farther. Also, side swimming would place both pelvic fins in a position where they could facilitate rapid left (now ventral) turns while possibly adding lift.

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CHROMOSOMAL ANALYSIS OF ALBACORE, *THUNNUS ALALUNGA*, AND YELLOWFIN, *THUNNUS ALABACARES*, AND SKIPJACK, *KATSUWONUS PELAMIS*, TUNA

Chromosomal analysis is being used as part of an investigation of the population stock structure of the North Pacific albacore, *Thunnus alalunga*. There is a growing body of evidence (Brock 1943; Laurs and Lynn 1977; Laurs and Wetherall 1981; Laurs 1983) that North Pacific albacore are not as homogeneous as usually assumed (Clemens 1961; Otsu and Uchida 1963). Results from recent tagging studies suggest that northern and southern substocks constitute the North Pacific albacore population and that these proposed substocks have different migratory patterns (Laurs and Nishimoto 1979[1]; Laurs 1983). Laurs and Wetherall (1981) also found that the growth rates were significantly different in the two proposed substocks. In addition, the differences in growth rate are consistent with differences in length frequencies of albacore caught in commercial fisheries off North America (Brock 1943; Laurs and Lynn 1977).

In this paper we report results from chromosomal analysis using C-banding for albacore (from the proposed North Pacific southern substock) and compare them with similar results obtained for yellowfin, *Thunnus alabacares*, and skipjack, *Katsuwonus pelamis*, tuna. We demonstrate that there is a chromosomal basis for placing the albacore and the yellowfin tuna in the genus *Thunnus* and that recognizable chromosomal differences exist between the genera *Thunnus* and *Katsuwonus*. These findings corroborate the taxonomy of the albacore and the yellowfin and skipjack tuna based on comparative anatomy (Gibbs and Collette 1967; Collette 1978).

The results reported here are from part of a larger study, which is helping us to evaluate if genetic heterogeneity exists in the North Pacific albacore population. Information on chromosome characteristics is scarce for fishes, and to our knowledge this is the first time chromosome analyses have been reported for scombrid fishes.

Materials and Methods

All blood samples were collected from freshly caught fish either aboard the NOAA RV *David Starr Jordan* (August 1983) or aboard fishing boats

[1]Laurs, R. M., and R. N. Nishimoto. 1979. Results from North Pacific albacore tagging studies. U.S. Dep. Commer., Natl. Mar. Fish. Serv., SWFC Admin. Rep. LJ-79-17, 9 p.

(October-November 1983). Because albacore have a high titer of red blood cells (Alexander et al. 1980), it was expedient to separate the lymphocytes from the erythrocytes. The lymphocytes were isolated from the blood on a density gradient of ficoll-sodium diatrizoate solution using a modification of the technique developed by Boyum (1968), which is specific for the concentration of lymphocytes. We found that it was necessary to isolate the lymphocytes and place them in culture within a couple of hours after blood samples were collected. The ficoll gradient procedure was not successful using undiluted heparinized blood that was retained for more than a few hours.

Two albacore, three skipjack tuna, and four yellowfin tuna were sampled. All fish were juveniles which have virtually no sexual dimorphic characteristics, and no sex determinations were made. The estimated fork lengths of the fish ranged from 65 to 85 cm for albacore, 80 to 120 cm for yellowfin, and 45 to 55 cm for skipjack.

From each fish, an 8-10 mL sample of blood was withdrawn via sterile intracardial puncture into a syringe coated with 1,000 units/mL of heparin. Two mL aliquots of blood were pipetted into each of the four 15 mL centrifuge tubes, and 4 mL of cell culture medium[2] was added. The mixture was centrifuged at 20 *g* for 5 min, and the white cells and plasma were transferred to another centrifuge tube. This procedure for the separation of the plasma and white cell mixture was repeated three times following the suggestions given by Blaxhall (1981).

Five mL of the white cell-plasma mixture were layered over 3 mL of ficoll-sodium diatrizoate solution and centrifuged at 572 *g* for 30 min. The overlaying plasma was removed carefully with Pasteur pipets, and the lymphocytes below were transferred to a culture tube containing 5 mL of marine teleost cell culture medium (Michael and Beasley 1973). This procedure resulted in an erythrocyte free culture of lymphocytes having a higher mitotic index. The cultures were incubated at 25°C for 3-5 d, at which time they were terminated and the cells harvested. The techniques for chromosomal analysis were patterned after those of Nowell (1960) for mammals because tuna are also endothermic (Graham and Dickson 1981). This work is an extension of the procedures developed by Kelly and Laurs (1983[3]).

Prior to harvesting the cells, 0.5 μg colcemid was added to 5 mL of culture medium and incubated for 2 h at 25°C. The culture was then centrifuged for 5 min at 180 *g* and the supernatant was replaced with 5 mL 0.075 M KCl for 10 min. The culture tubes were centrifuged again for 5 min at 180 *g*, and the supernatant was replaced with 3 mL of freshly prepared cold fixative which consists of 3 parts methyl alcohol to 1 part glacial acetic acid and mixed for 1 h. The tubes were again centrifuged at 180 *g* for 5 min, decanted, and fixed. The cell pellet plus 0.5 mL of fixative was retained for slide preparation.

Precleaned slides dipped in methanol and then in deionized water were used for slide preparations. Two drops of cell suspension were placed on the slide and 4 drops of fixative were immediately added. The slide was dried on a slide warmer at 37°C and stored at room temperature for 24-72 h prior to C-banding. The C-banding procedures were patterned after the work of Pardue and Gall (1970) and Arrighi and Hsu (1971).

In preparation for C-banding, the slides were placed in 0.2 N HCl for 15 min at 37°C, rinsed in deionized water, treated with saturated $Ba(OH)_2$ at room temperature for 7 min, and rinsed in deionized water. They were then immediately dipped again in 0.2 N HCl for 10 s and rinsed in deionized water. After the final rinsing the slides were incubated in 2× sodium chloride-sodium citrate solution at 60°C for 90 min and then stained for 90 min in Giemsa diluted with 1:10 Sorenson's buffer pH 6.8. Suitable metaphase figures were photographed at 1,008× magnification using a Zeiss[4] microscope equipped with a phase planapochromat 63/1.4 oil immersion lens.

Results

Chromosome Numbers

Kelly and Laurs (fn. 3) found that the diploid number of chromosomes for albacore was 48. We have confirmed this observation and have found that the diploid numbers for yellowfin and skipjack tuna are also 48. The modal frequencies of about 90 cells containing 48 chromosomes were 82.2% for albacore, 92.6% for yellowfin, and 80.5% for skipjack. Kelly and Laurs also observed that 85% of albacore cells had 48 chromosomes. Two polyploid cells with

[2]RPMI-1640 Sigma Cat. No. R6504.

[3]Kelly, Raymond M., and R. Michael Laurs. 1983. Summary of methods developed for investigations of albacore chromosomes and of findings made on number of chromosomes. Unpubl. field and laboratory notes and results (April 1983). [Raymond M. Kelly, School of Medicine, University of California, La Jolla, CA; R. Michael Laurs, National Marine Fisheries Service, NOAA, La Jolla, CA.]

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

96 chromosomes were observed in skipjack, and one polyploid cell with 96 chromosomes was observed in albacore. No polyploid cells were observed in yellowfin.

Chromosome Morphology

The albacore and the yellowfin and skipjack tuna were observed to have the same diploid chomosome number; however, their karyotype differed with respect to chromosome morphology. In this study, the chromosome pairs were arranged according to the morphology index (M), developed by Giannelli and Howlett (1967), which is obtained by dividing the length of the total haploid chromosome set p + q by the arm ratio (q/p). Based on our evaluation of 256 metaphase cells (Table 1), we found that the chromosome morphology of the yellowfin (Fig. 1) is more similar to that of the albacore (Fig. 2) than the skipjack (Fig. 3). The differences in chromosome morphology were most apparent in the three largest pairs of chromosomes (Table 1). The morphology index (M) places the metacentric and submetacentric chromosomes of the albacore and yellowfin in the number 1 and 2 positions respectively. Chromosome 3 of the albacore is also submetacentric while chromosome 3 of the yellowfin is referred to as subtelocentric. The subtelocentric category is used to describe chromosomes in which the centromeres are displaced more towards the telomere when compared with submetacentrics. The metacentric chromosome of the albacore was consistently larger than the metacentric of the yellowfin. The remaining 42 chromosomes were telocentric in the albacore and yellowfin. All of the chromosomes of the skipjack were telocentric.

C-Banding Patterns

C-banding determinations were done to differentiate individual chromosome characteristics among the three species of tunas (Table 2). The centromeric regions of most of the chromosomes of all three species contained C-band constitutive heterochromatin. However, there were differences in the intensities of staining on comparable chromosomes among the three species. Intercalary C-banding was observed only in the skipjack tuna and there was variability in terminal banding among the three species.

In the albacore all chromosomes, except pair 10, showed C-banding in the centromeric regions with intense, prominent bands notably apparent in chromosome pairs 2 and 3 (Fig. 2). Terminal banding was restricted to chromosome pair 1 which had obscure C-bands on one arm of each homologue. No intercalary C-banding was observed in the albacore. There were some minor differences in the C-banding patterns between albacore and yellowfin tuna. In the yellowfin, the centromeric regions of all chromosomes were banded, the intensity of the banding in the centromeric region was uniform

TABLE 1.—Classification of chromosome morphology for albacore and yellowfin and skipjack tuna.

Chromosome number	Albacore	Yellowfin	Skipjack
1	metacentric	metacentric	telocentric
2	submetacentric	submetacentric	telocentric
3	submetacentric	subtelocentric	telocentric
4-48	telocentric	telocentric	telocentric

TABLE 2.—Summary of C-banding characteristics for albacore and yellowfin and skipjack tuna.

Location of bands	Albacore	Yellowfin	Skipjack
Centromeric region	Present on all chromosomes except pair 10; intensely prominent on pairs 2 and 3	Present on all chromosomes with uniform prominent intensity	Present on all chromosomes except pairs 10 and 19, great variability in intensity most prominent on pairs 1, 3, 4, 7, and 18
Terminal bands	Present on one arm of each homologue on pair 1; weakly developed	Weakly developed on chromosome pairs 1, 3, 7, 8, 14, 15, 21, & 24	Notably prominent in pair 4
Intercalary	None present	None present	Present on all chromosome pairs except 17 and 24

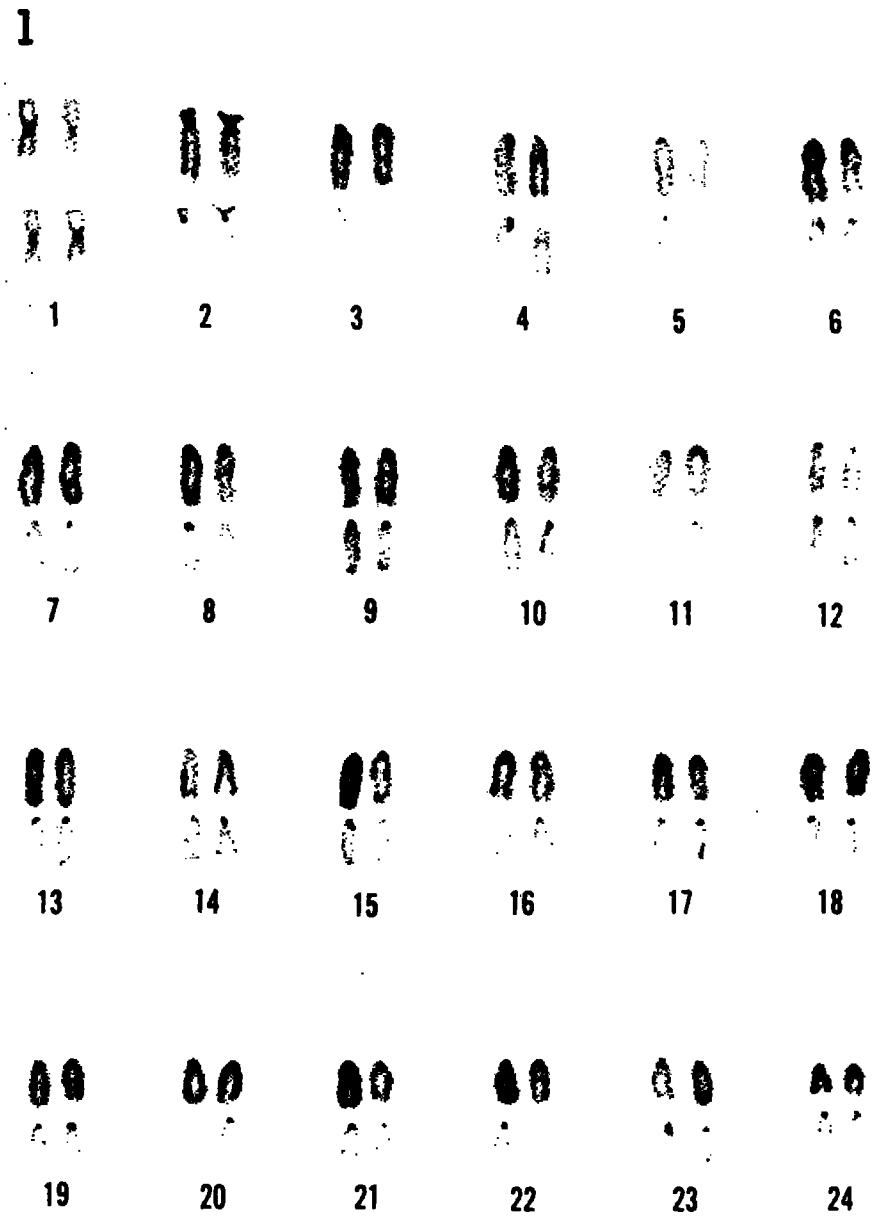

FIGURE 1.—Giemsa stained karyotype (upper row) and C-banding karyotype (lower row) of the same yellowfin tuna.

among all chromosomes, and terminal banding was weakly developed on eight pairs of chromosomes (Fig. 1). As in the albacore, no intercalary banding was observed in the yellowfin. The following significant differences were observed in the C-banding patterns between the skipjack and the other two species: 1) all chromosomes except 10 and 19 had C-banding in the centromeric region, 2) there was great variability in the intensity of staining in the centromeric region, 3) terminal banding was notably prominent in chromosome pair 4, and 4) there were intercalary bands on all chromosomes except pairs 17 and 24.

Discussion

Our results assist in understanding speciation processes that have occurred in the evolution of the

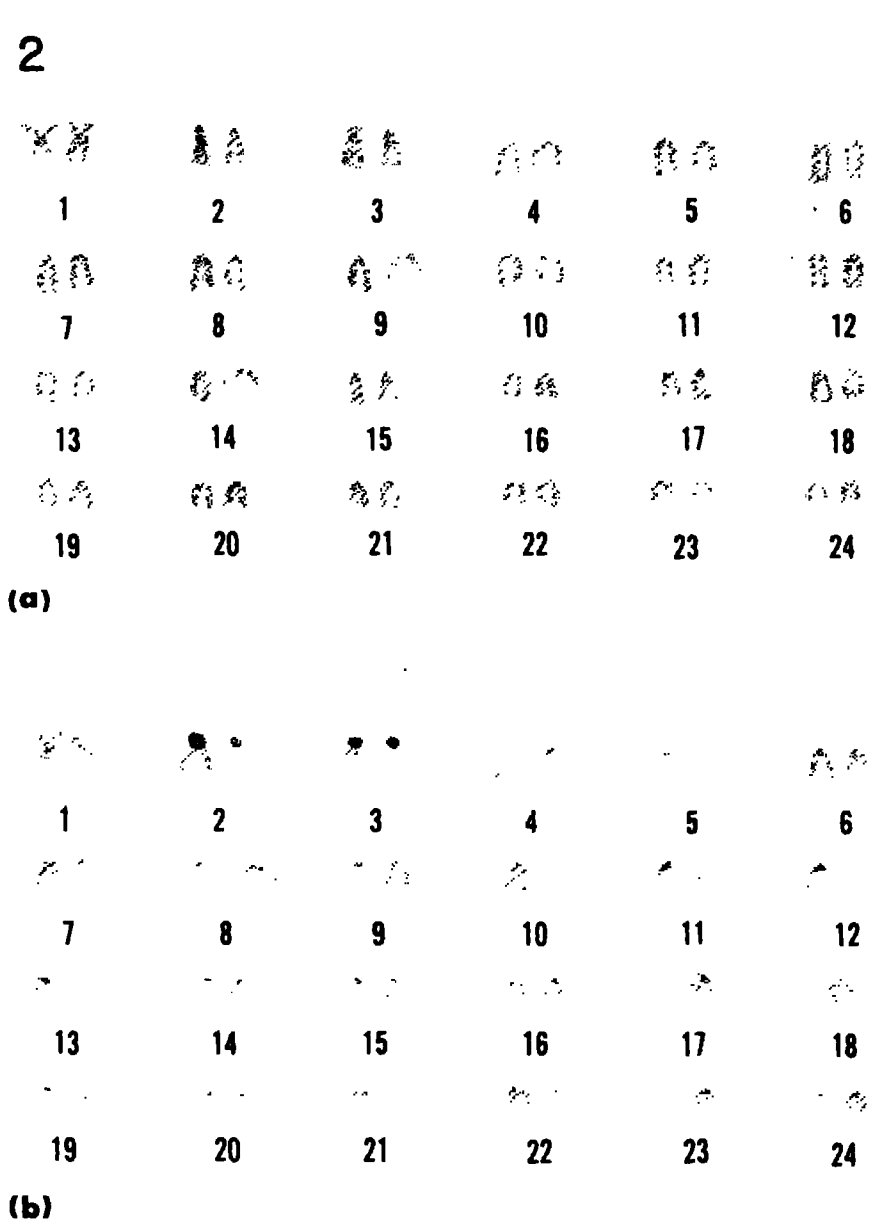

FIGURE 2.—Giemsa stained karyotype (a) and C-banding karyotype (b) from two different fish of the North Pacific albacore.

tuna. Gibbs and Collette (1967) proposed that seven species of tuna be included in the genus *Thunnus* on the basis of external morphological and internal anatomical characters. Our results demonstrate that there is a genetic basis for placing the albacore, *T. alalunga*, and the yellowfin tuna, *T. albacares*, in one genus *Thunnus* and the skipjack tuna, *Katsuwonus pelamis*, in a separate genus. These relationships are based on the assumption that closely related species will share certain karyotypic characteristics.

The determination that the albacore, yellowfin tuna, and skipjack tuna have the same number of chromosomes suggests that speciation of the genera of Thunnini might have occurred by intrachromosomal rearrangement as opposed to Robertsonian changes as hypothesized for the rainbow trout, *Salmo gairdneri* (Thorgaard 1976). If speciation had

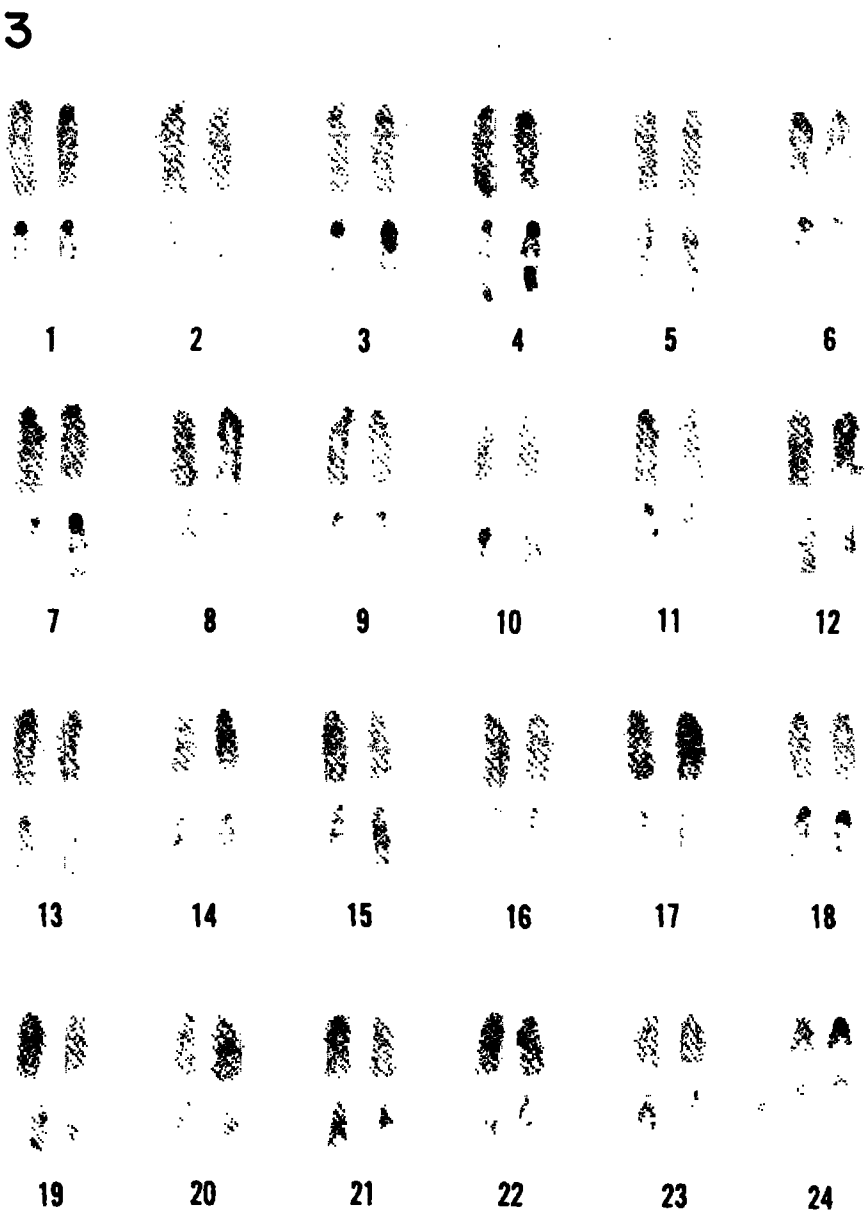

FIGURE 3.—Giemsa stained karyotype (upper row) and C-banding karyotype (lower row) of the same skipjack tuna.

involved a reduction in uniarmed chromosomes to form biarmed chromosomes, we would have expected to find a difference in the chromosome number between *Katsuwonus* and *Thunnus*.

It is probable that speciation within the genus *Thunnus* might also be related to chromosome rearrangement because the number of chromosomes is the same. Pericentric inversion is a type of intrachromosomal rearrangement that could result in the displacement of the centromere to convert a telocentric chromosome into a metacentric one. Zenzes and Voiculescu (1975) suggested that pericentric inversion was involved in the chromosomal organization of the brown trout, *Salmo trutta*. The extent to which this mechanism has been related to the speciation of genera *Thunnus* and *Katsuwonus* is

uncertain. However, the occurrence of terminal C-bands on chromosome 1 of the albacore and chromosomes 1 and 3 of the yellowfin tuna is consistent with the hypothesis that these biarmed chromosomes were derived from a uniarmed condition. Indeed, White (1951) believed that, in grasshoppers, telocentric chromosomes are more primitive than the metacentric condition. Absence of terminal bands on chromosomes 2 and 3 of the albacore and chromosome 2 of the yellowfin tuna does not preclude the suggested derivation of metacentric chromosomes. It is possible that in the metacentric chromosomes lacking terminal bands, centrometric heterochromatin either was not moved or was lost. It is also possible that chromosome rearrangement in the speciation of the albacore and yellowfin occurred through changes in the euchromatic portions of chromosomes. To test this hypothesis it will be necessary to use G-banding techniques (Rishi 1978) to conduct analysis of these portions of the chromosomes.

In contrast to the albacore and yellowfin tuna, the telocentric chromosomes of the skipjack tuna showed a variety of intercalary and terminal C-banding in addition to those of the centromeric regions. An interesting condition was the polymorphic terminal heterochromatic block that occurred in chromosome pair number 4 of the skipjack, but not in the albacore or yellowfin. While the four specimens of skipjack analyzed had this polymorphism, it is not possible to comment on the frequency with which it might occur in the population. This type of differential banding also occurs in other fishes as demonstrated by Zenzes and Voiculescu (1975) who observed a difference in the size of C-bands in *Salmo trutta*. The C-band polymorphism we observed in skipjack could be related to the sex determining mechanism of the fish. However, we do not have any information on the sex of the skipjack used in this study and most fish do not have heteromorphic sex chromosomes (Zenzes and Voiculescu 1975; Thorgaard 1976; Kligerman and Bloom 1977). An exception occurs in the eels which have highly heteromorphic sex chromosomes (Park and Grimm 1981).

Analysis of C-banding patterns associated with the morphological differences in chromosomes has permitted us to identify all of the chromosome pairs of the albacore, yellowfin tuna, and skipjack tuna. We have demonstrated that karyotype analysis may provide a chromosomal basis for placing albacore and yellowfin in *Thunnus* and skipjack in *Katsuwonus*. Although C-banding techniques did not allow a detailed evaluation of the *Thunnus* chromosomes, we believe that the use of multiple banding procedures could provide important information on the speciation and cytotaxonomy of the species of this commercially important genus. In addition, use of G-banding procedures will be an important next step in determining if genetic heterogeneity exists in the North Pacific albacore population.

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ABUNDANCE, SIZE, AND SEX RATIO OF ADULT SEA-RUN SEA LAMPREYS, *PETROMYZON MARINUS*, IN THE CONNECTICUT RIVER[1]

Populations of sea-run sea lampreys, *Petromyzon marinus*, occur in many rivers on the east coast of North America from Labrador to Florida (Bigelow and Schroeder 1953). The Connecticut River in the northeastern United States is believed to have the largest population (Beamish 1980). Although the historical, upstream range of the sea lamprey in the Connecticut River is not known, it probably was similar to American shad, *Alosa sapidissima*, which migrated 280 km upstream to Bellows Falls, VT (Moffitt et al. 1982).

Upstream migration of anadromous fish species in the Connecticut River main stem was first restricted in 1798 by the construction of Turners Falls Dam at km 197, and further in 1849 by the construction of Holyoke Dam at km 140. The first upstream fish passage facility for anadromous fish was a fish lift at Holyoke Dam that began operating in 1955. Until 1969 the sea lampreys using the fish lift were counted and either killed or thrown back. From 1969 to 1984, they have been passed upstream each year. Sea lampreys have also used the fish ladders that were completed in 1980 and 1981 at Turners Falls and Vernon Dams, respectively. With the completion of the fish ladder at Bellows Falls Dam in 1984, migrants now have access to 350 km of main-stem river and many additional tributaries (Fig. 1).

The present report summarizes the annual counts of sea lampreys from 1958 to 1984 at the two Holyoke fish lifts (a second fish lift was added in 1976). We also examined the sex ratio, total length, and weight of adults in 1981-82 and compared these characteristics with those of the population in the St. John River, New Brunswick. Beamish et al. (1979) sampled the St. John River population at km 140, at a fish lift located at Mactaquac Dam.

Methods

Sea lampreys that were lifted above the dam were counted each year from 1958 to 1984, except for the period from 1969 to 1974. From 1958 to 1968, sea lampreys were counted by personnel of the Holyoke Water Power Company (the owner of the dam), and

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