

Errata for *NOAA Technical Memorandum NMFS-NE-180*

1. Pg. 5, col. 2, para. 3: After “(Umminger and Mahoney 1972),” insert

“A more comprehensive analysis of Hudson-Raritan Estuary winter flounder hematological variables (Mahoney and McNulty 1992) showed hemoglobin and hematocrit values to be high from October to April and low from June through September; values in May were transitional.”

2. Pg. 6, col. 1, para. 1: After last sentence, insert

“Mean MCHC in winter flounder from the Hudson-Raritan Estuary was generally higher during the period October through April as compared to May through September (Mahoney and McNulty 1992).”

3. Pg. 9, col. 2: After “Lux” entry, insert

“Mahoney, J.B.; McNulty, J.K. 1992. Disease-associated blood changes and normal seasonal hematological variation in winter flounder in the Hudson-Raritan Estuary. *Trans. Am. Fish. Soc.* 12:261-268.”



NOAA Technical Memorandum NMFS-NE-180

**Variability in Blood Chemistry
of Yellowtail Flounder,
Limanda ferruginea,
with Regard to Sex, Season,
and Geographic Location**

U. S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

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NOAA Technical Memorandum NMFS-NE-180

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Variability in Blood Chemistry of Yellowtail Flounder, *Limanda ferruginea*, with Regard to Sex, Season, and Geographic Location

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^aRobins, C.R. (chair); Bailey, R.M.; Bond, C.E.; Brooker, J.R.; Lachner, E.A.; Lea, R.N.; Scott, W.B. 1991. Common and scientific names of fishes from the United States and Canada. 5th ed. *Amer. Fish. Soc. Spec. Publ.* 20; 183 p.

^bRobins, C.R. (chair); Bailey, R.M.; Bond, C.E.; Brooker, J.R.; Lachner, E.A.; Lea, R.N.; Scott, W.B. 1991. World fishes important to North Americans. *Amer. Fish. Soc. Spec. Publ.* 21; 243 p.

^cTurgeon, D.D. (chair); Quinn, J.F., Jr.; Bogan, A.E.; Coan, E.V.; Hochberg, F.G.; Lyons, W.G.; Mikkelsen, P.M.; Neves, R.J.; Roper, C.F.E.; Rosenberg, G.; Roth, B.; Scheltema, A.; Thompson, F.G.; Vecchione, M.; Williams, J.D. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. 2nd ed. *Amer. Fish. Soc. Spec. Publ.* 26; 526 p.

^dWilliams, A.B. (chair); Abele, L.G.; Felder, D.L.; Hobbs, H.H., Jr.; Manning, R.B.; McLaughlin, P.A.; Pérez Farfante, I. 1989. Common and scientific names of aquatic invertebrates from the United States and Canada: decapod crustaceans. *Amer. Fish. Soc. Spec. Publ.* 17; 77 p.

^eRice, D.W. 1998. Marine mammals of the world: systematics and distribution. *Soc. Mar. Mammal. Spec. Publ.* 4; 231 p.

^fCooper, J.A.; Chapleau, F. 1998. Monophyly and interrelationships of the family Pleuronectidae (Pleuronectiformes), with a revised classification. *Fish. Bull. (Washington, DC)* 96:686-726.

^gMcEachran, J.D.; Dunn, K.A. 1998. Phylogenetic analysis of skates, a morphologically conservative clade of elasmobranchs (Chondrichthyes: Rajidae). *Copeia* 1998(2):271-290.

^hISO [International Organization for Standardization]. 1981. ISO standards handbook 3: statistical methods. 2nd ed. Geneva, Switzerland: ISO; 449 p.

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Common and Scientific Names of Species Discussed

Atlantic halibut	<i>Hippoglossus hippoglossus</i>
Brook trout	<i>Salvelinus fontinalis</i>
Channel catfish	<i>Ictalurus punctatus</i>
English sole	<i>Parophrys vetula</i>
Eurasian perch	<i>Perca fluviatilis</i>
European flounder	<i>Platichthys flesus</i>
Lake trout	<i>S. namaycush</i>
Largescale blackfish	<i>Girella punctata</i>
Madai	<i>Pagrus major</i>
Mummichog	<i>Fundulus heteroclitus</i>

Pinfish	<i>Lagodon rhomboides</i>
Plaice	<i>Pleuronectes platessa</i>
Rainbow trout	<i>Oncorhynchus mykiss</i>
Redear sunfish	<i>Lepomis microlophus</i>
Rock bass	<i>Ambloplites rupestris</i>
Smallmouth bass	<i>Micropterus dolomieu</i>
Striped bass	<i>Morone saxatilis</i>
Striped mullet	<i>Mugil cephalus</i>
Summer flounder	<i>Paralichthys dentatus</i>
Windowpane	<i>Scophthalmus aquosus</i>
Winter flounder	<i>Pseudopleuronectes americanus</i>
Yellowtail flounder	<i>Limanda ferruginea</i>

Acronyms

MCHC	mean corpuscular hemoglobin concentration
NEFSC	Northeast Fisheries Science Center
NEMP	Northeast Monitoring Program
OPP	Ocean Pulse Program

ABSTRACT

Yellowtail flounder, *Limanda ferruginea*, were collected from six locations along coastal New England (*i.e.*, Cape Cod Bay, Georges Bank, Massachusetts Bay, mouth of the Merrimack River, Mud Patch (south of Marthas Vineyard), and New York Bight), and were monitored for blood chemistry and hematology. Plasma osmolality, sodium, potassium, calcium, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration (MCHC) were measured.

Osmolality and sodium concentrations were frequently reduced during spring. Potassium was generally elevated during spring and/or summer, and reduced during fall and/or winter. Calcium was usually highest during fall. The hematological indices of hematocrit, hemoglobin, and MCHC showed an overall elevation during winter as compared to fall. Fish from the inshore locations of Cape Cod Bay, Massachusetts Bay, Merrimack River, Mud Patch, and New York Bight differed significantly from fish from the offshore reference location of Georges Bank for particular blood parameters during certain seasons.

These data provide a baseline range for blood constituents of yellowtail flounder over an annual cycle and at key locations in the Northeast U.S. Shelf Ecosystem. These results present evidence of possible anthropogenic effects on the blood chemistry and hematology of yellowtail flounder collected from inshore areas. Variability in blood chemistry appears to be regulated by seasonally-induced physiological and/or environmental factors, and influenced by sex and capture location. This information may prove to be useful in monitoring the health of field-collected yellowtail flounder and in assessing the condition of aquacultured fish of this species.

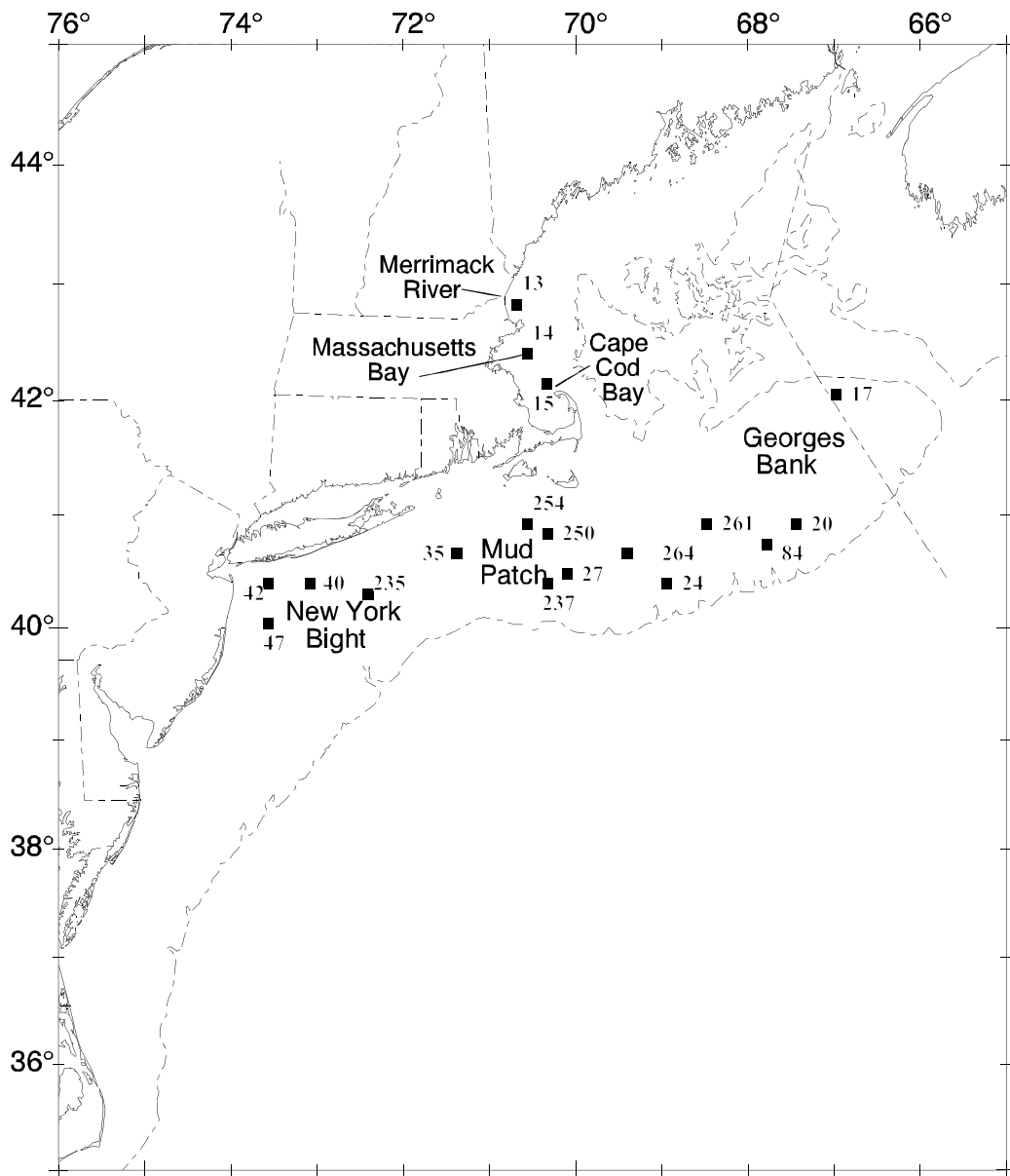


Figure 1. Stations for trawl collection of adult yellowtail flounder in the Northwest Atlantic during 1978-85. (Each station is assigned to one of five inshore (Merrimack River, Massachusetts Bay, Cape Cod Bay, Mud Patch, and New York Bight) or one offshore (Georges Bank) location.)

INTRODUCTION

Yellowtail flounder, *Limanda ferruginea*, is a commercially important flatfish inhabiting Northwest Atlantic waters ranging from the Gulf of St. Lawrence to Chesapeake Bay (Bigelow and Schroeder 1953; Collette and Klein-MacPhee 2002). Evidence suggests that yellowtail flounder from Cape Cod Bay, Georges Bank, and the region encompassing Southern New England to the Mid-Atlantic Bight represent three distinct stocks (Cadrin 2003; Cadrin and King 2003). Yellowtail flounder are fast growing, have high market value (Johnson *et al.* 1999), and have been identified as a candidate species for aquaculture (Rabe and Brown 2000). Despite the historical importance of yellowtail flounder as a commercial resource, relatively little information is available concerning the blood chemistry of this species.

Blood chemistry and hematological measurements can provide valuable tools for monitoring the health and condition of both wild and cultured fish. Physiological indices can offer critical feedback on rearing conditions and nutritional status, and can aid in the diagnosis of disease. These monitoring and feedback applications require an understanding of normal blood component levels specific to yellowtail flounder, as blood chemistry concentrations are known to vary among fish species (Larsson *et al.* 1976; Hardig and Høglund 1983; Folmar 1993). Natural changes in environmental conditions associated with season can affect blood chemistry and hematology (Bridges *et al.* 1976; Warner and Williams 1977; Lane 1979; Dawson 1990; Folmar 1993; Houston 1997; Luskova 1998; O'Neill *et al.* 1998; Edsall 1999). Physiological variation in fish may be influenced by both internal and external cues, including reproductive stage, water temperature, dissolved oxygen, nutrient availability, and photoperiod, all of which undergo annual cycles (Sandstrom 1989; Dawson 1990). Seasonal changes may also be influenced by nonenvironmental factors such as diet, metabolic adaptations, and activity levels (Denton and Yousef 1975).

Hematological measures, which can be affected by exposure to chemical pollutants (Heath 1995), are useful indicators of sublethal environmental stress in fish (Bridges *et al.* 1976; Warner and Williams 1977; Folmar 1993). Yellowtail flounder demonstrate strong site fidelity, are found both inshore and offshore (Royce *et al.* 1959; Lux 1964; Johnson *et al.* 1999; Cadrin 2003; Cadrin and King 2003), and live on or close to bottom sediments where contaminant loading occurs. Resident bottom-dwelling organisms, such as flatfish, often exhibit environmentally-induced disease (Ziskowski *et al.* 1987), making them potential indicator species for monitoring of anthropogenic effects. Before blood constituent data can be applied as a diagnostic tool, however, general patterns related to season, sex, and collection location need to be well documented and understood (Bridges *et al.* 1976; Courtois 1976; Hardig and Høglund 1983; Folmar 1993).

The goal of this study was to determine baseline levels of blood parameters for yellowtail flounder. Blood chemistry and hematological measurements of osmolality, potassium, sodium, calcium, hematocrit, hemoglobin, and mean corpuscular hemoglobin (MCHC) were determined for fish collected from various locations in the Northeast U.S. Shelf Ecosystem. Blood measurements were compared with regard to fish sex, season, and collection location.

MATERIALS AND METHODS

Yellowtail flounder were collected from Northwest Atlantic waters between 1978 and 1985 aboard National Marine Fisheries Service, Northeast Fisheries Science Center (NEFSC) cruises. Nearly all fish were collected on cruises associated with the NEFSC's Ocean Pulse Program (OPP) and Northeast Monitoring Program (NEMP); a few fish were collected on cruises associated with the NEFSC's Bottom Trawl Survey Program. (The OPP and NEMP were the same fundamental program; the name changed from the former to the latter early in the effort, though.) The focus of NEMP was assessment of ecological, genetic, pathological, and physiological changes in coastal and shelf organisms of the Northeast U.S. Shelf Ecosystem, which had been potentially exposed to the effects of contaminant stress (Pearce 1998).

Yellowtail flounder were collected using a "3/4 Yankee" (or "No. 36") otter trawl towed for a 30-min period (Survey Working Group, Northeast Fisheries Center 1988). Animals were obtained from five nearshore locations, including Cape Cod Bay, Massachusetts Bay, mouth of the Merrimack River, Mud Patch (south of Marthas Vineyard on the continental shelf), and New York Bight, as well as from an offshore location, Georges Bank (Figure 1). Table 1 shows the general coordinates (*i.e.*, latitude and longitude) of the collection stations, as well as the number of adult yellowtail flounder collected at each station. For the Georges Bank, Mud Patch, and New York Bight locations, fish caught at adjacent stations within a given location were pooled to increase sample size for statistical analysis. Environmental conditions (*e.g.*, depth, substrate, temperature) at these adjacent stations were fundamentally similar.

Fish were transferred to flowing seawater aboard ship and processed immediately to minimize stress-induced changes associated with capture (Larsson *et al.* 1976; Wedemeyer and Yasutake 1977; Dawson 1990). Changes in blood concentrations can occur within hours to days of collection (Umminger 1970; Wedemeyer and Yasutake 1977; Bourne 1986); therefore, it was critical that samples collected at sea be processed without delay. Blood was collected by cardiac puncture using a 3-ml plastic syringe with a 22-ga needle and transferred gently into an 8-ml glass vial coated with 150 units of dried ammonium heparin as an anticoagulant. Hemoglobin (g/100 ml) was determined by the cyanmethemoglobin method using Hycel reagents and

a Bausch and Lomb Spectronic 20 spectrophotometer. (Note: Use of trade names does not imply endorsement by NMFS.) Hematocrit was determined by collecting blood in micro-hematocrit tubes, which were then centrifuged at $13,500 \times g$ for 5 min and read. Hematocrit was expressed as a percentage of the volume of the whole blood sample. The remainder of each blood sample was centrifuged at $12,000 \times g$, and the resulting plasma was frozen until further analyses could be conducted. The MCHC, the ratio between hemoglobin and hematocrit, was expressed as g/100 ml of packed red blood cells, and was calculated by the formula: $MCHC = \text{hemoglobin/hematocrit} \times 100$. Plasma osmolality was determined using an Advanced 3L or 3C2 Cryomatic Osmometer and Advanced Instruments freezing-point calibration standards. Sodium, potassium, and calcium (mEq/L) were determined using a Perkin Elmer Coleman 51 Flame Photometer.

Sampling dates were assigned to a season according to calendar designations: winter (December 22 - March 19), spring (March 20 - June 20), summer (June 21 - September 22), and fall (September 23 - December 21). Some locations and seasons were sampled more frequently than others because of problems often associated with extensive field sampling at sea, such as equipment failure, inclement weather, and variation in fish availability. Occasionally, a sample was not obtained for a given season. Small sample sizes were included in a data set when information was available for other seasons at that location.

A Pearson product moment correlation was used to determine whether hydrographic characteristics such as temperature, salinity, and dissolved oxygen are associated with blood chemistry values. A multivariate analysis of variance was conducted to determine effects of sex, location, and season on osmolality, sodium, potassium, calcium, hemoglobin, hematocrit, and MCHC. These seven blood parameters served as the dependent variables. Independent variables for the model included sex (males and females), location (Cape Cod Bay, Georges Bank, Massachusetts Bay, Merrimack River, Mud Patch, and New York Bight), season (winter, spring, summer, and fall), sex \times location, sex \times season, and sex \times location \times season. Fish length was used as a covariate. Post-hoc tests consisted of multiple pairwise comparisons of least square means for significant effects ($P < 0.05$). Statistical comparisons were limited to seasons and locations containing a sample size of $N \geq 5$. The significant differences which have been reported, but based on small sample sizes, should be interpreted conservatively. All statistical analyses were performed using PC-SAS Software Version 8.3 (SAS Institute 1989).

RESULTS

Two of the three hydrographic parameters correlated highly with blood indices. Salinity correlated positively with MCHC (Table 2). Dissolved oxygen correlated positively with hemoglobin and MCHC, and negatively with osmolality and sodium. There were also relationships be-

tween certain blood parameters. Sodium correlated positively with osmolality, and negatively with MCHC. Hemoglobin correlated positively with hematocrit and MCHC. A significant difference was observed for sex \times location \times season for all seven blood parameters (Table 3). Following are results for differences related to sex, season, and location. Location-related differences are limited to a comparison of results from the five inshore locations versus those from Georges Bank, the offshore reference location.

SEX-RELATED DIFFERENCES IN BLOOD CHEMISTRY

Significant differences in blood parameters were observed between males and females within the same season at Georges Bank (8 instances), New York Bight (5), Mud Patch (5), and Merrimack River (2), and are shown underlined in Tables 4-10. These differences were observed most frequently during spring (8), but were also observed during summer (6), winter (4), and fall (2). Male and female blood chemistry concentrations differed significantly from one another for hemoglobin (6), osmolality (4), hematocrit (4), calcium (3), potassium (2), and MCHC (1).

Mud Patch males had significantly greater hematocrit during winter, and hematocrit, hemoglobin, and osmolality during spring, than females. Mud Patch females had significantly greater hemoglobin during summer than males. Merrimack River females had significantly greater calcium during spring and summer than males. New York Bight males had significantly greater hematocrit and osmolality during winter, hemoglobin and osmolality during spring, and potassium during fall, than females. Georges Bank males had significantly greater hemoglobin during winter, hematocrit and hemoglobin during spring, hemoglobin, potassium, and MCHC during summer, and osmolality during fall, than females; however, females had significantly greater calcium during summer than males.

SEASONAL DIFFERENCES IN BLOOD CHEMISTRY

Overall, osmolality showed few significant differences among seasons, although there was a minor trend toward reduced osmolality during spring, as observed in both males and females from Georges Bank (Table 4). Fall osmolality in Mud Patch fish was significantly greater than summer osmolality in males, and spring osmolality in females. Osmolality in New York Bight males was significantly elevated during winter and spring, and reduced during fall.

Generally, sodium concentrations were significantly lower during spring, as observed in males from Georges Bank, Merrimack River, Mud Patch, and New York Bight, and in females from Georges Bank and Mud Patch (Table 5).

Potassium concentrations were often significantly higher during spring or summer, and significantly lower during fall or winter (Table 6). Potassium showed signifi-

cantly higher levels during spring in males from Merrimack River, and in females from Georges Bank and Mud Patch. Summer levels were significantly higher in fish of both sexes from Georges Bank and Mud Patch. Potassium was significantly lower during fall than spring and/or summer in males from Georges Bank, Merrimack River, and Mud Patch, and in females from Georges Bank. Winter potassium was significantly lower than summer for females from Georges Bank and Mud Patch.

Calcium concentrations were generally higher during fall, as observed in fish of both sexes from George Bank and Mud Patch, and in females from New York Bight (Table 7). A highly elevated calcium concentration during spring was documented in females from Merrimack River.

Yellowtail flounder demonstrated a trend toward significantly higher hematocrit during spring, as compared with fall (Table 8). Hematocrit was significantly greater during spring than fall in fish of both sexes from Merrimack River, Mud Patch, and New York Bight, and in males from Georges Bank. Winter hematocrit concentration was significantly greater than fall concentration in females from Georges Bank.

Hemoglobin showed a similar pattern to that observed for hematocrit, but the results were less consistent (Table 9). Hemoglobin tended toward significantly greater concentrations during winter and/or spring than fall. Hemoglobin in fish of both sexes from Georges Bank and Merrimack River was significantly higher during winter than fall, while spring levels were significantly higher than fall levels in males and females from Merrimack River, Mud Patch, and New York Bight, and in males from Georges Bank.

MCHC was generally higher during winter than spring, as observed at Georges Bank and Merrimack River for males, and at Merrimack River and New York Bight for females (Table 10). Winter also tended to have higher MCHC than fall, as seen at Georges Bank and Merrimack River for males, and at Merrimack River and Mud Patch for females.

LOCATION-RELATED DIFFERENCES IN BLOOD CHEMISTRY AS COMPARED TO GEORGES BANK

Blood parameters in fish from the inshore locations were compared to such parameters in fish from Georges Bank, the reference location. During winter, osmolality in female yellowtail flounder from Merrimack River was significantly lower. During spring, osmolality in both sexes from New York Bight and in males from Cape Cod Bay was significantly higher. During summer, osmolality in both sexes from Merrimack River and in males from Mud Patch was significantly lower. During fall, osmolality in males from Merrimack River, Mud Patch, and New York Bight was significantly lower (Table 4).

During winter, Merrimack River, Mud Patch, and New York Bight sodium concentrations in females were significantly reduced. During spring, sodium in females from Massachusetts Bay, males from Cape Cod Bay, and both sexes from New York Bight was significantly elevated. Fall

sodium concentrations were reduced in both sexes from Merrimack River (Table 5).

Reduced potassium was observed during spring in males from Cape Cod Bay and in females from Massachusetts Bay and New York Bight. Merrimack River showed elevated potassium during spring in males. Significantly reduced potassium was measured in Merrimack River males during summer. Females from Merrimack River and Mud Patch and both sexes from New York Bight had elevated fall potassium (Table 6).

Females from Merrimack River and Massachusetts Bay had significantly higher calcium during spring. Summer calcium was reduced in female fish from Mud Patch. Calcium concentrations during fall were significantly lower in both sexes from Merrimack River and in males only from Mud Patch (Table 7).

During winter, hematocrit was significantly lower in males from Cape Cod Bay and in females from Mud Patch. During spring, hematocrit was significantly higher in both sexes from Merrimack River, and in females from New York Bight. During summer, hematocrit was significantly higher in females from New York Bight (Table 8).

During winter, hemoglobin in males from Cape Cod Bay and New York Bight, and in both sexes from Mud Patch, was significantly reduced. Merrimack River females had greater winter hemoglobin. During spring, hemoglobin was reduced in female fish from Massachusetts Bay and elevated in females from Merrimack River. Summer hemoglobin was reduced in Mud Patch males. Fall hemoglobin in New York Bight and Mud Patch males was significantly higher (Table 9).

Males from Cape Cod Bay and New York Bight had significantly lower winter MCHC, while in females from Merrimack River, MCHC was significantly greater. During summer, males from Merrimack River and females from New York Bight had significantly reduced MCHC. Male fish from Merrimack River, Mud Patch, and New York Bight had significantly higher MCHC during fall (Table 10).

DISCUSSION

SEASONAL AND SEX-RELATED DIFFERENCES IN BLOOD CHEMISTRY

Blood chemistry and hematological measurements are significantly affected by the wide range of natural environmental conditions experienced by fish over the course of a year (Bridges *et al.* 1976; Warner and Williams 1977; Lane 1979; Dawson 1990; Folmar 1993; Houston 1997; Luskova 1998; O'Neill *et al.* 1998; Edsall 1999). Changes in temperature (Powers 1980) and season (Shell 1961) are known to affect oxygen metabolism and hematology. Variations in blood constituents may be related to natural physiological cycles, environmental stimuli, or both (Bridges *et al.* 1976; Luskova 1998). Metabolic fluctuations in fish such as growth, activity, and feeding are strongly influenced by

environmental cues (Barnhart 1969; Fletcher 1977). Reproductive cycles, associated with season, can place fish under extremes of metabolism and osmoregulation (Courtois 1976), and may alter the content of fish blood (Nagler *et al.* 1987; Sandstrom 1989; Bjornsson *et al.* 1998; Luskova 1998).

Osmolality

Yellowtail flounder experienced only minor seasonal fluctuations. In several cases, osmolality values were reduced during spring. Windowpane (*Scophthalmus aquosus*) also experience significantly lower osmolality during spring than summer and fall (Dawson 1990). In a laboratory study, however, no significant differences were observed in the serum osmolality of winter flounder (*Pseudopleuronectes americanus*) held at -1° and 15°C (Umminger 1970).

The significantly elevated osmolality observed during winter and spring in males from New York Bight in our study has some adaptive significance. Pearcy (1961) suggests that elevated osmotic pressure in flounder during cooler weather may protect against freezing in fishes inhabiting cold shallow waters. Serum osmolality was also highest in winter flounder during the winter months, just prior to spawning (Pearcy 1961; Umminger and Mahoney 1972). Osmolality in mummichog (*Fundulus heteroclitus*) was found to increase significantly at colder water temperatures (Umminger 1969).

Seasonal differences in osmotic pressure may be caused by osmotic imbalance or other factors (Pearcy 1961). An increase in serum constituents other than the major ions, such as a buildup of organic and inorganic materials, can elevate osmolality during cold weather (Pearcy 1961; Umminger 1969; Umminger and Mahoney 1972). An increase in serum electrolytes may be a compensatory mechanism used by fish to maintain the total number of osmotically active particles in the plasma (Shell 1961). In several instances, males showed significantly greater osmolality than females during winter and/or spring. Perhaps females are undergoing osmotic changes, specifically hemodilution, related to preparations for late spring spawning.

In our study, osmolality and sodium were highly positively correlated. This high positive correlation would be expected, as sodium is an important osmotic constituent. In our study, both of these parameters had similar patterns of reductions during spring. Also in our study, dissolved oxygen and osmolality were highly negatively correlated, which suggests that the reduced osmolality during spring may be associated with the elevated dissolved oxygen levels following spring turnover.

Sodium

Marine fish exposed to low seawater temperatures often experience increased sodium (Umminger 1969; Murphy and Houston 1977; Sandstrom 1989). Increases in freezing

point depression of winter flounder blood serum during the winter months can be attributed in part to elevated plasma sodium (Fletcher 1981). Winter flounder sampled in Newfoundland had significantly higher sodium during winter than summer (Fletcher 1977), while sodium in winter flounder from New England was also higher during late winter (Pearcy 1961; Fletcher 1977). Winter-collected windowpane had significantly higher sodium than summer and autumn fish (Dawson 1990). Interestingly, Umminger and Mahoney (1972) noted no seasonal change in sodium among winter flounder collected from New Jersey.

In measurements on freshwater fish, sodium levels in the Eurasian perch (*Perca fluviatilis*) reached their maximum during mid-winter (Sandstrom 1989), and in rainbow trout (*Oncorhynchus mykiss*) were higher during both fall and winter than during summer (Houston *et al.* 1968). In our study, dissolved oxygen concentrations were correlated negatively with both sodium and osmolality. This correlation suggests a possible disruption of osmoregulatory function at reduced oxygen levels.

Potassium

The significantly reduced potassium levels during cold weather, which were observed in our study, have also been described in winter flounder, and may be related to a decline in metabolism and feeding at lower temperatures (Umminger and Mahoney 1972; Bentinck-Smith *et al.* 1987). Reduced blood potassium concentrations during winter may also be attributed to a loss of potassium from the cells of body tissues in response to the cold (Umminger 1969). Potassium in the windowpane was also found to be higher during summer than fall and winter (Dawson 1990).

Changes in blood potassium levels have also been observed in freshwater fish. Rainbow trout collected during fall-winter had reduced levels as compared to those fish collected during summer (Houston *et al.* 1968). Elevated potassium levels were also observed at high water temperatures in smallmouth bass (*Micropterus dolomieu*) (Shell 1961), rainbow trout (Murphy and Houston 1977), channel catfish (*Ictalurus punctatus*) (Ellsaesser and Clem 1987), Eurasian perch (Sandstrom 1989), and madai (*Pagrus major*) (Woo 1990).

Increased plasma potassium may result from disruption of potassium regulatory ability at either the external level (*i.e.*, between the fish and seawater) or internal level (*i.e.*, between the intracellular and extracellular fluid), with a resulting release of potassium into the blood. Serum potassium levels may increase in response to elevated tissue catabolism, or due to osmotic adjustment when compensating for a decline in other serum components (Shell 1961).

Calcium

Calcium plays an important role in osmoregulation (Shell 1961), and is a critical component in the reproductive pro-

cesses of fish. In our study, calcium concentrations were generally higher during fall and lower during spring. This trend toward calcium being elevated during fall and depressed during spring may reflect the spring and summer spawning pattern of yellowtail flounder, which peaks during late May (Bigelow and Schroeder 1953; Royce *et al.* 1959; Fahay 1983; Collette and Klein-MacPhee 2002; Cadrin and King 2003). Also in our study, a contrasting pattern was observed in females from Merrimack River; they showed significantly elevated calcium during spring which may be related to anthropogenic, rather than seasonal, influences.

In our study, female fish from Georges Bank and Merrimack River had significantly elevated spring and/or summer calcium levels as compared to males. These elevated calcium levels in females are most likely related to hormonal changes linked to spawning activity. In freshwater fish also, calcium concentrations in females during the spawning period were significantly higher than in males for lake trout (*Salvelinus namaycush*) (Edsall 1999), brook trout (*Salvelinus fontinalis*) (Booke 1964), and rock bass (*Ambloplites rupestris*) (Bidwell and Heath 1993).

Blood calcium has been shown to follow spawning patterns in numerous fish including brook trout (Booke 1964), rainbow trout (Nagler *et al.* 1987), English sole (*Parophrys vetula*) (Johnson *et al.* 1991), striped mullet (*Mugil cephalus*), pinfish (*Lagodon rhomboides*) (Folmar *et al.* 1992), rock bass (Bidwell and Heath 1993), and Atlantic halibut (*Hippoglossus hippoglossus*) (Bjornsson *et al.* 1998). Blood calcium in rainbow trout rises in connection with yolk synthesis and reaches maximum levels just prior to spawning (Hille 1982). High spring calcium levels observed in windowpane (Dawson 1990) appear to correspond with a late spring and summer spawning season (Bigelow and Schroeder 1953). Calcium varied irregularly in the Eurasian perch, with fluctuations increasing sharply before and after spring spawning (Sandstrom 1989). Variations in blood calcium of fish appear to be tied more closely to reproductive changes than to seasonality or hematopoiesis (*i.e.*, blood formation) (Luskova 1998).

Hematocrit, Hemoglobin, and MCHC

Hematocrit and Hemoglobin

Hematocrit provides a measurement of red blood cells (erythrocytes) in whole blood, while the hemoglobin within those erythrocytes is the main transport mechanism for oxygen and carbon dioxide in the blood. Alterations in blood oxygen capacity reflect seasonal adjustment in oxygen transport (Cameron 1970; Anderson *et al.* 1985). Elevations in spring hematocrit and hemoglobin are likely due, in part, to an increase in oxygen consumption and metabolic rates corresponding to a rise in water temperatures (Powers 1980; Dwyer *et al.* 1983; Zanuy and Carrillo 1985; Martinez *et al.* 1994). Spring hemoconcentration of the blood may also contribute to elevated hematological levels (Preston 1960).

In addition to these seasonal rhythms, sex also appears to play a role in hematocrit and hemoglobin levels. In our study, males sometimes showed higher hematocrit and hemoglobin levels than females during winter and spring. Lane (1979) also observed significantly higher hematocrit and hemoglobin levels in male versus female rainbow trout. These differences in hematology with regard to males and females may be related to differential oxygen demand by sex, which in turn may be related to reproductive activity. Elevations in hematocrit and hemoglobin of rock bass seemed to occur in relation to the onset of spawning (Bidwell and Heath 1993).

The fall reduction in yellowtail flounder hematocrit and hemoglobin levels observed in our study may reflect a corresponding hemodilution of the blood which results in decreased hematological parameters and/or a reduction in hemopoietic capacity (Preston 1960). Rhythms in hematocrit and hemoglobin concentration may also result from changes in plasma volume or erythrocyte volume (Sandstrom 1989), for example, an expansion of plasma volume reduces the density of circulating red blood cells, thereby decreasing hematocrit (Courtois 1976). Changes in metabolism and hormonal activity, triggered by cooler water temperatures and declining photoperiod during fall, may result in anemia (Lane 1979) and reduced erythropoietic production (Lane 1979; Zanuy and Carrillo 1985). Physiological stresses associated with fasting and spawning can trigger a decline in fish condition (Bridges *et al.* 1976; Sano 1960ab; Lane 1979; Zanuy and Carrillo 1985).

Seasonal hematological patterns vary among species. In plaice, *Pleuronectes platessa*, hematocrit was lowest during winter, increased through the spring into summer, and declined again into fall. Hemoglobin levels in that species were high at the end of winter, were lowest during spring, were again high during the summer, and had a further decline during late fall (Preston 1960). In windowpane, Dawson (1990) found significantly lower winter hematocrit. Winter flounder from Maine had the lowest levels of hematocrit and hemoglobin during winter and early spring (Bridges *et al.* 1976), while winter flounder from New Jersey had lower levels of hemoglobin during late winter (Umminger and Mahoney 1972). Tun and Houston (1986) found increased hematocrit and hemoglobin in rainbow trout exposed to summer versus winter conditions. An overall trend toward increased hemoglobin was observed in pinfish and striped mullet held at high temperatures (Cameron 1970). Hematocrit and hemoglobin in striped bass (*Morone saxatilis*) were highest during fall and winter and lowest during summer (Lochmiller *et al.* 1989). Haider (1969) found hemoglobin in rainbow trout to be highest during winter and lowest during fall.

MCHC

Changes in the hemoglobin content of the blood in response to the environment might come about either by a

change in the number of erythrocytes or by a change in the hemoglobin concentration of the individual cells (Anthony 1961). MCHC, the amount of hemoglobin in a given number of red blood cells, was generally higher during winter than spring and fall. Since MCHC values are directly related to erythrocyte maturation, the relatively high proportion of newly proliferated young erythrocytes during late spring -- when fish are in a state of rapid growth -- is associated with relatively low MCHC values (Denton and Yousef 1975; Hardig and Hoglund 1983). MCHC corresponded with hematocrit and hemoglobin in striped bass, being highest during fall and winter and lowest during summer (Lochmiller *et al.* 1989). MCHC in the largescale blackfish (*Girella punctata*), increased during spring and decreased during fall (Kakuno and Koyama 1994). Dawson (1990) observed no seasonal or location differences in MCHC among windowpane collected from various locations in Long Island Sound.

As hemoglobin is a measure of the amount of oxygen carried in the blood, the strong positive correlation observed in our study between dissolved oxygen and two hematological indices, hemoglobin and MCHC, would be expected. Similarly, Kakuno and Koyama (1994) observed a strong positive correlation between hematological parameters and dissolved oxygen in teleost fishes. Also in our study, MCHC correlated positively with salinity and negatively with sodium concentration. These results suggest that hematological indices may be affected by seasonal changes in hydrographic conditions.

LOCATION-RELATED DIFFERENCES IN BLOOD CHEMISTRY AS COMPARED TO GEORGES BANK

One of the most important potential uses of blood chemistry data from a toxicological perspective is to assess environmental quality by comparing animals from different locations (*i.e.*, reference versus contaminated locations) (Folmar 1993). Physiological biomonitoring, using hematological and metabolic indices to assess stress, could provide an alternative to traditional population-based approaches (Lochmiller *et al.* 1989). A knowledge of normal physiological values is necessary to evaluate the health of fish with respect to their physiological responses to a stimulus or stress which affects homeostasis (Luskova 1998). A variety of environmental pollutants, such as certain chlorinated hydrocarbons and heavy metals, have been shown to affect osmotic and ionic regulation in fish (Haux 1979; Heath 1995).

Differences in blood parameters between yellowtail flounder from inshore locations and those from Georges Bank, the offshore location, suggest a possible anthropogenic effect at several inshore locations. Blood constituents that are significantly elevated or reduced in inshore fish as compared to Georges Bank fish may indicate a physiologically stressed condition (Reid *et al.* 1987). By virtue of location, the Georges Bank location experiences minimal

input from anthropogenic sources, whereas the inshore locations are known to contain contaminated sediments (Boehm 1983). In a summary of the NEMP, Reid *et al.* (1987) documented elevated trace metals in the sediments from New York Bight, Mud Patch, and Massachusetts Bay, as well as at the mouths of coastal estuaries. New York Bight had the highest measurements of polychlorinated biphenyls, while the incidence of fin rot at that location was found to be significantly greater than at other Northwest Atlantic locations (Ziskowski *et al.* 1987).

Osmolality and Sodium

Significantly lower osmolality levels in Merrimack River, Mud Patch, and New York Bight male yellowtail flounder during fall, and significantly lower sodium levels in Merrimack River males and females during the same season, suggest reduced osmoregulatory capacity, and may indicate an anthropogenic effect, as chronic low osmolality levels are thought to result from exposure to contaminants (Wedemeyer and Yasutake 1977). Dawson (1990) documented significantly reduced osmolality in windowpane from a polluted harbor in Long Island Sound as compared to fish from other, less impacted areas. European flounder (*Platichthys flesus*) exposed to titanium dioxide industrial effluent experienced reductions in osmolality and blood sodium (Larsson *et al.* 1980). Reduced blood sodium was documented in rainbow trout exposed to chlorine (Zeitoun *et al.* 1977). Leakage of sodium and other ions from extracellular fluids, or decreased uptake of these ions through the gills, can contribute to impaired osmoregulatory ability (Pearcy 1961; Larsson *et al.* 1980).

The significantly elevated spring osmolality and blood sodium levels observed in New York Bight male and female yellowtail flounder may indicate a pollutant effect. Windowpane exposed to mercury in the laboratory showed elevated sodium concentrations (Dawson 1990). A marked increase in blood sodium was observed in seawater-acclimated rainbow trout exposed to bunker C oil, most likely resulting from direct interference of the oil with the sodium transport system of the gills (McKeown and March 1978).

Potassium

Significantly elevated potassium levels were observed during fall in yellowtail flounder males and females from New York Bight, and in females from Merrimack River and Mud Patch. These elevated levels may result from impaired uptake of potassium ions through the gills, disruption of food absorption in the intestinal mucosa, or defective renal function and impaired reabsorption of potassium ions in the renal tubes (Larsson *et al.* 1981).

Elevated potassium is also known to result from chlorine exposure in rainbow trout (Zeitoun *et al.* 1977). European flounder exposed to sublethal cadmium concentra-

tions showed a dose-dependent reduction in potassium concentration (Larsson *et al.* 1981).

Calcium

Calcium was significantly elevated in Massachusetts Bay and Merrimack River females during spring, while levels were significantly reduced in females from Mud Patch during summer, and in fish of both sexes from Merrimack River and males from Mud Patch during fall. These values differed significantly from Georges Bank, perhaps symptomatic of impaired physiological processes. Larsson *et al.* (1981) speculates that a disturbance in calcium metabolism of fish may be caused by defective calcium absorption or impaired calcium reabsorption in the renal tubes, or a combination of these effects.

In laboratory exposures, windowpane exposed to mercury (Dawson 1990) and European flounder exposed to cadmium experienced reduced calcium concentration (Larsson *et al.* 1981).

Hematocrit, Hemoglobin, and MCHC

With only one exception, yellowtail flounder from Cape Cod Bay had the lowest levels of hematocrit, hemoglobin, and MCHC by sex during winter. These levels for Cape Cod Bay fish were also among the lowest levels over all seasons and locations in our study. Mud Patch females had the lowest level of winter hematocrit; they also had the next-to-lowest level of winter hemoglobin. Similarly, anemic summer flounder (*Paralichthys dentatus*), winter flounder, and windowpane were also collected from Cape Cod Bay and Mud Patch during NEMP cruises (Reid *et al.* 1987). Reduced hematocrit and/or hemoglobin can indicate physiological stresses such as anemia, dehydration, and hemodilution resulting from either gill damage (Wedemeyer and Yasutake 1977) or increased red blood cell breakdown in the spleen (Larsson *et al.* 1980). Reduced MCHC suggests disruption of the hemoglobin-forming process as opposed to the rate of blood cell production (Heath 1995). Reduced hematocrit, hemoglobin, and MCHC were documented in redear sunfish (*Lepomis microlophus*) collected next to a selenium discharge site; the selenium appeared to interfere with normal hemoglobin formation, evidenced by the disproportionate reduction in hemoglobin as compared to red blood cells (Sorensen and Bauer 1983). European flounder exposed to cadmium in the laboratory experienced significant reductions in hematocrit and hemoglobin with no associated change in MCHC. In this case, the cadmium-induced anemic response may be due to increased blood plasma volume, accelerated loss or destruction of erythrocytes, and/or decreased rate of erythrocyte production (Johansson-Sjoberg and Larsson 1978).

During spring, elevated hematocrit was noted in yellowtail flounder males and females from Merrimack River, and females from New York Bight; it likely represents a swelling of cells in response either to high blood levels of carbon dioxide or to low pH rather than an increase in the number of circulating erythrocytes (Heath 1995), as no significant difference was noted in MCHC. Hematocrit may increase in response to stress at a different rate than that of the hemoglobin contained in the erythrocytes (Wells *et al.* 1986).

During fall, males from Mud Patch and New York Bight showed elevated hemoglobin, possibly a response to anthropogenic factors. Abnormally elevated hematocrit or hemoglobin may be related to hemoconcentration, dehydration stress, or polycythemia (Wedemeyer and Yasutake 1977), or to osmoregulatory dysfunction (Heath 1995). Also during fall, significantly elevated MCHC was measured in males from Merrimack River, Mud Patch, and New York Bight, and may indicate a greater amount of hemoglobin per unit of red blood cells and possibly swollen erythrocytes. Windowpane from a polluted harbor in Long Island Sound had significantly higher hematocrit and hemoglobin than other less-impacted areas, suggesting increased hematopoiesis (Dawson 1990). Larsson *et al.* (1980) also saw increased hematocrit and hemoglobin in female European flounder exposed to titanium dioxide industrial effluent in the field, and noted that flounder may try to compensate for impaired oxygen uptake by a release of erythrocytes from the spleen. Increased hematocrit was observed in rainbow trout stressed by chlorine exposure (Zeitoun *et al.* 1977).

CONCLUSIONS

Seasonally-induced variability was observed in yellowtail flounder blood constituent levels. This variability suggests that blood chemistry values are best defined in terms of a physiological reference range (Luskova 1998).

Seasonal patterns were not always consistent among the study locations and may reflect natural variability among locations (Bidwell and Heath 1993). This variability may result from inherent differences in regional oceanographic conditions, such as temperature and salinity, but may also be influenced by handling stress, and/or stock-related variations or a combination of these factors (McCarthy *et al.* 1973; Hille 1982).

Our results present some evidence for possible anthropogenic effects on certain blood chemistry parameters of yellowtail flounder collected from inshore locations. Perhaps the most valuable aspect of this study, though, is that it presents a benchmark for changes due to environmental factors or anthropogenic influences over an annual cycle at key locations in the Northeast U.S. Shelf Ecosystem.

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Table 1. General coordinates (latitude and longitude) of stations, and numbers of adult yellowtail flounder collected at those stations, during 1978-85				
Location	Station No.	Coordinates		No. of Fish
		Latitude	Longitude	
Georges Bank	17	42° 00'N	67° 00'W	35
	20	40° 58'N	67° 33'W	122
	24	40° 20'N	69° 00'W	11
	84	40° 48'N	67° 41'W	18
	261	40° 48'N	68° 38'W	20
	264	40° 35'N	69° 31'W	13
Merrimack River	13	42° 48'N	70° 45'W	184
Massachusetts Bay	14	42° 19'N	70° 36'W	34
Cape Cod Bay	15	42° 09'N	70° 25'W	34
Mud Patch	27	40° 29'N	70° 12'W	20
	35	40° 41'N	71° 22'W	143
	237	40° 43'N	70° 11'W	15
	250	40° 27'N	70° 14'W	20
	254	40° 50'N	70° 32'W	20
	40	40° 26'N	73° 11'W	48
New York Bight	42	40° 25'N	73° 46'W	10
	47	40° 01'N	73° 25'W	76
	235	40° 19'N	72° 07'W	20

Table 2. Pearson product moment correlations for Northwest Atlantic hydrographic conditions and blood constituent levels in trawl-collected adult yellowtail flounder during 1978-85. (Sample sizes ranged from 268 to 830. R-values > 0.4, shown in bold, were considered significant.)

	Temperature	Salinity	Dissolved Oxygen	Osmolality	Sodium	Potassium	Calcium	Hematocrit	Hemoglobin	MCHC
Temperature	-									
Salinity	0.11	-								
Dissolved Oxygen	-0.15	0.22	-							
Osmolality	-0.01	-0.12	-0.65	-						
Sodium	0.11	-0.38	-0.57	0.56	-					
Potassium	0.38	-0.08	-0.02	0.14	0.16	-				
Calcium	0.15	0.00	-0.03	0.33	0.27	0.01	-			
Hematocrit	-0.01	0.01	0.08	0.08	0.00	0.04	0.11	-		
Hemoglobin	-0.23	0.33	0.57	-0.12	-0.22	-0.03	0.06	0.74	-	
MCHC	-0.14	0.52	0.98	-0.36	-0.44	-0.03	-0.08	-0.23	0.53	-

Table 3. MANCOVA results for blood constituent levels in trawl-collected adult yellowtail flounder from the Northwest Atlantic during 1978-85

Variable	Constituent							
	Osmolality	Sodium	Potassium	Calcium	Hematocrit	Hemoglobin	MCHC	
Sex	0.1884	0.1475	0.8539	0.3480	0.2809	0.1525	0.6788	
Site	<0.0001	<0.0001	0.0110	0.0041	<0.0001	<0.0001	0.0012	
Season	0.8979	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	
Sex × Site	0.0183	0.6918	0.2616	0.0018	0.2501	0.1291	0.6750	
Sex × Season	0.5923	0.1028	0.1669	0.5396	0.0024	0.0179	0.0522	
Sex × Site × Season	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Length	0.0331	<0.0001	<0.0001	0.0218	0.0137	0.0063	0.4442	

Table 4. Blood osmolality (ls mean mOsm/kg \pm s \bar{x} (N)) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values (read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Offshore Station		Inshore Stations				
Sex	Season	Georges Bank	Merrimack River	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight	
Male	Winter	494.16 \pm 18.43 (4)	418.49 \pm 13.01 (8)	431.70 \pm 15.07 (6)	503.39 \pm 18.41 (4)	454.29 \pm 13.95 (7)	<u>463.40 \pm 9.98 (14)^a</u>	
	Spring	440.14 \pm 6.97 (28) ^a	429.47 \pm 16.47 (5)	430.09 \pm 26.19 (2)	485.76 \pm 10.21 (13)	<u>450.00 \pm 7.91 (22)</u>	476.44 \pm 7.22 (27)^a	
	Summer	472.57 \pm 10.21 (13) ^b	426.77 \pm 8.93 (17)	nd	nd	436.58 \pm 8.39 (20)^a	nd	
	Fall	<u>487.10 \pm 9.89 (14)^b</u>	412.47 \pm 6.54 (32)	nd	nd	461.67 \pm 6.67 (32)^b	436.02 \pm 10.27 (14)^b	
Female	Winter	447.68 \pm 7.80 (24) ^a	410.44 \pm 13.91 (7)	455.36 (1)	495.49 \pm 26.03 (2)	435.45 \pm 10.21 (13)	<u>434.33 \pm 9.22 (16)</u>	
	Spring	425.17 \pm 6.36 (35) ^b	436.45 \pm 16.51 (5)	454.18 \pm 13.93 (7)	488.45 \pm 18.57 (4)	<u>428.86 \pm 6.66 (32)^a</u>	453.83 \pm 8.24 (20)	
	Summer	450.51 \pm 5.62 (44)^a	423.31 \pm 10.30 (13)	nd	nd	440.05 \pm 8.67 (18)	448.31 \pm 21.45 (3)	
	Fall	<u>436.43 \pm 11.27 (11)</u>	415.25 \pm 5.23 (53)	nd	nd	451.65 \pm 5.15 (51) ^b	434.85 \pm 9.99 (14)	

Table 5. Blood sodium concentration (Is mean $mEq/L \pm s_{\bar{x}}(N)$) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values (read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Offshore Station		Inshore Stations					
Sex	Season	Georges Bank	Merrimack River	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight		
Male	Winter	248.84 ± 7.97 (4)	209.58 ± 5.63 (8)	206.71 ± 6.52 (6)	203.85 ± 7.96 (4)	227.16 ± 6.03 (7) ^a	221.64 ± 4.18 (15)		
	Spring	205.61 ± 2.55 (39) ^a	201.27 ± 7.12 (5) ^a	217.97 ± 11.32 (2)	235.02 ± 4.41 (13)	201.26 ± 3.41 (22) ^b	217.18 ± 3.07 (28)^a		
	Summer	218.72 ± 4.42 (13) ^b	220.44 ± 3.86 (17) ^b	nd	nd	214.28 ± 3.54 (21) ^a	nd		
	Fall	223.80 ± 4.01 (16) ^b	200.77 ± 2.79 (33)^a	nd	nd	215.21 ± 2.80 (34) ^a	229.90 ± 4.94 (11) ^b		
Female	Winter	253.99 ± 3.30 (25) ^a	209.03 ± 6.02 (7)	231.85 (1)	206.50 ± 9.20 (3)	225.14 ± 4.42 (13)^a	217.29 ± 3.99 (16)		
	Spring	204.15 ± 2.51 (42) ^b	205.63 ± 7.14 (5)	217.23 ± 5.65 (8)	242.67 ± 8.03 (4)	209.89 ± 2.79 (34) ^b	215.41 ± 3.57 (20)		
	Summer	213.15 ± 2.43 (44) ^c	216.68 ± 4.28 (14) ^a	nd	nd	218.68 ± 3.65 (19)	257.52 ± 9.27 (3)		
	Fall	216.82 ± 4.87 (11) ^c	203.65 ± 2.30 (50)^b	nd	nd	213.85 ± 2.23 (51) ^b	219.30 ± 4.48 (13)		

Table 6. Blood potassium concentration ($1s$ mean $mEq/L \pm s_{\bar{x}}(N)$) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values (read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Offshore Station		Inshore Stations					
Sex	Season	Georges Bank	Merrimack River	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight		
Male	Winter	4.60 ± 1.09 (4)	3.98 ± 0.77 (8) ^a	3.78 ± 0.89 (6)	3.56 ± 1.09 (4)	4.74 ± 0.83 (7)	2.90 ± 0.57 (15) ^a		
	Spring	4.54 ± 0.34 (39) ^b	6.59 ± 0.97 (5)^b	3.37 ± 1.27 (3)	2.95 ± 0.63 (12)	4.87 ± 0.48 (21) ^b	4.45 ± 0.44 (26) ^b		
	Summer	<u>6.98 ± 0.60 (13)^a</u>	5.24 ± 0.53 (17)	nd	nd	6.28 ± 0.48 (21) ^a	nd		
	Fall	4.30 ± 0.55 (16) ^b	4.31 ± 0.37 (35) ^a	nd	nd	4.93 ± 0.40 (31) ^b	<u>7.62 ± 0.65 (12)^c</u>		
Female	Winter	4.22 ± 0.45 (25) ^a	3.53 ± 0.82 (7)	4.65 (1)	4.74 ± 1.26 (3)	3.77 ± 0.60 (13) ^a	3.10 ± 0.55 (16)		
	Spring	5.30 ± 0.34 (42) ^b	4.16 ± 0.98 (5)	3.35 ± 0.77 (8)	2.45 ± 1.10 (4)	5.62 ± 0.39 (32) ^b	4.02 ± 0.50 (19)		
	Summer	<u>5.47 ± 0.33 (44)^b</u>	4.36 ± 0.59 (14)	nd	nd	6.40 ± 0.50 (19) ^b	11.69 ± 1.27 (3)		
	Fall	3.03 ± 0.67 (11) ^a	5.02 ± 0.32 (50)	nd	nd	5.36 ± 0.32 (46)^b	<u>5.68 ± 0.64 (12)</u>		

Table 7. Blood calcium concentration ($1s$ mean $mEq/L \pm s_{\bar{x}}(N)$) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values (read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Offshore Station		Inshore Stations					
Sex	Season	Georges Bank	Merrimack River	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight		
Male	Winter	4.19 ± 0.64 (4)	4.51 ± 0.45 (8)	4.53 ± 0.52 (6)	4.60 ± 0.64 (4)	4.52 ± 0.48 (7)	5.31 ± 0.34 (15)		
	Spring	4.50 ± 0.20 (39) ^a	<u>4.86 ± 0.57</u> (5)	5.76 ± 0.91 (2)	3.79 ± 0.37 (12)	4.24 ± 0.27 (22)	5.05 ± 0.25 (28)		
	Summer	<u>4.34 ± 0.35</u> (13) ^a	<u>4.22 ± 0.31</u> (17)	nd	nd	3.97 ± 0.28 (21) ^a	nd	nd	
	Fall	6.31 ± 0.34 (14) ^b	4.24 ± 0.24 (29)	nd	nd	4.78 ± 0.22 (34) ^b	5.33 ± 0.38 (12)		
Female	Winter	4.64 ± 0.26 (25) ^a	4.75 ± 0.48 (7) ^{b,c}	4.77 (1)	5.08 ± 0.74 (3)	4.25 ± 0.35 (13)	4.84 ± 0.32 (16)		
	Spring	4.44 ± 0.20 (41) ^a	<u>7.75 ± 0.52</u> (6) ^a	5.67 ± 0.45 (8)	4.66 ± 0.64 (4)	4.23 ± 0.22 (34) ^a	4.40 ± 0.29 (19) ^a		
	Summer	<u>5.27 ± 0.19</u> (44) ^b	<u>5.25 ± 0.34</u> (14) ^b	nd	nd	3.97 ± 0.29 (19) ^a	2.56 ± 0.74 (3)		
	Fall	5.64 ± 0.39 (11) ^b	4.28 ± 0.19 (47) ^c	nd	nd	4.99 ± 0.18 (51) ^b	5.71 ± 0.36 (13) ^b		

Table 8. Hematocrit (Is mean $\% \pm s_{\bar{x}}$ (N)) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values (read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Offshore Station		Inshore Stations				
Sex	Season	Georges Bank	Merrimack River	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight	
Male	Winter	31.60 ± 2.62 (5) ^{a,b}	27.59 ± 2.07 (8) ^{b,c}	29.05 ± 2.08 (8)	20.78 ± 2.40 (6)	<u>27.52 ± 2.22 (7)^a</u>	<u>30.53 ± 1.45 (17)</u>	
	Spring	<u>32.05 ± 0.94 (39)^a</u>	37.32 ± 2.22 (7)^a	17.84 ± 3.41 (3)	nd	<u>32.71 ± 1.20 (24)^b</u>	30.74 ± 1.09 (30) ^a	
	Summer	26.91 ± 1.57 (14) ^b	30.07 ± 1.42 (17) ^c	nd	nd	22.49 ± 1.97 (9) ^a	nd	
	Fall	23.68 ± 1.47 (16) ^b	23.21 ± 1.02 (33) ^b	nd	nd	25.04 ± 1.06 (32) ^a	26.97 ± 1.58 (15) ^b	
Female	Winter	27.01 ± 1.26 (23) ^a	26.81 ± 2.39 (6) ^a	25.62 (1)	22.23 ± 2.93 (4)	21.21 ± 1.62 (13)^a	<u>24.58 ± 1.47 (16)^b</u>	
	Spring	<u>25.54 ± 0.89 (46)</u>	34.26 ± 2.22 (7)^b	21.91 ± 2.08 (8)	nd	<u>26.82 ± 1.00 (36)^b</u>	29.20 ± 1.18 (25)^a	
	Summer	25.59 ± 0.87 (46)	26.23 ± 1.47 (16) ^a	nd	nd	26.07 ± 1.42 (17) ^{b,c}	33.76 ± 2.64 (5)^a	
	Fall	22.57 ± 1.87 (10) ^b	24.94 ± 0.86 (49) ^a	nd	nd	24.00 ± 0.85 (47) ^{a,c}	23.56 ± 1.58 (14) ^b	

Table 9. Hemoglobin concentrations (1s mean $g/100\text{ ml} \pm s_x$ (N)) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values (read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Inshore Stations					
		Offshore Station	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight	
Sex	Season	Georges Bank	Merrimack River				
Male	Winter	<u>6.40 ± 0.49 (5)^a</u>	6.18 ± 0.38 (8) ^a	5.87 ± 0.38 (8)	3.29 ± 0.44 (6)^a	5.00 ± 0.41 (7)^{ac}	5.23 ± 0.27 (17)
	Spring	<u>5.65 ± 0.17 (40)^a</u>	6.21 ± 0.41 (7) ^a	3.17 ± 0.63 (3)	4.98 ± 0.30 (13) ^b	<u>5.67 ± 0.22 (25)^a</u>	<u>5.48 ± 0.19 (35)^a</u>
	Summer	<u>5.54 ± 0.31 (12)^a</u>	5.02 ± 0.26 (17) ^b	nd	nd	4.01 ± 0.24 (21)^b	nd
	Fall	3.70 ± 0.27 (16) ^b	4.28 ± 0.18 (35) ^c	nd	nd	4.37 ± 0.20 (32)^c	4.80 ± 0.29 (15)^b
Female	Winter	<u>5.13 ± 0.22 (26)^a</u>	6.12 ± 0.41 (7)^a	6.32 (1)	3.72 ± 0.54 (4)	4.19 ± 0.30 (13)	4.60 ± 0.26 (17)
	Spring	<u>4.48 ± 0.16 (47)^b</u>	5.45 ± 0.41 (7)^a	3.42 ± 0.38 (8)	4.68 ± 0.49 (5)	<u>4.86 ± 0.19 (36)^a</u>	<u>4.86 ± 0.22 (25)^a</u>
	Summer	<u>4.74 ± 0.18 (36)</u>	4.41 ± 0.27 (16) ^b	nd	nd	<u>4.75 ± 0.25 (19)^a</u>	4.79 ± 0.49 (5)
	Fall	4.04 ± 0.35 (10) ^b	4.31 ± 0.15 (52) ^b	nd	nd	4.16 ± 0.15 (51) ^b	4.08 ± 0.28 (15) ^b

Table 10. Mean corpuscular hemoglobin concentrations (ls mean g/100 ml packed red bloodcells $\pm s_{\bar{x}}(N)$) in adult yellowtail flounder trawl-collected from six Northwest Atlantic locations during 1978-85. (Station-related differences (*i.e.*, between the five inshore stations and the Georges Bank offshore station) for a given season and sex are indicated by bold values(read horizontally). Season-related differences for a given station and sex are indicated by the presence or absence of superscripts for the values (read vertically); values with the same or no superscript are not significantly different; values with different superscripts are significantly different. Sex-related differences for a given station and season are indicated by underlined values (read vertically). For some stations and seasons, no data (“nd”) were available. For statistical significance, $P < 0.05$.)

		Offshore Station		Inshore Stations					
Sex	Season	Georges Bank	Merrimack River	Massachusetts Bay	Cape Cod Bay	Mud Patch	New York Bight		
Male	Winter	21.63 \pm 1.45 (5) ^a	22.47 \pm 1.14 (8) ^a	20.41 \pm 1.15 (8)	16.07 \pm 1.33 (6)	18.23 \pm 1.22 (7)	17.20 \pm 0.80 (17)		
	Spring	18.14 \pm 0.52 (39) ^b	16.66 \pm 1.22 (7) ^{b,c}	18.17 \pm 1.88 (3)	nd	17.34 \pm 0.66 (24)	17.61 \pm 0.60 (30)		
	Summer	<u>21.16 \pm 0.93 (12)^{a,c}</u>	16.73 \pm 0.78 (17)^b	nd	nd	19.72 \pm 1.09 (9)	nd	nd	
	Fall	15.39 \pm 0.81 (16) ^d	18.91 \pm 0.57 (32)^c	nd	nd	17.85 \pm 0.60 (30)	18.12 \pm 0.87 (15)		
Female	Winter	19.14 \pm 0.69 (23)	22.73 \pm 1.32 (6)^a	24.91 (1)	16.57 \pm 1.62 (4)	19.85 \pm 0.90 (13) ^a	19.27 \pm 0.81 (16) ^a		
	Spring	17.75 \pm 0.49 (46)	16.06 \pm 1.23 (7) ^b	15.45 \pm 1.15 (8)	nd	18.22 \pm 0.55 (36)	17.02 \pm 0.65 (25) ^b		
	Summer	<u>18.20 \pm 0.54 (36)</u>	17.44 \pm 0.81 (16) ^b	nd	nd	19.04 \pm 0.78 (17)	14.58 \pm 1.46 (5)^b		
	Fall	17.73 \pm 1.04 (10)	18.35 \pm 0.47 (49) ^b	nd	nd	17.72 \pm 0.47 (47) ^b	17.63 \pm 0.88 (14)		

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Organization: Manuscripts must have an abstract, table of contents, and -- if applicable -- lists of tables, figures, and acronyms. As much as possible, use traditional scientific manuscript organization for sections: "Introduction," "Study Area," "Methods & Materials," "Results," "Discussion" and/or "Conclusions," "Acknowledgments," and "References Cited."

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For spelling of scientific and common names of fishes, mollusks, and decapod crustaceans from the United States and Canada, use *Special Publications* No. 20 (fishes), 26 (mollusks), and 17 (decapod crustaceans) of the American Fisheries Society (Bethesda, MD). For spelling of scientific and common names of marine mammals, use *Special Publication* No. 4 of the Society for Marine Mammalogy (Lawrence, KS). For spelling in general, use the most recent edition of *Webster's Third New International Dictionary of the English Language Unabridged* (Springfield, MA: G.&C. Merriam).

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Manuscript Submission

Authors must submit one paper copy of the double-spaced manuscript, one disk copy, and original figures (if applicable). NEFSC authors must include a completely signed-off "NEFSC Manuscript/Abstract/Webpage Review Form." Non-NEFSC authors who are not federal employees will be required to sign a "Release of Copyright" form.

Send all materials and address all correspondence to: Jon A. Gibson (Biological Sciences Editor), NMFS Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543-1026.

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Publications and Reports of the Northeast Fisheries Science Center

The mission of NOAA's National Marine Fisheries Service (NMFS) is "stewardship of living marine resources for the benefit of the nation through their science-based conservation and management and promotion of the health of their environment." As the research arm of the NMFS's Northeast Region, the Northeast Fisheries Science Center (NEFSC) supports the NMFS mission by "planning, developing, and managing multidisciplinary programs of basic and applied research to: 1) better understand the living marine resources (including marine mammals) of the Northwest Atlantic, and the environmental quality essential for their existence and continued productivity; and 2) describe and provide to management, industry, and the public, options for the utilization and conservation of living marine resources and maintenance of environmental quality which are consistent with national and regional goals and needs, and with international commitments." Results of NEFSC research are largely reported in primary scientific media (*e.g.*, anonymously-peer-reviewed scientific journals). However, to assist itself in providing data, information, and advice to its constituents, the NEFSC occasionally releases its results in its own media. Currently, there are three such media:

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Northeast Fisheries Science Center Reference Document -- This series is issued irregularly. The series typically includes: data reports on field and lab studies; progress reports on experiments, monitoring, and assessments; background papers for, collected abstracts of, and/or summary reports of scientific meetings; and simple bibliographies. Issues receive internal scientific review, but no technical or copy editing.

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