PLANKTON VOLUME LOSS WITH TIME OF PRESERVATION

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The investigation of plankton volume decrease with time of preservation is an outgrowth of an investigation into the constituent composition of plankton samples collected in 1957, a year of marked change in the seasonal pattern of plankton volumes surveyed on cruises of the California Cooperative Oceanic Fisheries Investigations (Thrailkill 1959, pp. 4-8). The constituent study on the 1957 samples was begun about a year after the samples had been collected and the initial volume determinations made. It was soon noted that the earlier plankton measurements could not be duplicated. Most samples not only contained a lesser volume than that which had been obtained earlier, but percentage decreases were much greater in samples in which non-crustacean plankters predominated. The difficulty in duplicating plankton volume measurements had been noted as early as 1949, but the magnitude of the volume loss was not appreciated until the systematic remeasuring of volume was begun on the 1957 collections.

The non-replicability of volume measurements stimulated us to make a quantitative study of plankton volume change with time of preservation. The investigation was carried out on a group of 12 test samples, (Table 1) selected to provide a variety of constituent compositions. Volumetric measurements were made on the live collections, on the samples immediately after preservation, and at intervals thereafter until the samples showed little or no further decrease in volume with time; i.e. had reached their stable preserved volumes. The time spacing of observation was empirically determined by the rates of change observed (Table 2).

TABLE 1. COLLECTION DATA FOR PLANKTON HAULS USED IN VOLUME LOSS STUDY[1]

Cruise	Station	Position		Date (1959)	Hour P.S.T.		Vol. water strained ($m.^3$)	Depth of haul (m.)	Depth of water at station (fms.)	Temp. at 10 m. Depth °C
		Lat. N.	Long. W.		Start	End				
5902	110.33	29°50.5′	115°52.2′	II-14	1248	1257	279	0–84	50	16.3
	110.65	28°46.5′	117°59′	II-13	1911	1926	492	0–141	1800	17.3
	113.30	29°22.5′	115°17.5′	II-14	1732	1737	194	0–50	30	—
	113.40	29°02′	115°58.5′	II-14	2231	2246	475	0–142	960	16.8
	113.50	28°42′	116°37.5′	II-15	0526	0541	487	0–142	1750	17.1
	120.35	28°03′	114°54′	II-17	1253	1301	282	0–58	45	16.9
	123.42	27°17.2′	115°00.3′	II-19	1846	1901	465	0–140	650	18.3
	130.35	26°19′	113°48.5′	II-21	2216	2230	478	0–138	140	18.8
5903	133.30	25°55′	113°07.6′	III-22	0920	0934	472	0–141	104	19.0
5907	107.50	29°50.5′	117°23.5′	VII-27	0831	0846	513	0–137	1450	19.3
	107.55	29°40.3′	117°43′	VII-27	0546	0601	508	0–133	1600	20.2
	110.90	28°01′	119°36′	VII-26	0216	0231	544	0–130	2050	21.2

[1] Hauls taken obliquely with a standard CalCOFI plankton net, 1.0-meter in diameter at mouth, approximately 5 meters in length, and constructed of No. 30xxx grit gauze.

[1] This paper is a revised version of a manuscript presented at the ICES Symposium on Zooplankton Production, 1961 as contribution no. 12. Abstract in Rapp. et Proc.-Verb. 153:78.

TABLE 2. MEASUREMENTS OF VOLUMES OF 12 PLANKTON SAMPLES TO DETERMINE CHANGE IN VOLUME WITH TIME (in milliliters)

Cruise	Station	Before pres.	After pres.	Days after preservation							Months				
				1	2	3	7	10	16	20	1	2	4	12	24
5902	110.33	—	38	38	37	37	37	37	35	35	35	35	35	35	35
	110.65	57	47	30	30	30	—	24	24	24	24	24	24	20	20
	113.30	12	10	10	10	10	10	10	10	10	10	10	10	10	10
	113.40	50	42	40	39	—	—	35	35	35	34	34	34	32	32
	113.50	84	63	38	—	—	—	35	34	33	33	33	33	32	32
	120.35	13	12	12	12	—	11	11	11	11	11	11	11	11	11
	123.42	129	61	41	39	—	29	26	26	26	26	24	24	23	23
	130.35	25	19	15	15	—	13	13	13	13	13	13	13	12	12
5903	133.30	48	34	25	25	25	25	25	25	25	25	25	25	24	24
5907	107.50	93	60	37	—	30	23	21	20	18	17	15	14	13	*12
	107.55	42	26	16	16	16	15	14	13	13	13	13	12	10	*10
	110.90	77	48	38	35	33	31	31	31	31	30	30	29	28	*27

* 22 months after collection.

After the samples had attained comparative stability with respect to volume loss, they were intensively analyzed in order to relate volume loss to the constituent compositions of the test samples. The percentage composition by volume of the major groups of plankton organisms in each test sample was determined; the kinds (species) of organisms were identified and enumerated; the proportion of the displacement volume of each sample that was due to included interstitial liquid was determined; the amount of dry substances, organic substances, ash and nitrogen was determined per gram of plankton without interstitial liquid.

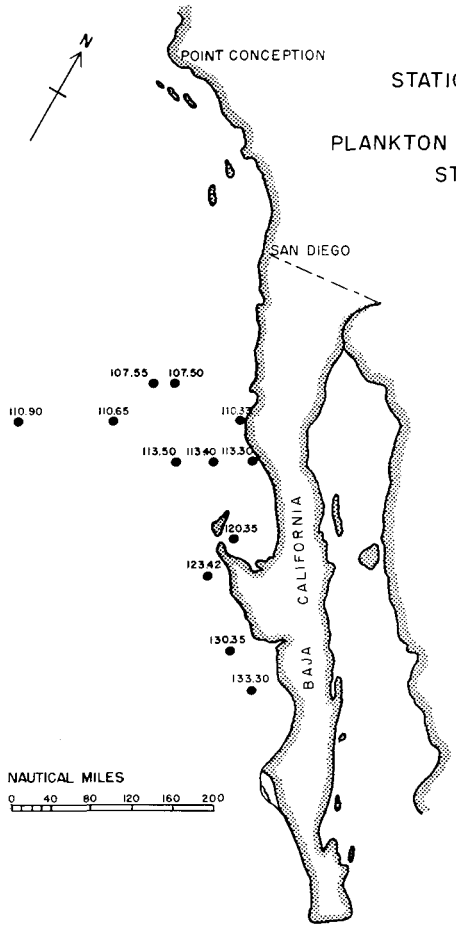

FIGURE 1. Stations at which samples were taken for plankton volume loss studies.

The test samples were collected off central Baja California (Fig. 1), eight by the junior author in February, 1959, one in March, 1959, and the remaining three in July, 1959. Water temperatures in 1959 were warmer than usual; plankton volumes were the smallest in a decade. Some groups of organisms that had been conspicuous constituents of the plankton in previous years were absent from the 1959 samples. Among these, the most interesting group was the pyrosomes. These tunicates had become the dominant organism in many hauls made off central Baja California during the mid-1950's particularly from August, 1955 through April, 1957. Previous to their emergence as a dominant plankton constituent, pyrosomes had been so rare as to be curiosities; by 1958 they again had become uncommon and none was taken in the samples reported upon in this paper. Ctenophores also were absent from the test samples. The three test samples taken in July, 1959, were selected in order to have an adequate representation of samples containing salps and doliolids in the volume-loss studies; these groups also were markedly less abundant in 1958 and 1959 than during previous years.

The method of measuring displacement volume of "wet" plankton was kept uniform throughout the experiment. The total volume, plankton with its preserving liquid was measured, the plankton was then separated from its preserving liquid, and the volume of the latter determined. The plankton volume, hence, was the difference between the two measurements. The consistency of the measurements is one gauge of their reliability. Problems associated with "wet" volume determinations are discussed in a later section.

The percentage composition by volume of the major constituents of the twelve test samples is given in

TABLE 3. PERCENTAGE COMPOSITION BY VOLUME OF MAJOR PLANKTON CONSTITUENTS

Constituents	Cruise 5902								Cruise 5903	Cruise 5907		
	110.33	110.65	113.30	113.40	113.50	120.35	123.42	130.35	133.30	107.50	107.55	110.90
Crustaceans												
Copepods	81	19	68	25	8	77	8	38	20	6	11	19
Euphausiids	*	19	*	11	11	1	18	12	1	2	3	30
Decapod larvae	9	*	4	2	*	11	9	30	25	1	1	17
Ostracods	—	*	*	*	*	—	*	*	*	*	1	*
Amphipods	—	*	—	*	*	—	*	—	—	1	*	*
Other Invertebrates												
Chaetognaths	10	9	25	42	5	10	8	8	45	10	10	16
Siphonophores	*	47	*	8	70	1	*	5	*	21	26	12
Medusae	—	*	—	—	—	—	1	—	—	4	5	—
Salps and Doliolids	*	*	*	*	*	—	48	—	—	52	40	3
Larvaceae	*	*	*	*	*	*	*	1	1	1	1	*
Molluscs	*	6	*	9	6	*	8	1	8	1	*	1
Fish Eggs	*	—	3	*	*	*	*	5	*	*	*	*
Fish Larvae	*	*	*	3	*	*	*	*	*	1	2	2

* Present, volume less than 0.5%;—no individuals of category observed.

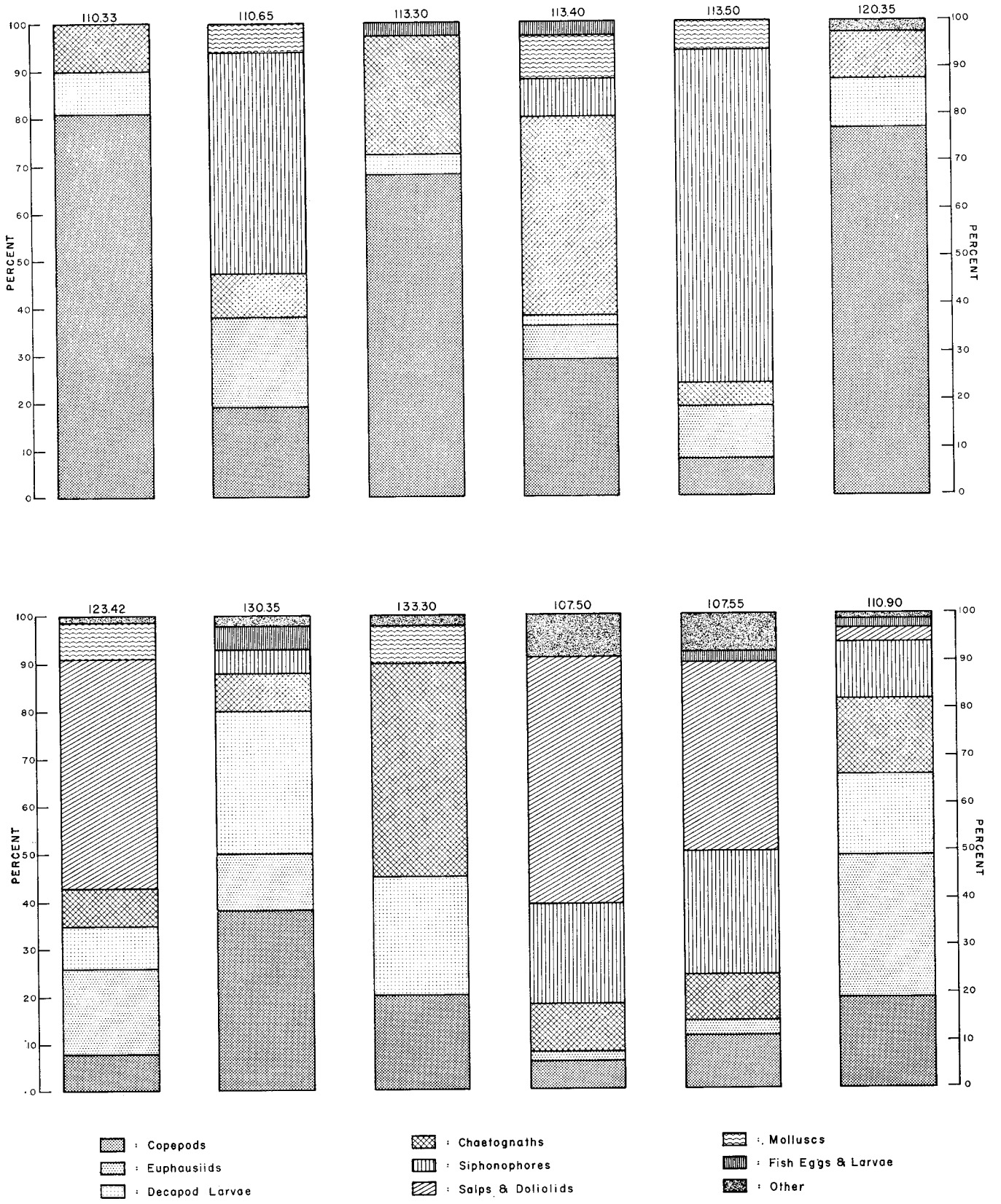

FIGURE 2. Diagrammatic representation of constituent compositions of test samples expressed as percentage of volume one year after collection.

table 3 and Figure 2. Crustacean constituents are grouped under five categories: copepods, euphausiids, ostracods, amphipods and decapod larvae. The important non-crustacean invertebrate constituents are placed in six categories: chaetognaths, siphonophores, medusae, salps and doliolids, larvaceae, and pelagic molluscs. The only vertebrate categories included are the planktonic stages of fish development—fish eggs and larvae. The volumetric determination of the major constituents was made a year or more after collection, at a time when the preserved volumes had attained comparative stability. This is an important point to keep in mind. Had the determination been made within the first few days of collection, the percentage composition would have been quite different—higher for salps and siphonophores, lower for crustacea, molluscs, and chaetognaths. Had the determination been made before preservation, it would have been more markedly different yet.

Volume shrinkage at preservation.

A striking change in the volume of plankton samples occurs at preservation (Fig. 3). The initial determination of wet volume was made on freshly collected material. Immediately following this measurement, the sample was preserved with three percent buffered formaldehyde solution. A measurement of the preserved volume was made within 10 to 15 minutes of preservation.

Information on volume loss at preservation is available for 11 of the 12 samples. Immediate shrinkage in volume at preservation ranged from seven percent to 53 percent of the live volumes. In only one sample was the percentage loss at preservation as little as ten percent, in only one was it as much as 50 percent; of the other nine samples, three had losses of 11 to 20 percent of the live volume, three had decreases of 21 to 30 percent, and three of 31 to 40 percent.

Volume loss during the first day after preservation.

The rapid shrinkage of the volume of many samples continued during the first 24 hours after preservation. Five samples showed a loss of between 32 percent to 40 percent of the initial preserved volume at the end of one day of preservation; in these, siphonophores and/or salps-doliolids predominated. Three samples showed no volume loss during this period: All three samples were composed almost entirely of crustacaens and chaetognaths. The remaining four samples showed intermediate losses of from five percent to 26 percent; in these samples crustaceans and chaetognaths predominated, but siphonophores and salps made up a part of the volume, except in the sample from station 5903—133.30. The rapid shrinkage of plankton organisms during the first day following preservation probably results from a water loss, especially marked in jelly-like constituents.

Several measurements were made on most samples during the first 24 hours of preservation, but the pattern of observation was not uniform enough from sample to sample to permit their incorporation into table 2. The sample from station 5907-107.50 had the most marked volume decrease of any in the series. After 78 hours of preservation, the volume of the sample was less than a third of the live volume. The decrease noted at intervals during this period was as follows:

Sample from 5907-107.50

Time of observation	*Vol. (ml.)*
Before preservation	93
After preservation ____ 10 min.	60
" " ____ 1 hour	56
" " ____ 4 hours	50
" " ____ 11 hours	41
" " ____ 24 hours	37
" " ____ 78 hours	30

This sample has shown a volume loss on each subsequent measurement. The final measurement (12 ml.), made 22 months after collection, was only 13 percent of the original live volume. Even after one year of preservation, salps and doliolids made up over 50 percent of the volume of this sample. Originally they may have constituted as much as 95 percent of the total volume (discussed in concluding section of paper).

The volume loss in the sample from station 5903-133.30 is different than that for any other sample in that there was a considerable shrinkage at and immediately following preservation but the sample soon reached an equilibrium volume:

Sample from 5903-133.30

Time of observation	*Vol. (ml)*
Before preservation	48
After preservation ____ 12 min.	34
" " ____ 3 hours	25
" " ____ 7 hours	25
" " ____ 24 hours	25
" " ____ 2 years	24

The equilibrium volume was reached within three hours of preservation. The change in volume of this sample during the succeeding two years amounted to only 1 ml. or four percent. With regard to the constituent composition of the sample—46 percent by volume consisted of crustacean plankton, 45 percent of chaetognaths and 8 percent pelagic molluscs. Since the exoskeleton of crustacea and the shells of pteropod molluscs prevent marked shrinkage (except for withdrawal of molluscs within their shells), the major adjustment in volume at this station must have occurred in the chaetognaths.

Volume loss subsequent to the first day of preservation.

The volume at one day after preservation is taken as the standard by which to gauge the subsequent shrinkage in plankton volumes of preserved samples. The rapid rate of shrinkage, observed for most samples at preservation and during the first 24 hours thereafter, was markedly slowed down after a day of preservation. Some samples showed little or no volume loss after the first day, others showed a continuing but decelerating decrease with time.

The percentage loss in plankton volumes from the reference volume at one day after preservation is shown for four time intervals in table 4: 10 days, 30

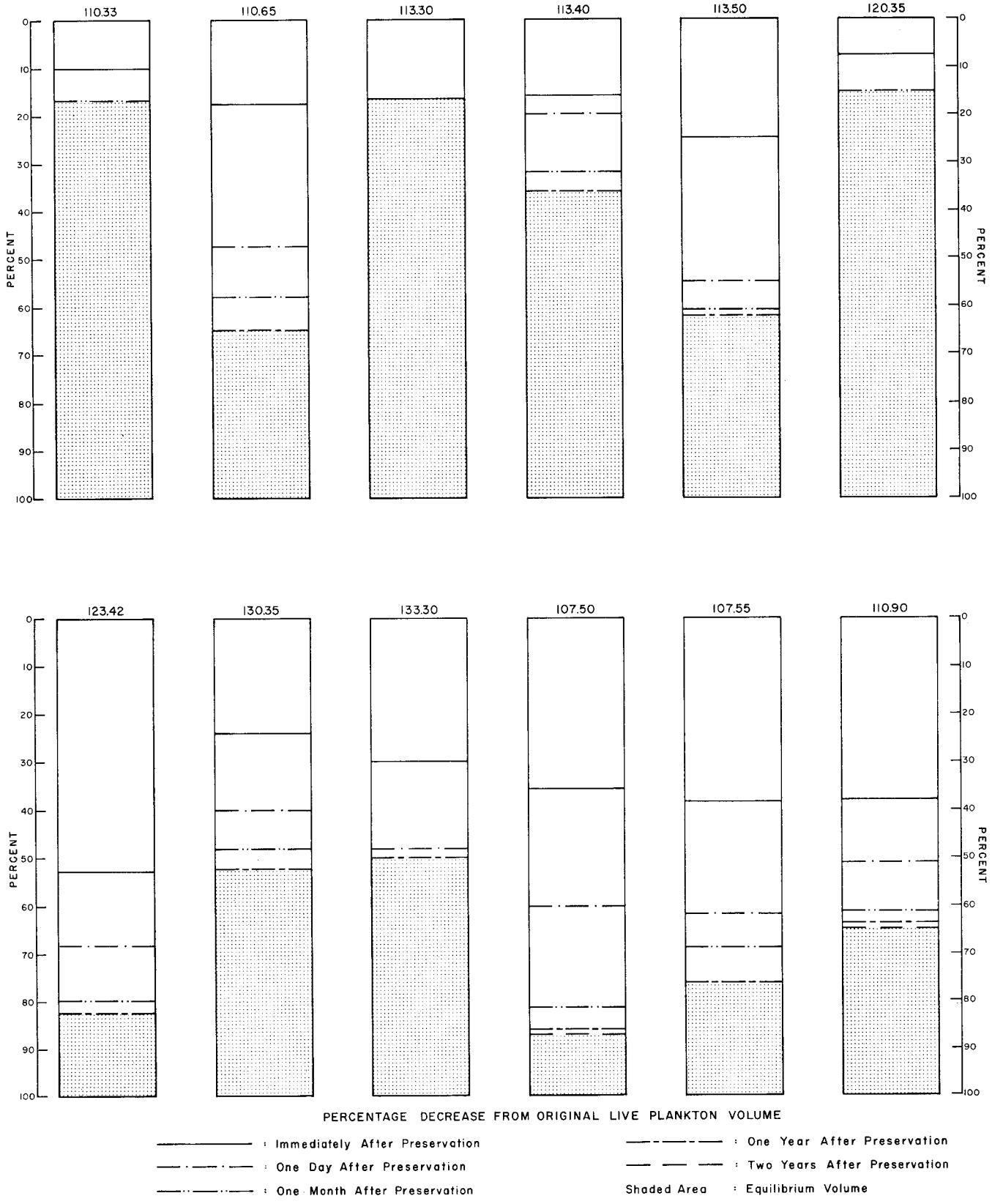

FIGURE 3. Temporal decrease in volumes of test samples, expressed as percentage of their live volumes.

days, one year, two years. The samples are arranged in this table according to increasing volume loss. In order to relate loss in volume to constituents, the gross composition of each sample is indicated under four categories: crustaceans, chaetognaths, coelenterate-thaliaceans and other constituents.

Four samples showed little or no volume decrease (zero to eight percent) over a two-year period. These samples contained less than one percent by volume of coelenterate-thaliacean constituents and 90 percent to nearly 100 percent of crustacean and chaetognath plankters.

At the other extreme, four samples showed losses of between one-third and two-thirds of their reference volumes. In these samples coelenterate-thaliacean constituents made up 47 percent to 78 percent of the volumes (after one year of preservation).

The samples that were intermediate in volume loss, had a preponderance of crustacean-chaetognath constituents, except for the sample from station 5902-113.50. This latter sample, in which siphonophores made up 70 percent of the volume, had only a moderate volume loss. It is commented upon more fully in a later section.

Constituents

The discussion thus far has dealt with the pattern of temporal decrease in plankton volumes as related grossly to dominant constituents. The specific composition of the samples is treated in table 5. It has been necessary to deal with numbers of individuals rather than their volumes in this table. The table has been assembled with the help of a number of scientists (see Acknowledgements). Specific identifications are not available for several minor constituent groups including ostracods, larvaceae, and some decapod larvae. Ctenophores were absent from the samples, annelids nearly so.

Copepods

Copepods were the dominant constituent in three of the four samples that showed the least volume loss with time (Table 3). The three "copepod" samples were collected near the coast, at stations having depths of 50 fathoms or less (Table 1).

Calanus helogolandicus was the most abundant species in two of the three samples (Table 5). In the sample from station 5902-110.33 it outnumbered the combined total of all other copepods by nearly five to one, while at station 5902-120.35 it was nearly as numerous as all other copepods. At station 5902-113.30, *Calanus helgolandicus* was outnumered by *Paracalanus parvus,* but was still the dominant species in volume. It is interesting to note that although these three samples contained a larger number of copepods than the other test samples, they contained fewer species per sample. Copepods made up 6 percent to 38 percent of the volumes in the other test samples.

Euphausiids

This group contributed significantly to the volumes of six samples taken at night (11 to 30 percent) but was a minor element in day hauls (one to three percent). Undoubtedly this resulted from the vertical movement of larger individuals into the stratum sampled at night. Although there is no reason to assume that euphausiids would decrease appreciably in volume, if at all, during preservation, they were associated with samples that showed moderate to heavy volume losses. *Euphausia eximia* and *Nyctiphanes simplex* were the most consistently abundant species; *Nematoscelis difficilis, Euphausia gibboides, Stylocheiron affine,* and *Euphausia recurva* were important constituents in one or more of the samples.

TABLE 4. COMPARISON OF PLANKTON VOLUME LOSS WITH CONSTITUENT COMPOSITION OF SAMPLES

Cruise and Station	Percentage Loss in Plankton Volume from Volume at 1 Day After Preservation — Time Interval				Constituent Composition Expressed as Percentage of Volume at 1 Year After Collection			
	10 Days	30 Days	1 Year	2 Years	Crust.	Chaet.	Coel.-Thal.	Other Const.
5902—113.30	0	0	0	0	74	26	*	*
5903—133.30	0	0	4	4	46	45	1	8
5902—110.33	3	8	8	8	90	10	*	*
5902—120.35	8	8	8	8	89	10	1	*
5902—113.50	8	13	16	16	19	5	70	6
5902—130.35	13	13	20	20	80	8	6	6
5902—113.40	12	15	20	20	38	42	8	12
5907—110.90	18	21	26	**29	66	16	15	3
5902—110.65	20	20	33	33	38	9	47	6
5907—107.55	12	19	38	**38	16	10	72	2
5902—123.42	37	37	44	44	35	8	49	8
5907—107.50	43	54	65	**68	10	10	78	2

* Present, volume less than 0.5%.
** 22 months.
Abbreviations in Table 4: Crust., Crustaceans; Chaet., Chaetognaths; Coel., Coelenterates; Thal., Thaliaceans; Other Const., Other Constituents.

TABLE 5. SPECIES COMPOSITIONS OF PLANKTON SAMPLES USED IN VOLUME–LOSS STUDY

Crustacean Constituents	Cruise 5902								Cruise 5903	Cruise 5907		
	110.33	110.65	113.30	113.40	113.50	120.35	123.42	130.35	133.30	107.50	107.55	110.90
Copepods												
Acartia negligens				100		250						100
A. tonsa			1200			1250						
Calanus gracilis					100							100
C. helgolandicus	33800	400	3800	3200		14500	300			100	100	600
C. minor		300	200	900	100	250	600	100	250			200
C. tenuicornis	250	200		200	300			200	250	100		
Calocalanus styliremis	250						200					
Candacia aethiopica										100	100	200
C. bipinnata										200	200	100
C. curta	1000			100		250		100	250			
C. simplex										100		
C. spp.		100		100							200	200
C. truncata				100								
Centropages elongatus												100
C. spp.											100	
C. violaceus											100	100
Clausocalanus furcatus		400	600	300		1750		500	250	300	100	300
C. spp.	2250	1500	1600	2200	900	2500	1200	2800	4500	900	200	200
Corycaeus spp.	500	300	200		100	1000	100	100		200	100	300
Ctenocalanus vanus		200	1400				300			200	100	100
Euaetideus australis		100		100					250	100	100	
Eucalanus californicus									250			100
E. crassus				100					250			
E. pileatus				200			200					
Euchaeta acuta				200	100			500				100
E. longicornis		100						400	500			
E. marina												100
E. media												100
E. spp.		200		200	100		200	200	500	100		
Euchirella pulchra												100
Gadius pungens				200							100	
Haloptilus acutifrons									250			100
H. longicornis				200						200		
H. spiniceps										100		100
Heterorhabdus papilliger				500	100		100	100				
H. spinifrons								100				
Heterostylites sp.								100				
Labidocera acutifrons								100				
L. trispinosa			200									
Lucicutia flavicornis		100							500			
L. ovalis		100										
Mecynocera clausi		300		100		250	200			100		
Metridia pacifica				100								
Oncaea spp.					100		600	200		500	400	400
Oithona spp.		200	200	100	200	3250	500	300	250	200	100	100
Paracalanus denudatus		400	200	500		250	200	2800	250			
P. parvus	2000		8200		300	4750	200	200	2000			
Pleuromamma abdominalis		400		700	300			700			200	800
P. borealis		900		1500	1900		100	100				1300
P. gracilis		400			100		200	400	1250			100
P. spp.		100		100	200		100	100				
P. xiphias												200
Rhincalanus nasutus	250							300				
Scaphocalanus echinatus							100				100	
Scolecithricella abyssalis												100
S. dentata												
S. subdentata		300		300								
Scolecithrix danae				100								
Scottocalanus persecans				100								
Temora discaudata				500		500						
Undeuchaeta sp.				100								200
Unid. Calanids	1000	700	2200	800	200	250	500	200	250	100		200
Copilia mirabilis		65	4	32	8						100	88
Sapphirina sp.											276	16
Euphausiids												
Euphausia eximia A.		8		0	8		40	0			20	96
" " J.		416		40	16		24	4				
" " L.		168		376	0		24	20		100	92	
E. gibboides A.		8			8		24					
" " J.		40					8	3			16	216
" " L.		40					0			228	92	8
E. hemigibba A.		80			1							
" " J.		40									16	
" " L.												16
E. recurva A.		32			40							304
" " J.		360			144						12	264
" " L.					8							0
Nematobrachion flexipes A.		1			1			4				8
" " J.								12				8
" " L.				8								
Nematoscelis difficilis A.		8		0	24		0	1				80
" " J.				40	16		24	11	16			24
" " L.		40		112	16		0	4				
N. gracilis L.		24										

TABLE 5. SPECIES COMPOSITIONS OF PLANKTON SAMPLES USED IN VOLUME—LOSS STUDY—Continued

Crustacean Constituents		Cruise 5902								Cruise 5903	Cruise 5907		
		110.33	110.65	113.30	113.40	113.50	120.35	123.42	130.35	133.30	107.50	107.55	110.90
Euphausiids—Continued													
N. tenella	A.					1							
" "	J.		1										8
" "	L.										4	12	
Nyctiphanes simplex	A.				16	2	4	32	73				
" "	J.		24		24	8	32		288	58	88		
" "	L.		56		216	32	8	64	216	49	40		
Stylocheiron affine	A.		40		56	8		40	2				8
" "	J.		96		88	88		8	12	8	12	4	24
" "	L.		40		48	48		0	4				0
S. maximum	L.				8								
Thysanoessa gregaria	A.		1		8	1							
" "	J.								4				
Thysanopoda aequalis	L.											8	16
Ostracods													
Conchoecia sp.		0	296	12	504	108	0	40	68	96	84	36	104
Other					24						28	164	40
Amphipods													
Eupronoe minuta					3	4							
Eusiropsis riisei			4										8
Hyperia schizogeneios											8		
" sp.											4		
Hyperoche mediterranea			4										
Paraphronima crassipes												4	
Phrosina semilunata					10								
Platyscelus serratulus			2										
Primno macropa					3	4							
Pronoe capito								8					
Streetsia challengeri											4		
Viblia armata			3					32					
Viblia stebbingi			3										
Decapod larvae													
Panilurus interruptus												1	
Pleuroncodes planipes		10400	24	3720	2320	24	3200	2728	640	4300	4		72*
Brachyuran								8	2	16			
Caridean						8					20	36	
Pasiphaeid									12*				
Sergestid											84	28	64
Stomatopod							4	8	15				
Other									1				
Other Invertebrates													
Chaetognaths													
Sagitta bierii		50	175	1	440	204		168	112	656	36	19	900
S. bipunctata								16			18	4	
S. enflata		500	251	232	780	131	241	456	111	872	21	154	400
S. euneritica		2100		633	50	1	210		2		2		
S. hexaptera					1	1					10	4	
S. minima		450	50	1	350	14	154	32	5	192	23	15	200
S. pacifica			5			8			3	4	6	40	50
S. pseudoserratodentata			18		250	31		160	9	6	197	189	900
Krohnitta subtilis			6		78	6			5	192	1	3	
Pterosagitta draco			4			2			3		2		
Siphonophores													
Abylopsis eschscholtzii											4		
Agalma okeni	Nect.		97										
" "	Br.		182								4	4	
Bassia bassensis	Sup. N.		17		10	1					118	108	51
Chelophyes appendiculata	Sup. N.				8						52	29	17
" "	Inf. N.										4		
Diphyes bojani	Eud.		78		96	4					79	407	992
Eudoxoides spiralis	Nect.		280		200				3		114	709	1513
" "	Eud.							16				8	8
E. mitra	Eud.												
Lensia campanella	Sup. N.				17	1		16	11	2	2		
L. challengeri	Sup. N.							8	1				
" "	Inf. N.								9				
" "	Eud.		9		8						10	6	10
L. subtiloides	Sup. N.		1									1	
" "	Inf. N.	8			9		80		2	8	8		
Muggiaea atlantica	Nect.			8			8			16			
" "	Eud.					73							
Nectodroma reticulata	Br.					50							
" "	Gon.					2							
" "	Polyg.							1					
Halistemma rubra	Br.				1						1	6	
Sulculeolaria sp.	Inf. N.												
Medusae													
Aglantha digitalis								72			24	120	
Liriope tetraphylla											504	84	
Rhopalonema velatum			8					8				8	

* Juveniles.

TABLE 5. SPECIES COMPOSITIONS OF PLANKTON SAMPLES USED IN VOLUME—LOSS STUDY—Continued

Crustacean Constituents	Cruise 5902								Cruise 5903	Cruise 5907		
	110.33	110.65	113.30	113.40	113.50	120.35	123.42	130.35	133.30	107.50	107.55	110.90
Salps and Doliolids (Thaliacea)												
Doliolid oozooid	3	20		33	26					216	97	274
Doliolum denticulatum Gonzo.										14		10
" " Phozo.										6		16
" " S.N.G.	3	4	2								16	
Doliolum gegenbouri S.N.G.										10	1	
Pegea confoederata Agg.							272					
Ritteriella picteti Agg.							24					
Thalia democratica Agg.										424	574	70
" " Sol.										72	72	8
Molluscs												
Cavolinia inflexa		2		6	5					2	4	
Creseis virgula		1		28		4			15	5		4
Cuvierina columella									1			1
Limacina inflata	8	5010	52	11860	4420	48	4000	352	2410	232	12	630
L. trochiformis		4		2		8	45	23	640			1
Peraclis sp.										1		
Desmopterus sp.										1		
Atlanta leusueri												6
A. peroni		11							55	35		12
A. sp.	8			42		8	3				12	
Pterygiotenthis sp.										1		
Pyrogopsis sp.										2		
Vertebrates												
Fish Eggs												
Sardinops caerulea	7		12			10						
Engraulis mordax	40	1	469	2		1			3			
Etrumeus acuminatus								40				
Leuroglossus stilbius	1			3								
Bathylagus wesethi										20	8	23
Argentina sialus				3			2					
Nansenia sp.					2					1		
Vinciguerria lucetia	39				2		5			122	16	162
Stomias atriventer				2	1		12			4	2	
Synodus lucioceps						1		1				
Cololabis saira										2		
Citharichthys spp.	79		8			74		8	1			
Paralichthys californicus			13					23				
Pleuronichthys verticalis	1		2			1		1				
Merluccius productus	117		9	15			11	115	4			
Trachurus symmetricus										2		
Chiasmodon sp.											1	
Lepidopus xantusi							6	152	4			
Other	3		11	3	5	1	6	7	13	5	21	3
Fish Larvae												
Sardinops caerulea	1		3	1				1				
Engraulis mordax	45		48	414		14	25	4	4			
Bathylagus wesethi										3		6
Nansenia sp.									1			
Vinciguerria lucetia		14		9	10		1	10	2	43	101	170
Cyclothone sp.				2	1		1				2	6
Argyropelecus sp.				2								
Stomias atriventer				1					1			
Macroparalepis macrurus											2	
Ceratoscopelus townsendi												7
Diogenichthys atlanticus				1								
D. laternatus		3		7	2			3		1		5
Electrona crockeri				4								2
Gonichthys tenuiculum							1				1	4
Hygophum atratum				1								1
Lampanyctus mexicanus		3		3			2			8	9	155
L. ritteri		1		9	1					1		
Myctophum californiense										2		
M. margaritatum											2	
Melamphaes spp.		2									3	3
Citharichthys spp.	2		1	5		2	1					
Paralichthys californicus	1											
Pleuronichthys verticalis			1									
Merluccius productus	9		1	4				7	37			
Trachurus symmetricus										1		
Cynoscion sp.			1									
Chiasmodon sp.		1		1								
Lepidopus xantusi				1								
Sebastodes spp.	10		9	26				1				1
Other					1							

Abbrevations in Table 5:

A—Adults
J—Juveniles
L—Larvae
Nect.—Nectophore
Sup. N.—Superior Nectophore
Inf. N—Inferior Nectophore
Br.—Bract
Eud.—Eudoxid
Gon.—Gonophore
Polyg.—Polygastric stage
Gonzo.—Gonozooid
Phozo.—Phorozooid
S.N.G.—Stage not given
Agg.—Aggregate
Sol.—Solitary

Decapod larvae

Decapod larvae were important constituents in the samples studied; they made up 9 percent to 30 percent of the volumes in half of the samples and were present in all. The four samples that showed the least change in volume with time contained 4 percent, 25 percent, 9 percent and 11 percent by volume of decapod larvae, with *Pleuroncodes planipes* contributing all or most of the volume in each instance. The sample in which decapod larvae made the largest percentage contribution to the volume (5902-130.35) contained young of pasiphaeid shrimp. In only this instance did a decapod other than *Pleuroncodes* contribute significantly to test sample volumes.

Chaetognaths

Chaetognaths were consistently important constituents in the test samples; in a third of the samples their volumetric contribution ranged between 16 percent and 45 percent, in another third it was ten percent, and in the remaining third the contribution was between five percent and nine percent.

Chaetognaths were important constituents in the four samples that showed the least volume loss subsequent to the first day of preservation. The sample from station 5902-113.30, containing 26 percent by volume of chaetognaths, showed no loss during this time period and the sample from station 5903-133.30, containing 45 percent by volume of chaetognaths, suffered only a four percent reduction in volume. Thus chaetognaths appear to conserve their "preserved" volume.

We have, however, previously commented upon the fact that there must be a rather marked volume loss in chaetognaths at preservation. At least this is our interpretation of the volume loss in the sample from station 5903-133.30 at preservation. The "preserved" volume is just slightly more than half the "live" volume of the sample.

Chaetognaths made up 45 percent of the preserved volume. The only important constituent groups in this sample other than chaetognaths were crustacea and molluscs, both of which have an external skeleton that precludes shrinkage. The most abundant species of chaetognath in this sample, *Sagitta enflata*, is also the largest and the flabbiest. Most of the initial volume loss in this sample may be attributed to this species.

Siphonophores

The physonectid siphonophores fragment on collection, and the counts of nectophores and bracts, consequently, represent only parts of colonies. The less complex calycophorid siphonophores invariably had the inferior nectophores (if developed) separated from the superior nectophore in the polygastric stage and usually had bracts separated from gonophores in the eudoxid stage. The two types of nectophores are separately tabulated but counts (with one or two exceptions) of the eudoxid stage are based on counts of gonophores.

Siphonophores were an important constituent in over half of the test samples and the dominant constitutent in two. These latter samples offer an interesting contrast. The dominant species in the sample from station 5902-110.65 was *Agalma okeni*. The nectophores of this species have now collapsed, the bracts have become limp. The sample had lost a third of its reference volume after 2 years of preservation. The sample from station 5902-113.50, containing a higher percentage of siphonophores, had lost only a sixth of its reference volume. The important species in this sample was *Nectodroma reticulata*, present in both the polygastric and eudoxid stages. Two polygastric specimens made up half the volume of this sample. These two specimens were removed and separately measured after the tenth day. They showed no subsequent volume loss. The bracts and gonophores of the eudoxid stage of this species occurred in much larger numbers, but occupied considerably less volume.

Of the remaining species of siphonophores in the test samples, some hold up less well in preservation than others. Nectophores of *Muggiaea atlantica* collapsed. On the other hand, *Eudoxoides spiralis* held its shape well. *Chelophyes appendiculata*, also stood up well, even with repeated handling. There can be little doubt that much of the volume loss in siphonophores results from collapse of nectophores, bracts, and gonophores, and that the amount of volume loss is variable from species to species.

Thaliacea

Salps and doliolids did not contribute significantly to the volumes of the test samples collected in February (5902), except at station 5902-123.42. For this reason, several additional samples containing salps and doliolids were selected and included in the test series during the July cruise.

The three samples which showed the largest volume loss during preservation contained from 40 percent to 52 percent by volume of salps and doliolids after one year of preservation. In two of these, 5907-107.50 and 5907-107.55, the dominant constituent was the salp, *Thalia democratica*, in the other sample, from 5902-123.42, the dominant constituent was the salp, *Pegea confoederata*.

Mollusca

The pelagic molluscs in the test samples were almost all shelled forms that retained their "live" volume, except for withdrawal into their shells. Only one species *Limacina inflata* was common to abundant in most samples, accounting for most of the volumes shown for mollusca. *Limacina trochiformis* was common in only one sample, although it occurred in half of the test samples. *Atlanta* spp. occurred in most samples but did not contribute significantly to the volumes.

Fish eggs and larvae

Fish larvae shrink on preservation, but soon come to an equilibrium volume. Fish eggs shrink but slightly, if at all. Neither would contribute significantly to volume loss after the first day of preserva-

tion. Fish larvae made up one percent to four percent of the volumes in four samples. Most of the contribution was made by *Engraulis mordax* larvae in sample 5902-113.40 while in the other three samples the important constituent was *Vinciguerria lucetia* larvae. Fish eggs made up three percent of the volume in station 5902-113.30 and five percent of the volume in station 5902-130.35. In the former, eggs of *Engraulis mordax* predominated, in the latter, those of *Lepidopus xantusi.*

"Wet" displacement volumes of plankton

Throughout the present study, "wet" plankton volumes were determined in a similar manner. The total volume of plankton plus preserving liquid was measured in a graduated cylinder. The sample was then poured into a funnel-shaped silk strainer, the plankton being retained, the preserving liquid being caught in another graduated cylinder. Each sample was allowed to drain for five minutes before a measurement was taken of the preserving liquid. The determination of the "wet" plankton volume was an indirect measurement, based on the difference in the volume of the sample plus its preserving liquid and that of the liquid alone. This type of measurement was utilized in order to keep the handling of the plankton itself to a minimum. In order to minimize loss of liquid by absorption into the screening material the strainer was dampened before use.

The plankton strainer is a cone of No. 56xxx grit gauze attached to a plastic rim. Early experiments had shown that for consistency in volumetric determination, it is essential that the silk strainer be free from contact with other objects. Such contact points often result in "water pockets" which prevent adequate draining.

There is a possibility that the periodic remeasurement of the volumes of the test samples hastens the volume loss in some samples. Volume loss currently is being followed in two collections of salps obtained off southern California in May, 1962. The salps from each collection were separated from the other constituents of the samples before preservation, and divided into two subsamples, one of which has been remeasured five times in four months, the other 24 times. The samples that have been repeatedly measured, show only one to two percent greater volume loss than the samples that have been measured only a few times.

	Percentage decrease in volume during first four months after preservation of sample when remeasured	
Organism	*24 times*	*5 times*
Salpa fusiformis—solitary form	51.5	49.7
Salpa fusiformis—aggregate form	51.2	50.0

Interstitial liquid

A measurement of a drained wet plankton volume consists of two quantities: the plankton itself and the adhering or interstitial liquid. Unfortunately, the latter quantity is far from negligible and is somewhat variable in relative amount from sample to sample.

Investigators have tried to minimize the amount of interstitial liquid by various techniques including blotting, air drying, and vacuum filtration. This problem has engaged the attention of a number of scientists: Ealey 1954, Yentsch and Hebard 1957, Frolander 1957, and Tranter 1960 are recent contributors. Most methods are devised for small samples and are impractical for the large volumes usually obtained on CalCOFI cruises.

We employed a colorimetric technique for determining the amount of interstitial liquid remaining in drained test samples. This technique is based on changes in optical density of an India ink standard that results from dilution by interstitial liquid when a wet plankton sample is added to it (Sutcliffe 1957). The "standard" solution is made up of one part of India ink in 6,000 parts of three percent formaldehyde solution. This is accomplished in two steps: first a stock solution is made up, consisting of one part by volume of India ink and 99 parts of three percent formaldehyde solution; a standard solution is made by mixing one part of the stock solution with 59 parts of a three percent formaldehyde solution. The standard solution is calibrated, using a Klett photoelectric colorimeter. Changes in optical density values that result from progressive dilution of the standard with three percent formaldehyde solution are determined, and a curve prepared.

The drained plankton sample is added to a known volume of standard, the plankton and standard are thoroughly mixed, and a quantity of the liquid is pipetted off. To prevent taking up plankton organisms in the supernatant liquid, the pipette is fitted with a small silk filtering cone. The optical density of the pipetted liquid is then obtained, and the amount of dilution by interstitial liquid is determined from the dilution curve of the standard.

Estimates of the amount of interstitial liquid in the twelve test samples are given in table 6 and Figure 4.

TABLE 6. DETERMINATION BY COLORIMETRIC METHODS OF THE AMOUNT OF INTERSTITIAL LIQUID IN DRAINED "WET" PLANKTON SAMPLES

Cruise & Station	Total volume of drained "wet" plankton (ml.)	Volume of interstitial liquid (ml.)	Volume of organisms only (ml.)	Percentage interstitial liquid
5902				
110.33	35	12.0	23.0	34
110.65	20	5.5	14.5	28
113.30	10	4.0	6.0	40
113.40	32	11.0	21.0	34
113.50	32	5.5	26.5	17*
120.35	11	5.0	6.0	45
123.42	23	7.5	15.5	33
130.35	12	5.0	7.0	42
5903				
133.30	24	8.0	16.0	33
5907				
107.50	12	4.5	7.5	38
107.55	10	4.0	6.0	40
110.90	27	9.0	18.0	33

* Samples from 5902-113.50 not strictly comparable with others (see text).

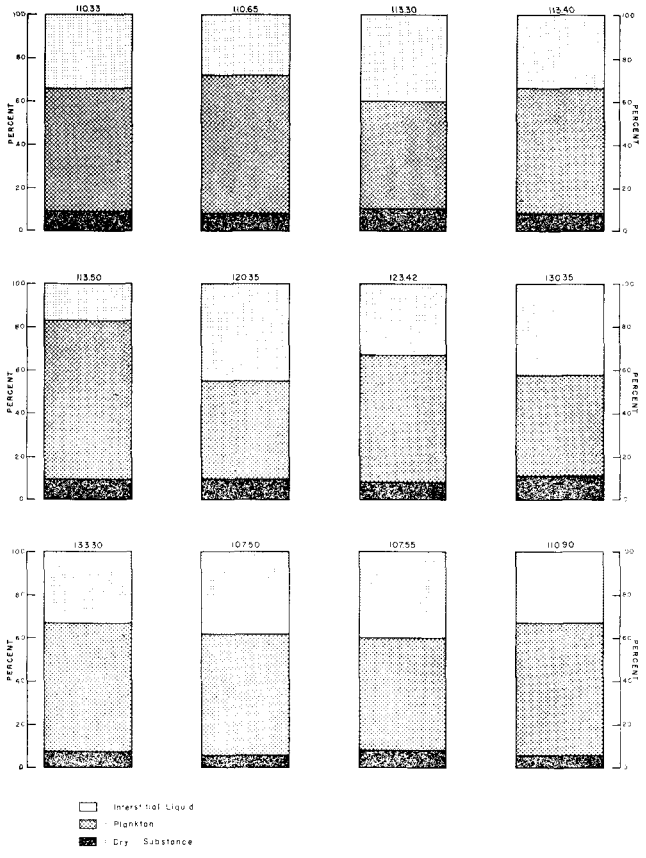

FIGURE 4. Diagrammatic representation of the percentage that interstitial liquid, plankton, and dry substances constituted of the stable preserved volume (weight) measurements of test samples.

The range in values is from 17 percent to 45 percent of the total wet volumes. Except for the sample from station 5902-113.50, no sample had less than one part of interstitial liquid to three parts of plankton and eight of the twelve samples contained between 31-40 percent of interstitial liquid. Several tests were made in order to eliminate adsorption as a possible source of error in our colorimetric determinations. Adsorption was found to be negligible.

The sample from station 5902-113.50 is not strictly comparable to the others, because of the circumstance that half its volume resulted from two specimens of the siphonophore, *Nectodroma reticulata*. Interstitial liquid was separately determined for these as follows:

	Wet vol. (ml.)	Amount of interstitial liquid in ml.	Vol. of plankton alone (ml.)	Percentage of interstitial liquid
Portion containing two specimens of Nectodroma	16.0	0.2	15.8	1
Remainder of sample	16.0	5.3	10.7	33

The portion of the samples containing the two specimens of *Nectodroma* caused practically no change in the optical density of the standard. This observation is interesting for two reasons: it shows how little interstitial liquid may adhere to larger specimens of "jellies", and it affords indirect evidence that adsorption of the colloidal carbon particles of the standard on the surface of the specimens was negligible. The remainder of the sample contained about 33 percent interstitial liquid, hence approximates the median value for interstitial liquid in the other test samples.

Our values of 28 to 45 percent interstitial liquid in the 12 test samples are only slightly higher than those reported by Riley, Stommel and Bumpus (1949) and Frolander (1957). The former investigators removed interstitial liquid by rolling plankton animals on filter paper. They obtained weight losses of between 20 and 30 percent in most samples tested in a group of about 20, but had losses as high as 45 percent. Frolander reported average volume losses of 19.4 percent for one group of 10 samples, 27.5 percent for a second group of 10 samples when interstitial liquid was removed by vacuum filtration.

Removal of interstitial liquid by blotting

The commonest techniques for removing interstitial liquid is by blotting. Determinations of interstitial liquid were made by both blotting and colorimetric measurement on a sample of 2,000 individuals of *Calanus helgolandicus*, separated from 5902-110.33. Dry weight and ash weight determinations also were made on this sample.

Determinations made on 2,000 *Calanus helgolandicus*

Wet volume	2.8 ml.
Interstitial liquid	1.5 ml.
Copepods only	1.3 ml.
Wet weight	2.68 g.
Blotted weight	1.15 g.
Dry weight	0.1694 g.
Ash weight	0.0077 g.
Percent ash/dry weight	4.55%

Interstitial liquid was determined by the colorimetric technique and found to be approximately 54 percent of the wet volume. The sample was washed with formaldehyde solution, allowed to drain for five minutes, and its wet weight determined. Interstitial liquid was then removed by blotting and air drying. The sample was again weighed and the weight loss attributable to interstitial liquid was determined to be approximately 60 percent of the wet weight. Considering the limits of precision of the two techniques, the resulting values for interstitial liquid are closely comparable. However, the values for interstitial liquid are greater than those obtained in mixed plankton samples.

In our experience the blotting technique is most reliable when used on organisms with an exoskeleton. Blotting of samples containing salps or siphonophores can give an overestimate of the interstitial liquid by

TABLE 7. DRY WEIGHT DETERMINATIONS OF PORTIONS OF TEST SAMPLES

Station	Wet[1] weight	Percentage of wet weight due to plankton	Estimated weight of plankton alone	Dry weight (grams)	Ash weight (grams)	Dry substance per gram of plankton (grams)
110.33	3.420	66	2.257	0.215	0.0130	0.095
110.65	4.250	72	3.060	0.260	0.0223	0.085
113.30	1.285	60	0.771	0.085	0.0061	0.110
113.40	6.240	66	4.118	0.340	0.0350	0.083
113.50	2.565	67	1.719	0.170[2]	0.0133	0.099
120.35	1.985	55	1.092	0.110	0.0097	0.101
123.42	2.475	68	1.683	0.140	0.0090	0.083
130.35	2.520	58	1.467	0.170	0.0164	0.116
133.30	1.810	67	1.211	0.095	0.0114	0.078
107.50	1.825	63	1.150	0.065	0.0116	0.056
107.55	1.115	60	0.669	0.055	0.0048	0.082
110.90	2.820	67	1.876	0.180	0.0188	0.096

[1] Wet weight of plankton plus interstitial water.
[2] Two polygastric specimens of **Nectodroma reticulata** excluded from determinations.

removing part of the body liquids from the organisms (overdrying). The volume of the two polygastric specimens of *Nectogroma reticulata* from station 5902-113.50 was reduced to from 17 to 14 ml. by overdrying with a blotter. When put back in preservative these specimens regained their former volume after several days. Overdrying of salps such as *Iasis zonaria* on the other hand, has resulted in a permanent decrease in preserved volume. Overblotting of some kinds of salps and ctenophores has resulted in the physical breakup of specimens. Physical rupture of specimens was more often observed when the technique of vacuum filtration was employed to remove interstitial liquid, however. Vacuum filtration also may induce overdrying of specimens. This technique has to be used with caution.

For most plankton samples, weight and volume are readily convertible: 1 ml. of plankton weighs approximately one gram. This comparison has been made on a number of plankton samples and constituents; of the common constituents only pteropods do not conform. Hence in some parts of the discussion that follows, we have used grams or milliliters of plankton interchangeably. In many of our experiments, determinations of both weight and volume were made.

Dry weight

A dry weight determination of plankton has certain definite advantages. Water is eliminated, both interstitial and that within the organisms, only organic and inorganic substances contained in the bodies of the plankters remain. This determination, therefore, is a more basic one for evaluating the potential food value of the standing crop of plankton than a determination based on wet volume or wet weight.

Dry weight determinations made on fractions of the 12 test samples are given in table 7. These determinations were made 23 to 26 months after collection of the test samples (in June, 1961). The plankton used for dry weight determinations had two categories of organisms removed, pteropods and fish eggs and larvae. The former category was excluded because it was felt that their calcareous shells would make the dry weight determinations hard to interpret. Riley and Gorgy (1948) have shown that the dry weight of pteropods was approximately 83 percent ash and only 17 percent organic matter.

The 12 test samples are arranged in table 8 in descending order with respect to amount of dry substances (D.S.) per gram of plankton. Also contained in this table are organic substances (O.S.) and nitrogen content (ΣN) expressed in mg. per gram of

TABLE 8. COMPARISONS OF DRY SUBSTANCES, ORGANIC SUBSTANCES, ASH, AND NITROGEN CONTENT OF TEST SAMPLES, WITH THEIR CONSTITUENT COMPOSITIONS

Cruise & Station	Per gram of plankton			Per 100 mg. D.S.			Constituent composition (% by vol.)			
	D.S. mg.	O.S. mg.	ΣN mg.	O.S. mg.	Ash mg.	ΣN mg.	Crustacea	Chaet-ognaths	Coelen-terates	Thaliacea
5902–130.35	116	105	13.3	90.4	9.6	11.4	85	9	5	1
5902–113.30	110	102	11.7	92.8	7.2	10.6	74	26	*	*
5902–120.35	101	92	9.7	91.2	8.8	9.6	89	10	1	*
5902–113.50	99	91	13.6	92.2	7.8	13.7	44	11	45	*
5907–110.90	96	86	10.6	89.6	10.4	11.0	68	17	12	3
5902–110.33	95	89	10.8	94.0	6.0	11.4	90	10	*	*
5902–110.65	85	78	9.3	91.4	8.6	10.9	40	10	50	*
5902–113.40	83	74	8.8	89.7	10.3	10.7	43	48	9	*
5902–123.42	83	78	9.5	93.6	6.4	11.4	38	9	1	52
5907–107.55	82	75	10.2	91.3	8.7	12.3	16	10	32	42
5903–133.30	78	69	8.3	88.0	12.0	10.7	50	49	*	1
5907–107.50	56	46	5.3	82.2	17.8	9.5	10	10	25	55

* Present, but less than 0.5% by volume.
D.S., Dry Substances; O.S., Organic Substances; ΣN, Nitrogen Content.

plankton, and in mg. per 100 mg. of dry substances (symbols adopted from Krey, (1958)). For ready comparison of the relation between D.S., O.S., and ΣN values, and constituent composition, the approximate percentage by volume of the four major groups of plankton organisms are listed for each sample.

A further summary of the material contained in table 8 follows:

Dominant organism	No. samples	Per 1 g. plankton		Per 100 mg. D.S.			Per 100 mg. O.S.
		D.S. ave. mg.	D.S. range mg.	O.S. ave. mg.	Ash ave. mg.	ΣN ave. mg.	ΣN ave. mg.
Crustacea	5	103.6	(95–116)	91.6	8.4	10.8	11.8
Crustacea-chaetognath	2	80.5	(78–83)	88.8	11.2	10.7	12.0
Crustacea-coelenterate	2	92.0	(85–99)	91.8	8.2	12.3	13.4
Thaliacean	3	73.7	(56–83)	89.0	11.0	11.1	12.5

The range in dry solids (D.S.) values is from 56 mg. to 116 mg. per gram of preserved plankton. The five samples in which crustaceans predominate (66 to 89 percent of sample volumes) averaged 103.6 mg. D.S. per gram plankton as compared to 73.7 mg. D.S. per gram plankton for samples in which salps and doliolids (thaliaceans) were dominant (i.e., made up 42 to 55 percent of sample volumes). The two samples containing crustacea and chaetognaths in about equal volumes averaged only 80.5 mg. D.S. per gram plankton, while the two samples in which crustacea and coelenterates each made up 40 to 50 percent of the volumes, averaged 92.0 mg. D.S. per gram plankton. The values for D.S. are strikingly more alike than had been anticipated. More of this later.

A number of authors give D.S. and O.S. determinations of plankton samples or of constituents. We have chosen for comment the following: Lovegrove (1961), Riley and Gorgy (1948), Tranter (1960), and Krey (1958).

Tranter (1960, table 6) reports D.S. values for plankton ranging from 24 mg. (salp swarm) to 160 mg. (sample predominantly euphausiids), with most values falling within the range given by us. His determinations on salp samples were made soon after collection, before the salps had "concentrated" to the extent that they had in our test samples.

Riley and Gorgy (1948) reported on D.S., O.S., and ash for representative plankton groups. The values they obtained for four constituent groups were as follows:

Constituent	Quantity in mg. per gram of constituent			Percent ash weight of dry weight
	D.S.	O.S.	Ash	
Copepods	173	147	26	15
Chaetognaths	134	98	36	27
Euphausiids	107	93	14	13
Thaliaceans	17	6	11	65

Their values are instructive in several respects. For one thing they point up the marked differences that obtain in both dry substances and organic substances between constituent groups at time of capture. The thaliaceans contain only four percent as much O.S. per gram of organism as do copepods, and six percent as much O.S. per gram of organism as do chaetognaths. Their values also show a higher inorganic (ash) content than was present in our test samples at equilibrium volume.

As noted previously, dry weight determinations were made on 2000 specimens of *Calanus helgolandicus*. The D.S. value of this sample is 147 mg. per gram of *Calanus;* ash content was only 6.7 mg., hence organic matter constituted 140 mg. per gram of *Calanus*. The latter value is quite similar to that given by Riley and Gorgy for copepods; the ash content is markedly less. The amount of biomass of *Calanus helgolandicus* required to yield one gram of organic matter is 7.15 grams. Lovegrove (1961) quotes a value of 6.8 grams of biomass per gram of organic matter for the closely related species, *Calanus finmarchicus*.

There appears to be both a water loss and an inorganic salt loss from the bodies of plankton organisms during preservation. The ash content of our samples ranged from 6.0 to 17.8 percent of the D.S. with only four values exceeding 10 percent. This is markedly less than the ash weights reported for the principal constituent groups by Riley and Gorgy. The three samples of our material in which salps dominated had ash contents that amounted to 6.4, 8.7, and 17.8 percent of the D.S. determinations. Based on Riley and Gorgy's determinations, the ash weight of these samples should have approximated 50 percent of the dry weight, had the inorganic salts been fully retained by the organisms during the period of volume concentration.

Krey (1958, table III) gives information on ΣN as well as O.S. and D.S. for various kinds of plankton samples. The ΣN values for four samples in which copepods predominated (80 to 100 percent copepods) ranged between 7.5 and 11.1 mg. per 100 mg. O.S. Our nitrogen values for "plankton" samples in which copepods dominated are somewhat higher, averaging 11.8 mg. N per 100 mg. O.S. Our higher values could result from a loss of some of the oils from the bodies of copepods, which would be shown as an increase in the protein content of O.S. Even so, our test samples with mixed constituents had a somewhat higher protein content (ΣN) than the samples in which crustacea were the dominant component.

Discussion

This study has drawn attention to the fact that the volume of organisms in a preserved sample is always less than their live volume. All of the test samples showed volume decreases subsequent to preservation.

The percentage decreases in volumes that were observed at selected time intervals are summarized in table 9. The time period required by the samples to reach their equilibrium volumes follows.

Equilibrium volume attained	No. samples
Immediately following preservation	1
1 day	0
10 days	1
1 month	1
1 year	7
2 years	2

Ten of the 12 test samples attained equilibrium volume within a year of collection. The remaining two samples showed only trivial losses during the second year. For all practical purposes, preserved plankton volumes can be considered to become stabilized within a year after collection.

The decreases in volume of plankton that occurred in test samples are from 15 to 87 percent of the live volumes. The amount of decrease is related to constituent composition, being least for samples in which crustacea made up most of the volume, greatest for samples in which salps were dominant.

The constituent compositions of samples when determined subsequent to preservation are not a measure of the amounts (by volume or weight) of the living constituents. Constituent determination of living material, however, is usually impractical. This cannot be done at sea on programs such as CalCOFI in which we are engaged. Hence this determination must be made at some subsequent time. The time to be preferred is when the samples have reached a relatively stable volume. Crustacean samples reach an equilibrium volume quite soon after preservation—salp samples slowly. Even salp samples show little change after one year of preservation, hence constituent volume determinations made one year after collection should be reproducible.

The constituent compositions (expressed as percentage by volume) of samples that have reached stable preserved volumes are useful, as long as their limitations are kept in mind. They would differ markedly from the original constituent compositions (percentage by volume) in some samples and would approximate them in others. In order to derive even moderately reliable estimates of the original constituent compositions of preserved samples, more information is needed concerning volume losses suffered by the various constituent groups and by the more abundant species within these groups. Several volume loss studies on individual constituents are underway at our laboratory.

Rough estimates of the original constituent compositions were derived for several of the test samples containing a mixture of crustacean-chaetognath and jelly-like constituents. In these computations a shrinkage of 50 percent was allowed for custacea, chaetognaths and molluscs. This is an "outside" estimate, for the crustacean-chaetognath samples showed a 15 to 50 percent decrease from their live volumes.

Sample from 5902-123.42

(Volume before preservation: 129 ml.; volume at time of constituent determination: 23 ml.)

	Crustaceans-Chaetognaths	*Thaliaceans-Coelenterates*
Percent by volume-one year after collection	51	49 (48% Salps)
Volume	11.7 ml.	11.3. ml.
Estimated original volume	23 ml.	106 ml.
Estimated percent by volume original collection	18	82

Sample from 5907-107.50

(Volume before preservation: 93 ml.; volume at time of constituent determination: 13 ml.)

	Crustaceans-Chaetognaths	*Thaliaceans-Coelenterates*
Percent by volume-one year after collection	23	77
Volume	3.0 ml.	10.0 ml.
Estimated original volume	6.0 ml.	87 ml.
Estimated percent by volume in original collection	6	94

The number of organisms in a sample does not change with time. Counts made several years after collection should not differ from counts made at the time of collection. Very few zooplankton constituents disappear from a preserved sample. Even ctenophores, such as *Pleurobrachia,* which break up in time and seemingly dissolve in the supernatant liquid, still leave

TABLE 9. PERCENTAGE DECREASE IN VOLUME OF PRESERVED PLANKTON SAMPLES FROM ORIGINAL LIVE VOLUME, GIVEN FOR SELECTED TIME INTERVALS

Cruise & Station	Orig. vol. ml.	Final vol. ml.	Percentage decrease from original live plankton volume					
			Imm. after pres.	1 day after pres.	10 days after pres.	1 mon. after pres.	1 year after pres.	2 years after pres.
5902-120.35	13	11	92.3	92.3	84.6	84.6	84.6	84.6
5902-113.30	12	10	83.3	83.3	83.3	83.3	83.3	83.3
5902-110.30	(44)	35	(87)	(87)	(85)	(80)	(80)	(80)
5902-113.40	50	32	84.0	80.0	70.0	68.0	64.0	64.0
5903-133.30	48	24	70.8	52.1	52.1	52.1	50.0	50.0
5902-130.35	25	12	76.0	60.0	52.0	52.0	48.0	48.0
5902-113.50	84	32	75.0	45.2	41.6	39.3	38.1	38.1
5902-110.65	57	20	82.4	52.6	42.1	42.1	35.1	35.1
5907-110.90	77	27**	62.4	49.4	40.3	39.0	36.4	35.1
5907-107.55	42	10	61.9	38.1	33.3	30.1	23.8	23.8
5902-123.42	119	23**	47.3	31.8	20.2	20.2	17.8	17.8
5907-107.50	93	12**	64.4	39.7	22.6	18.3	14.0	12.9

** 22 months after collection.
Values in parentheses are estimates.

TABLE 10. ESTIMATES OF NUMBERS OF COPEPODS, EUPHAUSIIDS, AND CHAETOGNATHS PER MILLILITER OF SAMPLE IN 12 TEST SAMPLES

Station	Sample vol. ml.	Copepods			Euphausiids			Chaetognaths		
		Est. vol. ml.	Est. number	No. per ml.	Est. vol. ml.	Est. number	No. per ml.	Est. vol. ml.	Est. number	No. per ml.
110.33	35	28.35	41,300	1455	*	80	—	3.50	3100	885
110.65	20	3.80	7,765	2040	3.80	1443	380	1.80	509	285
113.30	10	6.80	20,004	2940	*	240	—	2.50	867	347
113.40	32	8.00	13,932	1655	3.50	840	240	13.45	1949	145
113.50	32	2.55	5,108	2005	3.50	470	135	1.60	398	250
120.35	11	8.45	31,000	3665	0.11	68	620	1.10	605	550
123.42	23	1.85	5,900	3190	4.15	728	175	1.85	832	450
130.35	12	4.55	10,600	2330	1.45	261	180	0.95	250	265
133.30	24	4.80	12,000	2500	0.25	152	610	10.80	1922	180
107.50	12	0.72	3,600	5000	0.25	244	975	1.20	316	265
107.55	10	1.10	2,676	2430	0.30	272	905	1.00	418	430
110.90	27	5.10	6,804	1335	8.10	1080	133	4.30	2450	570

* Present, volume less than 0.5%.

behind tentacle sheaths and comb rows as evidence of their presence. Hence, counts of organisms in a sample constitute a more conservative value than volume or weight.

Nevertheless, counts of individuals, to have value in quantitative assessments of biomass of standing crop, must be related in a meaningful way to volume or weight. Unless this is done, counts are of dubious utility. Obviously, 1,000 *Paracalanus parvus* are not equivalent in any meaningful way, except in number, to 1,000 *Calanus helgolandicus*. The volume occupied by the *Calanus* would be many times that occupied by the *Paracalanus*. Still other complications arise when dealing with numbers of organisms. Each species has a series of developmental stages that differ markedly in size. It might take a hundred or more larvae of *Euphasia eximia*, for example, to equal the mass of one adult.

Estimates of the number of individuals per ml. of wet volume have been derived for three groups: copepods, euphausiids, and chaetognaths (Table 10). Similar estimates can be derived for the other important constituent groups in the test samples. Copepods range in number from 1,335 to 5,000 individuals per ml., euphausiids from 133 to 975 individuals per ml., and chaetognaths from 145 to 885 individuals per ml. There are over seven times as many euphausiids per ml. in the sample from 5907-107.50 as in the sample from 5902-113.50, over six times as many chaetognaths in the sample from 5902-110.33 as in the sample from 5902-113.40. Obviously, data on numbers of individuals of a constituent group have limited value until they are related to volume or weight.

Although wet plankton volumes are over-estimates of the volume occupied by the organisms, due to included interstitial liquid, the amount of the latter can be determined with some precision. From determinations on test samples, interstitial liquid ordinarily makes up from 28 to 45 percent of the volumes of wet plankton samples, hence the amount is variable from sample to sample. Even so, a fairly adequate "average" value for interstitial liquid in our test samples would be 35 percent of the wet plankton volume.

The dry weight of a plankton sample is less subject to change with time than the "wet" weight (or volume). The major change in weight (volume) of "wet" plankton is due to water loss, which has the effect of concentrating the organic constituents. However, water loss is accompanied by an inorganic salt loss, which lowers the ash content of preserved samples. Organic substances appear to be more fully retained in the bodies of preserved plankton organisms. We have found only traces of nitrogen in the preserving liquids of undisturbed plankton samples that have been stored for considerable periods of time. Oils, however, are less fully retained. A verification of this loss is readily made on *Calanus*-rich samples in which the extracted oil can be seen as free-floating droplets.

Lovegrove (1961) has discussed some of the problems that arise in drying plankton for D.S. and O.S. determinations. Higher D.S. values are obtained when samples are dried by desiccation than when water is removed by oven drying, especially if the drying is done at temperatures above 60° C.

Most workers have related dry weights to wet weights of organisms. There has been little consistency in use of fresh or preserved material, perhaps because it was not appreciated that the volume measurements of live and preserved materials are not comparable. As is evident from our studies, volume determinations of preserved material will be less than "live" volumes by a variable amount depending on constituent composition and the time interval intervening since preservation. Obviously, dry weights should be related to the weight of freshly collected material, from which interstitial water has been removed. There is considerable merit in the oft-made suggestion that a plankton sample should be partitioned on collection, one part to be preserved, the other to be used for a dry weight determination. Technical difficulty in carrying out this suggestion has prevented its adoption as a standard technique.

A dry weight determination can be expressed as the amount of organic substances in a standard volume of water (1000 m^3, for example). This determination bypasses most of the technical problems associated with relating dry weight to wet weight of plankton.

We have been intrigued by the strikingly similar dry weight determinations obtained on our test samples after two years of preservation, despite the marked differences in constituent compositions. The range in values of 56 mg. to 116 mg. per gram of preserved plankton (without interstitial liquid) constitutes a range of only 2X; furthermore, most values were grouped much more closely together. Apparently the nutrient compositions of samples have been made roughly comparable per unit volume (or weight) of plankton through the process of concentration of the volumes of jelly-like constituents relative to that of crustaceans and other constituents with an exoskeleton. An implication of this finding is that plankton volume determinations, *per se,* made on plankton samples that have reached their equilibrium volumes after a year of preservation, constitute meaningful measures of the standing crop of zooplankton.

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