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Morphology and morphometric vatiations of larval Anisakis sp. (Nematoda) from Atlantic salmon (Salmo salar) and Atlantic herring (Clupea harengus)

by

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INTRODUCTION

Variations in the incidence and intensity of infection of marine fishes with larvae of the nematode Anisakis sp. have been observed by several authors (Bishop and Margolis, 1955; Templeman, Squires and Fleming, 1957; Khalil, 1969; Parsons and Hodder, 1971; Young, 1972, and Davey, 1972). These variations may be used to obtain information on the homogeneity or heterogeneity of fish stocks from different areas or among different age classes of fish from the same areas. In the past, no attempt has been made to define the degree of homogeneity among the Anisakis larvae themselves. Thus, in 1969 a detailed study of the morphology and morphometric variations among Anisakis larvae in salmon was initiated. Preliminary studies on these larvae indicated significant variations existed and in 1970 the work was extended to include larvae from Atlantic herring. This report presents a summary of the results obtained to date.

MATERIALS AND METHODS

All specimens came from the body cavity or the walls of visceral organs of the host.

Larvae to be used in general anatomical studies were removed from fresh hosts, heat relaxed and studied immediately, or were fixed in hot glycerine alcohol and cleared in pure glycerine before examination. Larvae to be used for histological studies were fixed in Alcoholic Bouin's solution for 48 hours and then passed through four 24 hour changes of 70% ethanol before processing. Specimens were cut in half, dehydrated in ethanol, cleared in chloroform, and mounted in "Paraplast" (Fisher Scientific Cat. No. 12-646-105). Treatment with "Molliflex" (BDH) for 24 hours before sectioning

each 1.5 mm portion was used to soften the cuticle. Sections were cut 10 μ thick and stained with Ehrlich's hematoxylon and eosin. Specimens to be used for general morphometric studies came from previously frozen hosts. After thawing they were placed in a drop of tap water on a microscope slide and photographed at magnifications suitable for future measurements (low magnification for whole worms and higher magnifications for head and tail portions). Strips of labeled negatives were later projected onto a screen and measurement made from the projected images.

When only the total lengths of the larvae were to be recorded, freshly thawed larvae were placed on a glass slide and a mid-line drawing made with the aid of a camera lucida attached to a dissecting microscope. Total lengths of the larvae were obtained from these drawings.

Autofluorescence was studied with a fluorescence microscope with an Osram HBO-200 high pressure mercury lamp. Ultraviolet light was transmitted to specimens through Schott exciter filters BG3 and BG38. Specimens were viewed and photographed with bright field illumination through Carl Zeiss barrier filters 44 and 50.

RESULTS

Description of Anisakis sp. larvae

General morphology

Specimens were from 12.5 to 35.1 mm long and from 0.32 to 0.71 mm in diameter at the middle of the body. The anterior quarter of the body narrowed gradually while the posterior end tapered abruptly near the anus (Fig. 1A). The tail was from 0.100 to 0.319 mm long, was usually rounded but sometimes conical, and ended with a retractable tail spine about 0.029 mm long (Fig. 1B).

The cuticle was from 0.0096 to 0.0154 mm thick. Striations were present near the anterior and posterior ends of most specimens (Fig. 1B and 1C) and were present along the entire body of some specimens.

The mouth was triangular, the dorsal lip trilobed, and the ventrolateral lips bilobed. A single indistinct papilla occupied each of the ventrolateral lips; these papillae could not be observed on many specimens.

The anteroventrally projecting boring tooth was located ventral to the mouth and anterior to the groove between the ventrolateral lips (Fig. 1D). It was controlled by three pairs of muscles (Fig. 1C); one pair had its origins near the antero-ventral extremity of the esophagus and their insertions on each side of the dorsal surface of the tooth; the second pair had its origins in the body wall dorsal to the mouth and the insertions near the tip of the tooth; and the third pair had its origins on the inner edges of the ventrolateral lips and its insertions on the ventral edge of the tooth.

The esophagus, 1.24 to 5.59 mm in length was slightly narrower at its anterior end than at its posterior end. Very fine transverse striations were visible at the extreme anterior end of the esophagus and the area immediately behind this was granular in appearance (Fig. 1C). The brush border lining the esophagus was visible in most specimens (Fig. 1C). The ratio of the length of the esophagus to body length varied from 1:12.82 to 1:3.29 (from Table 1) and its length was related to total body length (Table 2, Treatment 10). The dorsal esophageal gland extended to just anterior to the nerve ring and its posterior end was near the posterior extremity of the esophagus. The two ventrolateral glands were small and located near the posterior end of the esophagus (Fig. 1A).

The ventriculus was from 0.46 to 1.88 mm long and from 0.078 to 0.360 mm in diameter at its midpoint. Changes in the length and diameter of the ventriculus of live specimens (Table 3) appeared to be related to the dorsoventral flexures of the body. Its juncture with the esophagus was perpendicular to its long axis whereas it usually joined the intestine at an angle of about 69° (Range: 48°-89°). The ventral side of the ventriculus was slightly longer than the dorsal side. The wall of the ventriculus did not always have a uniform thickness (Fig. 1A); the lumen was generally irregular in cross-section. The intestine opened into a short intestino-rectal valve lined internally by a thick layer of cuticular material and externally by four epithelial cells. There were three rectal glands: two dorsal and one ventral (Fig. 1B).

The nerve ring was 0.120 to 0.499 mm from the anterior extremity. Lateral cervical papillae (Fig. 1A) were located about 0.14 mm behind the nerve ring.

The excretory pore was situated ventrally between the two ventrolateral lips. The single excretory duct extended from the excretory pore to the excretory cell (Fig. 1A). The main body of the excretory cell extended posteriad from just anterior to the ventriculus to 6.76 to 8.40 mm from the anterior end of the body. Except at the extremities, where its cross-section was rounded, the excretory cell was elongate in cross-section and lay with its concave proximal side against the alimentary canal and its convex distal side against the somatic musculature. A single duct passed through the anterior half of the cell (Fig. 1A and 1E), bifurcated, and continued to the posterior extremity of the cell as two smaller ducts (Fig. 1A and 1E). Numerous fine tubules extended from the main ducts throughout the length of the cell. These branched many times and permeated most areas of the cell (Fig. 1E). A single large excretory sinus filled with an acidophilic substance was located in the excretory cell from the vicinity of the posterior end of the ventriculus to the vicinity of the bifurcation of the excretory duct (Fig. 1E). The anterior end of this reservoir had four or five openings into the pseudocoel; the two most anterior openings were the largest. The nucleus was near the posterior end of the reservoir and the cell tapered rapidly behind this.

The dorsal and ventral hypodermal cells were much larger than the inconspicuous central cells. They were paired so that the transverse cell boundaries of the ventral cells were close to those of the dorsal cells and the number of ventral cells present was the same as the number of dorsal cells (Fig. 1A). Each side of each of two larvae examined had twelve pairs of large hypodermal cells posterior to the ventriculus (accurate counts were not possible anterior to the intestino-ventricular junction). These cells ranged in length from 0.065 to 3.68 mm and had a mean length of 1.10 mm (Table 3).

The genital primordrium was not seen in any whole mounts but was visible in sectioned material. It could be distinguished as a group of cells lying in the pseudocoel about 1 to 2 mm posterior to the ventriculus and surrounded by the alimentary canal, hypodermal cells, the somatic musculature and the excretory cell.

Almost all tissues, except the cuticle, fluoresced bluish-white when illuminated with ultraviolet light. Bright fluorescence was observed only in previously frozen or fixed (in formalin or ethanol) specimens. Live specimens emitted a very pale fluorescence. The fluorescence of the striated and granular portion of anterior extremity of the esophagus (Fig. 1C) varied from pale bluish-white to bright bluish-white or bright orange.

Morphometric variations

The mean coefficient of variation of the various measurements made on the larvae was 17.27%. The coefficients varied from 11.41% for the diameter of the larvae to 25.36% for the width of the ventriculus (Table 4). The highest variation noted for combined dimensions was 38.46% for the product of the length and width of the ventriculus. The coefficient of variation for the width of the ventriculus (25.36%) was greater than that of the length of the ventriculus (18.63%) among over 200 specimens from herring and salmon. A similar difference was not apparent among individual living larvae measured at different times (Table 5). A comparison of the coefficients of variation for the length and width of the ventriculus in Table 3 with those in Table 5 suggests that there is considerably less variation in the size of the ventriculus within the same larvae than among different larvae.

Differences in the dimensions of different parts of the body with increasing length of the larvae were studied. Regression lines of various dimensions on total lengths of the larvae were calculated (Table 2) and the correlation and regression coefficients of all variables were found to be low but statistically significant (Table 2, Treatments 3, 6, 9, 12, 15 and 18). Thus, longer Anisakis larvae tended to have a greater diameter, greater distance between the anterior end and the nerve ring, larger ventriculus, and a longer esophagus and tail. Since the sizes of the various parts of the larvae were related to some extent to the length of the larvae, the length of the larvae was chosen to study variations with host sex and age. However, because preliminary analyses of the data suggested a somewhat more complex relationship in morphometry between larvae from different host species, variations with host species were dealt with in some detail. Special

attention was given to the relationship of the morphometry of the larvae to the species of host in which they were found.

Variations with host sex

The mean lengths of larvae from male and female 2-sea-winter salmon were similar for 378 larvae from Canada ($.1 < P < .2$) and 231 from the United Kingdom ($.7 < P < .8$) (Table 6). This justified combination of data on larvae from male and female salmon. It was assumed a similar pattern existed for larvae from herring.

Variations with host species

The length of the larvae from herring (23.5 mm) was significantly greater ($P < .01$) than the length of those from salmon (22.3) (Table 1). Similarly the body diameter, length, and width of the ventriculus were also greater in larvae from herring. The distance of the nerve ring from the anterior end, the length of the esophagus and the length of the tail were similar in larvae from both host species.

Coefficients of variation for the distance from the anterior end to the nerve ring, length of the esophagus, ventriculus and tail, and the width of the tail were all greater among larvae from salmon than among those from herring. However, the variation in the length and diameter of larvae from salmon was less than that in those from herring (Table 4). Larvae from salmon appeared to be of a more uniform size but have less uniform body proportions than did larvae from herring.

The combined length of the esophagus and ventriculus was greater in larvae from herring than in those from salmon (Table 1). If one assumes the ventriculus to be roughly cylindrical, the larger product of the length and width of the ventriculus in larvae from herring indicated the overall volume of the ventriculus was slightly greater in larvae from herring. The ratio of the product of the length and width of the ventriculus to the total length of the larvae was also greater among specimens from herring. Similarly, the ratio of the length of the ventriculus to the length of the esophagus was different for the two groups of larvae. However, the ratio of the sum of the length of the esophagus and the length of the ventriculus to the total body length was the same in larvae from both hosts.

Regression lines of the various dimensions on total lengths were calculated for larvae from both herring and salmon (Table 2). The correlation coefficients of the regressions of the diameter, anterior end to nerve ring, length of the esophagus, length of the ventriculus, width of the ventriculus and length of the tail on the total body length of larvae from herring (.49, .22, .55, .57, .30, and .34 respectively) were all low. However, they were significantly different than zero (Table 2, Treatments 3, 6, 9, 12, 15, and 17). Similarly the regression coefficients for these treatments were significantly different from zero ($P < .05$). In contrast, only half the correlation coefficients and regression coefficients of similar treatments for larvae from salmon (Treatments 2, 5, 8, 11, 14, and 17) were significantly different from zero (diameter ($r = .21$), length of the esophagus ($r = .28$) and length of the ventriculus ($r = .41$)); there was no apparent relationship between the

distance of the nerve ring from the anterior end, the width of the ventriculus, and the length of the tail with the total length of the body of larvae from salmon (Treatments 5, 14 and 17).

Combined dimensions were compared with the total length of the larvae (Table 2, Treatments 19-30). The product of the length and width of the ventriculus increased with the total length of larvae from herring (Treatment 21) but did not increase among larvae from salmon (Treatment 20). Also, there was a tendency for the sum of the length of the esophagus and the ventriculus to increase with the total length of larvae from both herring ($r = .60$) and salmon ($r = .35$) (Treatments 23-24).

There was a positive correlation between the length and width of the ventriculus among larvae from herring (Treatment 27) but no correlation among those from salmon (Treatment 26). A positive correlation was noted in the regression of the length of the esophagus and the length of the ventriculus in both herring ($r = .70$, $P < .01$) and salmon ($r = .52$, $P < .01$).

Tests for homogeneity of variance (Snedecor, 1956) for the various dimensions and body proportions of larvae from salmon and herring (Table 1) indicated the samples were drawn from populations with common variances.

Analyses of covariance (Snedecor, 1956) were performed to examine differences between regression lines (with significant correlation coefficients) for larvae from herring and salmon (Table 2). In these analyses the F-value derived from the ratio of mean square for regression coefficients to mean square within samples gave a measure of the variation in regression coefficients, i.e. whether or not the regressions were parallel. The F-value derived from the ratio of mean square for adjusted mean to mean square for common regression measured the variation in intercepts, or vertical separation of the regression lines.

Both the slopes and the elevations of the regression of the length of the ventriculus on the length of the esophagus were different among larvae from herring and salmon (Table 2, Treatment e). The slopes of the regressions of the diameter on the total lengths of the larvae from salmon and herring were similar but the elevations were different (Treatment a and Fig. 2A). Also, slopes of the two regressions of the length of the ventriculus on the total length were similar but the elevations different (Treatment c and Fig. 2B). The only measurements made which did not indicate any difference were those of the length of the esophagus and the length of the esophagus plus the length of the ventriculus (Treatments b and d). Thus, the only structure measured, the size of which did not appear to be related to the host species, was the esophagus. Since the larvae from herring were larger than those from salmon, one might have expected a significant difference between the length of the esophagus of larvae from the two hosts. The lack of such a difference is likely related to the low correlation coefficients involved ($r = .55$ and $.28$ respectively). Similarly, low or insignificant correlation coefficients may also be related to the lack of significant differences between many of the means given in Table 1.

Apparently, the tendency of the diameter, the distance from the anterior end to the nerve ring, the length and width of the ventriculus and

the length of the tail to increase with increasing total length of the larvae is greater among larvae found in herring than among those from salmon (compare r for Treatments: 2 and 3, 5 and 6, 8 and 9, 11 and 12, 14 and 15, and 17 and 18 in Table 2).

Geographic variations

Variations were observed in the dimensions of larvae from the same host species from widely separated geographic localities. The length frequency distribution of larvae from herring from Canadian waters was different from that of larvae from European herring (Fig. 3). The mean lengths of both groups were also significantly different ($P < .01$). As might be expected from the foregoing results, frequency distributions of the length of the esophagus, ventriculus, and tail were different among larvae from the two areas. However, there were no obvious differences in the distributions of the width of the ventriculus and the distance of the nerve ring from the anterior end (Fig. 3).

Among larvae from Atlantic salmon of the same sea age (2-sea-winters), those of European origin had a significantly higher ($P < .01$) mean length (mean of 231 = 22.29 ± 2.47) than those of North American origin (mean of 378 = 21.25 ± 2.27; other dimensions were not compared for these larvae; Table 6).

Variations were also observed among larvae from herring from relatively close localities. Analysis of variance on the lengths of 3839 larvae from herring from six Canadian areas indicated there were significant differences among the samples ($P < .01$ for $F = 176.96$ with 5 and 3833 degrees of freedom). Duncan's multiple range test (Kramer, 1956) on the means indicated that the samples from Banquereau and Hawke's Bay areas were drawn from populations with similar means and that all other samples had means different from these and different from each other. The differences in the size distributions of the samples from St. Paul's and Canso Bank areas are dramatic but differences among the other distributions were less so (Fig. 4). These differences were also apparent when the mean lengths of the larvae were compared with hosts' ages (Fig. 5).

Variations with host age

The ages of herring from which our samples were taken varied from 3 to greater than 10 years. Significant differences in the mean lengths of larvae from different age classes of herring were found among larvae from herring in the St. Paul's area. However there was no consistent increase or decrease in these differences; this indicated that the differences were related to some factor other than age of the host. Also, no trends in the mean length of larvae with the age of the hosts were noted among larvae from the five areas (Fig. 5). Similar observations were made among larvae from salmon. The mean length of larvae from 2-sea-winter salmon (20.74 mm) was significantly higher ($P < .01$) than that of larvae from 1-sea-winter salmon (21.13 mm). Also, the mean length of those from 3-sea-winter salmon (21.80 mm) was higher ($P < .01$) than the mean length of larvae from 2-sea-winter salmon. This apparent trend of a higher mean length of larvae with greater host age of salmon was apparent when all samples were combined but was not obvious in all the individual samples (Table 7).

DISCUSSION

Berland (1961) reported two morphologically distinct forms of Anisakis larvae: Anisakis sp. larva (I) and Anisakis sp. larva (II). Except for a description of part of the excretory system, Berland did not describe larva (I) but rather referred to descriptions by Grainger (1959) and Punt (1941). The description and drawing of the cephalic structures of the Anisakis sp. larvae by Grainger do not conform with the drawings of Berland. Grainger describes and illustrates the edge of the lip mass as being "covered with large numbers of minute teeth or hairs". These are not shown in Berland's illustrations. Grainger described "a few scattered rather flat papillae" on the lip mass but suggested they may be artifacts resulting from fixation. Berland does not show similar papillae in his figures and it is not clear in the text if he observed them in his specimens. Grainger's figure 4 shows a prominent boring tooth which appears to project dorsally rather than ventrally as is indicated in the text and in his figure 5. Berland's figures of Anisakis larva (I) conform with the illustration of the anterior extremity and the general description of Anisakis larvae given by Punt. However, Punt's description of the tail, "Se termine par un bouton terminal typique", implies that the tail spine was papilliform rather than pointed. Punt did not illustrate the posterior extremity. Presumably the tail spines of Punt's specimens were all partially retracted giving the impression of papilliform processes. With this general exception, which is of doubtful significance, and Punt's single specimen with a forwardly directing intestinal caecum, which undoubtedly was not Anisakis, Berland's illustrations of Anisakis larva (I) conform with Punt's description of Anisakis sp. Berland's larva (I) may therefore be described as one which fits the general description of Anisakis sp. as given by Punt, has a pointed tail spine, and does not have an intestinal caecum. Apparently, this was Berland's intentions when he referred to Punt's description. With the above reservations, the specimens examined in this study have been identified as Anisakis sp. larva (I) of Berland.

Berland's larva (II) was rare, had a shorter ventriculus, the junction between the intestine and the ventriculus was not oblique and the tail, which did not have a tail spine, was conical instead of rounded. All dimensions, including that of the ventriculus, given by Berland for larva (II) were within the ranges given here for larva (I). The angle between the long axis and the posterior end of the ventriculus of 13 of 100 larva (I) specimens examined here was between 85 and 90°. All of these 13 larvae possessed tail spines, (seven extended, six retracted) and one had a conical tail typical of Anisakis larva (II). Thus, the present description of larva (I) includes all characteristics, except the absence of a tail spine, previously described as typical of larva (II). The presence of a tail spine on all specimens examined here precludes their identification with Berland's larva (II).

Punt (1941) gives the dimensions of 55 Anisakis larvae from 12 teleost hosts (Punt's table XIX actually presents data on 56 specimens but

the specimen from Osmerus eperlanus cannot be considered a valid inclusion because it possessed an intestinal caecum: "chez des exemplaires dont la longeur dépasse 28 mm., l'intestin peut être prolonge en forme de caecum, longeant le ventricule et à peu près de même longeur"). The ranges for Punt's dimensions are within those cited for the present specimens indicating morphometric similarity between our specimens and Punt's. However, when Student's t tests were performed between the means of the various dimensions given by Punt and those presented here, significant differences ($P < .01$) were found. In view of the heterogeneity found among samples examined in the present study, the difference between our specimens and Punt's cannot be regarded as evidence that we are dealing with a different species of Anisakis.

Banning (1971) successfully reared larvae from herring, mackerel (Scomber scombrus) and Norway haddock (Sebastes marinus). However, he did not study the morphology of the adults he cultured and simply referred to them as Anisakis marina. This identification was based on the proposal by Thiel (1966) that all adult species of Anisakis in sea mammals in the North Sea and South Atlantic belong to the same species, Anisakis marina, and that the specific name for the larvae in herring should therefore be A. marina. However, Khalil (1969) rejected Thiel's proposal because he did not provide any evidence in support of his view. Davey (1971) recognized three valid species of Anisakis and rejected Thiel's "marina" because it depended on acceptance of Gordius marinus of Linnaeus 1767, the description of which Baylis (1944) stated was inadequate. Banning's (1971) study cannot be considered as having solved the problem of the identity of the larvae. Positive identification will only be possible after a detailed study of adults reared in the laboratory.

Of particular interest among our specimens is the amount of variation observed between samples of larvae from different host species, the same host species in both distant and nearby areas, and between larvae from different age classes of hosts in the same areas. Despite the significant differences between samples, these larvae undoubtedly belong to the same species. Indeed, Nyman and Pippy (1970) studied the population genetics of these larvae and concluded that those from herring practically all belong to the same breeding population. Larvae from salmon had slightly different gene frequencies but these were again in genetic equilibrium. During our investigation it was hypothesized that the length of the larvae might be related to oceanic temperatures. A search through the literature and an examination of surface temperature data collected since the early 1900's (from the Canadian Oceanographic Data Centre) did not provide suitable data for a comparison of the mean lengths of the larvae with oceanic temperatures. The authors feel such a comparison would be worthwhile should the necessary oceanographic data become available.

With respect to the possibility that Anisakis larvae grow within the fish host, two apparently conflicting finds have been presented. There did not appear to be any increase in the mean length of larvae throughout the life span of the herring regardless of the mean length of larvae in the younger herring in the samples (Fig. 4). In contrast, there were significant differences in the regression lines describing the relationships of various body dimensions to the lengths of the larvae from salmon and herring hosts

(Table 2). These differences in regression lines suggest that the larvae grow while in the fish host, either before or during the infective stage. If this is so, differences in mean sizes of the larvae resulting from differences in the times spent in the fish hosts by the larvae must be insignificant when compared with differences resulting from other factors (for example, temperature, as mentioned in the previous paragraph). Another possible explanation for the differences in regression lines for larvae from the two hosts is that the differences are related to utilization of the larvae by different invertebrate hosts. Larval Anisakis have been found in amphipods (Caprella septentrionalis), decapods (Hyas araneus), and euphausids (Thysanoessa raschii, T. longipes, T. inermis and T. longicaudata) (Smith, 1971). If the growth of the larvae and the relative growth of the various organs is dependent to some extent on the invertebrate species utilized, and if salmon and herring feed mainly on different species of these invertebrates, the apparent effects on growth characteristics might be similar to those presented in Table 2. If this latter suggestion is true there is no conflict between the results presented in Table 2 and those presented in Fig. 4.

The absence of precise knowledge of the factor or factors which contribute to the variations in the morphometry of Anisakis larvae complicates using these variations as aids to separating fish stocks. However, insofar as the variations are detectable, it seems likely that they may be of some value for this purpose. Their prime importance may be as additional parameters in a description of host stocks (in addition to factors such as size and age composition or abundance of parasites).

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Table 1. Summary of dimensions (in mm) of Anisakis sp. from Atlantic salmon and Atlantic herring: N is the number of larvae in each sample; R is the minimum and maximum values observed; $\bar{x}$ is the mean; and SD is the standard deviation. A raised d indicates a significant difference ($P < .01$) between mean values for larvae from salmon and for those from herring.

	From Atlantic salmon			From Atlantic herring			From salmon and herring		
	N	R	$\bar{x}$±1SD	N	R	$\bar{x}$±1SD	N	R	$\bar{x}$±1SD
[d]Total length	101	(16.5-35.1)	22.3±2.8	152	(22.5-31.1)	23.5±3.7	253	(12.5-35.1)	23.0±3.5
[d]Diameter*	89	(.323-.690)	.496±.060	101	(.421-.714)	.544±.066	190	(.323-.714)	.522±.063
Anterior end to nerve ring	95	(.240-.499)	.339±.046	131	(.120-.419)	.346±.038	226	(.120-.499)	.343±.042
Length of esophagus	99	(1.240-5.590)	2.823±.496	116	(1.805-4.790)	3.897±.410	215	(1.240-5.590)	2.867±.447
[d]Length of ventriculus	97	(.460-1.880)	.803±.155	113	(.582-1.250)	.887±.023	210	(.460-1.880)	.848±.158
[d]Width of ventriculus**	97	(.078-.338)	.179±.039	118	(.089-.360)	.234±.050	215	(.078-.360)	.209±.053
Length of tail***	100	(.103-.319)	.160±.036	120	(.100-.255)	.169±.033	220	(.100-.319)	.163±.035
Length esophagus / Total length	89	(.086-.304)	.128±.025	101	(.077-.171)	.124±.018	190	(.078-.304)	.126±.022
Length ventriculus / Total length	89	(.024-.075)	.036±.006	101	(.023-.056)	.038±.006	190	(.023-.075)	.037±.006
[d]Esophag. + Vent.	89	(2.258-6.460)	3.632±.575	101	(2.477-5.930)	3.797±.521	190	(2.258-6.460)	3.720±.551
Esophag. + Vent. / Total length	89	(.115-.341)	.165±.028	101	(.108-.226)	.162±.022	190	(.108-.341)	.163±.025
[d]Length x Width Vent.	89	(.057-.376)	.144±.046	101	(.090-.441)	.215±.072	190	(.057-.441)	.182±.070
[d]Length x Width Vent. / Total length	89	(.003-.015)	.007±.002	101	(.004-.016)	.009±.002	190	(.002-.016)	.008±.003
[d]Length Ventriculus / Length esophagus	89	(.121-.410)	.286±.039	101	(.214-.417)	.307±.038	190	(.121-.417)	.297±.039

*Diameter determined at mid-point of total body length.
**Width of ventriculus determined at its mid-point.
***Length of tail determined from a lateral viewpoint; measurements were made from a curved line following the cuticle from the tip of the tail (excluding the mucron) to the anterior lip of the anus.

Table 2. Regression lines and results of tests of significance on various dimensions of larval Anisakis sp. from Atlantic salmon and herring. Fe = ratic of mean square for adjusted means to mean square for the common regression (vertical separation); Fm = ratio of mean square for regression coefficients to mean square within samples (slope); H = herring; r = regression coefficient; S = salmon; t = t value for test of significance of slope > 0; * indicates 0.05 > P > 0.01 and ** P < 0.01.

Treatment #	Total length vs	Host	Number larvae	Regression line	r	t	Analysis of covariance: Treatment #	Fm DF (slope)	Fe DF (vert. sep.)	Regression line comparisons (S vs H)
1		S + H	190	y = .3624 + .0057x	.42**	6.42**				
2	Diameter	S	89	y = .4028 + .0042x	.21*	1.96*	a	$2.41_{1,186}$	$18.11^{**}_{1,187}$	different
3		H	101	y = .3482 + .0082x	.49**	5.57**				
4		S + H	190	y = .3085 + .0015x	.14*	1.97*				
5	Ant.-nerve ring	S	89	y = .3249 + .0007x	.04	0.44				different
6		H	101	y = .3040 + .0018x	.22*	2.29*				
7		S + H	190	y = 1.6392 + .0533x	.43**	6.60**				
8	Length esophagus	S	89	y = 1.8061 + .0458x	.28**	2.76**	b	$.41_{1,186}$	$.01_{1,187}$	same
9		H	101	y = 1.5457 + .0571x	.55**	6.67**				
10		S + H	190	y = .3040 + .0236x	.52**	8.42**				
11	Length ventriculus	S	89	y = .3167 + .0218x	.41**	4.14**	c	$.002_{1,186}$	$7.07^{**}_{1,187}$	different
12		H	101	y = .3644 + .0221x	.57**	6.84**				
13		S + H	190	y = .1293 + .0035x	.23**	3.26**				
14	Width ventriculus	S	89	y = .2266 - .0021x	-.15	1.45				different
15		H	101	y = .1469 + .0038x	.30**	3.11**				
16		S + H	190	y = .1002 + .0027x	.29**	4.13**				
17	Length tail	S	89	y = .1181 + .0017x	.16	1.50				different
18		H	101	y = .0969 + .0029x	.34**	3.58**				
19		S + H	190	y = -.0120 + .0084x	.43**	6.44**				
20	Length x width ventriculus	S	89	y = .0888 + .0025x	.16	1.52				different
21		H	101	y = .0104 + .0086x	.46**	5.26**				
22	Length esophagus	S + H	190	y = 1.9432 + .0769x	.50**	7.86**				
23	+	S	89	y = 2.1227 + .0677x	.35**	3.44**	d	$.29_{1,186}$	$.48_{1,187}$	same
24	Length ventriculus	H	101	y = 1.9100 + .0792x	.60**	7.38**				
25	Width ventriculus	S + H	190	y = .1001 + .1298x	.39**	5.73**				
26	vs	S	89	y = .1699 + .0120x	.05	0.44				different
27	Length ventriculus	H	101	y = .0968 + .1581x	.48**	5.48**				
28	Length ventriculus	S + H	190	y = .2060 + .2242x	.61**	10.58**				
29	vs	S	89	y = .3052 + .1759x	.52**	5.82**	e	$5.05^{*}_{1,186}$	$15.72^{**}_{1,187}$	different
30	Length esophagus	H	101	y = .1123 + .2676x	.70**	9.86**				

Table 3. **Dimensions of the 12 most posterior** ventral hypodermal cells from one side only of two Anisakis sp. larvae.

Cell # (Posterior Anterior to)	Length of cell (mm)	
	Larva #1	Larva #2
1	2.13	3.68
2	1.25	0.147
3	1.73	1.70
4	1.22	1.62
5	0.065	0.662
6	1.09	1.54
7	0.765	1.005
8	0.600	0.515
9	1.17	1.03
10	0.570	1.23
11	0.600	0.490
12	0.870	0.784
Mean	1.01	1.20

Table 4. Coefficients of variation (expressed as percents) of the dimensions of Anisakis sp. larvae from Atlantic salmon and Atlantic herring. Calculated from data in Table 1.

	Larvae from salmon	Larvae from herring	Both
Total length	13.56	15.75	15.21
Diameter	12.10	12.13	11.41
Anterior end to nerve ring	13.57	10.98	12.25
Length of esophagus	17.16	14.15	16.64
Length of ventriculus	19.68	17.19	18.63
Width of ventriculus	21.79	21.37	25.36
Length of tail	22.50	20.00	21.47
Length esophagus / Total length	19.53	14.52	17.46
Length ventriculus / Total length	16.67	15.79	16.21
Esophagus + ventriculus	15.83	13.72	14.81
Esophagus + ventriculus / Total length	16.97	13.58	15.34
Length x width ventriculus	31.94	33.49	38.46
Length x width ventriculus / Total length	28.57	22.22	37.50
Length ventriculus / Length esophagus	13.64	12.38	13.13

Table 5. Changes in length (L) and width (W) of ventriculus of Anisakis sp. larvae measured at 30 second intervals. All measurements in millimetres.

Time (seconds)	Larva #1		Larva #2		Larva #3		Larva #4		Larva #5	
	L	W	L	W	L	W	L	W	L	W
0	.74	.180	.75	.220	.74	.218	1.02	.221	.85	.212
30	.71	.159	.78	.221	.74	.240	.96	.218	.88	.280
60	.71	.168	.77	.221	.82	.218	1.00	.250	.90	.284
90	.67	.168	.77	.221	.80	.225	.92	.220	.90	.280
120	.62	.170	.71	.220	.82	.220	1.00	.220	.89	.278
150	.65	.162	.82	.230	.84	.221	.95	.216	.84	.272
180	.66	.166	.77	.238	.82	.213	1.02	.218	.92	.268
210	.66	.178	.85	.218	.82	.216	.99	.230	.88	.273
240	.65	.165	.77	.223	.83	.210	.94	.273	.89	.220
270	.65	.173	.83	.224	.80	.220	.97	.224	.87	.275
300	.63	.200	.82	.230	.84	.220	1.00	.220	.89	.257
330	.70	.195	.82	.240	.81	.213	.95	.222	.92	.257
360	.73	.184	.81	.231	.85	.211	.98	.223	.95	.260
390	.69	.190	.73	.231	.85	.212	1.09	.240	.94	.260
420	.70	.200	.85	.240	.84	.218	1.00	.230	.92	.263
Mean	.678	.177	.790	.227	.815	.218	.986	.228	.896	.262
Std. dev.	.036	.014	.042	.008	.034	.007	.041	.004	.031	.021
Coef. var.	5.31	7.91	5.32	3.52	4.17	3.21	4.16	1.75	3.46	8.02
Std. error	.009	.004	.011	.002	.009	.002	.011	.004	.008	.005

Table 6. Summary of total length data on Anisakis sp. larvae from male (M) and female (F) 2-sea-winter Atlantic salmon from Canada and the United Kingdom.

	Canada			United Kingdom		
	M	F	M + F	M	F	M + F
Number	64	314	378	35	196	231
Mean	20.92	21.32	21.25	22.16	22.31	22.29
Variance	6.375	4.853	5.116	9.702	5.506	6.106
Standard deviation	2.525	2.202	2.262	3.115	2.347	2.471
Standard error	0.316	0.124	0.116	0.527	0.168	0.163

Table 7. Mean lengths of 762 Anisakis sp. larvae from Atlantic salmon from 7 sampling stations arranged according to the sea age of the hosts, 1969. Number examined in brackets.

Sampling station	Mean length of larvae		
	1 sea-winter	2 sea-winters	3 sea-winters
3	-	21.2(75)	21.4(14)
4	20.2(22)	20.5(45)	21.5(5)
5	20.9(21)	21.5(50)	22.2(21)
6	19.9(7)	19.6(50)	20.8(17)
7	-	21.2(72)	21.1(19)
8	-	21.7(156)	23.3(24)
10	21.1(39)	21.0(111)	21.3(14)
N	(89)	(559)	(114)
Mean	20.74	21.13	21.80
Std. dev.	0.439	0.591	0.892
Std. error	0.047	0.025	0.084

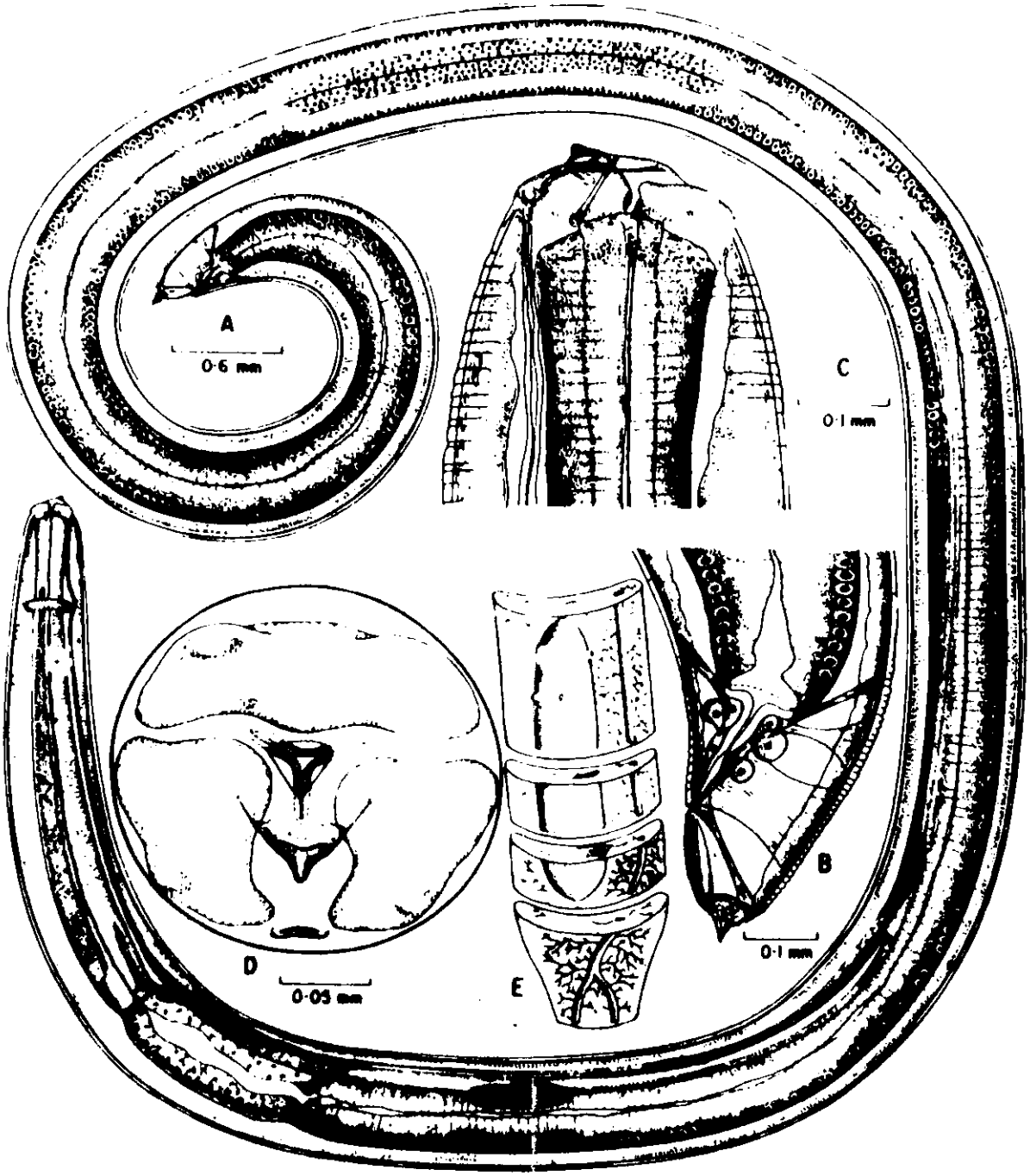

Fig. 1. Morphology of Anisakis sp. larvae: A, Entire worm; B, Tail end; C, Head end; D, *En face* view; E, Diagrammatic presentation of the structure of the excretory cell. All figures except E were drawn with the aid of a camera lucida.

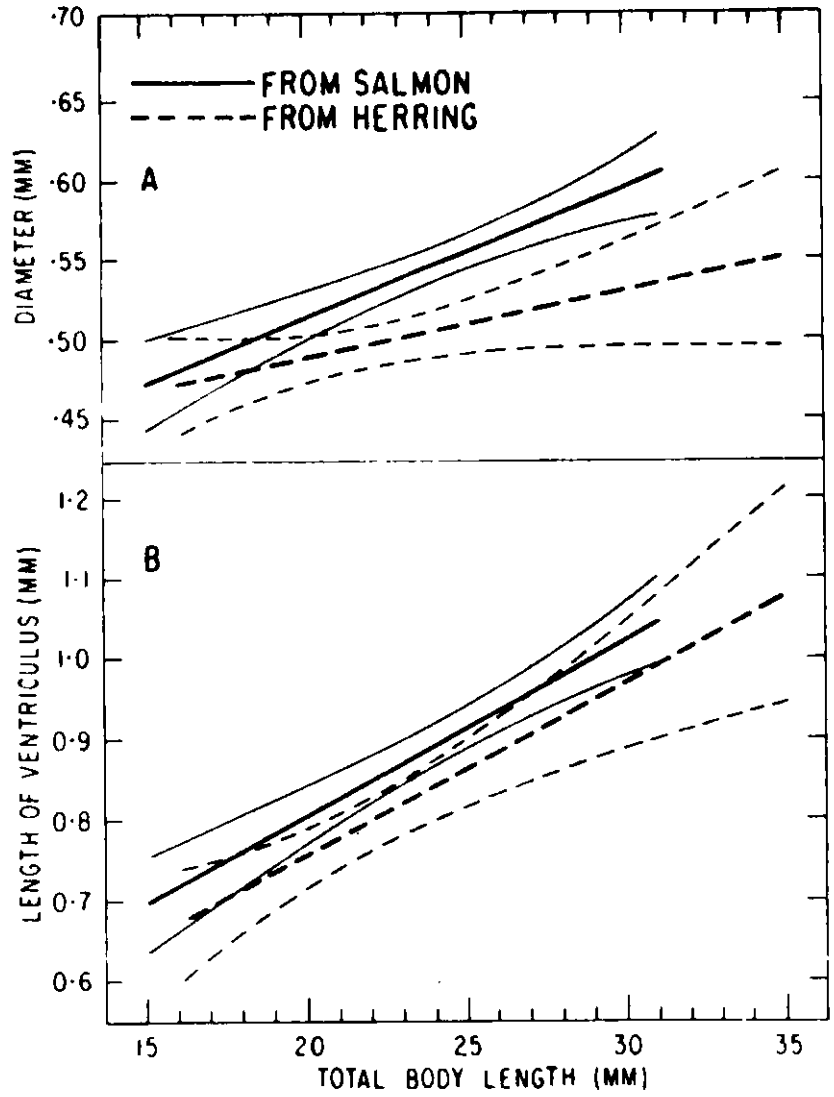

Fig. 2. Least squares regression lines for regressions of the diameter (A) and the length of the ventriculus (B) of Anisakis sp. larvae on the total body length of the larvae. Heavy straight lines are the regression lines and lighter curved lines represent 95% confidence bands.

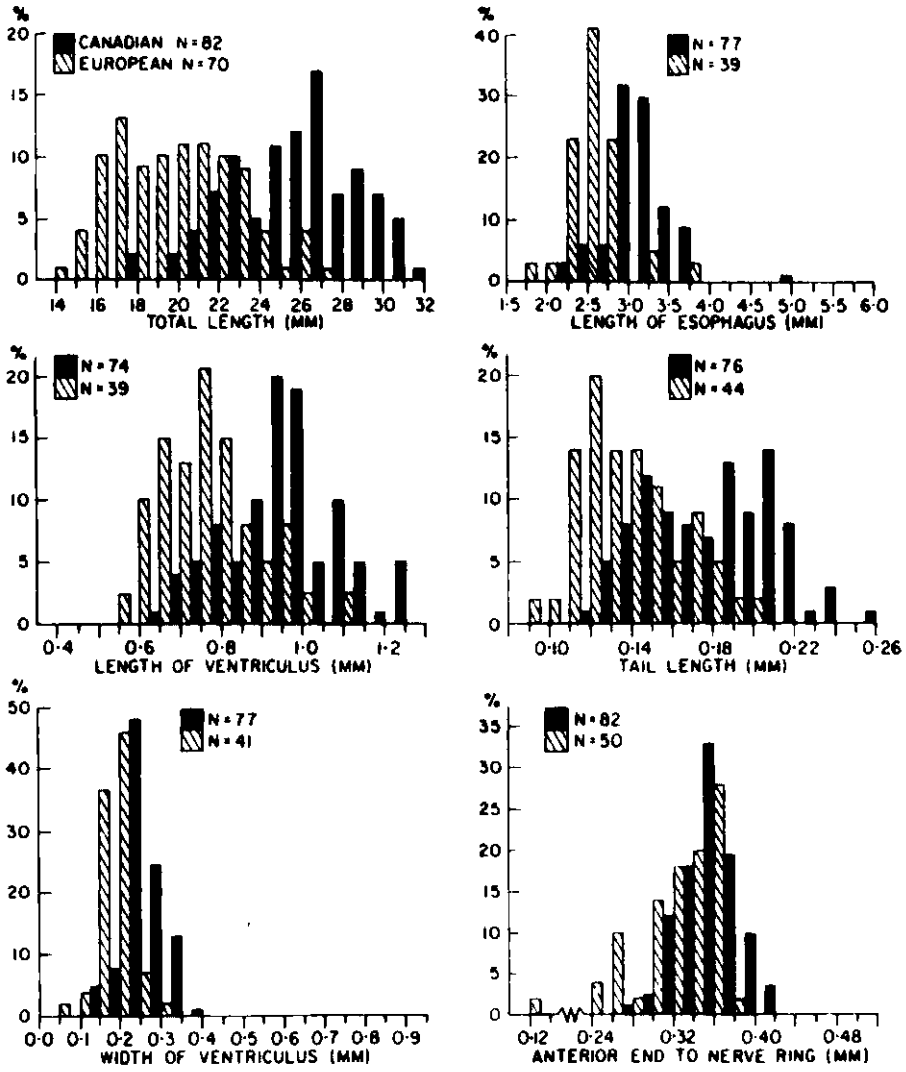

Fig. 3. Frequency distributions of the total length, length of the esophagus, length of the ventriculus, length of the tail, width of the ventriculus and the distance of the anterior end to the nerve ring of Anisakis larvae from herring taken in Canadian and European waters. N = Number of larvae in sample.

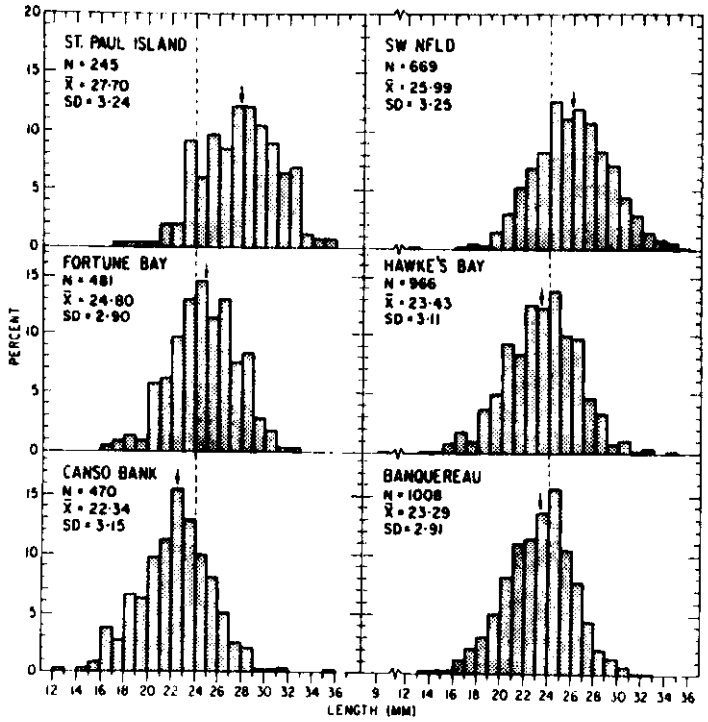

Fig. 4. Length frequency distributions of six samples of Anisakis sp. larvae from herring taken near the Canadian coast; all ages combined. N = Number of larvae examined, $\bar{x}$ = mean length of the larvae and SD = standard deviation of the distribution; arrows indicate sample means and vertical broken lines indicate the mean of all six samples combined.

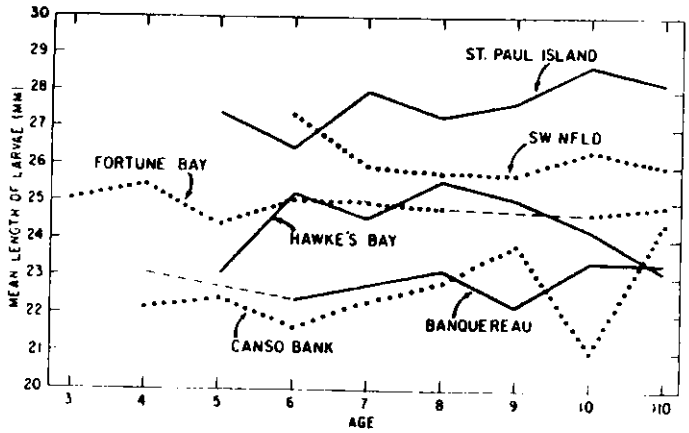

Fig. 5. Mean length of Anisakis sp. larvae compared with the age of the herring in which they were found. Age classes represent a breakdown of samples used in Fig. 4. Broken lines indicate no larvae for that age class. Note that there is no apparent increase in the length of the larvae with the age of the host.