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Shrinkage and Weight Loss of Marine Fish Food Organisms Preserved in Formalin*1

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餌料性生物のホルマリン固定による収縮と重量変化

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海産生物を固定するために広く用いられている4％ホルマリン水溶液が数種の餌料生物の長さおよび重さに与える影響を観察した。

長さの変化は *Eurytemora*, *Artemia*, *Neomysis*, 重量の変化は *Neomysis*, *Nereis*, *Gammarus* についてそれぞれ固定後100日まで調べた。体の長さの収縮は *Neomysis* で4％, *Artemia* で1.6％であったが, *Eurytemora* では大きな変化はなかった。固定による重量の減少は *Neomysis* で17.4％, *Nereis* で10.1％ *Gammarus* で10.3％であった。固定による長さの収縮および重量の減少は, いずれも固定後30日以内で大きかった。この期間における観察, 測定には注意を要する。

Abstract

The shrinkage of *Eurytemora affinis*, *Artemia*-nauplii and *Neomysis integer* and wet weight changes of *Neomysis integer*, *Nereis virens* and *Gammarus locusta* after fixation in 4% formalin-seawater solution were followed over a period of 100 days. The mean length reduction of *Neomysis* and *Artemia*-nauplii was 4% and 1.6% (days 25-100) of the initial length. The cephalothorax-length of *Eurytemora* did not change significantly. The wet weight reduction of *Neomysis*, *Nereis* and *Gammarus* due to preservation amounted to 17.4, 10.1 and 10.3% of the initial weights at the end of the observation period (100 days). Storage time less than 30 days caused variable weight changes.

Formalin preserved samples of aquatic organisms exhibit often different length-weight relationships than unpreserved specimens. The effect of preservatives on length and body weight varies considerably with concentration and exposure time. Length and weight determination of planktonic and benthic invertebrates usually are undertaken with preserved

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material.

Correction factors for original size and weight of preserved specimens have recently been described for adults and larvae of several fish species (AMOSOV, 1960; BLAXTER, 1971; DAVIS, 1973; PARKER, 1963; LOCKWOOD and DALY, 1975; ROSENTHAL and v. WESTERNHAGEN, 1976; ROSENTHAL *et al.*, 1978; SCHNACK and ROSENTHAL, 1978; FUKUHARA, 1979). Only a few investigators describe quantitatively the changes in length or weight of planktonic crustacean (AHLSTROM and THRAILKILL, 1962; LASKER, 1966; HOPKINS, 1968; OMORI, 1970, 1978; DURBIN and DURBIN, 1978; LANDRY, 1978; CHAMPALBERT and KERAMBRUN, 1979) and macro-benthic invertebrates (HOWMILLER, 1972) when preserved in formalin for various periods of time.

This study was carried out at the Aquaculture Experimental Station Kiel-Bülk to determine the influence of the preservative formaldehyde on the food organisms *Eurytemora*, *Artemia*, *Neomysis* and *Gammarus*, which play an important role in the fish rearing process as well as in fish stomach analyses and estimates of the standing stocks.

Materials and Methods

The food organisms *Artemia* (newly hatched nauplii), *Eurytemora affinis* (ripe female), *Neomysis integer* (adults), *Nereis virens* (adults, 50 mm) and *Gammarus locusta* (adult males, 15 mm) used, were collected in outdoor tanks of the Aquaculture Experimental Station Kiel-Bülk.

The length of freshly caught animals was measured using a binocular in connection with a "Wild Censor" and their weight was determined using a "Mettler" balance (H 54 AR). The length accuracy was 0.01 mm for *Artemia*-nauplii and *Eurytemora* and 0.1 mm for *Neomysis*. The weight accuracy was 0.01 mg for *Neomysis*, *Gammarus* and *Nereis*, respectively. For weight measurement the following standard handling procedure was employed: Specimens were removed from the individual sample, blotted on absorbant paper for 30 sec., and weighted immediately in the following 30 sec., so that the whole procedure for each individual took not more than one minute.

The samples were preserved by the standard preservation of a 4% formalin-non-buffered seawater solution (17‰ S ambient salinity, 18 ± 2°C). Of the mysids, gammarids and polychaetes 5 adult specimens each were held separately in 100 m*l* bottles and 10 copepods and 10 *Artemia*-nauplii in 10 m*l* tubes. The identical individual of specimen was measured at each observation.

For length measurements the cephalothorax-length for *Eurytemora* was used. *Neomysis* was measured from the top of the eyes to the end of the telson. Bend specimens were straightened by tweezers. For *Artemia*-nauplii total length was determined. For *Nereis* and

Gammarus weight changes were employed as criteria for the effect of formalin preservation.

Results and Discussion

The effects of a 4% formalin-seawater solution on changes in body length of *Eurytemora*-females, *Artemia*-nauplii and adults of *Neomysis* over a total time of 100 days are described in Fig. 1 and Table 1. The length of *Artemia*-nauplii and copepods increased after preservation between day 0 and 5 and between day 0 and 1, respectively. After that period measurable size reductions occurred, which finally were stabilized at mean values of 1.66% (day 25-100) and 4.4% of the initial length for *Artemia* and *Neomysis*, respectively. Although stabilized size reduction for *Eurytemora* deviated by 0.27% from the initial length of the cephalothorax, these values are not significantly different from the unpreserved specimens. The major changes in wet-weight of *Gammarus*, *Nereis* and *Neomysis* occurred within the first 30 days (Fig. 2, Table 2). *Gammarus* specimens gained weight during the first 7 days after preservation. Similarly the loss of weight of *Neomysis* and *Nereis* showed strong fluctuations during the first month. The average weight

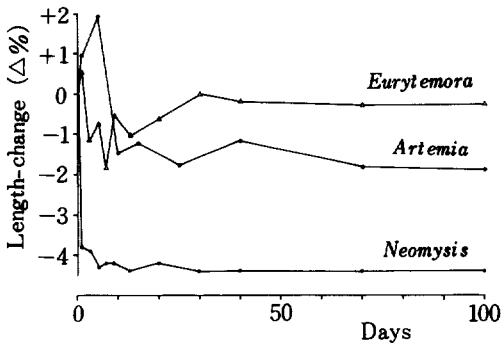

Fig. 1. Mean length changes in *Neomysis*, *Eurytemora* and *Artemia*-nauplii due to preservation in a 4% formalin seawater solution.
△% = per cent length change relative to day 0.

Table 1. Mean length changes (in mm) in *Neomysis* (n=5), *Eurytemora* (n=10) and *Artemia*-nauplii (n=10) due to preservation in a 4% formalin seawater (17‰ S) solution. $\bar{X}$ = mean length (mm) ; SD = standard deviation ; △% = per cent length change relative to day 0.

Species		Storage time (days) after preservation											
		0	1	3	5	7	9	13	20	30	40	70	100
Eurytemora affinis	$\bar{X}$	0.98	0.99	0.97	0.97	0.96	0.98	0.97	0.98	0.98	0.98	0.98	0.98
	SD	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.04	0.05	0.04	0.03
	△%		+0.51	−1.12	−0.71	−1.83	−0.51	−1.02	−0.61	0	−0.20	−0.28	−0.25
Neomysis integer	$\bar{X}$	11.9	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4
	SD	1.05	1.02	1.06	1.09	0.90	0.98	1.04	1.03	1.01	1.01	1.05	1.11
	△%		−3.8	−3.9	−4.3	−4.2	−4.2	−4.4	−4.2	−4.4	−4.4	−4.4	−4.4
		0	2		5		10	15	25		40	70	100
Artemia-nauplii	$\bar{X}$	0.41	0.42		0.42		0.41	0.41	0.41		0.41	0.41	0.40
	SD	0.019	0.019		0.015		0.016	0.014	0.015		0.016	0.016	0.017
	△%		+0.97		+1.92		−1.46	−1.21	−1.79		−1.16	−1.82	−1.86

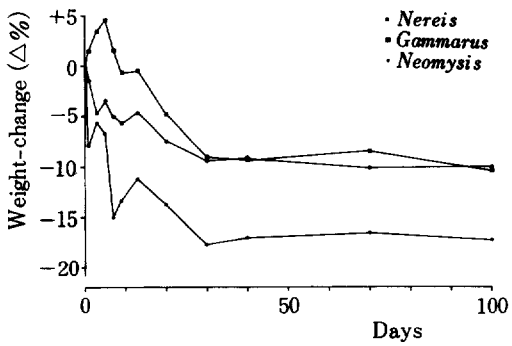

Fig. 2. Mean weight changes in *Nereis*, *Gammarus* and *Neomysis* due to preservation in a 4% formalin seawater solution. △%=per cent weight change relative to day 0.

reduction after 100 days was 17.4, 10.1 and 10.3% of the initial weight of *Neomysis*, *Nereis* and *Gammarus*, respectively. Shrinking rate became constant in all three species after 20-30 days of fixation. As indicated by the standard deviations (Table 2) the individual weights of the preserved animals were quite variable.

However analysis of variance indicated that from day 30 to 100 no significant difference exist between the 5 individually maintained specimens of each species on the 5% level, according to the individual relative length in relation to day 0 (comparison after arcsin$\sqrt{\%}$ transformation).

The length of *Eurytemora* proved to be fairly constant in both preserved and unpreserved specimens, which agree with observations also reported by DURBIN and DURBIN (1978) for *Acartia clausi*, showing no significant changes in length in 3% formalin preservation over a period of 41 weeks. Both results support, that because of its consistance, the cephalothorax-length is the most useful measure size of copepods (CORKETT and MCLAREN, 1978).

In contrast to the unsignificant length-change OMORI (1970) described a 54% loss of dry weight in *Calanus cristatus* and DURBIN and DURBIN (1978) also stated a loss of 29.5% dry weight of *Acartia clausi*. HOWMILLER (1972) found that tubificid worms lost 24% and chironomid larvae 42% of their initial wet weight after 44 days fixation in 4% formalin, respectively.

From our observations it seems obvious, that shrinking rate determination is not necessary

Table 2. Mean weight changes (in mg) in *Neomysis*, *Nereis* and *Gammarus* (n=5 each) due to preservation in a 4% formalin-seawater (17‰ S) solution. $\bar{X}$=mean wet weight (mg); SD=standard deviation; △%=per cent change relative to day 0.

Species		Storage time (days) after preservation 0	1	3	5	7	9	13	20	30	40	70	100
Neomysis integer	$\bar{X}$	13.4	12.4	12.6	12.5	11.4	11.6	11.9	11.6	11.1	11.1	11.2	11.1
	SD	3.9	3.9	4.2	4.3	3.8	4.0	4.2	4.0	4.2	4.2	3.7	4.0
	△%		−8.0	−5.7	−6.7	−15.1	−13.4	−11.3	−13.8	−17.8	−17.1	−16.6	−17.4
Nereis virens	$\bar{X}$	681	671	649	657	646	642	649	630	617	617	612	612
	SD	239	261	253	267	255	261	247	230	239	240	243	242
	△%		−1.5	−4.7	−3.5	−5.1	−5.7	−4.7	−7.5	−9.4	−9.4	−10.1	−10.1
Gammarus	$\bar{X}$	79.5	80.6	82.2	83.1	80.7	79.0	80.0	75.6	72.2	72.0	72.8	71.3
	SD	19.3	18.4	19.7	20.0	17.4	19.7	19.2	17.4	18.8	17.2	18.2	17.3
	△%		+1.4	+3.4	+4.5	+1.52	−0.69	+0.65	−4.9	−9.2	−9.4	−8.4	−10.3

for preserved specimens of *Eurytemora* as long as cephalothorax or other parts of the exoskeleton are used for size characterization. Weight reduction stabilizes after a certain period in most of the species observed, so that preserved samples can be characterized at any time by the initially determined correction factor.

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