JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

V. EXPERIMENTAL INVESTIGATIONS WITH *ZOARCES VIVIPARUS* L.

BY

JOHS. SCHMIDT.

I. Introduction.

In a previous paper[1]) the principal results of my statistical investigations with *Zoarces viviparus* were summarized. I there expressed the following view, with regard to the nature of "races" in *Zoarces* and other fishes as characterized by our population analyses: "A fish "race" is largely a statistical conception. It implies a mixing of different genotypes, and the average values characterising the "race" are primarily dependent upon the quantitative proportion between these; only secondarily on the environment." It was further pointed out that we could not expect to arrive at any final decision before the results of those experimental investigations which were carried out along with the statistical investigations with the same species since 1914, were available. This is the case now, at least with regard to some of the experiments which are so advanced as to be suitable for publication. I propose to give here a summary of some of the most important results.

Two *Zoarces* populations are to be dealt with here, both from Danish waters, viz the Ise Fjord (Station 31) and Roskilde Fjord, north coast of Sealand (Station 34), the latter being a branch of the former (see Fig. 1, p. 8). Both populations were mentioned in the earlier papers quoted above and graphs representing the number of vertebrae were given[1]).

[1]) See Johs. Schmidt: *Zoarces viviparus* L. and local races of same (Comptes-rendus du Laboratoire Carlsberg, **13**, p. 389, 1917), and »Statistical investigations with *Zoarces viviparus* L.« (Journal of Genetics, VII, No. 2, 1918).

In addition to the ordinary population-analyses individual offspring analyses have been carried out each autumn since 1914, the offspring of each gravid female being separately investigated. From each female, 10 embryos (a "decade") were taken at random and their number of vertebrae etc. determined[1]).

For the sake of brevity I shall restrict myself to mentioning here a single character, viz. the number of vertebrae. This character has proved to be of great importance in distinguishing local races in *Zoarces*, where the number has been found to vary from 99 to 126. A great material of many thousands of specimens was analysed and statistically treated. The countings were made by myself (1914—1917) and by Mr. Vilh. Ege, M. Sc. (1917—1919).

II. Comparison of the different annual classes within a population.

For this purpose the decade investigations, consisting in an analysis of ten embryos taken a random from each gravid female are excellently suited. By examining the embryos instead of the adult specimens all doubt is of course excluded that the individuals belong to the same annual class.

Table I p. 11 shows the results of offspring analyses (decade investigations) within the population from Station 31 during the years 1914—1919. Altogether 871 decades or 8710 individuals rather evenly distributed between the six years have been examined.

It is seen from this table that the population-averages for the different annual classes agree very well. By means of the present great material of observations it has thus not been possible to point out any difference between the various annual classes with regard to the average number of vertebrae. In other characters such a difference can, however, be pointed out, e. g. for the number of pectoral rays[1])

In Table II p. 11 the same material is arranged in another way. If it is represented by a curve it will be seen that this is very regularly shaped, as might be expected when the material is so large.

Table III p. 12 also deals with the same material. It shows the average values of ten decades, each figure representing a

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[1]) Johs. Schmidt: Constancy investigations continued, in Comptes-rendus du Laboratoire Carlsberg. **14**. No. 1, p. 2—11, 1917.

hundred individuals. It will be seen that the highest value is 114.17, the lowest 112.28 which are respectively 0.97 above and 0.92 under the average of the population which is 113.1963. The values of Table III are especially interesting when compared with the corresponding figures for individuals coming from the same population, but developed under different outer conditions (see section V, p. 7).

III. Comparison of mothers and their offspring. — Significance of internal factors.

This part of the investigations is directed to the question whether the character examined, viz. the number of vertebrae, is hereditary, i. e. due to internal factors. As the father in each single case is unknown it is only possible to compare the offspring with the mother. As the material is so large we can however assume that the paternal number of vertebrae is on an average equal to the average of the population, i. e. abt. 113.2. Provided the number of vertebrae is a hereditary character our investigation should show that mothers with a great number have offspring with a great number. To test this we arrange the material given in Tables I—III so that for each number of vertebrae occurring in mothers we indicate the frequency of it and the average number of vertebrae in the offspring derived from these mothers.

From Table IV p. 12, representing a very considerable material namely no less than 857 mothers and 8570 individuals of their offspring, it clearly appears that the average number of vertebrae in the offspring depends upon the number of vertebrae of the mothers since the values of the offspring continuously increase as the maternal numbers of vertebrae increase from 107 to 119. A conformity like that could not appear if the number of vertebrae in an individual were alone determined by the environmental conditions during the development.

The investigations with *Zoarces* thus show that there are differences of hereditary nature between the various individuals, and they confirm the results of R. C. Punnett, arrived at by investigation of the viviparous shark *Spinax niger*[1]), as well as

[1]) R. C. Punnett: Merism and Sex in *Spinax niger* (»Biometrika«, vol. III Part IV, 1904).

those obtained from my experiments with *Lebistes* and the common trout[1]).

The Table thus shows that the number of vertebrae in *Zoarces* is a hereditary character. We must, however, not expect that the mothers with for instance 119 vertebrae have offspring with on an average 119 vertebrae. As will be seen from the table that is not the case, the average being only 116.3. In the first place the paternal number is not 119, but abt. 113.2 (the average of the population), a circumstance that causes the offspring to "regress" towards the average value of the population. Further regression is due to the fact that the maternal numbers of vertebrae, in casu 119, are personal values, while their generative values in all probability will deviate less from the average of the population than do the personal values[2]). Provided the material be sufficiently great, the following relation must presumaly hold:

$^1/_2$ (Average ♀♀ 119 + 113.2) = Average Offspring ♀♀ 119,

where "Average ♀♀ 119" is the average of the generative values of mothers with 119 vertebrae and "Average Offspring ♀♀ 119" is the average of the offspring originating from those mothers.

I am not going to discuss the results of Table IV closer here. As the present large material, however, seems to deserve a more thorough statistical treatment than I am prepared to give it I have asked the statistician Miss Kirstine Smith, D. Sc. to deal with it and I hope the results will before long be ready for publication in the "Comptes-Rendus du Laboratoire Carlsberg".

IV. Comparison of two different populations developed under the same environmental conditions. — Significance of internal factors, continued.

The two populations in question differ essentially in the average number of vertebrae, which for the first of them, the population dealt with above, from the mouth of the fjord (Station 31) is abt. 113.2, and for the other population, from the innermost

[1]) Johs. Schmidt: Experiments with *Lebistes reticulatus* (Peters) Regan (Comptes-rendus du Laboratoire Carlsberg, **14**. Nr. 5, 1919) and Diallel crossings with trout (*Salmo trutta* L.) (Journal of Genetics, Vol. IX, No. 1, 1919).

[2]) About the concepts "personal" and "generative" value see Johs. Schmidt: "La valeur de l'individu à titre de générateur, appréciée suivant la méthode du Croisement diallèle (Comptes-rendus du Laboratoire Carlsberg, **14**, No. 6, 1919).

part of the fjord (Station 34, see figure 1, p. 8) abt. 108.0. The experiment was carried out in the way that in June of 1916, i. e. at a time of year when the sexual products of *Zoarces* are not yet ripe, abt. 300 specimens of each population were caught. They were removed to an embanked area in the neigbourhood to Station 31 and put into big perforated wooden boxes, placed on the bottom near each other, projecting above the water so that feeding could easily be carried out. The pairing and the development of the embryos thus took place in captivity and under the same external conditions for both population-samples. In December 1916, i. e. at a season when *Zoarces* contains large embryos, the boxes were lifted out of the water and their contents preserved. In the population-sample from Station 31 was found 27 females with embryos, and in that from Station 34 a number of 11.

Under natural conditions the average number or vertebrae in the population at Station 31 is abt. 113.2 (see Table I), while in the population at Station 34 it is abt. 108.0 (see my first papers from 1917 and 1918). The values for the two populations thus differ by abt. 5 vertebrae, a difference which the annual investigations since 1914 have proved constant. In spite of the small distance of abt. 50 kilometers between the two stations, their natural conditions differ widely, e. g. at the first of them the salinity of the water is abt. 21 $^0/_{00}$, and at the other only abt. 12 $^0/_{00}$. It might therefore be conceivable that the cause of the difference in the number of vertebrae would be found in a direct action of the different environmental conditions. If this was the case, it was to be expected that the difference would disappear when the two populations were brought under similar outer conditions. This was done in our transplantation experiment, and it is the question now, whether the difference of 5 vertebrae, appearing under normal conditions, has disappeared in the offspring, all of which have developed in our experiment under the same external conditions.

The result of the experiment is given in Table V p. 13. For comparison it may be stated that the average number of vertebrae in offspring developed under natural conditions in 1916 for Station 31 was 113.101 $\pm$ 0.447[1]) (125 decades), and for Station 34 was

[1]) The uncertainty of the average is here and in the following indicated by the fluctuation equal to 5 times the probable error.

107.768 ± 0.468 (116 offspring-samples each consisting of 5 specimens), showing as usual a difference of abt. 5 vertebrae.

It is seen from the table that the offspring of the transplanted population-sample from Station 31 had on a average 114.819 ± 1.059 vertebrae, while the corresponding value for the sample from Station 34 was 108.283 ± 1.363. In other words the difference between the two populations has not disappeared although the development took place under the same circumstances. The result of our transplantation experiment with *Zoarces* thus agrees with the experiment with *Lebistes* previously described and confirms that the difference, proved to exist between the two populations, is of hereditary (genotypical) nature.

V. Comparison of two samples of the same population developed under different environmental conditions. — Significance of external factors.

The transplantation experiments just mentioned were continued in 1917 with material from Station 31. A great number of *Zoarces* individuals were in early summer, and therefore when the sexual organs were still unripe, brought into four large wooden boxes placed in the same embanked area near Station 31 as in 1916.

When the experiment was brought to a conclusion the total number of gravid females was 92. The number of offspring samples examined from transplanted specimens was, including the 27 decades from the 1916 experiment, altogether 119.

The investigation showed that the average number of vertebrae of the 119 decades was 114.535 ± 0.493[1]). The corresponding figure for the non-transplanted samples of the same population was in 1916 113.101 ± 0.447 and in 1917 113.251 ± 0.372. It is therefore evident that the outer conditions which were altered through the transplantation have decidedly raised the average number of vertebrae in the population.

The same thing becomes apparent when the material is arranged in another way. With the same arrangement of the material from the transplantation experiment as was used in Table III,

[1]) For the sake of completeness it is stated that the average for the transplanted mothers was 112.74 ± 0.65, and for the males (111 specimens), found in the boxes at the conclusion of the experiment, 112.95 ± 0.61.

we arrive at the following figures, each of which represents 100 individuals (10 decades):

114.63	114.08
114.55	114.59
114.65	114.65
114.09	114.46
114.09	114.42
113.88	114.42 (average of 9 decades only).

These figures have to be compared with the figures of Table III p. 12 for non-transplanted samples. Such a comparison does not leave any doubt that the number of vertebrae has really been raised by the transplantation: out of the 12 values above 11 are greater than 114, while of the 87 values in Table III only 5 are greater than 114.

It is further shown in Table VI p. 13 that the average number of vertebrae in offspring of mothers with a given number of vertebrae e. g. 116, is greater for transplanted than for non-transplanted specimens.

It is therefore proved to a certainty that the tranplantation from the natural conditions at Station 31 to the embanked area raises the average number of vertebrae in the population. It is not possible to decide for certain what factor or factors are at work to that effect. But it is a fact that the temperature in the boxes, placed in quite shallow water, about $2^1/_2$ meters deep, was higher than at Station 31 and on the whole probably higher than for any natural population of *Zoarces* in Danish waters. On the other hand the salinity was lower in the boxes than a Station 31 viz. abt. 12 ‰ against abt. 21 ‰ at Station 31. In the embanked area, where the boxes were placed, and which is used as an eel-farm, *Zoarces* is only found occasionally and in extremely small numbers, whereas it abounds outside the dam, at Station 31.

VI. The *Zoarces* populations of the Ise Fjord - Roskilde Fjord area.

Beside the yearly analyses of the populations from Stations 31 and 34 we in 1919 carried out analyses of a greater number of populations from the same fjord area. As will be

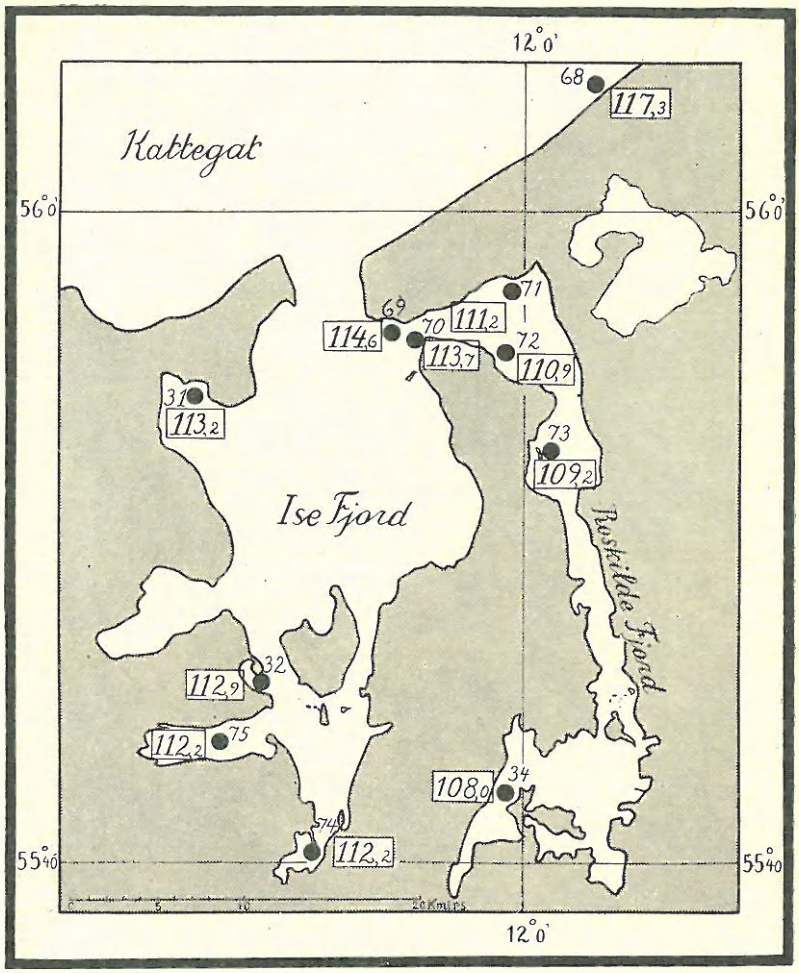

Fig. 1. — Average number of vertebrae in 11 *Zoarces* populations from the Ise Fjord - Roskilde Fjord area, Sealand, Denmark.
The figures at the black spots indicate station numbers, those within the rectangles the average number of vertebrae of the population concerned.

seen from Fig. 1, the Ise Fjord opens into the Kattegat (at the north coast of the isle of Sealand), and Roskilde Fjord is a branch of Ise Fjord. In both fjords *Zoarces* occurs in abundance right from the mouth to the base.

Table VII p. 14 shows the result of our population-analyses which are also represented in Figure 1, p. 8. It will be seen that the populations living outside the two fjords, in the Kattegat, have the average number of vertebrae which is characteristic of the

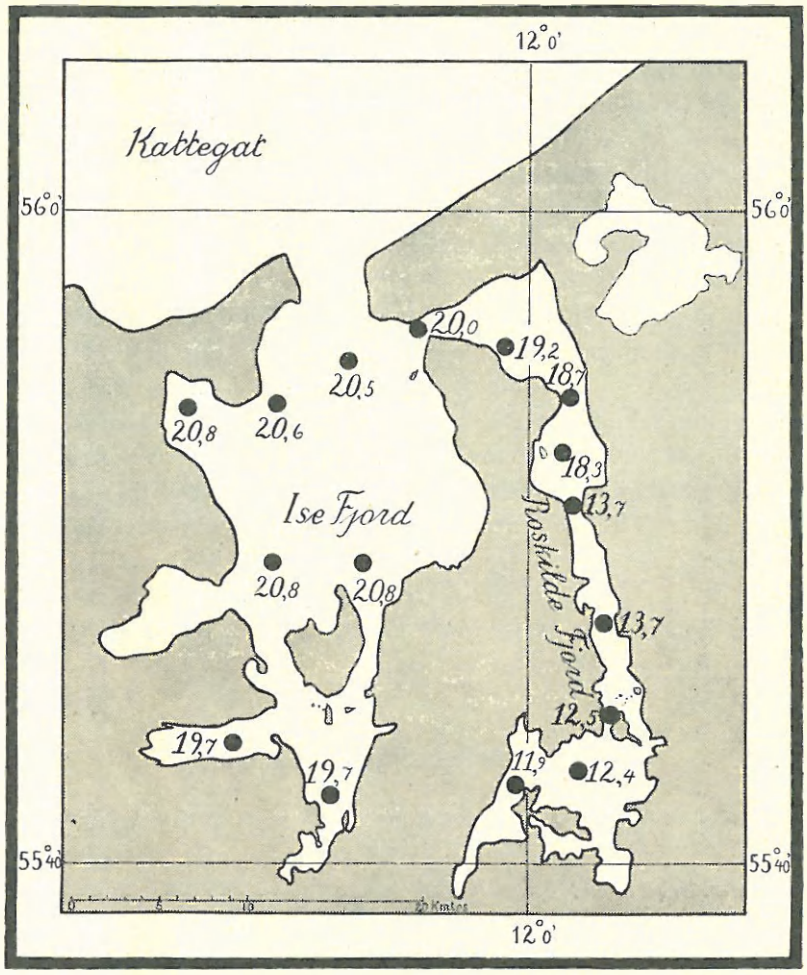

Fig. 2. — Ise Fjord · Roskilde Fjord area, Sealand, Denmark. Showing the salinity of the water in per mille (‰) at the end of November 1919.

populations of the open sea in the Baltic area, viz. 117—118. In the mouth of the fjord the number is 114—115. From this the value falls quite evenly in the Ise Fjord until 112.2 which is the lowest value for that fjord. In the Roskilde Fjord it falls suddenly to 111.2 (Station 71) and 110.9 (Station 72), reaching near the base of this fjord, at Station 34, the lowest average value hitherto known for any *Zoarces* population, viz. abt. 108.0.

This detailed investigation thus quite confirms the results of

my previous investigations (C. R. Labor. Carlsberg 13, 1917) and shows strikingly in all details how the average number of vertebrae decreases from the coast and the mouth of the fjord towards the base of the fjord. Here *Zoarces* has a short and plump shape, while the populations living farther out consist of individuals slender and elongated in shape (see fig. 16—17, p. 309 in C. R. Labor. Carlsberg, 13, 1917). This quite regular gradation of the average number of vertebrae has now been found in all Danish fjords where investigations have been undertaken and it must therefore be considered a definitely established fact.

The chemical and physical conditions of our fjords are not closely investigated. Among the conditions which are best elucidated the salinity of the water exhibits the most characteristic picture. As will be seen from Fig. 2 this factor shows a gradation which strikingly corresponds to the gradation in the average number of vertebrae. From that to conclude that this character is directly determined by the salinity or by a factor acting parallel to it — which several investigators have been inclined to do — is however not justifiable as I have already explained in my first paper on *Zoarces* from 1917.

VII. Concluding Remarks.

The investigations and experiments with *Zoarces* described above have rendered new evidence of the correctness of the view that the race-characters in fishes are of a hereditary nature i. e. that they are first and foremost determined by internal factors (Sections III and IV). That they are however on the other hand to a certain extent influenced by external factors, is here for the first time experimentally proved for one of our northern species of fish. We have thus seen that the average number of vertebrae which under natural conditions remained very constant in 6 consecutive annual classes of the same population (Section II) was altered, if not strongly, at any rate perceptibly, by a transplantation of the population (Section V).

In view of the investigations hitherto undertaken the gradation of the average number of vertebrae has to be interpreted not as a direct effect of the external conditions (the salinity or another factor acting parallel to it), but as the result of a selection.

Table I. — Station 31, Nakkehage, Ise Fjord, Denmark, 1914—1919. — Decade investigations.

Average number of vertebrae *(a)* in the six annual classes 1914—1919.

(*n* number of decades, *a* average number of vertebrae, *σ* standard deviation for average value of a decade. P. E. A. Probable error of average, P. F. A. Probable fluctuation of average.)

Year	1914	1915	1916	1917	1918	1919	1914—1919
n	139	177	125	178	154	98	871
a	**113.180**	**113.275**	**113.101**	**113.251**	**113.248**	**113.124**	**113.196**[2])
σ[1])	1.452	1.459	1.480	1.472	1.453	1.265	
P.E.A.[1])	0.083	0.074	0.089	0.074	0.079	0.086	0.032
P.F.A.[1])	0.415	0.370	0.447	0.372	0.395	0.431	0.160

Table II. — Station 31, Nakkehage, Ise Fjord, Denmark, 1914—1919. — Decade investigations.

Number of vertebrae in 857 decades. Same material as in Table I[3]).

Number of vertebrae	Number of specimens
122	1
121	1
120	6
119	22
118	100
117	269
116	647
115	1109
114	1616
113	1785
112	1397
111	893
110	463
109	172
108	65
107	17
106	6
105	1

[1]) calculated from exact decade averages.

[2]) average of the six averages (1914—1919).

[3]) 14 decades — offspring of 14 mothers in which the number of vertebrae could not be determined due to accidental damage — were excluded.

Table III. — Station 31, Nakkehage, Isefjord, Denmark, 1914—1919. — Decade investigations.

Each figure represents 100 individuals (average of 10 decade averages).

114.09	113.34	113.42	112.71	113.85
113.51	113.77	112.41	113.83	113.63
112.65	113.69	113.17	113.55	112.77
112.54	113.04	113.56	113.65	113.03
113.00	113.34	112.59	114.17	112.62
112.82	113.55	113.13	113.96	112.75
112.82	113.36	113.52	112.75	113.25
113.65	114.08	112.70	113.49	112.38
113.12	113.69	113.05	112.44	113.34
113.39	112.28	112.74	113.45	113.52
113.59	112.95	112.98	113.45	112.99
113.12	112.82	112.59	113.24	113.23
112.74	112.45	112.91	113.31	113.47
113.48	112.33	113.05	113.42	113.40
114.07	113.38	113.11	113.56	112.68
113.93	112.94	113.43	113.22	
113.22	113.73	113.86	114.08	
112.70	112.86	112.76	112.93	

Average of population ca. 113.196.

Table IV. — Station 31, Nakkehage, Isefjord, Denmark, 1914—1919. — Decade investigations.

Number of vertebrae in 857 mothers as compared with average number of vertebrae in their offspring (857 decades). — Average of population ca. 113.196.

Maternal number of vertebrae	Frequency of mothers or decades	Average number of vertebrae in offspring
119	3	116.300
118	6	115.617
117	31	114.794
116	65	114.155
115	109	113.851
114	151	113.515
113	185	113.121

Maternal number of vertebrae	Frequency of mothers or decades	Average number of vertebrae in offspring
112	135	112.948
111	105	112.523
110	47	111.596
109	11	111.245
108	7	110.400
107	2	109.250

Table V. — Transplantation of population samples from Station 31 and Station 34, 1916. — Decade investigations.

	Station 31	Station 34
Average number of vertebrae in offspring of transplanted sample	**114.819 ± 1.059** (27 decades)	**108.283 ± 1.363** (11 decades)]
Average number of vertebrae in transplanted mothers	113.115 (26 specimens)	107.00 (9 specimens)

Table VI. — Station 31, Nakkehage, Ise Fjord, Denmark. — Transplantation Experiments, 1916—1917. — Decade investigations.

Average number of vertebrae in offspring of transplanted specimens and in offspring developed under natural conditions.

Maternal number of vertebrae	Average number of vertebrae in offspring of Transplanted specimens	Not transplanted specimens
116	116.14 (7)	114.16 (65)
115	115.22 (17)	113.85 (109)
114	114.59 (18)	113.52 (153)
113	115.34 (17)	113.12 (185)
112	114.14 (18)	112.95 (135)
111	113.91 (22)	112.52 (105)
110	112.59 (9)	111.60 (47)

The figures in brackets indicate the number of mothers or decades.

Table VII. — The *Zoarces* populations of the Ise Fjord - Roskilde Fjord area. — Population analyses 1919.

	Average number of vertebrae.
Station 68, Kattegat (outside fjord)	117.330
— 69 (mouth of fjord)	114.563
— 70 (— - —)	113.732
— 31, Ise Fjord	113.2 (1914—19)
— 32, —	112.939
— 74, —	112.243
— 75, —	112.225
— 71, Roskilde Fjord	111.168
— 72, — —	110.892
— 73, — —	109.152
— 34, — —	108.0 (1914—19)

Carlsberg Laboratory, 22. February 1920.

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II. JOHS. SCHMIDT: The rotational movement of hop stems and its diurnal periodicity. Ibidem, vol. 10, page 267—283 (1913).

III. Ö. WINGE: The pollination and fertilization processes in *Humulus lupulus, L.* and *H. Japonicus* Sieb. et Zucc. Ibidem, vol. 11, page 1—44 (1914).

IV. Ö. WINGE and J. P. H. JENSEN: A method for quantitative determination of resins in hops. Ibidem, vol. 11, page 116—147 (1914).

V. JOHS. SCHMIDT: On the aroma of hops. Ibidem, vol. 11, page 149—163 (1915).

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I. JOHS. SCHMIDT: *Zoarces viviparus L.* and local races of the same. Comptes-rendus des travaux du Laboratoire Carlsberg, vol. 13, page 277—397 (1917).

II. JOHS. SCHMIDT: Constancy investigations continued. Ibidem, vol. 14 no. 1, page 1—19 (1917).

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V. JOHS. SCHMIDT: Experiments with *Zoarces viviparus L.* Ibidem, vol. 14, no. 9, page 1—14 (1920).

VI. KIRSTINE SMITH: Statistical investigations on inheritance in *Zoarces viviparus L.* Ibidem, vol. 14, no. 11 (1921).