

## Effect of Formaldehyde on the Gelatinous Zooplankton (*Pleurobrachia pileus*, *Aurelia aurita*) During Preservation

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**Abstract:** The medusae, *Aurelia aurita*, and the cydipid ctenophoran, *Pleurobrachia pileus*, collected from the Black Sea were preserved in 2% borax-buffered formaldehyde solution for two months. Changes resulting from shrinkage and weight loss were calculated. *Pleurobrachia pileus* lost weight and shrunk more than *Aurelia aurita*. Factors for changes in body size and wet weight were calculated to be  $1.45 \pm 0.03$  ( $26 \pm 1.8\%$  Standard deviation) and  $4.6 \pm 0.4$  ( $70 \pm 4\%$ ) for *A. aurita* and  $3.5 \pm 0.03$  ( $71 \pm 4\%$ ) and  $54 \pm 2.4$  ( $97 \pm 1\%$ ) for *P. pileus*, respectively. The rate of dissolution of *A. aurita* in formalin was found to be dependent on size groups. Covariance analysis showed that there was no significant difference in length-weight relationship between living and preserved *A. aurita*, but this was highly significant for *P. pileus*.

**Key Words:** Formaldehyde, preservation, jellyfish, ctenophore

### Formoldehidin Jeli Organizmaları (*Pleurobrachia pileus*, *Aurelia aurita*) Üzerine Etkisi

**Özet:** Karadeniz'den toplanan deniz anası, *Aurelia aurita* ve taraklı jeli, *Pleurobrachia pileus*, 2 ay boyunca borax ile tamponlanmış % 2'lik formoldehid içerisinde saklanmıştır. Ağırlık ve boydaki değişimler hesaplandı. *Pleurobrachia pileus*, *Aurelia aurita*'dan daha çok ağırlık kaybetmiştir. Ağırlıkta ve boydaki değişim faktörü sırasıyla, *A. aurita* için  $1.45 \pm 0.03$  ( $26 \pm 1.8\%$  Standart sapma) ve  $4.6 \pm 0.4$  ( $70 \pm 4\%$ ) *P. pileus* için  $3.5 \pm 0.03$  ( $71 \pm 4\%$ ) ve  $54 \pm 2.4$  ( $97 \pm 1\%$ ) hesaplanmıştır. *A. aurita*'nın çekilmesi oranı boy sınıflarına göre farklılık göstermiştir. Kovaryans analizi canlı ve saklanmış *A. aurita*'nın ağırlık ve boy ilişkisinin farklı olmadığını ve *P. pileus*'un farklı olduğunu göstermiştir.

**Anahtar Kelimeler:** Formoldehit, saklama, deniz anası, taraklı jeli

### Introduction

There are 4 species of gelatinous zooplanktonic forms (*Rhiosstoma pulma*, *Aurelia aurita*, *Mnemiopsis leidyi* and *Pleurobrachia pileus*) currently living in the Black Sea (1-2). Of these forms, the last three species are very common in the Black Sea. *M. leidyi* was introduced to the Black Sea in the 1980s and has been living there for the last decade (3-4). There are speculations about the effect of *M. leidyi* on changes in the ecosystem and on stock depletion of some commercial fishes, e.g., anchovy (5-7). To determine the importance of gelatinous forms on the consumption of the copepods in the Black Sea, stomach content analysis was considered. Regarding stomach content after preservation of *A. aurita* and *P. pileus* in 2% formaldehyde, except for *Mnemiopsis leidyi*, both animals' stomachs whole in the solution. Only the gastrovascular

apparatus of *Mnemiopsis leidyi* was not deformed in the fixative solution.

A study concerning the quantification of *Mnemiopsis leidyi* from formalin-preserved plankton samples has been conducted by Purcell (8), and the population dynamics of *Aurelia aurita* in Kiel Bight has been investigated by Moller (9).

Due to difficulties in measuring live-weight on board, the biomass of such organisms might be misestimated. In the field, successive stations are so frequent that it is difficult to measure the size of all organisms precisely on board. In this case, preservation of them in formalin solution is necessary. This study aims to contribute to the conversion of the losses in size and weight of the preserved sample to actual live size and weight of the organisms, *P. pileus* and *A. aurita*.

## Materials and Methods

Materials were collected with the Hensen egg net (300  $\mu$  mesh size and 70 cm opening) from 50 stations along the Turkish coastal waters of the Black Sea. Vertical hauls were made from the depth of  $\sigma_t = 16.2$  upwards ( $\sigma_t = 16.2$  indicates the upper limit of the anoxic zone in the Black Sea) (10). Gelatinous forms, other than the samples, were sorted out and sized on board the R/V Bilim. Individuals were then preserved in 2% (= 5% Formalin) borax buffered-formaldehyde solution. Preserved organisms were re-measured (sized and weighed) after 2 months. Bell diameter (cm) for *Aurelia aurita* and body length (mm) for *Pleurobrachia pileus* were recorded. In order to establish the relationship between the length and weight of the living forms, additional samples were taken off the coast of Trabzon in September 1993. Measurements for size and weight were made in the laboratory of the Aquatic Resources Research Institute in Trabzon. The length-weight relationship of live specimens was then applied to calculate the empirical live weight of those sized and

preserved on board the R/V Bilim during its August cruise.

The losses in size and weight of preserved samples were estimated from the ratio of weight and length of live individual to preserved individual. Basic statistics (mean, variance, standard deviation and standard error) were computed for all measurements. Analysis of covariance was used to test the significance of the differences between regression coefficients (11-12).

## Results and Discussion

The complete body was readily apparent in preserved specimens. The relationship between the bell diameter of *A. aurita* in centimeters (d) and its wet-weight in grams (W) and the body length in millimeters (l) to wet-weight in milligrams of *P. pileus* are defined by the following equations given in Table 1 and Figures 1 and 2.

Preservation in 5% formalin shrunk the jelly-like-organisms and caused a considerable loss in weight.

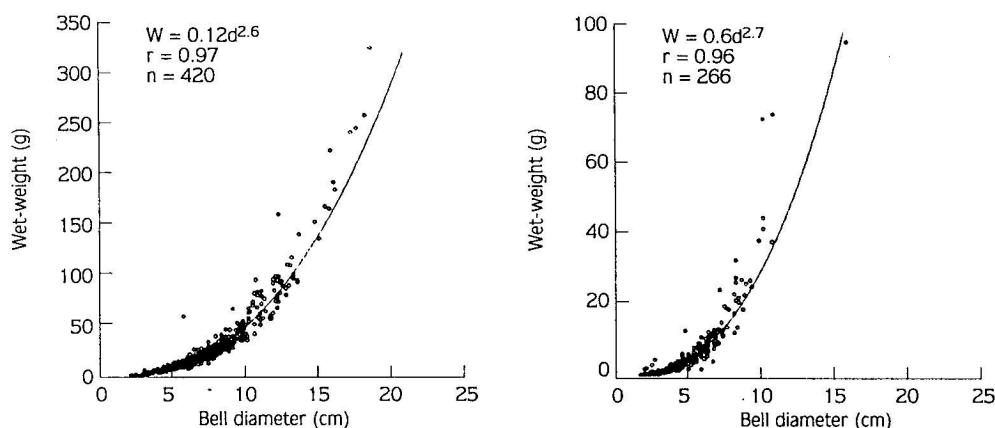


Figure 1. Relationship of length and wet-weight of the living (left) and preserved (right) specimens of *Aurelia aurita*.

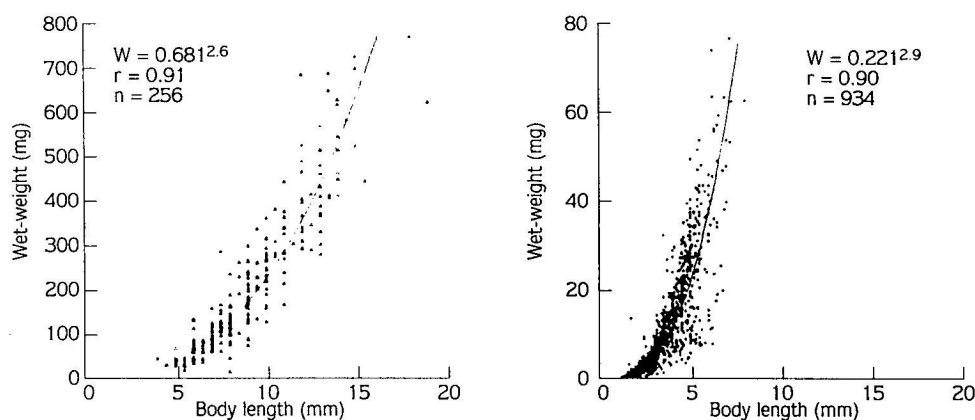


Figure 2. Relationship of length and wet-weight of the living (left) and preserved (right) specimens of *Pleurobrachia pileus*.

Table 1. Summary of the regression analysis between length and wet-weight of the living and preserved organisms (Bold number: highly significant)

	<i>Aurelia aurita</i>		<i>Pleurobrachia pileus</i>	
	Live	Preserved	Live	Preserved
Equation (W = )	$0.12d^{2.6}$	$0.06d^{2.7}$	$0.68l^{2.5}$	$0.22l^{2.9}$
r	0.97	0.96	0.91	0.90
n	420	266	256	934
$P_{\alpha} = 0.05$	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>

Measurements were different in the living and preserved specimens of the cydipid and scyphozoan jelly-like substances. Mean shrinkage in body size and loss in wet-weight were calculated to be  $1.45 \pm 0.03$  and  $4.6 \pm 0.4$  for *A. aurita* and  $3.5 \pm 0.03$  and  $54 \pm 2.4$  for *P. pileus* (Table 2 and Figures 2 and 3). In other words, the diameter and wet-weight of *A. aurita* was reduced up to  $26\% \pm 1.8\%$  SD and  $70\% \pm 4\%$  (Figure 3) depending on the size of the jellyfish, and *P. pileus* to  $71\% \pm 4\%$  and  $97\% \pm 1\%$  (Figure 4), respectively. In live individuals with a diameter of less than 5 cm, reduction in size and weight was only 20% and 62%, respectively. In medusae having a bell diameter between 5-10 cm, this was about 27% and 74%, respectively. In individuals larger than 10 cm, this was around 28% and 67%. Moller (9) found that the diameter was reduced by up to 30% and wet weight of 1 liter medusae was equated with 1 kg. By this method, he found that a loss in volume of 62.5% had to be taken into account. In the present study, mean loss in weight was about 70% comparing the volumetric loss to Moller results. To obtain the live diameter and wet-weight, the above mentioned percent reduction should be added to the measurements of the formalin-preserved gelatinous forms. Factors for the loss in size and wet-weight of comb-jelly were higher than those

Table 2. Coefficients (X) for shrinkage and loss in weight for the comb-jelly and jellyfish preserved in 5% formalin solution and percent reduction (%r) ( $W_{lp}$ : ratio of live weight to preserved weight).

	<i>Aurelia aurita</i>				<i>Pleurobrachia pileus</i>			
	$d_{lp}$	d%r	$W_{lp}$	w%r	$d_{lp}$	d%r	$W_{lp}$	w%r
X	1.4	26	4.6	70	3.5	71	54	97
S	0.25	1.8	3.5	31	0.5	4	44	1
S <sub>err</sub>	0.03	2	0.4	4	0.03	0.2	2.4	0.07
n	75		75		322		322	

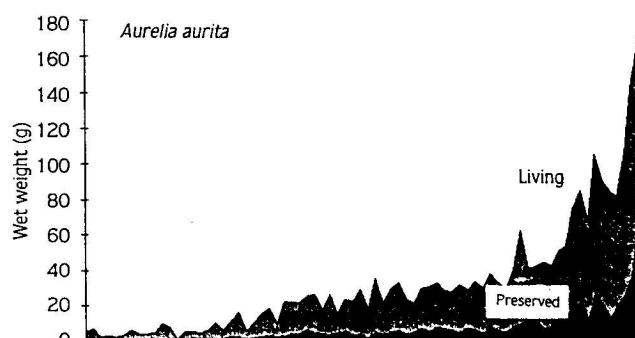
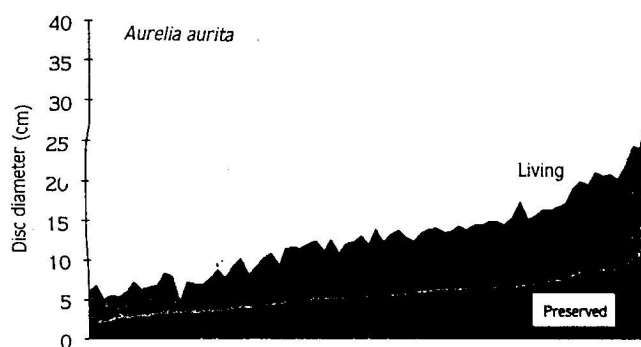


Figure 3. Losses in size (upper) and wet-weight (lower) for *Aurelia aurita*.

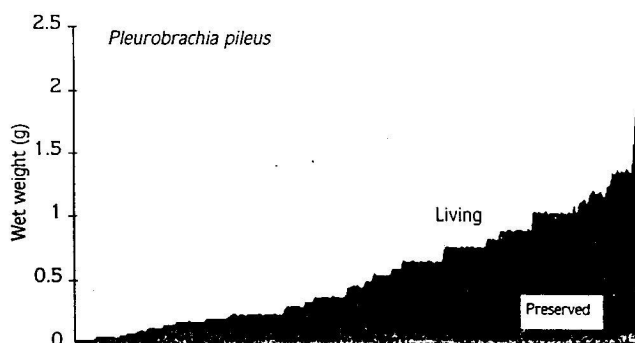
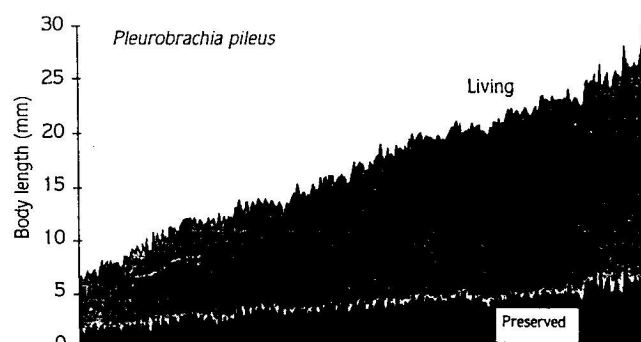


Figure 4. Losses in size (upper) and wet-weight (lower) for *Pleurobrachia pileus*.

of jellyfish. This loss in weight is related to the water content in the body. The ctenophoran species contains 96-97% (13-14) of body water where *A. aurita* holds above 93-95% water in the body tissue (15). Regarding the total content of body water, *P. pileus* as a species of ctenophore diffused almost all of its body water into solution during preservation.

The results of covariance analysis are given in Table 3. The results indicate that although there is only an insignificant difference between the length-weight re-

lationship of the living and preserved *A. aurita*, a highly significant difference for *P. pileus* exists.

### Conclusion

*Pleurobrachia pileus* lost weight and shrunk more than *Aurelia aurita*. Factors for changes in body size and wet-weight were calculated to be  $1.45 \pm 0.03$  (26%±1.8 SD)  $4.6 \pm 0.4$  (70%±4%) for *A. aurita* and  $3.5 \pm 0.3$  (71%±4%) and  $54 \pm 2.4$  (97%±1%) for *P. pileus*, respectively. To obtain the live diameter and wet-weight. The above mentioned percent reduction should be added to the measurements of the Formalin-preserved gelatinous forms. The rate of dissolution of *A. aurita* in formalin was found to be dependent on size groups. Covariance analysis showed that there was no significant difference between the length-weight relationship of the living and preserved *A. aurita*, but there was a highly significant difference for *P. pileus*.

### Acknowledgement

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Table 3. Results of covariance analysis for the length-weight relationship of the living and preserved specimens (Bold number: highly significant).

Species	differences among slopes				
	Source	df	SS	MS	F
<i>A. aurita</i>	among b	1	0.00558	0.00558	0.449
	sum of grp. dev	196	2.43484	0.01242	
<i>P. pileus</i>	among b	1	0.33101	0.33101	<b>7.557</b>
	sum of grp. dev	196	8.58526	0.04380	
Table F value $F_{0.05} (200,1) = 3.89$ $F_{0.01} (200,1) = 6.76$					

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