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Pretreatment of Spores as a Factor in Dimensional Analysis of Two Species of Histozoic Myxosporida

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Pretreatment of Spores as a Factor in Dimensional Analysis of Two Species of Histozoic Myxosporida[1]

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Abstract. Spores of *Henneguya exilis,* taken during the summer of 1968 from the gills of *Ictalurus punctatus* caught in the Des Moines River near Knoxville, Iowa, and of a species of *Myxosoma* apparently closely related to *Myxosoma ovalis,* taken from the gills of *Carpiodes velifer* from the same locality, were subjected to storage at room temperatures, 4° C, and deep freezing. Repeated measurements of spores under different pretreatment showed that the dimensions of the spores and polar capsules remained remarkably stable, as did ratios of the dimensions of spore parts. Abnormalities in the details of spore architecture were seen under conditions under which dimensions remained stable. It is recommended that, wherever possible, unfixed spores be used for measurements, and preserved spores be used for other observations on which species descriptions are to be based.

In his monograph on Myxosporida, Kudo (1920) pointed out that the most stable features of a species is its spore, and based his classification and characteristics of species primarily on spore form. This practice has been followed, generally. The pretreatment of spores before measurement, however, has a considerable effect on spore dimensions. Fixed and stained spores mounted in balsam are significantly smaller than fresh spores, and fixed spores suspended in an aqueous medium are intermediate in size (Kudo, 1921; Meglitsch, 1937). Different fixatives shrink spores differently, and it is reasonable to to suppose that species differences occur with regard to reactions to fixatives. Accordingly, it was recommended that, wherever possible, spore dimensions be taken from fresh, unstained spores, and that where this could not be done, the conditions under which measurements were taken be specified. Most investigators have followed these recommendations, but many species have been described without adequate specifications of the conditions of observation.

An advantage of using preserved material is that spore form remains stable, once the spore is preserved. The assumption that fresh, unstained material is more reproducible depends on the answers to several questions. How stable are fresh spores? How long do spore dimensions remain unchanged at room temperatures? Does chilling or freezing affect spore form significantly? During the

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summer of 1968, a study of the effects of spore pretreatment was undertaken to seek answers to such questions.

Materials and Methods

Henneguya exilis Kudo, 1929, from the gills of *Ictalurus punctatus* taken in the Des Moines River, near Knoxville, Iowa, was used as a subject for study. A species of *Myxosoma* from the gills of *Carpiodes velifer,* taken from the same locality, was also used. This species is apparently identical with *Myxosoma multiplicata,* as characterized by Rice and Jahn (1943). A marked discrepancy between the dimensions of spores studied by Rice and Jahn and those seen by Davis (1923), who originally described *M. ovalis,* leaves the precise species diagnosis in doubt.

Host fishes were brought into the laboratory immediately after their removal from the river. Some were brought back alive and kept alive until studied. Others died on the journey; these were dissected immediately, were chilled at 4° C, or deep frozen, until used.

Hanging drop mounts of spore suspensions were kept at room temperatures, chilled or frozen. Spore dimensions almost universally used for species characterization were measured repeatedly under oil immersion, with the aid of an ocular micrometer. Means, standard deviations, and coefficients of variation were calculated for all of the measurements taken, as well as for several of the ratios between measured dimensions.

Effects of Pretreatment on Spore Dimensions

Room Temperatures. Spores kept in hanging drop mounts at high, summer room tempertures are surprisingly stable, insofar as the dimensions of spores and polar capsules are concerned (Tables 1, 2). Some shrinkage of the polar capsules of *Henneguya exilis* occurs by the end of four weeks, but the coefficient of variation does not rise significantly. Apparently the capsule shrinkage is relatively uniform. It should be noted that the capsules of *H. exilis* are very narrow; the scale used was too large to permit an accurate reflection of the true variability of this dimension. A trend toward greater variability appears in the second and third weeks, especially in the length of the spore proper, but this is not borne out in the fourth week. Possibly spores not fully mature were deteriorating at this time, and are omitted in later samples because fragments were not measured.

The polar capsules of *Myxosoma* sp. vary more than those of *H. exilis.* They undergo some shrinkage in the second and third weeks, and a trend toward increased variability is also seen. The length and breadth of the spore itself is not significantly changed by exposure to room temperatures for three weeks, and spore variability does not rise significantly.

Table 1. Dimensions of spores of *Henneguya exilis* Stored at Room Temperatures

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficent of Variation
		Length of Polar Capsule		
24 hrs.	8.7-8.9 μ	0.453-0.826 μ	5.0-9.5	6.58
3 days	8.4-8.5	0.434-0.448	5.2-5.3	5.25
1 week	7.6-8.8	0.546-1.007	6.6-11.5	9.00
2 weeks	8.50-8.60	0.468-0.583	5.0-6.9	6.13
3 weeks	8.5-8.5	0.431-0.505	5.3-6.1	5.65
4 weeks	7.5-7.7	0.447-0.690	5.9-9.0	7.73
		Breadth of Polar Capsule		
24 hrs.	1.40-1.53 μ	0.000-0.28 μ	0.0-13.0	5.93
3 days	1.40	0.01	5.0	5.0
1 week	1.19-1.58	0.01-0.26	5.0-16.2	11.7
2 weeks	1.40-1.43	0.00-0.15	0.0-11.1	7.6
3 weeks	1.40-1.40	0.00-0.068	0.0-2.2	1.1
4 weeks	1.05-1.06	0.00-0.071	0.0-6.7	3.35
		Spore Breadth		
24 hrs.	4.34-4.48 μ	0.303-0.334 μ	6.8-7.7	7.25
3 days	4.17	0.284	6.8	6.8
1 week	4.16-4.40	0.291-0.485	7.0-11.0	8.5
2 weeks	4.20-4.27	0.262-0.349	6.3-8.2	7.25
3 weeks				
4 weeks	4.19-4.50	0.321-0.339	7.1-7.9	7.5
		Length of Spore Proper		
24 hrs.	18.0-19.5 μ	1.228-1.240 μ	6.3-6.9	6.6
3 days	18.0	0.769	4.3	4.3
1 week	17.8-18.9	0.969-0.933	5.3-5.8	5.5
2 weeks	17.0-17.4	0.326-3.164	2.1-18.4	10.75
3weeks	16.6	3.132	18.9	18.9
4 weeks	17.7-17.9	1.430-1.475	8.0-8.3	8.15
		Total Spore Length		
24 hrs.	63.0-66.8 μ	6.283-6.777 μ	9.4-10.8	10.1
3 days	64.0	7.322	11.4	11.4
1 week	58.45-65.1	6.062-0.231	9.3-15.6	12.1
2 weeks	61.4-62.6	5.943-6.516	9.1-10.4	9.8
3 weeks	67.2	5.526	8.2	8.2
4 weeks	59.8-62.7	8.168-9.011	13.0-15.1	14.1

Chilling and Freezing. Tables 3 to 6 show the effects of chilling or freezing the spores. It is evident that spores are not materially altered by freezing or chilling. Dimensions remain substantially the same, and variability is not significantly altered, although changes in the dimensions of the polar capsules are less than in spores held at room temperatures.

Ratios Based on Spore Dimensions

The stability of spore dimensions suggests that the shape of the spore and its capsules may not be affected severely by pretreatment. This is of some importance in species recognition, for in several of

Table 2. Dimensions of Spores of *Myxosoma* sp. Stored at Room Temperatures

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficent of Variation
		Length of Polar Capsule		
24 hrs.	3.8-4.7 μ	0.588-1.040 μ	13.0-22.3	16.5
2 weeks	4.4-4.5	0.598-0.712	13.4-16.2	14.8
3 weeks	3.5-3.6	0.645-0.725	17.8-20.8	19.3
		Breadth of Polar Capsule		
24 hrs.	3.2-3.5 μ	0.337-0.568 μ	10.1-17.0	14.1
2 weeks	3.1-3.2	0.372-0.412	12.0-13.1	12.6
3 weeks	26-2.7	0.495-0.535	18.1-20.2	19.2
		Spore Breadth		
24 hrs.	8.6-9.4 μ	0.760-1.292 μ	8.8-14.0	11.7
2 weeks	9.4	0.574	6.1	6.1
3 weeks	9.0	0.981	10.9	10.9
		Spore Length		
24 hrs.	10.2-10.7 μ	0.535-0.679 μ	5.2-6.4	5.6
2 weeks	10.6	0.539	5.0	5.0
3 weeks	10.1	1.159	11.4	11.4

Table 3. Dimensions of Spores of *Henneguya exilis* Stored at 4° C

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficent of Variation
		Length of Polar Capsule		
24 hrs.	8.5-8.7 μ	0.319-0.343 μ	5.7-4.1	3.9
2 days	8.8-8.9	0.714-0.748	8.1-8.4	8.3
1 week	8.0-8.6	0.429-1.840	5.0-22.3	13.4
2 weeks	8.4-8.7	0.560-0.722	6.4-8.6	7.2
3 weeks	8.4-8.7	0.298-0.522	4.2-6.1	4.7
4 weeks	8.3-8.3	0.537-0.610	65-7.4	7.0
		Breadth of Polar Capsule		
24 hrs.	1.4-1.4 μ	0.000 μ	0.0	0.0
2 days	1.8-1.8	0.245-0.280	13.4-15.3	14.4
1 week	1.3-1.4	0.000-0.341	0.0-233	10.3
2 weeks	1.3-1.4	0.069-0.187	4.8-14.1	7.4
3 weeks	1.4-1.4	0.000-0.069	0.0-48	2.4
4 weeks	1.2-1.2	0.189-0.272	14.7-23.4	19.5
		Spore Breadth		
24 hrs.	4.3 μ	0.334 μ	7.7	7.7
2 days	4.7	0.400	8.6	8.6
1 week	4.3-4.4	0.322-0.638	7.5-14.5	11.0
2 weeks	4.1-4.3	0.348-0.433	8.8-9.7	9.3
3 weeks	4.4	0.462	8.1-10.3	9.5
4 weeks	6.32	0.659	10.4	10.4

Table 3, Cont.

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficient of Variation
		Length of Spore Proper		
24 hrs.	17.8 μ	0.497 μ	2.8	2.8
2 days	19.0	1.157	6.1	6.1
1 week	17.5	0.824-1.717	4.7-9.8	7.3
2 weeks	16.3-17.6	0.846-3.241	4.8-19.0	11.9
3 weeks	16.3-18.3	0.666-1.088	3.8-5.9	4.9
4 weeks	18.7	1.208	6.4	6.4
		Total Spore Length		
24 hrs.	61.6 μ	6.562 μ	10.7	10.7
2 days	62.1	8.836	14.3	14.3
1 week	62.9-63.8	7.385-11.820	11.6-18.8	15.2
2 weeks	62.9-63.9	5.020-6.155	8.0-9.6	8.8
3 weeks	60.7-61.7	5.32-8.583	8.6-14.5	10.8
4 weeks	62.86	6.738	10.1	10.1

Table 4. Dimensions of Spores of *Myxosoma* sp. Stored 4° C

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficent of Variation
		Length of Polar Capsule		
36 hrs.	4.6-4.7 μ	0.510.0.545 μ	10.9-11.9	11.4
2 days	4.5	0.747-0.757	16.6-16.9	16.8
5 days	5.3	0.325-0.439	6.2-8.3	7.3
2 weeks	4.2-4.3	0.307-0.313	7.2-7.4	7.3
4 weeks	4.4-4.5	0.487-0.561	10.9-127	11.7
		Breadth of Polar Capsule		
36 hrs.	3.4 μ	0.348-0.363 μ	10.3-10.7	10.5
2 days	3.2-3.3	0.400-0.440	12.4-13.5	13.0
5 days	2.8-3.1	0.192-0.310	6.7-10.0	84
2 weeks	3.5	0.137	3.9	3.9
4 weeks	3.1-3.4	0.303-0.416	9.0-13.3	11.3
		Spore Breadth		
36 hrs.	9.4 μ	0.425 μ	4.5	4.5
2 days	9.0	0.948	10.5	10.5
5 days	8.5	0.469	5.5	5.5
2 weeks	8.7	0.508	5.8	5.8
4 weeks	9.2-9.3	0.417-0.597	4.5-6.5	5.5
		Spore Length		
36 hrs.	10.8 μ	0.446 μ	4.1	4.1
2 days	10.7	0.613	5.7	5.7
5 days	10.2	0.446	4.3	4.3
2 weeks	10.2	0.404	2.6	2.6
4 weeks	10.2-10.3	0.543-0.687	5.2-6.6	5.9

the larger genera, ratios have been suggested as a useful adjunct to the means of distinguishing between species otherwise about the same size. On *a priori* grounds, it seems safe to say that unless

Table 5. Dimensions of Spores of *Henneguya exilis* Stored in Frozen Condition

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficent of Variation
		Length of Polar Capsules		
1 week	8.1-8.4 μ	0.657-0.891 μ	8.1-10.7	9.6
2 weeks	8.3-8.4	0.428-0.499	5.1-5.9	5.5
3 weeks	8.3	0.510-0.547	6.1-6.5	6.3
4 weeks	7.9	0.557-0.666	7.1-8.4	7.8
		Breadth of Polar Capsule		
1 week	1.3-1.5 μ	0.116-0.259 μ	8.5-16.9	12.4
2 weeks	1.4	0.067	4.8	4.8
3 weeks	1.4	0.095	6.8	6.8
4 weeks	1.2-1.3	0.171-0.181	13.4-14.9	14.2
		Spore Breadth		
1 week	4.3-4.5 μ	0.382-0.601 μ	8.9-13.4	11.2
2 weeks	4.3	0.297	6.8	6.8
3 weeks	4.1	0.248	6.0	6.0
4 weeks	4.3	0.534	12.5	12.5
		Length of Spore Proper		
1 week	18.3-18.9 μ	1.062-1.068 μ	5.6-5.8	5.7
2 weeks	18.0	0.901	5.0	5.0
3 weeks	17.6	0.988	5.6	5.6
4 weeks	17.9	1.873	10.4	10.4
		Total Spore Length		
1 week	62.0-65.1 μ	8.556-9.430 μ	13.1-15.2	14.2
2 weeks	62.4	7.320	11.7	11.7
3 weeks	65.0	4.730	7.2	7.2
4 weeks	63.0	9.531	15.1	15.1

Table 6. Dimensions of Spores of *Myxosoma* sp. Stored in Frozen Condition

Storage Time	Range of Sample Means	Range of Standard Deviations	Range of Coefficient of Variation	Average Coefficent of Variation
		Length of Polar Capsules		
1 day	4.1-4.4 μ	0.630-0.790 μ	15.1-18.1	16.9
3 weeks	4.0-4.2	0.488-0.496	11.7-12.3	12.0
4 weeks	4.3-4.4	0.539-0.573	12.5-13.1	12.8
		Breadth of Polar Capsules		
1 day	3.0-3.2 μ	0.468-0.500 μ	14.9-16.2	15.4
3 weeks	3.2	0.391-0.444	12.4-13.9	13.2
4 weeks	3.3	0.321	9.6-9.7	9.7
		Spore Breadth		
1 day	8.8-9.1 μ	0.567-0.677 μ	6.2-7.7	7.5
3 weeks	9.2	0.481	5.3	5.3
4 weeks	9.1	0.617	6.7	6.7
		Spore Length		
1 day	10.1-10.6 μ	0.956-1.026 μ	9.4-9.7	9.6
3 weeks	10.5	0.657	6.3	6.3
4 weeks	10.3	0.929	7.1	7.1

dimensions are more or less independent from proportionality as revealed by ratios, this kind of trait is meaningless for species recognition.

A small series of ratios was calculated: capsule length to capsule breadth (CL/CB); capsule length to spore length (CL/SL); spore breadth to spore length (SB/SL); and in the case of *Henneguya exilis,* the length of the spore proper to total spore length (SL/TSL). As a general rule, such ratios have been calculated from the means of spore dimensions. In this study, ratios were calculated for each spore, and the ranges, means, and standard deviations of the ratios were determined. The results are summarized in Tables 7 and 8.

Table 7

Ratio of Spore Dimensions of *Henneguya exilis* after Various Pretreatments

Time	Ratio	Pretreatment	Range	Mean	Ave. STD	Ave. V
1 day	CL/CB	Room Temperature	1.500- 3.000	6.10175	0.57825	9.525
4 weeks	CL/CB	Room Temperature	1.333- 4.166	7.210	0.652	9.25
1 day	CL/SL	Room Temperature	0.132- 0.136	0.474	0.032	6.75
4 weeks	CL/SL	Room Temperature	0.178- 0.233	0.426	0.046	10.8
1 day	SB/SL	Room Temperature	0.092- 0.096	0.236	0.022	9.35
4 weeks	SB/SL	Room Temperature	0.103- 0.125	0.248	0.0265	10.7
1 day	SL/TSL	Room Temperature	0.116- 0.157	0.02915	0.0345	11.8
4 weeks	SL/TSL	Room Temperature	0.189- 0.234	0.2955	0.046	15.6
1 day	CL/CB	Chilled	0.750- 1.000	6.115	0.2375	3.9
4 weeks	CL/CB	Chilled	3.166-16.250	7.4075	2.348	30.5
1 day	CL/SL	Chilled	0.096	0.476	0.024	5.0
4 weeks	CL/SL	Chilled	0.125	0.433	0.038	8.8
1 day	SB/SL	Chilled	0.087	0.242	0.020	8.3
4 weeks	SB/SL	Chilled	0.107	0.235	0.027	11.5
1 day	SL/TSL	Chilled	0.165	0.294	0.037	12.6
4 weeks	SL/TSL	Chilled	0.126	0.302	0.034	11.3
1 week	CL/CB	Frozen	2.666- 4.500	0.25175	0.78975	13.55
4 weeks	CL/CB	Frozen	2.666- 3.666	6.4335	0.830	12.85
1 week	CL/SL	Frozen	0.185- 0.188	0.444	0.0455	10.25
4 weeks	CL/SL	Frozen	0.170	0.440	0.047	10.7
1 week	SB/SL	Frozen	0.096- 0.194	0.235	0.0305	12.85
4 weeks	SB/SL	Frozen	0.107	0.239	0.031	12.9
1 week	SL/TSL	Frozen	0.159- 0.266	0.299	0.050	16.7
4 weeks	SL/TSL	Frozen	0.166	0.288	0.037	12.8

Table 8

Ratio of Spore Dimensions of *Myxosoma* after Various Pretreatments

Time	Ratio	Pretreatment	Range	Mean	Ave. STD	Ave. V
1 day	CL/CB	Room Temperature	0.400-0.888	1.3105	0.134	10.115
3 weeks	CL/CB	Room Temperature	0.666-1.400	1.350	0.2675	19.8
1 day	CL/SL	Room Temperature	0.183-0.487	0.414	0.068	16.234
3 weeks	CL/SL	Room Temperature	0.269	0.359	0.061	16.9
1 day	SB/SL	Room Temperature	0.416-0.645	0.854	0.118	13.8
3 weeks	SB/SL	Room Temperature	0.294	0.887	0.089	10.0
1 day	CL/CB	Chilled	0.500-0.583	1.387	0.163	11.7
4 weeks	CL/CB	Chilled	0.228-0.466	1.37775	0.09825	7.125
1 day	CL/SL	Chilled	0.169	0.433	0.048	11.1
4 weeks	CL/SL	Chilled	0.133-0.200	0.4265	0.0415	9.75
1 day	SB/SL	Chilled	0.120	0.873	0.036	4.2
4 weeks	SB/SL	Chilled	0.227-0.332	0.8895	0.0625	7.05
1 day	CL/CB	Frozen	0.600-1.000	1.34725	0.319	13.725
4 weeks	CL/CB	Frozen	0.400-0.550	1.3025	0.1165	8.95
1 day	CL/SL	Frozen	0.233-0.433	0.4135	0.072	17.4
4 weeks	CL/SL	Frozen	0.270	0.424	0.060	14.2
1 day	SB/SL	Frozen	0.395-0.572	0.8725	0.107	12.25
4 weeks	SB/SL	Frozen	0.200	0.886	0.057	6.4

As in the case of spore dimensions, a marked stability was observed; only the intital and final ratios are provided although ratio analysis was carried out for all observations made during the study. As the tables show, no evidence was obtained to suggest that the proportions of capsule to spore size, or of spore breadth to spore length, undergo significant changes under the range of conditions of pretreatment used in the study.

The authors are aware of no serious consideration of the stability of spore proportions in past literature. As ratios calculated on the basis of individual spores have not been presented, the ranges as well as the standard deviations and coefficients of variation are of interest. Ranges of ratios tend to be a little wider than the ranges of the individual measurements on which they are based, and the coefficient of variation tends to be a little higher. This indicates that lengths and breadths of both spores and capsules exhibit some degree of independence, but are not wholly independent. Thus the likelihood of the longest polar capsule to be the narrowest also, is somewhat less than might have been predicted in a completely random universe, but is somewhat higher than

would be predicated if spore proportions were more directly under the control of causative factors than individual spore dimensions.

Spore Form

The stability of spore dimensions and of ratios between dimensions under the range of conditions tested is not matched by complete morphological stability. A variety of abnormalities were seen, especially in spores held for more than two weeks at room temperatures. Polar capsules tend to become displaced, so that even though their sizes remain relatively constant, their positioning tends to become assymetrical. Optical properties of both polar capsules and sporoplasm undergo changes, and the iodinophile vacuole, if present, also changes. Thus the dimensions of parts may be measured with considerable confidence in unfixed material several days old, but the intimate details of spore structure may be altered.

Discussion

The results indicate that insofar as dimensional analysis of spores is concerned, species with heavy spore membranes, like *Myxosoma* and *Henneguya,* do not undergo significant changes under any conditions that are likely to be considered acceptable for the descriptions of species. In such spores, unfixed material is quite reproducible; certainly more so than spores that have been treated by unspecified fixatives and stains. For the purpose of measurement, fresh material deserves preferment. Measurements based on fresh material in the past can be presumed to be relatively dependable.

The same cannot be said of the details of internal spore construction. Spores are adapted to survive for a period outside of the host, a period necessary for transmission from host to host. The period of survival varies with species. Bond (1938) estimated that the life of spores of *Myxosoma subtecalis* was about 12-15 days, while no great changes could be seen in the spores of *Myxobolus bilineatum* after 28 days. Thélohan (1895) reported similar findings, and after longer periods of holding spores, found that all eventually degenerated. As spores deteriorate, abnormalities of placement and basic properties occur. Quite fresh material, or material that is preserved while quite fresh, is necessary for describing the details of spore form.

As in the case with Bond and Thélohan, nothing was seen that would suggest that the sporoplasm had emerged from the spore under the range of conditions used. As information accumulates, it seems ever more probable that spore germination, in most if not all species, depends upon some interaction between the parasite and

the host. Ingestion of the spore seems to be the most probable stimulus for the majority of species.

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