpendicularly to the north-south current in water depths

of 2.4–9.8 m and spanned a distance of approximately 1.6 km out to the dredged channel on the east side of the river. Stretched mesh sizes were 11.4, 11.8, 12.1, 12.7, 13.3, and 14.0 cm. Water temperatures during the sampling period ranged from approximately 6° to 19.5°C, and the salinity was essentially that of freshwater, rarely rising above 300 mg/liter. Sampling dates included the following periods in each year: 28 March–9 May 1973, 8 April–20 May 1974, and 6 April–19 May 1975. Fish were collected from the nets at 8–12 h intervals, placed on ice, and taken directly to a laboratory for total length (TL) measurements and stomach content analysis. Prey items were identified with the aid of a binocular dissecting scope and frequency of occurrence was noted.

## Results

#### Stomach Content Analysis

A summary table of stomach content data, pooled for the three years of analysis (Table 1), shows that adult, prespawning striped bass were highly piscivorous and that 89% of the stomachs containing identified food items contained fish and 21% contained invertebrate remains, primarily those of *Crangon* species. Clupeid species were the most prevalent fish, with blueback herring, *Alosa aestivalis*, predominating. Most of the clupeids were adult, prespawning herring, approximately 200 mm TL.

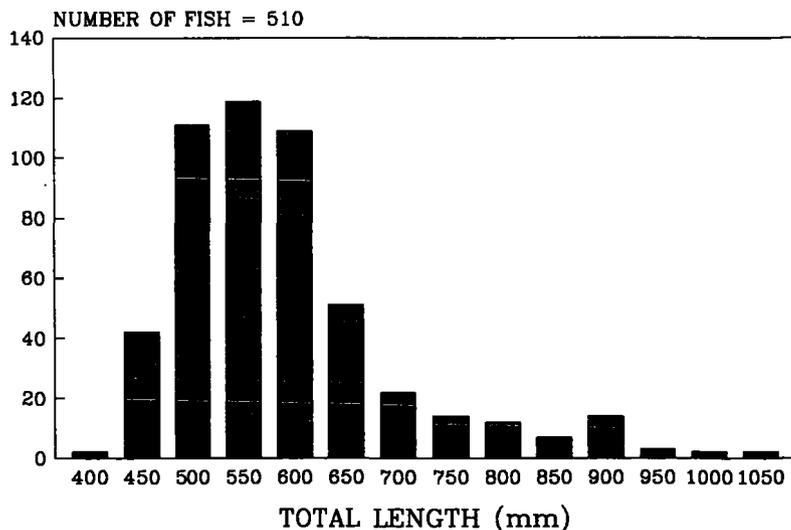


FIGURE 1.—Length distribution of Hudson River striped bass examined for stomach contents.

TABLE 1.—Stomach contents of adult prespawning striped bass collected by commercial gill net during spring 1973, 1974, and 1975.

Food item	Number of stomachs in which item occurred	% Frequency of occurrence based on total stomachs analyzed (n = 510)	% Frequency of occurrence based on number of stomachs containing identified food items (n = 201)
Fish	179	35.1	89.1
Clupeidae	73	14.3	36.3
<i>Alosa pseudoharengus</i>	13	2.5	6.5
<i>Alosa aestivalis</i>	29	5.7	14.4
<i>Brevoortia tyrannus</i>	3	0.6	1.5
Clupeidae	28	5.5	13.9
<i>Morone</i> spp.	9	1.8	4.5
<i>Morone americana</i>	6	1.2	3.0
<i>Morone saxatilis</i> <sup>1</sup>	1	0.2	0.5
<i>Morone</i> spp.	2	0.4	1.0
Other fish	29	5.7	14.4
<i>Ammodytes americanus</i>	14	2.7	7.0
<i>Microgadus tomcod</i>	7	1.4	3.5
<i>Osmerus mordax</i>	2	0.4	1.0
<i>Syngnathus fuscus</i>	2	0.4	1.0
<i>Anchoa mitchilli</i>	2	0.4	1.0
<i>Ictalurus catus</i>	1	0.2	0.5
<i>Scomber scombrus</i>	1	0.2	0.5
Unidentified fish remains	68	13.3	33.8
Invertebrates	43	8.4	21.4
<i>Crangon</i> spp.	38	7.5	18.9
<i>Polychaeta</i>	5	1.0	2.5
Unidentified contents	11	2.2	Not applicable
Total stomachs analyzed			510
Number (percent) containing identified food items			201 (39.4)
Number (percent) containing food material (identified and unidentified)			212 (41.6)
Number (percent) empty			296 (58.4)

<sup>1</sup>Two yearling striped bass in female collected 15 April 1974.

The next most abundant fish prey species in the composite 1973–75 sample was the American sand lance, *Ammodytes americanus*. Sand lance were present in striped bass stomachs only during 1975, when the frequency of occurrence was about 11% (Fig. 2). Atlantic tomcod, *Microgadus tomcod*, were found in striped bass stomachs only during 1973 when the frequency of occurrence was about 20%, somewhat higher than the frequency of clupeid species for that year (Fig. 2). These fish were yearling, postspawning Atlantic tomcod averaging about 125 mm TL. The *Morone* species, white perch, *M. americana*, and striped bass, had a frequency of occurrence of 4.5% for the composite sample and were present in striped bass stomachs every year (Fig. 2).

### Discussion

Similar to findings in this study, Trent and Hassler (1966) found that blueback herring were predominant in the diet of adult, prespawning striped bass from the Roanoke River, NC, and Manooch (1973) found that herring ranked second after Atlantic menhaden, *Brevoortia tyrannus*, in

the spring diet of adult striped bass from Albe-marle Sound, NC. Data from Gardinier and Hoff (1982) do not indicate that blueback herring or clupeids were important in the diet of prespawning Hudson River striped bass collected during 1976 and 1977, but fish remains in bass stomachs were generally not identified to the family or species level in their study.

American sand lance are rarely found over muddy bottoms (Bigelow and Schroeder 1953; Leim and Scott 1966) or within estuaries (Massman 1960; Norcross et al. 1961), but they ranked second only to blueback herring in the diet of Hudson River striped bass. Sand lance were not reported from the Hudson River prior to 1975 when adults and larvae were relatively abundant in February–April ichthyoplankton collections south of the Tappan Zee Bridge (Dew and Hecht 1976). Data from the present study (Fig. 2) and from early spring ichthyoplankton sampling during 1976 (Dew, unpub. data), indicate that sand lance are not abundant every year in the Hudson, but it is probable that during some years adult sand lance serve as important alternate prey for prespawning striped bass.

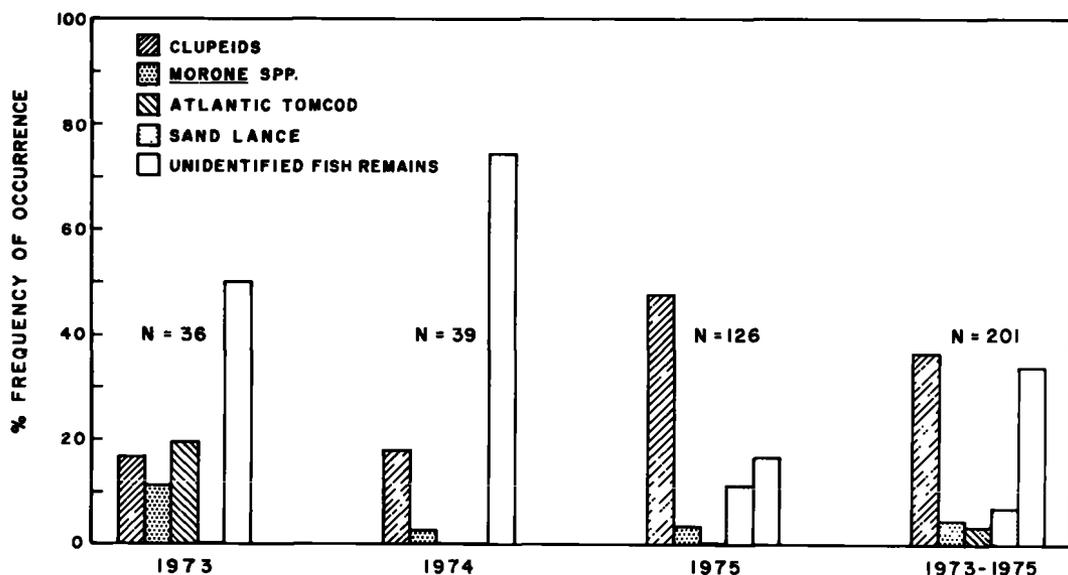


FIGURE 2.—Percent frequency of occurrence of fish prey categories based on number of stomachs containing identified food.

The fact that Atlantic tomcod were found only during 1973 may be due to a sampling artifact because 1973 was the only year in which March samples were taken, and all tomcod prey were found during March 1973. In March, gravid alewives, *Alosa pseudoharengus*, and blueback herring have not yet moved into the estuary and, prior to the arrival of these apparently preferred prey species, Atlantic tomcod may serve as an alternate forage fish. Thus the winter-spawning Atlantic tomcod may be a regular diet item at a time when alternate prey species are not available.

It is evident from Table 1 that there are several alternate fish prey for striped bass in the Hudson River (e.g., blueback herring, sand lance, alewife, Atlantic tomcod, white perch, and striped bass), and it is expected that striped bass predation upon these species would be of a compensatory nature. In a simple predator-prey situation where there are no alternate prey, predation is often compensatory (Neave 1953) because a population of predators would tend to seek out and consume a high proportion of small year classes and a lower proportion of strong year classes. In a more complex community where there are several species of prey, predation may be compensatory if predators change their feeding habits in response to the availability of food (Ivlev 1961; Forney 1971). That is, if several equally suitable prey species were available within a system, the predator spe-

cies would tend to feed more heavily on the most abundant species, thus acting in a compensatory manner.

The ratio of white perch to striped bass in stomach samples identified to the species level is 6:1 (Table 1). This ratio is based on a small sample size, but it closely approximates the long-term average ratio of 5.9:1 for annual CPUE values for white perch and striped bass collected in several hundred bottom trawls from the Haverstraw Bay region (Milepoint 36) during 1971-77 (Lawler, Matusky and Skelly Engineers unpubl. data). In other words, yearling and older white perch and yearling striped bass may be equally attractive prey species, and their frequency of occurrence in adult striped bass stomachs may depend primarily upon the frequency of random encounters rather than active prey selection. If this were true, the frequency of cannibalism should be greatest during those years when the abundance of yearling striped bass relative to white perch (and other species) was greatest.

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## ACCUMULATION OF AGE PIGMENTS (LIPOFUSCIN) IN TWO COLD-WATER FISHES

In fisheries management, age structured models are the preferred method for meeting the key objectives of estimating optimal yields and determining the effect of fishing on stock structure (Gulland 1978). However, few species of commercial marine fishes exist in which age can be determined with certainty (Boehlert 1985). The concentration of age pigments (lipofuscin) (Ettershank 1984) in fish tissues (Agius and Agbede 1984) may be a measure of fish age that could be used to validate other ageing techniques and might also improve estimates of age of long-lived species where other techniques are difficult to apply.

Lipofuscin originates in biological membranes through lipid peroxidation (Tappel 1975). Lipofuscin accumulation has been documented for a wide variety of animals, from mammals to the bread-mold *Neurospora* (Ettershank et al. 1983 and references therein). The rate of accumulation has been shown to be constant during the lifetime of laboratory-raised mice (Reichel 1968; Miquel et al. 1978), dogs (Munnell and Getty 1968), flesh-flies (Ettershank et al. 1983); man (Strehler et al. 1959); and also wild populations of mice (Dapson et al. 1980). On the other hand, the rate of lipofuscin accumulation has been shown to vary with level of activity and lifespan (Sohal and Donato 1978). It is expected that the rate in which lipofuscin accumulates with age in a natural population of fishes, or stock, with its free genetic interchange and likely common habitat, would be fairly uniform (but see Smith 1987). In addition, if measurements are made in nonmitotic and constantly metabolizing tissues such as brain or myocardium, the variation in concentration due to environmental effects is least likely.

In this paper we present the results of a preliminary study designed to assess the usefulness of extracted lipofuscin as a method of ageing fishes. Two species of cold-water fishes are included: the rainbow trout, *Salmo gairdneri*, reared in captivity, and of known age; and the Dover sole, *Microstomus pacificus*, a long-lived fish, in which age is not known with certainty. We present spectral characteristics of the extracted lipofuscin from several tissues and the change in concentration of lipofuscin with fish age.

## Materials and Methods

Specimens of Dover sole were collected on December 1986 off Point Conception. Ages were estimated by J. Butler, E. Lynn, and M. Drawbridge of the Southwest Fisheries Center using otolith sections. Ages ranged from 2.7 to 44.7 years (average of three readings). Specimens of the species *Salmo gairdneri* were collected in July 1986 at Hot Creek Hatchery. Ages ranged from 3 months to 3 years.

Samples were frozen after collection and kept at  $-80^{\circ}\text{C}$  until analysis. Studies by Nicol (1987) showed that this form of preservation yielded the lowest fluorescence when compared with ethanol and formalin-preserved samples. There was no indication that the level of fluorescence changed as a function of time of preservation or by interaction with the extracted lipofuscin, as in the case for formalin. To excise the brain, the top of the skull was opened and the four brain lobes were removed as a unit, without the optic nerve. To excise the heart, we separated the muscle from connective tissue, blood vessels, and fat deposits.

Three methods were compared for maximum lipofuscin extraction. The first two methods (Tappel 1975; MacArthur and Sohal 1982) were specifically developed for lipofuscin extraction and employed chloroform:methanol (2:1) as the extractive solvent; they differ in the optimal volume-to-weight ratio (30:1 and 20:1, respectively), temperature of extraction, and number of times the chloroform phase is washed with water. The third method was developed for lipid extraction in fishes (Bligh and Dyer 1959) and uses chloroform:methanol:water (1:2:0.8) as the extractive solvent. Subsamples ( $n = 3$ ) of cerebellum of *Stenella* sp. collected at the eastern tropical Pacific and kept at  $-30^{\circ}\text{C}$  were defrosted, dried with lint-free paper, weighed, and extracted following the three methods as described originally.

Tissues from Dover sole were extracted basically following the MacArthur and Sohal (1982) technique, with two additional steps to ensure a complete washing out of flavoproteins and photooxidation of retinol when present (Fletcher et al. 1973). Tissue was freeze dried prior to analysis. Ten mL of chloroform:methanol (2:1, v/v) was added to a homogenizer containing the sample for a final solvent:sample ratio of about 120:1 (vol/dry weight). The sample was ground with a teflon pestle attached to an electric drill and later submerged in a  $40^{\circ}\text{C}$  water bath for 1 minute. A 2 mL

subsample was taken from the homogenate. Three mL of deionized water was added, the sample was shaken, and the emulsion centrifuged 10 minutes at  $1,912\text{ g}$  and  $0^{\circ}\text{C}$ . After centrifugation, the hyperphase was decanted and a second rinse performed. After decanting the hyperphase a second time, 1 mL of the hypophase (chloroform containing lipofuscin) was sampled and transferred to a polypropylene tube. Three mL of chloroform were added to the sample (for a total of 4 mL) which was then exposed to UV irradiation (254 nm) for 1–2 minutes to photooxidize retinol. This last step was routinely performed for liver tissue.

Samples were then transferred to glass tubes, sealed, and kept in the refrigerator until analysis. Sample fluorescence was measured at the emission peak (430–440 nm) in a quartz cuvette with a Perkin Elmer Fluorescence Spectrophotometer<sup>1</sup> Model MFP-44A. The sample was excited at the peak of fluorescence excitation ( $\sim 360$  nm). The intensity of the fluorescent emission (at 430 nm) was normalized to the intensity of the quinine solution standard ( $1\text{ mg L}^{-1}$  in 1N sulphuric acid) and expressed in fluorescence units.

Lipofuscin in Dover sole is expressed as 1) total lipofuscin content per organ and 2) weight-specific lipofuscin concentration, calculated by dividing the total lipofuscin content by the dry weight of the entire organ.

Lipofuscin from rainbow trout tissues was extracted following the Bligh and Dyer (1959) technique, without modifications. Whole tissues were ground in water with a tissue homogenizer to give a final concentration of 100 mg of tissue (wet weight) in 0.8 mL of homogenate. A sample of 0.8 mL was taken and solvents were added to give a final ratio of 1:2:0.8 (chloroform:methanol:water) with a solvent to sample ratio of 20:1 (vol/w). The sample was then filtered through a 2.4 cm glass fiber filter (Whatman GF/C), the tissue re-extracted with 1 mL of chloroform and refiltered. The extract was then washed with 3 mL of water, shaken, and centrifuged for 10 minutes at  $1,912\text{ g}$  and  $0^{\circ}\text{C}$ . One mL of the chloroform hypophase was subsampled and fluorescence estimated as described above.

Lipofuscin in rainbow trout is expressed as 1) total lipofuscin content per organ and 2) weight-specific lipofuscin concentration (total lipofuscin content of the organ divided by its wet weight).

<sup>1</sup>Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

## Results

### Efficiency of Extraction

Lipofuscin was successfully extracted and quantified from three different fish tissues: brain, heart, and liver. Wavelengths of fluorescence excitation and emission maxima of extracted lipofuscin in chloroform are presented in Figure 1. All maxima are within the range cited by Sheldahl and Tappel (1973); fluorescence excitation maxima were between 340 and 370 nm and fluorescence emission maxima between 420 and 470 nm.

Retinol (wavelengths of maximum fluorescence excitation at 325–340 nm and maximum fluorescence emission at 475 nm) was photooxidized by exposing the chloroform extract to UV irradiation (254 nm). As expected, retinol was found in liver and sometimes it was present in brain and heart tissues. For example, wavelengths of fluorescence excitation and emission maxima in liver of Dover sole shifted from 352 to 365 nm and from 470 to 440 nm, respectively, after UV irradiation (see Figure 1C). Mullin and Brooks (1988) also found that this UV irradiation is effective in oxidizing retinol although they did not find significant retinol interference in fish tissue. It seems liver tissues may require UV irradiation to oxidize retinol while brain and heart tissue should be checked for retinol presence before irradiation.

The extractive efficiency of the three methods tested are compared in Table 1. All methods extracted similar fluorescent compounds from the brain of *Stenella* sp., as the fluorescence excitation and emission spectra were similar. The MacArthur and Sohal (1982) method extracted

TABLE 1.—Comparison of the three methods of lipofuscin extraction in brain tissue. Fluorescence excitation at 360 nm and fluorescence emission at 440 nm. Results are presented as fluorescence units per mg of wet tissue, where the fluorescence emission signal is normalized to the intensity of emission of a standard quinone sulfate solution (1 mg L<sup>-1</sup> in 1N sulphuric acid). The three methods were significantly different from each other,  $P < 0.05$ . Newman-Keuls range test (Zar 1974). ANOVA:  $F$  ratio = 21.81, 2 df,  $P < 0.05$ .

	Fluorescence units			Fluorescence maxima	
	Average 10-3	SD 10-3	<i>n</i>	Excitation (nm)	Emission (nm)
MacArthur and Sohal (1982)	4.30	0.39	3	362	445
Tappel (1975)	2.83	0.37	3	362	440
Bligh and Dyer (1959)	1.98	0.45	3	360	445

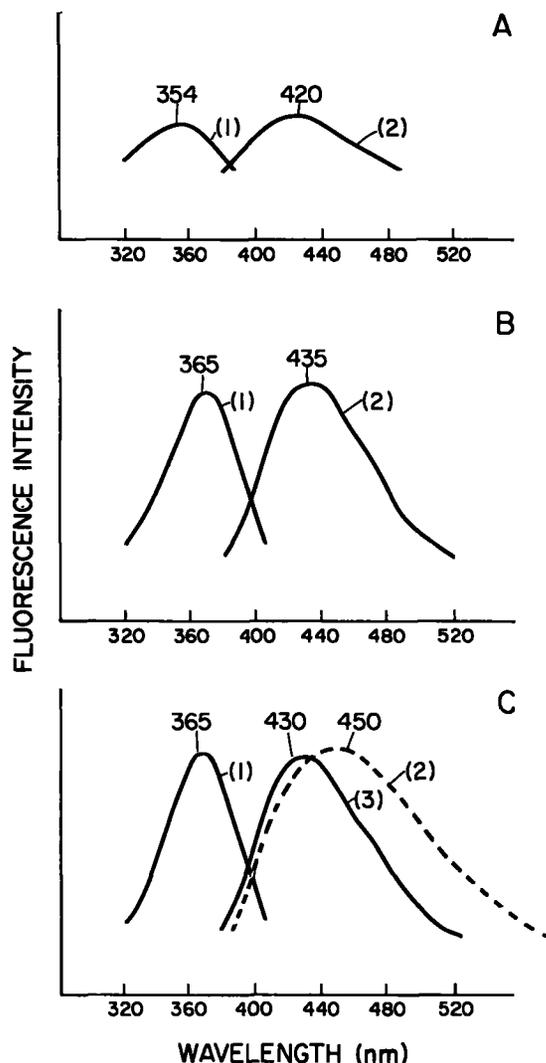


FIGURE 1.—Spectral characteristics of lipofuscin extracted from Dover sole, *Microstomus pacificus*, tissues (uncorrected spectra). In chloroform. (A) Brain: (1) Lipofuscin fluorescence excitation spectrum (emission wavelength 440 nm); (2) lipofuscin fluorescence emission spectrum (excitation wavelength 365 nm). (B) Heart: (1) Lipofuscin excitation spectrum (emission wavelength 430 nm); (2) lipofuscin fluorescence emission spectrum (excitation wavelength 365 nm). (C) Liver: (1) Lipofuscin fluorescence excitation spectrum (emission wavelength 430 nm); (2) lipofuscin emission spectrum (excitation wavelength 365 nm) before UV radiation; (3) Same as (2) after UV radiation (254 nm).

significantly more fluorescent pigment, than did either the Tappel (1975) or the Bligh and Dyer (1959) method which extracted 66% and 46% of maximum extraction, respectively). Thus all three methods are useful for quantitative estimation of extractable lipofuscin in fish tissue but

comparisons of amount of concentrations between tissues or species cannot be made if different extraction methods are used.

### Lipofuscin Concentration

The total concentration of extracted lipofuscin in Dover sole brain tissue was positively correlated with fish length (Fig. 2A) ( $Y = -15.4 + 0.516 X$ ,  $r^2 = 0.43$ ;  $b \neq 0$ ,  $P < 0.01$ ,  $t = 5.1$ ,  $df = 34$ ). Furthermore, total extracted lipofuscin content in the brain was positively correlated with age in organisms estimated by annu-

lar increments in otoliths to be from 2 to 15 years old (Fig. 3A) ( $Y = -2.5 + 1.0 X$ ,  $r^2 = 0.75$ ;  $b \neq 0$ ,  $P < 0.01$ ,  $t = 5.5$ ,  $df = 10$ ). On the other hand, in older Dover sole (estimated age from 20 to 45 years;  $n = 26$ ) the total extracted lipofuscin in the brain did not increase with their estimated age. The concentration of lipofuscin in the brain (extracted lipofuscin concentration per unit of g dry weight) followed a similar pattern (Fig. 3B). The concentration in the brain increased linearly with age for fish 2–15 years old ( $Y = 30.39 + 14.85 X$ ,  $r^2 = 0.79$ ,  $b \neq 0$ ,  $P < 0.01$ ,  $t = 6.66$ ,  $df = 10$ ) but fishes older than 15 years did not show an increase in pigment concentration with age.

The total extracted lipofuscin content extracted from rainbow trout brains increased with age (Fig. 4A) ( $Y = 0.14 + 0.18 X$ ,  $r^2 = 0.37$ ;  $b \neq 0$ ,  $P < 0.01$ ,  $t = 3.75$ ,  $df = 23$ ), as did the content of the heart (Fig. 4C) ( $Y = -0.54 + 0.82 X$ ,  $r^2 = 0.67$ ;  $b \neq 0$ ,  $P < 0.01$ ,  $t = 7.36$ ,  $df = 23$ ), and liver (Fig. 4E) ( $Y = -2.86 + 5.81 X$ ,  $r^2 = 0.62$ ;  $b \neq 0$ ,  $P < 0.01$ ,  $t = 7.54$ ,  $df = 34$ ) from 3 months to 3 years. The concentration of extracted lipofuscin per unit wet weight of heart tissue did not change with age (Fig. 4D) whereas that of brain and liver tissue decreased with age (Fig. 4B, F).

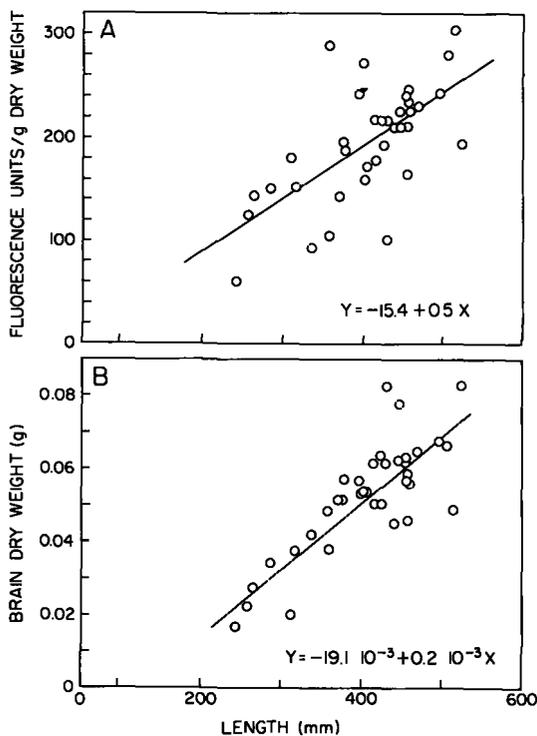
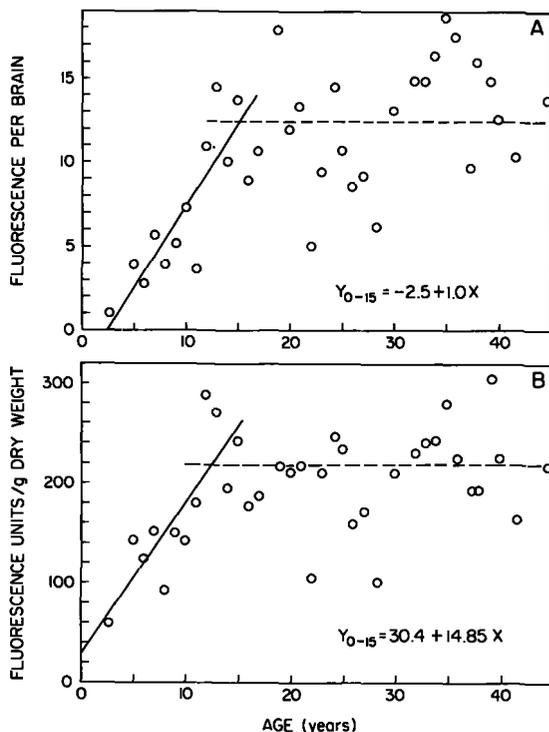


FIGURE 2.—Dover sole, *Microstomus pacificus*, brain: (A) Extracted lipofuscin per unit of dry weight as a function of fish length (mm) ( $n = 36$ ); (B) brain dry weight (g) as a function of fish length (mm). Fluorescence units: the intensity of fluorescence normalized to a standard solution of quinine sulfate ( $1 \text{ mg L}^{-1}$  in  $1 \text{ N}$  sulphuric acid).

FIGURE 3.—Lipofuscin in Dover sole, *Microstomus pacificus*, brain tissue as a function of age ( $n = 36$ ). (A) Total lipofuscin content per brain; (B) weight-specific (g dry weight) lipofuscin in brain.



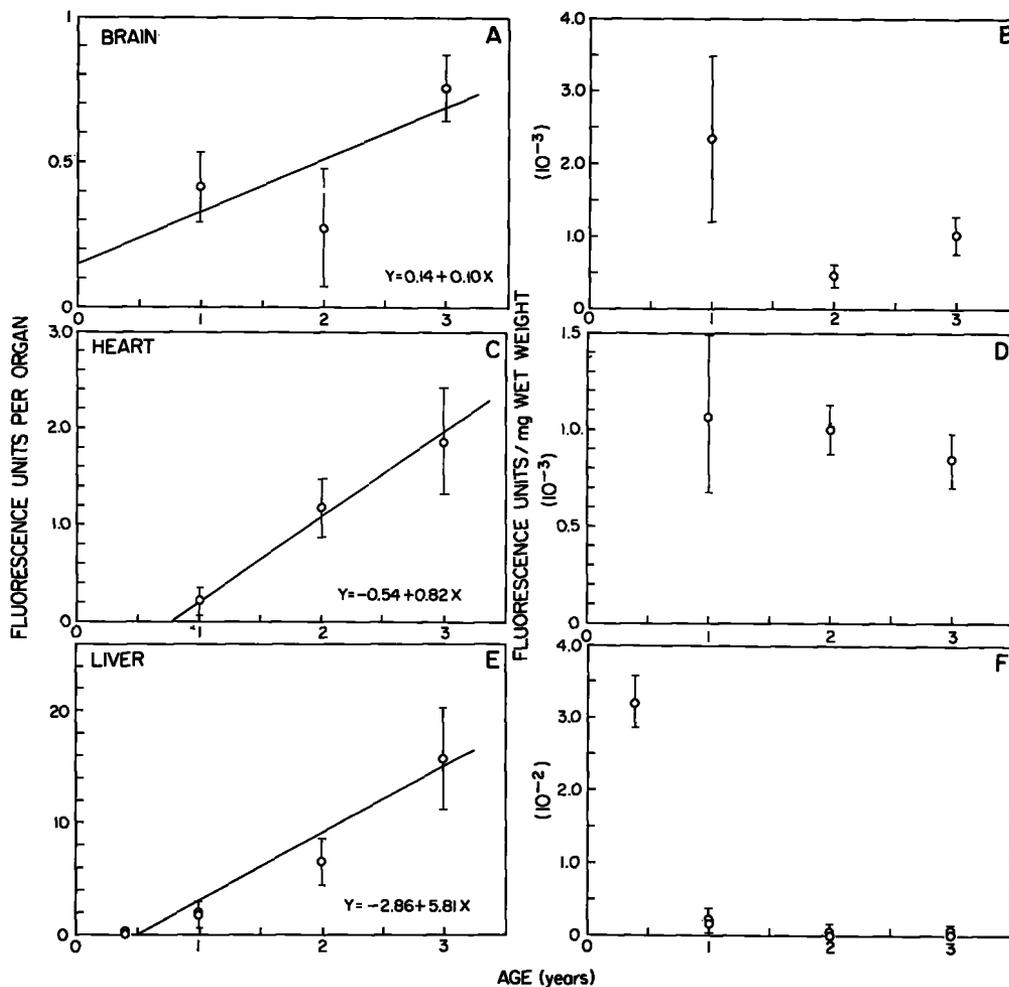


FIGURE 4.—Lipofuscin in rainbow trout, *Salmo gairdneri*, as a function of age ( $n = 21$ ). (A) Total lipofuscin in brain; (B) weight-specific (mg wet weight) lipofuscin in brain; (C) total lipofuscin in heart; (D) weight-specific (mg wet weight) lipofuscin in heart; (E) total lipofuscin in liver; (F) weight-specific (mg wet weight) lipofuscin in liver.

### Discussion

Our results indicate that lipofuscin accumulated in the brain of Dover sole with time (Fig. 2A). The concentration of lipofuscin increased over a wide range of fish lengths and with estimated age of 15 years but did not increase with older fish. Several explanations exist for the lack of change in concentration in older fish: 1) older fish were incorrectly aged; 2) growth of brain tissue masked the actual rate of accumulation; and 3) the rate of accumulation changed during the lifespan of this species due to changes in metabolic activity. Either one or several of these

factors may cause the lack of lipofuscin accumulation in older fish. We consider each of these issues below.

We do not know the accuracy of the age determination in Dover sole but believe it is unlikely that 3 readers would confuse fish aged 30–40 years with those of 20 years. Although future research will shed more light on this controversial subject, we think that grossly inaccurate age determination is the least likely an explanation.

A key difference between fishes and other organisms in which lipofuscin accumulation has been clearly documented as a function of chronological age (mammals and invertebrates) is that

fishes have indeterminate growth. For example, Figure 3B indicates that the brain of Dover sole continues to grow long after this fish begins reproduction (450 mm). Thus, unlike mammals and invertebrates which have determinate growth, the total content of extractable lipofuscin in any fish organ cannot be used as a measure of age but rather concentration (content per unit of weight) must be used. Furthermore, even the concentration of lipofuscin in the brain, a slow growing tissue, is a function of both the rate of lipofuscin accumulation and the rate of tissue growth. It follows then, that if the rate of brain growth is such that it masks the actual rate of lipofuscin accumulation in older organisms, lipofuscin concentration will not increase with time.

A critical assumption underlying the use of lipofuscin as a determinant of age is that metabolic rate, and hence lipofuscin accumulation rate, remains fairly constant over the portion of the life history of interest. The rainbow trout were reared in a hatchery where no net change in the environment occurred from 1983 to 1985. Dover sole, on the other hand, gradually migrate into deeper, colder, and less oxygenated waters as they age. Presumably the metabolism of fish under these conditions would be lower as might be the rate of lipofuscin accumulation in the brain.

The literature on fishes provide no conclusive evidence that lipofuscin is an accurate index of age over the entire lifespan. Hill and Radtke (in press) reported that the extracted lipofuscin per unit dry weight in the brain of the tropical fish *Dascillus albisella* accumulates exponentially with age. The relationship is driven by a single point for an 11-year-old individual and it would probably be linear without that single point, if only fish 1–7 years old were considered. In hatchery-reared *Cyprinus carpio* the total extracted lipofuscin per unit of dry weight in the brain of fish of the same age (6 years) increased with weight over 8-fold range in weight (Griven et al.<sup>2</sup>). About 45% of the lipofuscin content could be explained by difference in weight among fish. Thus, in fish of the same age there was a strong size effect on lipofuscin concentration. These results are very similar to those found in this study. Aloj Totaro et al. (1985) found that lipofuscin increased over a range of 0–2 years. But the method

was different as they measured lipofuscin granules present in the electric lobe of *Torpedo marmorata* brains rather than using extractable lipofuscin of the entire brain. Thus, all studies to date seem to indicate that accumulation occurs in the brains of fishes, but results are not conclusive owing to small sample sizes, limited age ranges, and failure to identify the effects of brain growth on rates of accumulation.

Additional research is required to evaluate lipofuscin as a method of age determination in fishes. The effect of brain growth on lipofuscin accumulation rates must be considered in such studies. A promising approach in this regard may be to estimate lipofuscin on a per cell basis instead of on a weight basis. This could be accomplished by either expressing extracted lipofuscin relative to DNA concentration or by histological techniques.

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### EXTRACTABLE LIPOFUSCIN IN LARVAL MARINE FISH

The "age pigment", lipofuscin, is thought to be a biochemically heterogeneous byproduct of the peroxidation of polyunsaturated lipids which accumulates in dense, intracellular inclusions called ceroid bodies. The material has been studied both microscopically and biochemically in tissues of several species (Miquel et al. 1977; Shimasaki et al. 1980; Dowson 1982), and some portion of it is quantitatively extractable with organic solvents (Fletcher et al. 1973).

Flies prevented from flying by putting baffles in the bottles in which they were raised, accumulated lipofuscin (as assessed by solvent extraction) more slowly than did free-flying flies but had a longer lifespan, so that at the ends of the respective lifespans the body contents of lipofuscin were similar in the two groups (Sohal and Donato 1978). Extractable lipofuscin thus appears to accumulate as a function of cumulative oxidative metabolism; it could be an indicator of physiological (rather than strictly chronological) age.

Additionally, if lipofuscin represents an integral of oxidative metabolism since birth and weight represents an integral of growth over the same period, the ratio of lipofuscin to organic weight should be proportional to the reciprocal of cumulative net growth efficiency [ $K_2 = \text{growth/assimilation} = \text{growth}/(\text{growth} + \text{respiration})$ , hence  $1/K_2 = 1 + \text{respiration/growth}$ ].

Ettershank (1984a) introduced the fluorometric measurement of extractable lipofuscin as a measure of physiological age in growing marine crustaceans, based on the work with insects, and (1984b) recommended a simple method for routine use in marine work. He also argued (without presenting extensive evidence) that preservation