Habitat and Morphological Correlates to Vertebral Number as Shown in a Teleost, *Gasterosteus aculeatus*

T. E. REIMCHEN AND J. S. NELSON

Vertebral counts were made on 3257 *Gasterosteus aculeatus* (threespine stickleback) from Drizzle Lake, Queen Charlotte Islands, Canada, and their frequency distribution tested for randomness with respect to sex, body length, body depth, and lake habitat (littoral, limnetic). Males had a higher vertebral number than females and had a smaller adult body size, a pattern inconsistent with the phenomenon of pleomerism. Mean vertebral number decreased from juvenile to pre-adult size classes (40–60 mm body length) and then increased among adult fish over 70 mm and reached the highest counts in the largest fish (>80 mm). Vertebral number was inversely correlated to relative body depth independent of sex and size class of fish. These data parallel trends found in higher taxonomic comparisons. Increased frequency of higher vertebral counts in littoral areas was principally the result of a higher proportion of males and larger bodied (older) fish of both sexes occurring in littoral areas. This emphasizes the importance of considering sampling biases before drawing conclusions on small differences in vertebral number between samples from different populations.

FISHES are notable for their degree of variation in vertebral number within populations, among populations, and among species. While developmental temperature is known to influence vertebral number, a major component of the variation is described by the phenomenon of pleomerism, the correlation, among different poikilotherms, between number of meristic parts (e.g., vertebrae) and their characteristic body size (e.g., maximum body length) (Lindsey, 1975). Lindsey found positive correlations of vertebral number with size (pleomerism) occurring at different taxonomic levels (e.g., species within a family) and even that it can occur between populations within a species (*Gasterosteus aculeatus*) and in several taxa, *Ecsenius* and *Etmopterus*, between sexes within a population. Major departures or exceptions from these associations are related to body depth, with deep-bodied taxa having fewer vertebrae than shallow-bodied taxa of equivalent size (C. C. Lindsey, pers. comm.).

We examine whether these general correlations between vertebral number and morphology exist within populations and whether microgeographical differences in frequencies are present among vertebral phenotypes. Such a study is useful as it provides an analysis of vertebral number where confounding differences in habitat, developmental temperature, and body shape would be slight in comparison to 'between-species' studies. We consider here body length, body depth, sex, and habitat parameters in a freshwater population of *G. aculeatus* (threespine stickleback) and test for associations with vertebral number. Our data provide new insight into the sources of population variability in vertebral number.

METHODS AND MATERIALS

Collections of stickleback were made from Drizzle Lake, Queen Charlotte Islands, British Columbia; habitat description is given in Reimchen and Douglas (1980). As part of a general investigation of interactions between stickleback morphology and habitat at this locality (Reimchen, 1983; Reimchen et al., 1985), 96 sites, which provided a representative three-dimensional profile of the lake, were sampled every 10 wk over a period of 3 yr. Numbers of fish per trap were low (often <5) and grouping of samples was necessary. For the purposes of this paper, samples were grouped into divisions representing three horizontal areas: littoral (from shoreline outwards to the 2 m depth contour), intermediate (2.5–3.5 m depth contour), and limnetic (4–18 m). Two vertical habitats were also defined: surface (from lake surface to 2 m depth) and deep (≥3 m depth). Depths of 2 m broadly correspond to the limits of light penetration in this deeply stained lake. Most fish were captured in bottom traps in shallow water (<5 m).
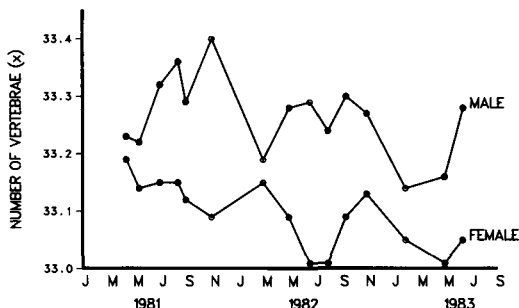
Fig. 1. Mean vertebral number against time for male and female *Gasterosteus aculeatus* from Drizzle Lake, Queen Charlotte Islands. Empty circles represent small samples (N = 20–50).
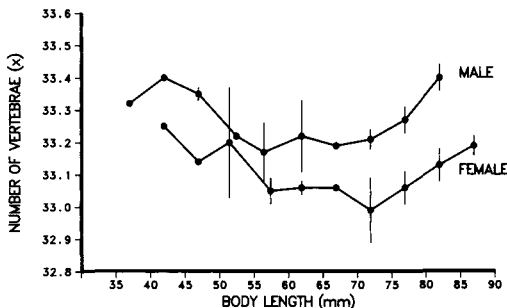

Fig. 2. Mean vertebral number against length class (standard length) for males and females of *Gasterosteus aculeatus.* Means and SE calculated on samples grouped for year.

Of 14,000 stickleback captured in the traps, 3297 were retained for analysis. These included random sub-samples of adults, most juveniles and sub-adults, trap mortalities (from sporadic storms), and fish which had predator injuries (broken spines, body lacerations). Sex, body length (standard length), and body depth were recorded (Reimchen et al., 1985). Length measurements (±0.1 mm) were made with vernier calipers. Radiographs were made (Kodak Industrex M film) and numbers of vertebrae were scored under a dissecting microscope with transmitted light. The associations of habitat, season, and sex with mean vertebral frequencies were analyzed with paired t-tests. Relative body depth (depth/body length) and vertebral number were compared. To overcome some of the limitations of ratios, body depth was plotted against body length (gravid females excluded) for each vertebral group, and slopes were compared by analyses of covariance (BMDPIV). Multi-way tables were constructed for the three major vertebral groups (V32, V33, V34), habitat (horizontal and vertical trap locales), season (spring, summer, winter), sex, body length (40–65 mm, 65–90 mm), and relative body depth (depth/body length). These tables were analyzed with log-linear models (BMDP4F) to test for interactions.

RESULTS

Vertebral number ranged from 31–35, of which two phenotypes, V33 and V34, comprised the majority of individuals (frequencies of 0.67 and 0.25, respectively). Sexual dimorphism was evident with males having 0.5% more vertebrae than females (x_{male} = 33.27; x_{female} =

33.09; paired t = 7.99, $P < 0.001$, df = 14). The dimorphism persisted during various temporal fluctuations in mean vertebral number over the study period (Fig. 1).

In both sexes, mean vertebral number decreases from juvenile to pre-adult size classes reaching a minimum value in samples of fish near 70 mm body length (2–3 yr of age). This corresponds to the minimum size at first reproduction in this population. Thereafter, among samples of larger and older fish, there is a consistent increase in mean vertebral number with the largest fish (>85 mm) exhibiting the highest mean vertebral count (Fig. 2). These fish usually show major deterioration of fins and skin and
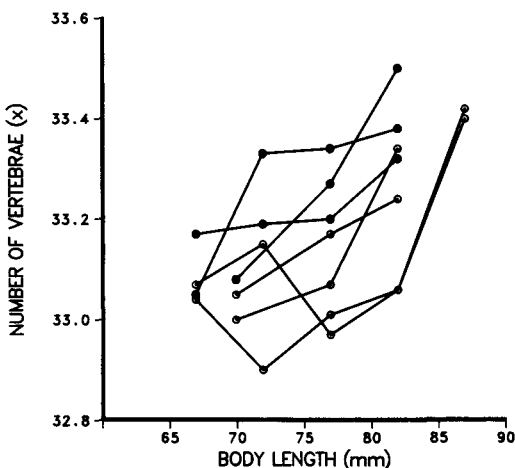

Fig. 3. Mean vertebral number of adult *Gasterosteus aculeatus* in relation to body length (standard length). Each line represents a single collection where minimum sample size is 100 individuals (minimum of 15 per point). Point—male, circle—female.
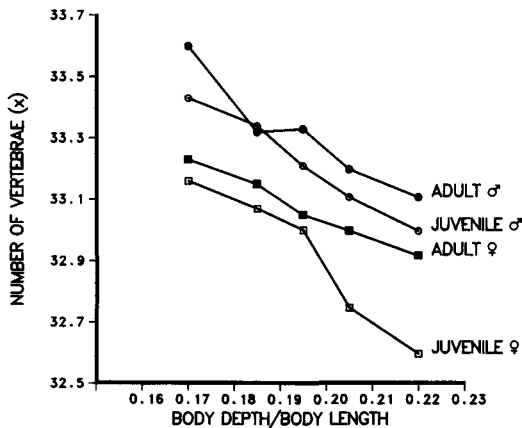
Fig. 4. Mean vertebral number in relation to body depth ratio of *Gasterosteus aculeatus*. Standard lenghs for juveniles 40–65 mm, adults 65–90 mm.

Fig. 5. Season and habitat effects on mean vertebral number ($\bar{x}$ + SE) of *Gasterosteus aculeatus*.

are probably 4 or 5 yr of age. Slopes of the two size classes (<70 mm, >70 mm) are significantly different from each other for each sex (ANOVA—male, F = 9.63, $P = 0.002$; female, F = 6.96, $P = 0.008$). This trend is not an artifact of grouping temporally disparate samples because similar trends were evident in individual collections (Fig. 3).

We have tested for possible relationships between number of vertebrae and relative body depth. Samples were separated for sex and for two major size classes (40–65 mm, 66–95 mm) and mean vertebral number was calculated for different depth ratios. The resulting plot (Fig. 4) yields highly significant correlations, with deeper-bodied fish having lower vertebral counts. This relationship was similar in both sexes and in both length groups and approximated a 1.2% reduction in vertebral number for a 30% increase in body-depth ratio. While these data show that relative body depth is an important vertebral correlate, the differences in regression lines between the juveniles and

adults of each sex suggest allometric effects to length ratios. To overcome some of the limitations of ratios, body depth was regressed against body length for each vertebral phenotype. Analysis of covariance shows similarity in slopes ($P > 0.5$) but significant differences in adjusted group means for the three vertebral phenotypes. Fish with few vertebrae (V32) have the greatest body depth, intermediate counts (V33) have intermediate body depth, and those with a high number (V34) have the lowest body depth. This pattern occurs in both sexes. All

TABLE 1. MATRIX OF *t*-TEST VALUES FOR ADJUSTED GROUP MEANS ON BODY DEPTH FOR THREE VERTEBRAL PHENOTYPES FOR MALE (ABOVE DIAGONAL) AND FEMALE (BELOW DIAGONAL) *Gasterosteus aculeatus*.

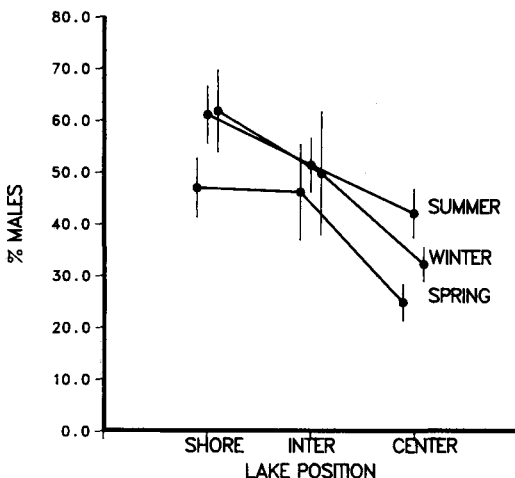|	V32	V33	V34
V32	—	3.52**	5.58**
V33	4.74**	—	4.76**
V34	5.81**	2.55*	—

*$P < 0.05$; **$P < 0.001$.

Fig. 6. Sex ratio (% males + SE) of *Gasterosteus aculeatus* for season and habitat.

TABLE 2.   FREQUENCY TABLE OF VERTEBRAL PHENOTYPES OF *Gasterosteus aculeatus*, STRATIFIED FOR HABITAT, SEASON, AND SEX.

Sex	Time	Horizontal position	Depth	Vertebra			N
				V32	V33	V34	
Male	Spring	Shore	Bottom	6.6	62.1	31.3	182
		Center	Surface	3.1	68.8	28.1	64
			Bottom	7.5	64.2	28.3	159
	Summer	Shore	Bottom	4.1	58.2	37.7	340
		Center	Surface	4.6	63.3	32.1	196
			Bottom	5.3	61.7	33.0	227
	Winter	Shore	Bottom	5.6	65.8	26.7	161
		Center	Surface	5.3	56.1	38.6	57
			Bottom	4.3	72.4	23.3	116
Female	Spring	Shore	Bottom	11.8	63.9	24.3	169
		Center	Surface	12.7	74.2	13.1	267
			Bottom	10.8	60.3	19.8	232
	Summer	Shore	Bottom	7.2	75.2	17.6	210
		Center	Surface	10.6	70.2	19.2	292
			Bottom	11.6	67.6	20.8	259
	Winter	Shore	Bottom	5.6	73.3	21.1	90
		Center	Surface	6.6	64.5	28.9	76
			Bottom	10.0	70.0	20.0	160

pairwise comparisons of the adjusted group means are significantly different from each other (Table 1).

Frequencies of vertebral phenotypes were compared among horizontal and vertical lake habitats. Grouping of fish into three vertebral classes (V32, V33, V34) shows differences between habitats with V34 contributing the major trends. Frequency of this phenotype decreased from 0.28 in littoral areas to 0.23 in limnetic regions (G = 10.4, df = 2, P < 0.01). These data were further separated for season as offshore movement during winter could obscure potential segregation in spring and summer. This produces more distinct trends with V34 decreasing during spring from 0.28–0.18 between littoral and limnetic regions (G = 14.3, df = 2, P < 0.001) but showing no change during summer and winter seasons (P > 0.3). Calculation of mean vertebral number for littoral and limnetic habitats from the 15 transects indicates a corresponding reduction in spring (paired t = 3.63, 3 df, P < 0.05), a modest reduction during summer (paired t = 2.61, 5 df, P < 0.05), and a marginal but non-significant increase during winter (Fig. 5).

Since there is sexual dimorphism in vertebral number, some of the differences in vertebral frequencies could result from habitat differences in the sexes. Sex ratios were plotted against habitat for each season and it was evident that males decrease in relative abundance from littoral to limnetic habitats (Fig. 6). Males are least abundant in limnetic regions during spring, which also coincides to when that area had the lowest mean vertebral count.

Data were grouped into a contingency table (horizontal distance from shore—H, water depth—D, and season—T) to partition the effects of sex and identify interactions with vertebral number (Table 2). This log-linear analysis yields significant vertebral interactions with sex (VS) (G = 68.4, df = 2, P < 0.001), with horizontal position and season (VHT) (G = 10.5, df = 4, P = 0.03), and with depth and season (VDT) (G = 9.6, df = 4, P = 0.05). During winter, V34 was more prevalent in surface than in benthic waters in both sexes; yet during summer, vertebral phenotypes were uniformly distributed with depth. In spring, V34 females occurred more frequently in benthic regions, the opposite to that in winter. These three factor interactions indicate a unique contribution of VHT and VDT to the log-linear model which is independent of the vertebral relationships with sex.

The correlation between body size and vertebral number could result in a relationship with

habitat if body size was related to habitat. Mean adult body length and body depth were greater in females than in males (78.9 mm vs 74.6 mm for length; 15.1 mm vs 14.9 mm for body depth), although size-adjusted body depth was greater in males (15.3 mm vs 14.7 mm, t = 18.1, $P < 0.001$). For example, an increased proportion of shallow-bodied fish near shore within each sex would produce an increased vertebral count. Adult male body length (≥ 65 mm) was regressed against body depth for three horizontal habitats. Although equality of slopes was present (F = 0.02, $P = 0.98$), there was a difference (0.7%) in adjusted means with littoral fish having the greatest body depth (F = 3.23, $P = 0.04$). Similar analysis for adult females (≥ 65 mm) (slope equality, F = 0.45, $P = 0.67$) also shows an increase (1.7%) in body depth of littoral fish (F = 16.1, $P = 0.001$). Therefore, this would lead to decreased rather than the observed increased vertebral counts in littoral fish. In addition, because of the correlation of vertebral number with adult body length (Fig. 2), an increased proportion of large adults in littoral areas within each sex would produce higher vertebral counts. Adult body length (≥ 65 mm) for each sex was plotted against horizontal habitats (from lake center to shoreline). Both sexes exhibit an increase in adult body size with increasing proximity to shore. Slopes, which are similar between the sexes (ANOVA: F = 3.1, $P = 0.08$), are significantly different from zero (F = 131.9, $P = 0.001$). Inclusion of the ratio of adult body depth to body length in a six factor contingency table (VHTSLR) produces significant interactions for HL (G = 134.3, df = 9, $P < 0.001$), VL (G = 13.0, df = 6, $P = 0.04$), and VS (G = 85.2, df = 2, $P < 0.001$), but the unique VHT interaction observed in the previous model is no longer significant (G = 7.8, df = 12, $P = 0.80$). Equivalent analysis for associations of vertebral number with water depth (VDTSLR) retains the VDT interaction (G = 9.63, df = 4, $P = 0.05$), indicating a unique contribution of water depth and season which is not a function of body length.

DISCUSSION

Given the possible importance of vertebral number for swimming performance, the degree of interpopulation variation in vertebral number, and the diverse habitat types, it might be expected that the position of individual fish in a body of water would be influenced, either actively by choice or passively by selection. Higher vertebral counts occur more frequently in littoral and in benthic areas during spring but in limnetic and surface waters during winter. These seasonal associations suggest partitioning of vertebral phenotypes by habitat and temperature. Comparable differences in microhabitat preferences are suspected to occur among lateral plate and spine phenotypes in *G. aculeatus* (Moodie, 1972; Reimchen, 1980) and may contribute to the fine-scale differences in allozyme frequencies observed in some other teleosts (Kennedy et al., 1985; White and Turner, 1985). However, correlations between vertebral number, sex, body length, and body depth confound efforts to identify the causal relationships in vertebral and habitat associations. While there may be functional aspects to increased vertebral counts near shore, the present data suggest that such habitat correlations are the consequence of older and post-reproductive fish occurring in littoral areas. Such associations within populations introduce potential sources of sampling error which should be considered in studies on geographic variation in vertebral number.

The positive relationship between maximum body length and vertebral number in fishes is widespread with exceptions correlated with departures in body depth (Lindsey, 1975). Our data show comparable trends within a population and within each sex; that is, in large individuals there can be a positive correlation between vertebral numbers and body length and an inverse correlation with relative body depth. As a functional response, this suggests a finely tuned developmental morphology in which variation in vertebral number cannot be considered to be independent of the correlated variables. For example, there may be a graded response in which the genetic components to body length and body depth in adult fish (in all taxa) are correlated with numbers of vertebrae formed during ontogeny. With this interpretation, one could expect to see regular differences in mean vertebral counts between small and large individuals within an age cohort which should persist as the cohort moves towards older age classes. Alternatively, the associations between vertebral number, body length, and body depth could result from differential survival of phenotypes to predators, possibly due to different swimming capabilities (Spouge and Larkin, 1979). Experimental evidence for differential predator susceptibility among vertebral phe-

notypes has been shown in *G. aculeatus* (Swain and Lindsey, 1984). Under these conditions, as opposed to those under the developmental hypothesis, the associations with vertebrae and morphology would change among age classes. Specifically, Lindsey (1975) suggests that if the optimal vertebral number occurs at adult size, then mean vertebral counts should decrease between hatching and adult size until the appropriate body size and associated vertebral count are reached. With further increments of body growth, there should be selection for increased vertebral number so as to track the close association with increasing body size. Our data (Fig. 2), which represent only a narrow range of age classes, are consistent with this prediction. There is extensive mortality from avian piscivores in this lake (Reimchen and Douglas, 1984) and there is evidence (Reimchen, in prep.) that fish with and without predator injuries differ in numbers of abdominal vertebrae, further suggesting a role for selective predation in the variation of vertebral number among size classes. Recent experimental work indicates that swimming performance of stickleback fry and their susceptibility to predation are related to the ratio of abdominal to caudal vertebrae (Swain, 1986).

Sexual dimorphism in vertebral number, which has been reported for only a few fish species, has previously provided further support for pleomerism as the larger of the sexes has had higher vertebral counts (Lindsey, 1975). Our data, with *G. aculeatus*, are not consistent with this since females, which have the lower vertebral counts, reach a larger adult size than males. To test whether this might be anomalous, we examined vertebral data collected in a previous study from 10 surrounding populations of *G. aculeatus* (Reimchen et al., 1985) and analyzed (ANOVA) for vertebral and size dimorphism between the sexes. These populations, which came from diverse habitats (streams, ponds, and lakes), showed a pattern similar to that at Drizzle Lake. Males had on average 0.3 more vertebrae than females ($F = 16.43, P < 0.001$) and were 6.6% smaller than females ($F = 38.73, P < 0.001$). Conceivably, non-heritable effects could produce the vertebral sexual dimorphism since environmental temperature during larval development of fishes is known to influence vertebral number (Lindsey and Arnason, 1981) and the potential exists for the role of temperature in sex determination (Conover, 1984; Conover and Fleisher, 1986). Direct experimental data on *Gasterosteus* (Lindsey, 1962) shows higher vertebral counts in females compared to males among clutches reared at cool temperatures (<20 C). Summer water temperatures at Drizzle Lake are cool, ranging from 12.5–19 C, yet females have a deficiency of vertebrae relative to males. Until rearing experiments are carried out, phenotypic induction of the sexual dimorphism remains a possibility for this apparent exception to pleomerism.

In conclusion, frequencies of vertebral phenotypes within a lake are not randomly distributed with season, habitat, length of fish, depth of fish, and sex. Our observations may represent rather crude summations of what at any instantaneous time could be more distinct differences in these associations. The positive relationship between adult body length and vertebral number suggests either a complex developmental phenomenon or differential survival. Experimental work designed to examine temperature preferences of different vertebral phenotypes would help in understanding the significance of these findings. It is clear from this study and from the work of Swain (1986), who shows that the ratio of abdominal to caudal vertebrae is subject to selection, that intrapopulation variation in vertebral number may constitute a more complex relationship between morphology and habitat than hitherto presumed.

LITERATURE CITED

CONOVER, D. O. 1984. Adaptive significance of temperature-dependent sex determination in a fish. Am. Nat. 123:297–313.

———, AND M. H. FLEISHER. 1986. Temperature-sensitive period of sex determination in the Atlantic Silverside, *Menidia menidia*. Can. J. Fish. Aquat. Sci. 43:514–520.

KENNEDY, P. K., M. L. KENNEDY AND M. H. SMITH. 1985. Microgeographic genetic organization of

populations of largemouth bass and two other species in a reservoir. Copeia 1985:118–125.

LINDSEY, C. C. 1962. Experimental study of meristic variation in a population of threespine sticklebacks, *Gasterosteus aculeatus*. Can. J. Zool. 40:271–312.

————. 1975. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J. Fish. Res. Bd. Can. 32:2453–2469.

————, AND A. N. ARNASON. 1981. A model for responses of vertebral numbers in fish to environmental influences during development. Can. J. Fish. Aquat. Sci. 38:334–347.

MOODIE, G. E. E. 1972. Morphology, life history, and ecology of an unusual stickleback (*Gasterosteus aculeatus*) in the Queen Charlotte Islands, Canada. Can. J. Zool. 50:721–732.

REIMCHEN, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? *Ibid.* 58:1232–1244.

————. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). Evolution 37:931–946.

————, AND S. DOUGLAS. 1980. Observations of loons (*Gavia immer* and *G. stellata*) at a bog lake on the Queen Charlotte Islands. Can. Field-Naturalist 94: 398–404.

————, AND ————. 1984. Seasonal and diurnal abundance of aquatic birds on the Drizzle Lake Reserve, Queen Charlotte Islands, British Columbia. *Ibid.* 98:22–28.

————, E. M. STINSON AND J. S. NELSON. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Can. J. Zool. 63:2944–2951.

SPOUGE, J. L., AND P. A. LARKIN. 1979. A reason for pleomerism. J. Fish. Res. Bd. Can. 36:255–269.

SWAIN, D. P. 1986. Adaptive significance of variation in vertebral number in fishes: evidence in *Gasterosteus aculeatus* and *Mylocheilus caurinus*. Unpubl. Ph.D. dissert., University of British Columbia, Vancouver, British Columbia, Canada.

————, AND C. C. LINDSEY. 1984. Selective predation for vertebral number of young sticklebacks (*Gasterosteus aculeatus*). Can. J. Fish. Aquat. Sci. 41:1231–1233.

WHITE, M. M., AND B. J. TURNER. 1985. Intralacustrine differentiation in two species of goodeid fishes. Copeia 1985:112–118.

DEPARTMENT OF ZOOLOGY, THE UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA T6G 2E9, CANADA.