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Morphometric variations among larval *Anisakis simplex* (Nematoda: Ascaridoidea) from fishes of the North Atlantic and their use as biological indicators of host stocks

Mary Beverley-Burton[1] & John H. C. Pippy[2]

[1] *Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada*
[2] *Department of the Environment, Fisheries and Marine Service, Biological Station, St. John's, Newfoundland, Canada*

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Synopsis

Analysis of variance on mean dimensions of samples of larval *Anisakis simplex* from Atlantic salmon (*Salmo salar*) and Atlantic herring (*Clupea harengus harengus*) indicated that highly significant morphometric differences existed. Subsequent analyses were simplified by using only the total body length of the larvae which was easily measured. Also the multiple range tests indicated that length showed the highest degree of heterogeneity. No difference ($P > 0.2$) was detected in the lengths of larvae from male and female salmon and most analyses suggested there was no change in length of the larvae with an increase in age of the host. There was, however, significant heterogeneity in the length of larvae in salmon which had spent more than one winter at sea and this heterogeneity was related in some way to the geographic localities in which the samples were taken. Possible reasons for these differences and the use of morphometric variants of *Anisakis* as a biological indicator are discussed.

Introduction

Differences in morphometric characters have been used to identify and define the geographic distribution of various free living organisms (Dannevig 1933, Fisher 1936, Runnström 1941, Hill 1959). Scott (1969) observed similarities in length-frequency distributions of *Lecithophyllum botryophorum* (Trematoda: Hemiuridae) in Atlantic argentine (*Argentina silus*) taken in the same season but from different areas and this is probably an expected result if the samples originated from the same stock infected with the same population of parasites. However, Scott did not consider the possibility of more than one population of host or parasite. Parasites, in common with free-living organisms, are undoubtedly morphometrically variable animals and parasites associated with one host population could have a different mean size from those in another population. Also, parasites in the same host population could have different mean sizes related to the sex, age of the host, intensity of infection or some other host dependent factors.

The present observations were made to determine if significant morphometric variations occur among larval *Anisakis simplex* (identified by Beverley-Burton et al. 1977) in Atlantic salmon (*Salmo salar*) and Atlantic herring (*Clupea harengus harengus*) and, if so,

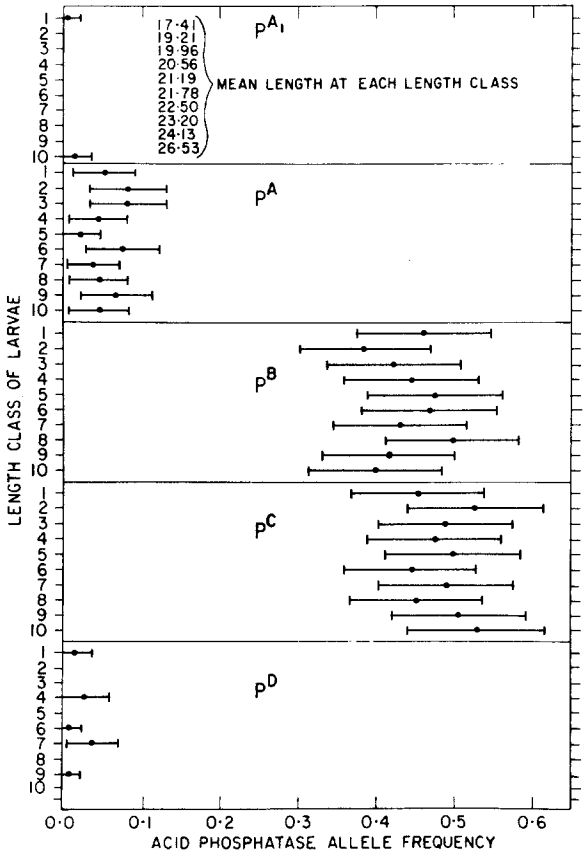

Fig. 1. 95% confidence limits of frequencies of 5 acid phosphatase alleles of 10 different length classes of 3rd-stage larvae of *Anisakis simplex*.

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to determine which morphometric character is best suited for subsequent population analyses; to examine these variations with respect to the host's sex, age and geographic location; and to determine if the variations are related to the presence of more than one population in the samples. Documentation and explanation of such variations would be valuable in any study designed to use *A. simplex* as a biological tag.

Data concerning distribution within the host, prevalence and mean numbers of larval *A. simplex* in Atlantic salmon will be presented subsequently (Berverley-Burton & Pippy, in press).

Materials and methods

Larval *Anisakis simplex* were obtained from hosts collected at 21 sampling stations in the North Atlantic as previously described (Beverley-Burton et al. 1977 (Fig. 1)).

Specimens were usually obtained from frozen fish. These larvae were covered with tap water in labelled vials and held at approximately −20° C. Larvae from North Sea herring (station 21) were a subsample from van Banning's (1971) in vitro cultures and were kept alive for some 4 days before freezing. For examination larvae were thawed, immediately placed on a microscope slide and photographed. The negatives were subsequently screened and the following measurements were made from the images: total length, distance from the anterior end to the nerve ring, length of esophagus, length and mid-point diameter of the ventriculus, length of the postanal tail and diameter of the worm at its mid-point. Total body length measurements were occasionally made from camera lucida drawings.

Larvae used to determine the best morphological feature for comparisons between different samples were the same as those used for multivariate statistical analyses. Larvae used to study morphometric variations in relation to the sex of the host were some of those used for electrophoretic analyses (Beverley-Burton et al. 1977). Larvae used for comparative purposes with host's age and area of capture were examined solely for that purpose. The sea-age of the salmon was determined using procedures outlined by Jones (1959). Salmon which had spent 1 winter at sea

Table 1. F ratios (from analyses of variance) indicating heterogeneity in size of different structures among samples of 3rd-stage larvae of *A. simplex*, and multiple range tests showing samples with similar mean dimensions (sampling stations underlined by the same line). A prime (') indicates Atlantic salmon (*Salmo salar*) hosts, otherwise hosts are Atlantic herring (*Clupea harengus harengus*); * = statistically significant at the 95% level; ** = significant at the 99% level; α = probability of a Type I error. Sampling stations as in Fig. 1, Beverley-Burton et al. (1977).

Treatment	Structure	F	DF within samples	Multiple range test (α = .05)
1	Total length	12.35**	239	21 1'16 13'8'17 11'7 6 15 10
2	Diameter	9.08**	213	1'21 15 11'8'13'7 6 10
3	Anterior end to nerve ring	2.36*	219	21 8'13'11'1'17 7 15 6 10
4	Esophagus length	3.75**	202	15 21 1'8'11'7 13'6 10 17
5	Ventriculus length	5.27**	197	13'21 15 1'8'11'10 6 17 7
6	Ventriculus width	14.86**	202	11'8'13'1'21 15 10 17 6 7
7	Tail length	5.26**	207	21 15 11'13'1'6 8'7 17 10

were referred to as age I salmon, etc. Data used in analysis of variation in length of larvae with population composition were derived from Beverley–Burton et al. (1977).

Results

General morphometric variations. Analyses of variance on the arithmetic mean dimensions of larval *A. simplex* indicated that highly significant differences were present among samples collected in different geographical areas of the North Atlantic (Table 1). Multiple range tests on mean body lengths of the larvae suggested 4 distinct groupings (Table 1, Treatment 1) while only 3 were apparent when the esophageal data were analysed (Treatment 4). There was little similarity in the order in which the samples of the 2 analyses were grouped. Groupings in the other 5 analyses (Treatments 2, 3, 5, 6 and 7) were even more complex and, again, there was little similarity in the orders in which the sample means were grouped. It was not determined if the differences in groupings were related to the low correlations between body dimensions (Table 2).

Further analyses were simplified by considering only the total body length of the larvae which was easily measured, so that large numbers of specimens could be handled. Also, the multiple range tests (Table 1) indicated that length showed the highest degree of heterogeneity and there were highly significant correlation coefficients between the larval length and other body dimensions, except the distance from the anterior end to the nerve ring (Table 2).

Morphometric variations and host's sex. Student's t tests on the mean lengths of 117 larvae from male salmon and 212 larvae from female salmon indicated there was no difference ($P > 0.2$) in the lengths of larvae from male and female fish (Table 3). This justified combining length data on larvae from male and female salmon.

Morphometric variations and host's age. A total of 760 larvae from both male and female salmon which had spent up to 3 winters at sea were used to determine if length of the larvae was related to host sea-age. Analyses of variance on larval length versus age suggested there was no change in length of the larvae with age of the host except in one sample from sta-

Table 2. Correlation matrix showing correlations between dimensions of 173 3rd-stage larvae of *A. simplex* from Atlantic herring (*Clupea harengus harengus*) and Atlantic salmon (*Salmo salar*). * = significant at the 95% level; ** = significant at the 99% level.

		a	b	c	d	e	f	g
a	Total length							
b	Diameter	0.43**						
c	Anterior end to nerve ring	0.15	0.16*					
d	Esophagus length	0.64**	0.44**	0.18*				
e	Ventriculus length	0.60**	0.39**	0.13	0.71**			
f	Ventriculus width	0.26**	0.61**	0.14	0.25**	0.44**		
g	Tail length	0.36**	0.22**	0.002	0.34**	0.35**	0.19**	

Table 3. Similarity of mean length of 3rd-stage larvae of *A. simplex* from male and female Atlantic salmon (*Salmo salar*) as demonstrated by results of student's t tests. N = number of larvae in sample, $\bar{x}$ = arithmetic mean length; SD = standard deviation; NS = not statistically significant at the 95% level. Sampling stations as in Fig. 1, Beverley-Burton et al. (1977).

		Male host			Female host			
Station	Age	N	$\bar{x}$	SD	N	$\bar{x}$	SD	t
4	I	24	20.23	1.89	16	19.79	2.81	0.79^{NS}
4	II	13	20.45	2.35	82	21.39	2.10	1.27^{NS}
8	II	3	23.50	1.80	13	23.04	1.56	0.42^{NS}
9	II	77	20.86	2.09	101	20.82	2.37	0.19^{NS}

Table 4. F ratios (from analyses of variance) indicating similarities in mean lengths of 3rd-stage larvae of *A. simplex* in 6 of 7 samples of Atlantic salmon (*Salmo salar*) with different ages. N = number of larvae in sample; DF = degrees of freedom; NS = not statistically significant at the 95% level; * = significant at the 95% level. Sampling stations as in Fig. 1, Beverley-Burton et al. (1977).

Treatment	Sampling station	Age	N	Mean length	F	DF within ages
1	3	II	75	21.15	0.04^{NS}	87
		III	14	21.29		
2	4	I	22	20.27	0.26^{NS}	69
		II	45	20.52		
		III	5	21.30		
3	5	I	21	20.93	1.20^{NS}	89
		II	50	21.48		
		III	21	22.26		
4	8	I	5	19.93	1.38^{NS}	71
		II	50	19.66		
		III	17	20.85		
5	9	II	72	21.19	0.053^{NS}	89
		III	19	21.03		
6	12	II	156	21.71	4.23*	178
		III	24	23.33		
7	14	I	39	21.04	0.11^{NS}	161
		II	111	20.95		
		III	14	21.36		

tion 12 (Table 4) where the mean length of the larvae was greater in older salmon. It could not be determined if some factor other than the age of the host was involved in producing this anomaly. Nevertheless, because of this, larval length data from hosts of different ages were not combined in further statistical analyses.

Geographical variations in morphometry. There was no significant variation in the mean length of larvae in age I salmon from 4 different sampling stations but the mean lengths of larvae from age II and III salmon fell into 3 and 2 groups respectively (Table 5). There was little similarity in the groupings of the mean lengths when considered from a geographical point of view. These analyses indicated there was significant heterogeneity in the length of larvae in salmon which had spent more than 1 winter at sea and that this heterogeneity was related in some way to the geographic localities in which the samples were collected.

Variation in length with population composition. There was no evidence that samples of larger

Table 5. F ratios (from analyses of variance) indicating heterogeneity in the mean lengths of 3rd-stage larvae of *A. simplex* in Atlantic salmon (*Salmo salar*) from different stations and multiple range tests showing samples with similar mean lengths (sampling stations underlined by the same line). DF = degrees of freedom; NS = not statistically significant at the 99% level; ** = significant at the 99% level; α = probability of Type I error. Sampling stations as in Fig. 1, Beverley-Burton et al. (1977).

Age	Sampling station	N	Mean length	F	DF within stations	Multiple range test ($\alpha = .05$)
I	4	22	20.27	0.41^{NS}	85	–
	5	21	20.93			
	7	7	19.93			
	10	39	21.04			
II	3	58	21.15	3.19**	582	8 4 13 14 3 9 5 12
	4	45	20.52			
	5	50	21.48			
	8	50	19.66			
	9	72	21.19			
	12	156	21.71			
	13	31	20.89			
	14	111	20.95			
III	3	14	21.29	2.47**	107	8 9 3 4 14 5 12
	4	5	21.30			
	5	21	22.26			
	8	17	20.85			
	9	19	21.03			
	12	24	23.33			
	14	14	21.34			

larvae tended to have different frequencies of the $P^{A}1$, P^{A}, P^{B}, P^{C} and P^{D} alleles (Fig. 1). Evidently, the observed variation in the lengths of the larvae in different samples of salmon is not related to the presence of more than one population of *A. simplex* in the samples.

Discussion

Although the significance of the relationship between the length of the larvae and the age of the host remained uncertain it appeared to be relatively unimportant. The geographic location of the sampling stations was, apparently, the most important factor contributing to the morphometric variations among larval *A. simplex*.

The possibility that differences in larval lengths were related to geographic variations in oceanic temperatures was considered. A literature search and an examination of surface temperature data collected since the early 1900s (from the Canadian Oceanographic Data Centre) did not provide adequate data for this comparison.

Van Cleave & Mueller (1934) studied morphometric variations of *Azygia longa* (Trematoda: Azygidae) from the American eel (*Anguilla rostrata*), bowfin (*Amia calva*) and brown trout (*Salmo trutta fario*) in Cross Lake, New York. The largest specimens from the eel were about 28 mm long while those from the bowfin were about 19 mm long. In contrast, specimens from brown trout were less than 5 mm long. Such variability could be compared with the relative sizes of parasites grown in vitro and McClelland (1971) found that preadult *Phocanema* (=*Terranova*) *decipiens* reared in vitro had a mean length of 27.4 mm (range: 21.6–36.8 mm) while infective larvae recovered from cod (Templeman et al. 1957) had a mean length of 38.9 mm (range: 15–58 mm). Variation in larval length could not be related to the presence of more than one population of *A. simplex* in the present study. Variations among larvae in both natural and in vitro situations are likely related to nutritonal, physiological, or other factors. Differences in such contributing factors among different stocks of salmon and herring have probably resulted in variations in length of larval *A. simplex*.

The results suggest that comparisons of the mean lengths of larval *A. simplex* in different samples of fish may be used to test hypotheses that 2 or more samples of fish were drawn from the same statistical population. Any doubt concerning the possible relationship of the lengths of the larvae to the age of the host may be overcome by stratifying the samples according to the age of the hosts.

Morphometrics as a biological indicator. The small differences in mean lengths of larval *A. simplex* in Atlantic salmon limits their value in stock identification. However, mean lengths which occur at extreme end of the length range can be used to compare samples. Larval *A. simplex* in age II salmon caught off northern Newfoundland (station 4) were significantly shorter than those in salmon caught in Miramichi Bay (station 12) (Table 5). This suggested that the salmon caught off northern Newfoundland had a different stock composition than those in the Miramichi area. Apparently, proportionately few age II salmon from the Gulf of St. Lawrence (station 12) were represented in catches off northern Newfoundland (station 4). Similar deductions could not be made from specimens collected from age I and age III salmon because larvae in these fish tended to be more homogeneous with respect to total body lengths.

Larvae in both age II and age III salmon were shorter in salmon caught off Newfoundland's east coast (station 8) than in those caught in Miramichi Bay (station 12). This suggested that proportionately few salmon from the Miramichi area were present in catches off eastern Newfoundland.

Morphometric variations were greater among larval *A. simplex* in herring than in salmon. This, together with the fact that different stocks of herring tend to remain separate in the sea, suggests that a study of the morphometry of these larvae in herring may be a fruitful avenue of research in the field of stock indentification.

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