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SCOR

REPORT ON

INTERCALIBRATION OF PIGMENT METHODS
AT HONOLULU 1961 By G.F. Humphrey

Three comparisons were made. One on "Vityaz" (September 3 at 1200) and two on "Gascoyne" (September 7 at 0630 and 8 at 0700). On each occasion a carboy was filled with water from each of the depths indicated. Each participant then took a subsample from each carboy.

Australian Method

The subsamples (3 to 5 litres) were taken by N. Dyson. They were filtered at once through millipore filters (HA, 47 mm, white, plain) to which about 0.1 gm of $MgCO_3$ had been added. Filtration was carried out at about half complete vacuum. The filter papers were folded and placed in glass tubes (5 x 1 cm) without stoppers. The tubes were kept over silica gel in a metal desiccator for 6 weeks while "Gascoyne" returned to Sydney.

The filters were then placed in nylon centrifuge tubes and 4 ml 90% acetone added. The mixture was stirred with a glass rod and kept overnight in the dark in a closed container. The mixture was again stirred and centrifuged for 10 minutes at 4,300 g. The supernatant was decanted into a graduated tube, the volume was then read and the optical density determined at 750, 665, 645, 630, 510, and 480 mμ in a 1-cm cell in a Unicam SP 500 spectrophotometer. The blank was 90% acetone. Comments on this method are given in Humphrey (1960).

The data are in Table 1.

Japanese Method

A measured volume of the subsample (usually 3 to 5 litres) was filtered through a Membranfilter No. 1. The filter was removed from the holder and exposed to steam for about 40 seconds. The filter was folded in half, placed within a filter paper which had been folded in half and fastened with a paper clip. The filters were kept over silica gel in a plastic desiccator and sent to Japan.

The edge of the filter was cut off and the centre with the phytoplankton was placed in a 30 ml conical flask. 4 ml 90% acetone was added, the mixture stirred and the flask closed with a glass stopper. The flask was kept in the dark in a refrigerator for 20 to 24 hours. The mixture was stirred again and transferred to a glass centrifuge tube, washing the flask with 90% acetone. After centrifugation for 10 minutes at 3,000 r.p.m., the supernatant was decanted into a graduated tube and the volume adjusted to 5 ml with 90% acetone. The optical density was determined at 750, 665, 645, 630, 510, and 480 mμ in a 5-cm cell in a Hitachi EPU-2 spectrophotometer. The blank was 90% acetone.

The data are in Table 2.

U.S. Method

The subsamples (1.6 to 5 litres) were taken by M. Oguri or V. Alvarez. They were filtered at once through millipore filters (HA, 47 mm, white, plain) with a very few millilitres of $MgCO_3$ (about 0.2 gm) suspension in water added as a wash after the sample had been deposited on the filter pad. Filtration was carried out at about half an atmosphere. The filter pads were folded and jammed into the bottom of numbered nylon centrifuge tubes and returned to the shore laboratory in a metal desiccator over silica gel and stored in a refrigerator for two days before extraction. Extraction was done overnight after adding about 10 ml of 90% acetone from a recently redistilled lot. The preparation was centrifuged the next morning for 10 minutes at 3400 g and the contents decanted into a 5-cm cuvette. The cuvette was filled to capacity (14 ml) with more of the same 90% acetone, the contents mixed and the per cent. transmittance of this pigment sample read at each of 750, 665, 645, 630, 510, and 480 mμ. The same readings were made for a 90% acetone combined reagent and cell blank for each cell each day.

The data are in Table 3.

Each method includes a reading at 750 mμ. This reading is made to check the turbidity of the acetone extracts. Each method uses the equations of Richards with Thompson (1952). The methods differ in the type of reagent blank.

In the Australian method glass cells are used which, when filled with 90% acetone, give the same readings as each other at all wave-lengths. The acetone extract of a filter treated according to the method (but with no sea-water filtered through it) gives negligible optical densities (about 0.001) at all wave-lengths when read against 90% acetone. In the present experiments, the 750 mμ readings were from 0.001 to 0.005. The uncorrected results (Table 4) were obtained by neglecting these values. This is the usual Australian procedure when the 750 mμ reading is not greater than 0.005. The corrected Australian results were calculated by subtracting the 750 mμ reading from each of the other readings.

In the Japanese method cells are used which, when filled with 90% acetone, give the same readings as each other at all wave-lengths. In the present experiments, the 750 mμ readings were from 0.000 to 0.022 using the 5-cm cells. The uncorrected results (Table 4) were taken by neglecting these values. The corrected results were calculated by subtracting the 750 mμ reading from the readings at 665, 645, and 630 mμ and twice the 750 mμ readings from the readings at 510 and 480 mμ. This is the usual Japanese procedure.

In the U.S. method a correction is determined for each day's analyses by preparing an acetone extract of a filter treated according to the method (but with no sea-water filtered through it) and placing this extract in the 5-cm cell to be used later for the pigment samples. The optical density of the cell containing the filter extract is then determined at each wave-length against an open pathway between the light source and the phototube. For each wave-length, these optical densities are subtracted from those found with the pigment samples. Therefore, there are no uncorrected U.S. results.

Comparison of Results

The water samples contained only small quantities of pigments (Table 4). Apart from chlorophyll c, the differences found were within the limits of chemical precision (± 0.06 mg/m^3 for chlorophyll a and ± 0.05 MSPU/m^3 for chlorophyll c, astacin and non-astacin) as given by McAllister et al. (1959) and within the present limits of biological usefulness (0.5 mg/m^3) as given by Humphrey (1962). In these respects, then, there were no differences between the results, each method giving the same picture of the water samples, i.e. they contained insignificant amounts of pigment.

However, it should be noted that the Australian corrected results for chlorophyll a were always lower than the Japanese which were usually lower than the U.S. ones. The uncorrected results (Australian and Japanese) were very close to each other. For astacin, the U.S. results were usually the lowest, then the Australian, and then the Japanese. As noted above, these differences are within the limits of chemical precision.

The results for chlorophyll c were much higher than for any other pigment. The corrected results were lowest for the Australian method, followed by those for the U.S. method, and then those for the Japanese method. The uncorrected results show that the Australian method usually gave lower results than the Japanese one. Although these differences are outside the limits of chemical precision, the Richards-Thompson method of calculation sometimes produces large errors when the spectrophotometer readings are low.

Correcting the Japanese results by the Australian method gave almost the same result as correcting by the Japanese method.

Suggested Work

The present work has shown that each of the three laboratories makes different assumptions about blanks in applying the Richards-Thompson equations.

It is necessary to repeat the intercalibration with samples containing higher concentrations of pigments before the effects of these assumptions and the effects of differences in method can be determined.

Humphrey, G.F. (1960).- C.S.I.R.O. Aust. Div. Fish. Oceanogr. Tech. Paper No.9.

McAllister, C.D. et al. (1959).- Fish. Res. Bd Canada M.S. Rep. Ser. No.55.

Richards T.A., with Thompson, T.G. (1952).- J. Mar. Res. 11, p.156.

TABLE 1

AUSTRALIAN DATA

A 1 cm cell was used in the spectrophotometer

The figures given for optical density are in units of 0.001

Sample		Optical Density						Extract volume ml	Sample volume ml
		750	665	645	630	510	480		
					mμ				
Sept. 3,	0m	1	4	3	4	15	26	4.2	5000
	10	1	4	4	4	11	16	4.3	5000
	50	3	4	3	3	9	13	5.8	5000
	75	4	6	6	6	20	30	4.3	5000
Sept. 7,	0	5	6	6	6	11	15	4.5	4000
	10	4	5	5	5	11	18	4.2	3000
	25	5	6	4	5	9	14	4.6	3000
	75	5	7	7	7	11	15	4.7	3000
Sept. 8,	0	5	6	5	5	10	11	4.9	5000
	10	1	3	2	1	4	8	4.5	4000
	25	3	4	1	1	4	6	4.4	4000
	50	1	4	5	3	15	17	4.6	4000
	75	2	4	3	2	4	7	4.6	4000

TABLE 2

JAPANESE DATA

A 5 cm cell was used in the spectrophotometer

The figures given for optical density are in units of 0.001

Sample		Optical Density						Extract volume ml	Sample volume ml
		750	665	645	630	510	480		
					mμ				
Sept. 3,	0m	22	35	38	41	101	140	5.0	3600
	10	16	27	30	30	96	130	5.0	4000
	50	15	27	30	34	87	121	5.0	4000
Sept. 7,	0	5	18	18	17	60	87	5.0	4000
	10	13	24	24	26	69	97	5.0	3000
	25	0	14	13	14	51	73	4.5	3000
	75	5	20	20	20	63	92	5.2	3000
Sept. 8,	0	10	23	20	18	63	98	5.0	5000
	10	0	14	15	17	55	83	5.0	4000
	25	7	17	16	16	52	81	5.0	4000
	50	9	17	15	15	50	73	5.7	4000
	75	13	22	18	18	56	85	5.0	4000

TABLE 3

UNITED STATES DATA

A 5 cm cell was used in the spectrophotometer

The figures given are in % transmission

Sample		Optical Density						Extract volume ml	Sample volume ml
		750	665	645	630	510	480		
				mμ					
Sept. 3,	0m	89.5	91.9	91.7	91.5	90.0	88.0	14	1900
	10	89.8	91.9	91.8	92.0	91.0	89.0	14	1900
	50	89.5	91.2	91.5	91.3	90.0	88.0	14	1600
	75	89.5	92.2	91.8	91.8	90.2	89.0	14	1900
Sept. 7,	0	89.8	92.0	92.0	91.7	91.0	89.8	14	4000
	10	89.8	92.0	92.0	91.8	91.0	89.5	14	3000
	25	89.5	91.7	92.0	91.8	90.0	89.0	14	3000
	75	89.5	91.2	92.0	91.3	90.3	89.2	14	3000
Sept. 8,	0	89.5	91.0	91.2	91.0	89.0	87.0	14	5000
	10	89.5	90.9	90.9	90.9	88.8	87.0	14	4000
	25	89.3	91.5	91.8	91.5	90.0	88.0	14	4000
	50	89.8	90.8	90.8	90.8	89.0	87.0	14	4000
	75	89.5	91.0	91.2	91.0	89.8	88.1	14	4000
Reagent blank (for all samples)		89.5	92.5	92.0	92.0	91.5	91.0		

TABLE 4

PIGMENT CONCENTRATIONS

A, J, and U denote Australian, Japanese, and U.S. methods. A blank space means sample not measured. Corrected results are those where allowance has been made for turbidity readings; no allowance has been made in the uncorrected ones. The figures in brackets in the column for corrected Japanese results are the results obtained when the Australian method of correction was used. ≠ = negative. Units are mg or MSPU/m^3. 03 = 0.03

		CHLOROPHYLL a					CHLOROPHYLL b					CHLOROPHYLL c					ASTACIN					NON-ASTACIN				
		Corrected			Uncorrected		Corrected			Uncorrected		Corrected			Uncorrected		Corrected			Uncorrected		Corrected			Uncorrected	
		A	J	U	A	J	A	J	U	A	J	A	J	U	A	J	A	J	U	A	J	A	J	U	A	J
Sept.3	Om	03	04(04)	06	04	12	01	04(04)	01	01	12	20	40(42)	34	25	77	06	09(13)	04	07	02	02	01(≠)	04	02	00
	10	03	03(03)	06	04	08	03	04(04)	06	04	09	18	28(25)	≠	23	52	05	10(12)	00	05	14	00	00(≠)	07	≠	04
	50	02	04(04)	15	06	08	≠	03(03)	03	03	07	≠	38(37)	29	22	66	04	08(11)	05	06	12	01	00(≠)	05	01	≠
	75	02		13	07		02		01	06		12		13	35		08		03	10		01		03	≠	
Sept.7	0	01	04(04)	02	09	07	01	04(04)	≠02	07	07	08	19(19)	14	46	20	04	07(08)	00	07	09	01	00(≠)	01	≠	≠
	10	02	05(05)	03	09	11	02	03(03)	≠03	07	09	10	32(32)	18	47	56	05	08(11)	00	08	14	04	01(≠)	03	01	≠
	25	02	05(05)	06	12	06	≠	04(04)	≠04	04	05	≠	29(29)	16	54	31	03	09(09)	04	07	09	03	≠(≠)	0	00	00
	75	04	06(07)	09	14	09	03	05(05)	≠05	12	07	21	34(34)	24	74	47	05	10(11)	02	10	13	01	00(≠)	0	≠	≠
Sept.8	0	02	04(04)	05	08	07	≠	03(02)	01	05	04	≠	09(08)	19	32	26	03	06(06)	04	06	07	≠	02(01)	01	04	00
	10	03	04(04)	06	05	05	02	04(04)	03	03	04	≠	31(31)	22	02	25	01	08(08)	05	02	08	≠	≠(≠)	≠	02	00
	25	02	03(03)	05	07	05	08	02(02)	00	≠	04	08	15(15)	14	03	25	00	05(06)	02	02	11	01	01(01)	02	01	00
	50	04	04(04)	08	06	06	≠	02(02)	04	09	05	≠	13(13)	17	15	29	11	06(08)	05	11	08	05	02(00)	00	≠	00
	75	03	03(03)	07	06	06	02	01(01)	01	04	05	≠	07(07)	24	09	34	01	03(06)	03	02	08	02	02(01)	01	01	00