USE OF DIAGNOSTIC X-RAY FOR DETERMINING VERTEBRAL NUMBERS OF FISH

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EXPLANATORY NOTE

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by

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X-ray photograph of an Atlantic menhaden,
90 millimeters fork length.

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ABSTRACT

Diagnostic X-ray equipment and supplies are described and methods given for resolving vertebral centri of menhaden larger than approximately one-half inch in length. Only a minimum amount of technical knowledge is necessary to produce satisfactory radiographs from which vertebral counts can be made, thus reducing the time required to determine the vertebral numbers of large samples of fish and forming a fixed impression of the vertebrae which can be conveniently stored for future reference.

USE OF DIAGNOSTIC X-RAY FOR DETERMINING VERTEBRAL NUMBERS OF FISH

Use of vertebral numbers to delineate races, or populations, has had wide application in fishery research (Clark, 1947; McHugh, 1951; Tester, 1948, et al.). Oftentimes, however, this character is ignored in such studies because of the great amount of time and effort required in dissection of vertebral columns in order to determine accurately the number of vertebrae. Furthermore, investigators frequently are reluctant to commit a valuable collection to dissection as the specimens subsequently are of little value for further study. Radiographs, therefore, not only furnish a means of rapidly determining vertebral counts of individual fish, but provide a permanent record of the vertebral column for future reference.

The adaptability of X-rays to the study of fish was first emphasized in this country by Gosline (1948) who produced satisfactory radiographs of several freshwater species (*Ameiurus melas melas*, *Ictalurus furcatus*, *Ictalurus lacustris puncatus*, *Notemigonus crysoleucas*, and *Percinaeaprodes*) with a used radiographic unit obtained from the War Assets Administration. Since 1948 several research papers note the use of X-rays in determining the vertebral numbers of fish (McHugh, 1951; Bonham and Bayliff, 1953; Howard 1954; Bailey and Gosline, 1955), but little detailed information concerning X-ray technique is given.

It is the purpose of this paper to (1) describe the types of X-ray machines commonly used in fish radiography, (2) describe necessary accessory equipment and supplies, (3) present techniques for successful resolution of fish vertebrae by radiography, (4) show a cost analysis of investment and operation of an X-ray machine, and (5) furnish a parts list and a circuit diagram from which an efficient, inexpensive machine may be assembled. The material presented is the result of nearly two year's work with X-ray methods in routine determinations of vertebral counts of many thousands of Atlantic menhaden (*Brevoortia tyrannus*).

TYPES OF X-RAY MACHINES

Two main types of X-ray machines have been commonly used in fish radiography; these may be classified as either soft or hard ray emitters, depending on the applied kilovoltage (kv.). Soft-ray machines operate from approximately 10 kv. (Grenz-rays) to 25 kv. (diffraction units) and are most suitable for examining small specimens. X-ray tubes operating on low kilovoltages have small focal spots, or anodes, from which radiation is emitted as a narrow beam for short distances. Thus, greater accuracy and photographic sharpness is to be expected as there is less likelihood of interference from scattered or reflected radiation. This type of radiography, however, has not proven advantageous over the higher kilovoltage radiographic types for routine examination of fish vertebrae. Bonham and Bayliff (ibid.), for example, reported having successfully radiographed specimens of *Gambusia* measuring 9 mm. standard length with a soft-ray machine, but were unable to resolve the vertebrae of small salmon (*Oncorhynchus*) and eel pouts (*Lycodes brevipes*) less than 35 mm. standard length. The probable reason for this apparent inconsistency of results is that vertebrae of slow-growing fish become more dense at a relatively smaller body length than those of relatively larger, fast-growing species. It follows then that the density of the vertebrae imposes limitations on soft-ray as well as hard-ray machines for successful resolution.

Hard-ray emitters, or diagnostic machines, operate on applied kilovoltages of approximately 25 to 100 kv. The focal spots on such machines are necessarily larger than on lower kilovoltage tubes as the energy imput is potentially much greater (Clark, 1940). Unless the energy is spread over a greater surface, melting and destruction of the anode would result. More interference from scattered or reflected radiation could be expected due to the increased size of the diametric field of effective radiation produced by the larger focal spot. However,

Figure 1.--Diagnostic X-ray machine used in menhaden radiography.

interference is held to a minimum by virtue of the penetrating qualities of hard radiation which requires shorter time exposures than are necessary when using soft-rays to make radiographs of fish of comparable sizes. Prolonged exposures at high kilovoltages would increase the likelihood of interference from reflected radiation by objects extraneous to the X-ray tube as most materials will absorb and re-emit radiation to some extent. Ordinarily, such prolonged exposures are not necessary in fish radiography, therefore, the effects of interference are not of serious consequence. Limitations of diagnostic units are similar to those inherent in soft-ray machines, viz., the vertebrae of small specimens are difficult to resolve satisfactorily. For large specimens the diagnostic unit is preferable since less time is required to complete the filming process.

The diagnostic machine assembled for use by the Menhaden Investigations was installed in a plywood cabinet, $24\frac{1}{2}$" X 59" X $41\frac{1}{2}$", with controls situated at one end. The X-ray tube was suspended $28\frac{1}{2}$" from the top of the cabinet by means of metal framework welded to a $1\frac{1}{2}$" diameter pipe. A 24" X 28" X 3/16" steel plate was fastened to the top of the cabinet beneath the tube to absorb most of the direct radiation. In addition, a second steel plate, 24" X $31\frac{1}{2}$" X 3/16", was mounted between the tube and the controls to protect the operator from scattered radiation. High voltage wires leading from the transformer, inside the cabinet, to the X-ray tube were enclosed in 1/4" conduit pipes as a safeguard against arcing when the machine is in operation (fig. 1).

Initially, the machine was equipped with a preset electronic timing device, calibrated from one-half second to three minutes, for precise exposure, but atmospheric corrosion caused frequent failures of the timer. It subsequently was replaced by an open-type electric push button control. Exposure time was determined by means of an electric exposure timer.

X-RAY FILMS

In general, standard fine grain X-ray film, either 10" X 12" or 14" X 17", has been found to be satisfactory for radiographing menhaden specimens larger than approximately 35 mm. In specimens which had not undergone metamorphosis, grain size of standard film was found to be too course and overly sensitive to radiation to show good contrast between incompletely ossified vertebrae and comparatively dense surrounding body tissues. In metamorphosed specimens, where ossification had been completed, density differences were sufficiently great for successful resolution.

Menhaden specimens smaller than 35 mm. have been X-rayed successfully for vertebral determinations by means of fine grain photographic film designed to produce sharply contrasting black and white prints. Exposures made with Kodalith film, for example, indicate that acceptable radiographs may be obtained of specimens ranging down to 24 mm. fork length, providing metamorphosis is at least partially completed. Results of some of these exposures are shown in figure 2. The relation of exposure time to fish length using Kodalith film is shown in figure 3.

As mentioned earlier, the density of vertebrae apparently varies greatly between

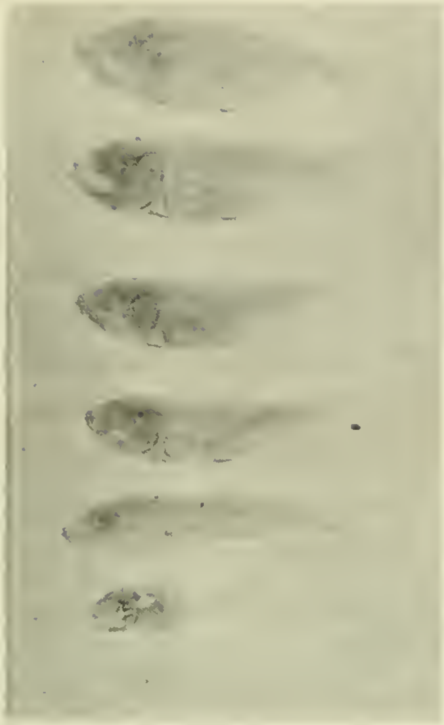

Figure 2.--X-ray photographs of larval fish using Kodalith film.

Figure 3.--Relation of exposure time to fish using Kodalith film at 65 kv.

species of young fish. We found, for example, in making test exposures of small mullet (Mugil cephalus), approximately 18 mm. standard length, and a small pompano (Trachinotus carolinus), 13 mm. standard length, there was sufficient contrast between vertebrae to permit accurate determinations in both species. The pompano showed exceptionally good contrast, indicating that acceptable radiographs of even smaller specimens could be made.

CASSETTES

A cassette is a paper or cardboard envelope which holds the X-ray film, protecting it from exposure to unfiltered light from the time it leaves the darkroom until returned for developing and fixing. Various features, such as radiation filters and intensifiers, may be incorporated into the construction of the cassette for special radiographic purposes, but are not essential for routine examination of fish vertebrae. Cardboard or paper of good quality can be used with very satisfactory results.

Commercial cardboard cassettes are recommended for X-raying specimens larger than approximately 55 mm. because of their sturdy, lightproof construction. In smaller specimens, however, the amount of radiation absorbed or scattered by fibrous cardboard becomes more critical. In such cases, a thin, lightproof paper is suggested. In either case, excessive fogging of the film by secondary or reflected radiation may be reduced by placing lead stripes between the cassette and the X-ray table.

DEVELOPING AND FIXING SOLUTIONS

The following chemicals are necessary for developing and fixing X-ray film:

1. X-ray developer (two-powder form)

2. X-ray fixer (single powder)

3. Acetic acid, 28 percent solution

The developer and fixer solutions are prepared according to directions given on the labels of containers.

Development time at specific temperatures are included for newly prepared solutions, however, prolonged exposure to air reduces the speed of the solutions so that a longer period of development is required after each use.

Submission of the film in the fixer solution for 10 minutes usually is sufficient to arrest all chemical action caused by the radiation and developer and harden the protective film emulsion. A longer fixing time is required as the solution becomes oxidized. After the fixing process has been completed, the film should be immersed in a flowing water bath for at least an hour to remove all traces of chemicals before being dried and examined.

The strength of the fixer can be prolonged by using a "short stop" bath of 28 percent acetic acid to rinse the film after it is taken out of the developer solution; the chemical strength of either solution may be kept longer by storing in lightproof, airtight glass or stainless steel containers while not in use.

X-RAY PROCEDURE

The procedure which we developed for X-raying menhaden followed the usual pattern of trial and error experimentation. Initially, a number of test exposures was made on a single array of specimens of various sizes, altering the kilovoltage, or exposure time, or both, until a satisfactory negative was produced. This approach led to considerable difficulty in interpretation of results as three variables were involved, kilovoltage, exposure time, and size of fish. It was noted, however, that consistent results were obtained by either increasing the kilovoltage and lowering the exposure time, or lowering the kilovoltage and increasing the time. This discovery let to the consideration of only two variables, viz., kilovoltage and fish length with time held constant. Interpretation of the variability of the data thus was greatly simplified, and radiography of acceptable to excellent quality became routinely possible.

The relation of kilovoltage to fish length is shown in figure 4. The minimal, optimal, and maximal curves were derived from exposures of several hundred menhaden specimens of various lengths to all kilovoltages which would produce a dark film background at 10 ma. for 10 seconds at a focal distance of 30 inches. Each film was then graded for contrast between vertebrae and surrounding tissue. All exposures lacking sufficient contrast for clear resolution of vertebral centri were considered unacceptable, the remainder was graded as acceptable or excellent. Examples of the exposures of varying contrast are shown in figure 5.

The relative simplicity of fish radiography is demonstrated in figure 4 by the large tolerence limits between the maximum and minimum kilovoltages required for specimens of particular size and emphasize the adaptability of the method for processing random samples. For example, a kilovoltage of 55 would suffice for X-raying a sample of fish ranging in size from approximately 55 mm. to 180 mm., a size difference of 125 mm. To determine the appropriate kilovoltage for a sample, it is necessary to consider only the smallest and largest specimens in the lot; and since the smallest fish are the most difficult to resolve, exposures suited to these should be selected. If size variation is great, it is suggested that the sample be segregated into lots of fish of similar size. Optimum exposures for fish of various sizes may be determined from the figure by inspection. All exposures were purposely made at 10 ma. for reasons of thermal dissipation, otherwise, a higher milliamperage setting would have been justified, especially where large fish were involved, as the time of exposure could be reduced. The X-ray tube can be operated continuously for several minutes at 10 ma. without an excessive build-up of heat beyond the thermal capacity of the tube.

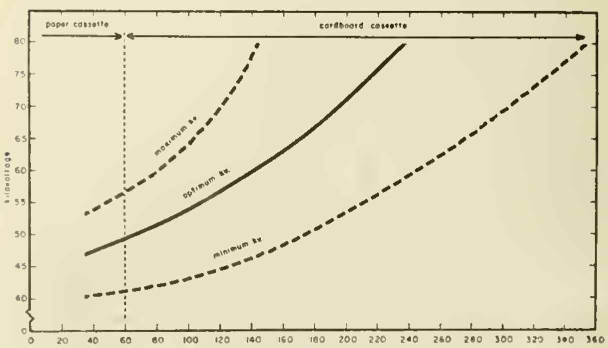

Figure 4.--Relation of kilovoltage to fish size for standard X-ray film.

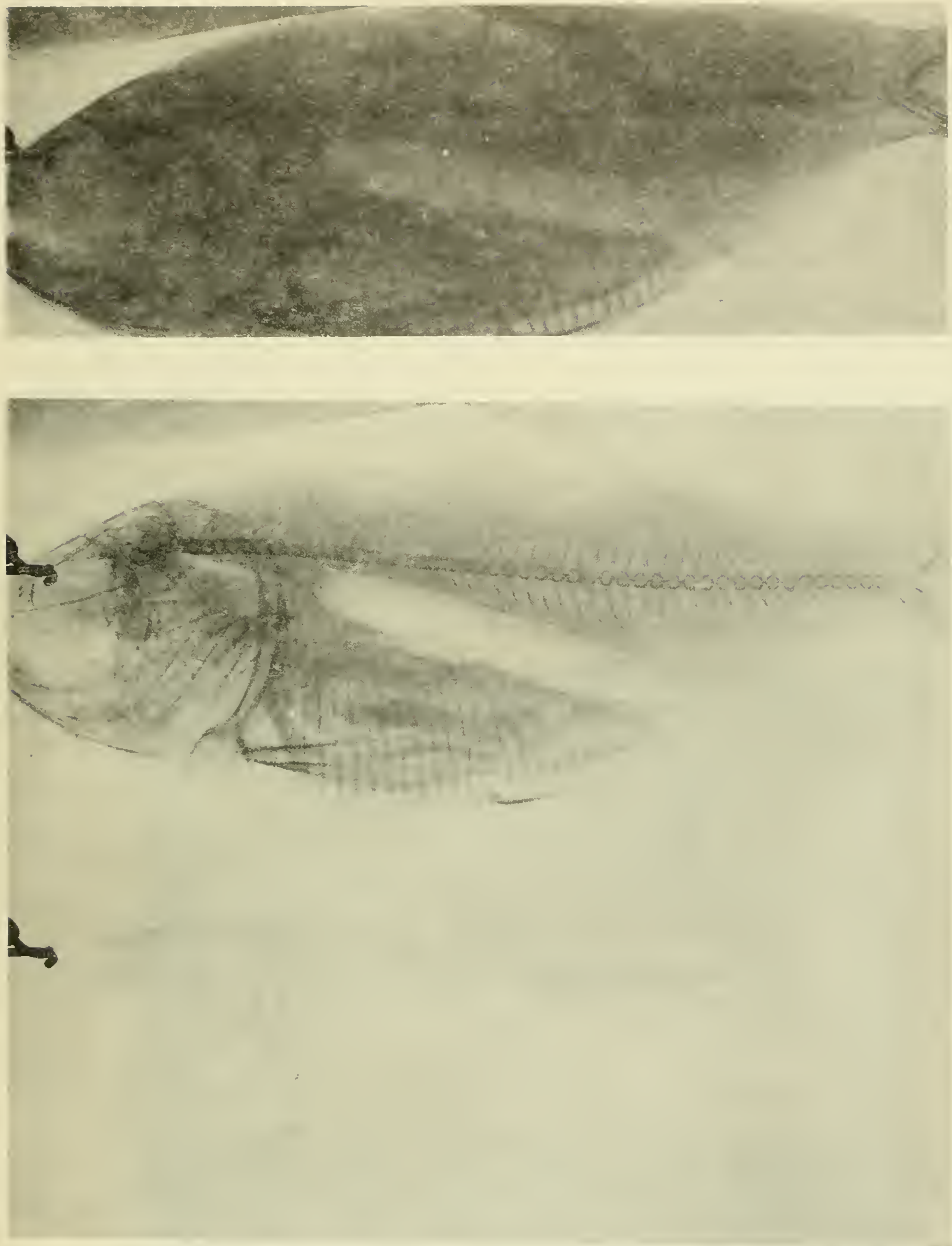

Figure 5.--X-ray photograph showing examples of under, optimum, and over-exposure.

The procedure for radiographing a sample of fish requires preliminary arranging of the developing and fixing solutions in open trays, or tanks, in a darkroom. The cassette, or X-ray film holder, is then loaded in the darkroom, exposing the film to yellow or red filtered light only. The cassette is placed in position on the X-ray machine so that it is centered beneath the X-ray tube and covered with wax paper to prevent any damage from moist specimens.

The specimens to be radiographed are arranged on the wax paper and identified serially by placing a lead number near the first and last fish. The film also may be identified by including lead numbers representing the collection number. After exposure, the cassette is returned to the darkroom where the film is removed and developed.

As a routine precaution, dental films are placed in the area immediately surrounding the operator to detect possible stray radiation during exposures. The films are developed and examined after 5 to 10 minutes of accumulated exposure. Results of all tests thus far have been negative.

Viewing the finished negative can best be accomplished by projecting diffused light through the film in the direction of the viewer. The difference in transparency of the film, resulting from differences in density of the various parts of the fish, become intensified by the diffused light, and, consequently, facilitate the process of counting vertebrae.

A suitable viewer for standard size X-ray films (8" X 10" and 14" X 17") was constructed of plywood and four 20-watt neon fluorescent tubes. This consists of rectangular box, 20" X 31" X $8\frac{1}{2}$", covered with a ground glass plate measuring 18" X 29". The fluorescent tubes, which are fastened to the inside bottom of the box, provide uniform illumination of suitable intensity (fig. 6). The film is placed directly on top of the glass plate for viewing. When counting vertebrae, it is advisable to cover the film with a clear glass plate to prevent unnecessary smudging and scratching of the film.

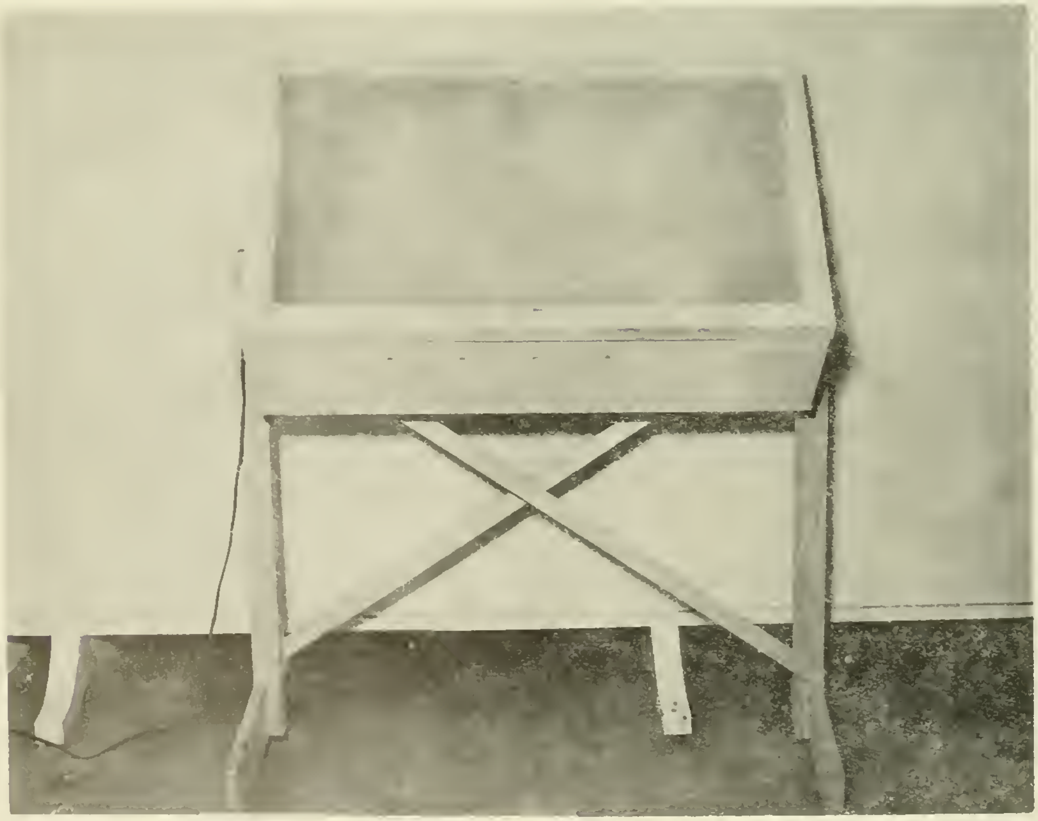

Figure 6.--Lightbox for viewing X-ray films of menhaden vertebrae.

When fine-grain photographic films are used, a binocular microscope is most suitable as the grain size of the film permits greater magnification of the vertebrae without loss of contrast.

COST ANALYSIS

The X-ray machine used by the Menhaden Investigations was assembled from used components at a total cost of approximately $550.00. Standard diagnostic machines with an equivalent kilovoltage range (25 to 100 kv.) from X-ray equipment manufacturers would cost somewhere between $1,000 and $1,500 new. This price would preclude cost of installation and necessary shielding.

In fish radiography, the expense of filming and developing is incidental, providing usual darkroom facilities are available. For example, the cost of radiographing menhaden specimens by this investigation is estimated at about $25.00 per 1,000 fish. This includes the purchase of X-ray film, film holders (cassettes), developing and fixing chemicals, and lead wire for identifying exposed films.

ACKNOWLEDGMENTS

The construction and technical development of the X-ray machine used by the Menhaden Investigations was accomplished by Mr. G. G. Barton, Electronics Engineer, Machine and Supply Company, Beaufort, North Carolina.

Thanks are due to various members of the staff of the Menhaden Investigations for the many valuable suggestions offered in the development of successful radiographic techniques.

Note: Those interested in adopting the X-ray method are urged to read the following references for further information on the use of low-voltage radiation for various research problems:

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SUMMARY

1. Radiographs provide an efficient means of determining vertebral numbers in fish.

2. Diagnostic X-ray machines having a kilovoltage capacity of approximately 25 to 100 kv. appear to be most suitable for fish radiography.

3. Standard X-ray film was found to be satisfactory for X-raying menhaden specimens larger than approximately 35 mm. fork length.

4. Fine-grain photographic film, such as Kodalith, used in combination with a paper cassette, was found to be satisfactory for resolving incompletely ossified vertebral columns of larval menhaden approximately 24 mm. in length. Also, successful resolution of vertebral columns of pompano, 13 mm. standard length, and mullet, 18 mm. standard length, was achieved with this type film.

5. The X-ray machine used by the Menhaden Investigations was assembled from used components at a cost of approximately $550.00.

6. The cost of routine X-raying of menhaden specimens for vertebral counts is estimated at about $25.00 per 1,000 fish.

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APPENDIX

PARTS LIST

Symbol	Item	Manufacturing Source
F_1	Fuse, 20 amp.	Common
M_1	Voltmeter, 0-150 v.a.c.	Common
M_2	Milliammeter, 0-15 ma.	Common
R_1	Rheostat, 300 o., 100 w.	Ohmite, General Electric, Westinghouse
R_2	Resistor, 12 o., 25 w.	Common
Re_1	Contactor, heavy duty, 2 pole	Allen Bradley, Potter Brumfield
S_1	Line switch, single pole, 15 amp.	Common
S_2	Push button, normal, open	Common
T_1	Auto-transformer, 110 v., 15 amp.	Variac
T_2	X-ray high voltage transformer with integral filament transformer, 110 v., 85 kvp., 30 ma.	General Electric, Westinghouse, Picker
V_1	X-ray tube, 85 kvp., 30 ma.	General Electric, Westinghouse, Eureka

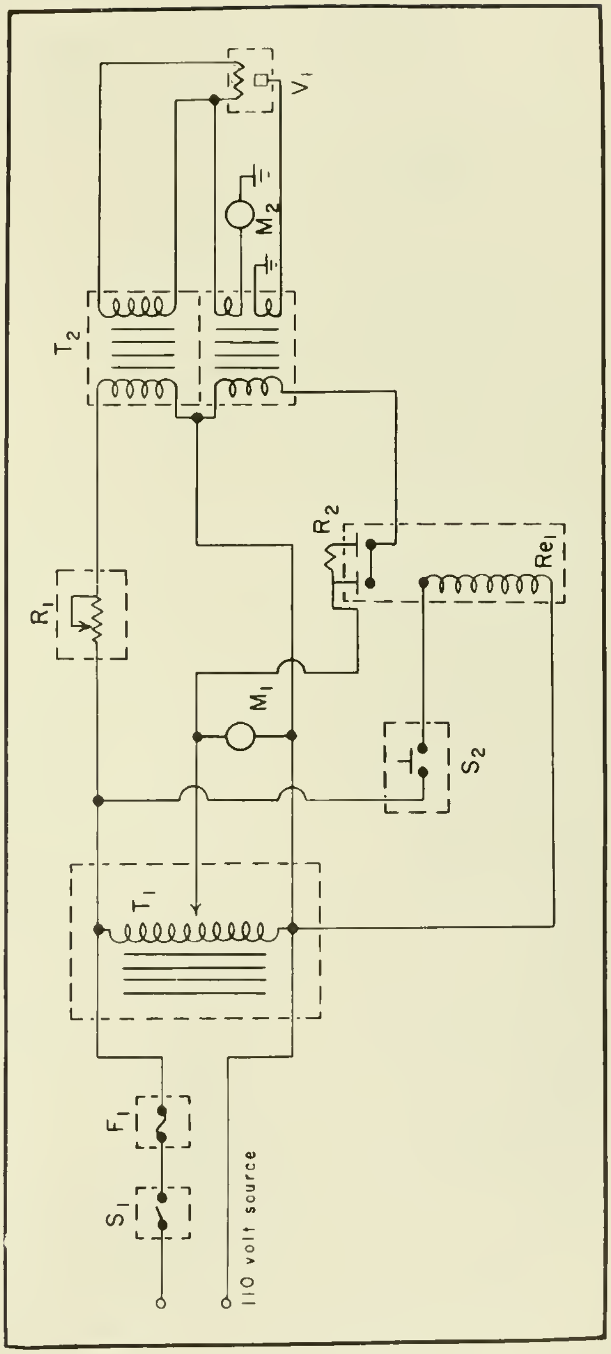

Figure 7.--Schematic diagram of X-ray emitter circuit.

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