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NOTE

Quantification of *Mnemiopsis leidyi* (Ctenophora, Lobata) from formalin-preserved plankton samples

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ABSTRACT: Lobate ctenophores were thought to be impossible to quantify using standard plankton collection and preservation techniques. I show that the tentacle bulbs of the lobate species *Mnemiopsis leidyi* provide a direct measure of numbers and sizes of the ctenophores in formalin-preserved plankton samples. The tentacle bulbs persist intact after preservation in 5 % formaldehyde solution for at least 9 mo. Bulb lengths of live and preserved specimens were closely correlated with ctenophore wet weights. This method is more accurate and convenient than measures of abundance previously used for *M. leidyi*, and it may be applicable to other species of ctenophores.

Ecological studies of ctenophores, other than a few species of cydippids (i.e. *Pleurobrachia* spp.), have been hampered because of the difficulty in sampling them quantitatively. Standard plankton collection and preservation methods cause the destruction of lobate ctenophores in the class Tentaculata. This has led to special techniques of collection (Harbison et al. 1978) and preservation (Adams et al. 1976) that are very labor intensive.

Lobate ctenophores in the genus *Mnemiopsis* form conspicuous population blooms during spring and summer in estuaries of the eastern United States. These ctenophores are so abundant that methods have been developed to exclude them from plankton nets and to dissolve them if collected (Heinle 1965 and Burrell & Van Engel 1970, respectively). The most common method of measuring the abundance of *Mnemiopsis* spp. ctenophores has been to measure their total live volume from plankton tows (Table 1). Total volumes have been converted to approximate numbers from size measurements of the ctenophores (Table 1). Alternatively, live ctenophores have been counted and measured immediately following plankton tows (Table 1). This method is extremely tedious, and potentially inaccurate for small ctenophores. Le Blanc & Straw (1972) and Kremer & Nixon (1976) used luminescent flashes of the ctenophores to measure abundance.

In the present study, I show that tentacle bulb length increases directly with size (wet weight) in *Mnemiopsis leidyi*. Metamorphosis from the cydippid larva to the adult form in lobate ctenophores involves disappearance of the tentacle sheaths, reduction of the tentacles, and elongation of the tentacular canals and bulbs (bases) as the tentacles shift orally in position (Hyman 1940). These structures remain after preservation in 5 % formaldehyde solution, and can be used to quantify the abundance and biomass of this lobate ctenophore species.

Table 1. Studies in which measures of biomass, size, and abundance of *Mnemiopsis* spp. ctenophores were made. All data were from live specimens from net tows

	Volume (ml)	Length (mm)	Number
Nelson 1925	×		×
Zeigenfuss & Cronin 1958	×		
Herman et al. 1967, 1968	×[a]		
Burrell 1968	×	×	
Miller 1970	×	×[b]	×
Miller & Williams 1972	×[c]		
Miller 1974	×	×[b]	×
Heinle 1974	×[d]		
Reeve & Baker 1975	×	×[b]	×
Burrell & Van Engel 1976	×	×	
Kremer & Nixon 1976	×	×	×
Kremer 1976	×[e]		
Kremer 1979	×[e]		
Mountford 1980			×
Lonsdale 1981	×		
Deason 1982	×	×[b]	×
Deason & Smayda 1982		×	×
Feigenbaum & Kelly 1984	×		

[a] Total volume of ctenophores and cnidarians. Data in 1968 are 1000× too large; [b] Separation of size classes by sieves; [c] From Herman et al. 1968; [d] From Herman et al. 1967; [e] From Kremer & Nixon 1976

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Materials and Methods. Ctenophores *Mnemiopsis leidyi* were collected individually from the Choptank River subestuary of the Chesapeake Bay, USA, in August 1987. The lengths of both tentacle bulbs in each living ctenophore were measured using a dissecting microscope with ocular micrometer. Live ctenophore length, displacement volume, and wet weight were measured. Wet weight, measured with an electronic balance, was most accurate and is reported here. The ctenophores were preserved individually in 5 % formaldehyde solution, and the lengths of the tentacle bulbs measured after 5 mo.

Plankton tows were made using a 1 m diameter net with flow meter. The net was made from 1.6 mm soft nylon mesh and had a bag cod end in order to minimize damage to the ctenophores during collection. Double oblique tows were made at 0 to 10 m depth at 09:00 h in mid-channel in Chesapeake Bay. Ctenophores were separated from the samples using a plastic colander and a 1 mm mesh sieve. Total live volume of the ctenophores was measured in graduated cylinders. All ctenophores were preserved by adding full-strength (37 %) formalin to create a 5 % final solution. The tentacle bulbs in these samples were counted and measured after 5 and 7 mo.

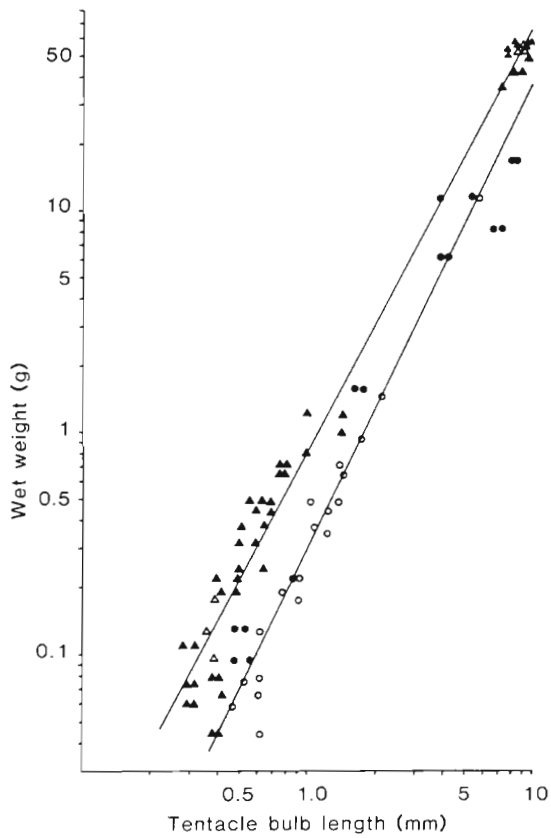

Fig. 1. *Mnemiopsis leidyi*. Relations of tentacle bulb length to wet weight. Circles: living specimens; triangles: specimens preserved in formalin for 5 mo. Open symbols indicate 2 bulbs of the same length in 1 ctenophore. Closed symbols represent 1 bulb. In specimens of >50 g wet weight, 8 bulbs at 8 to 9 mm and 16 bulbs at 9 to 10 mm preserved length were not drawn on the figure because of symbol overlap. The regressions, which included all data points, are given in the text

Results. The tentacle apparatus was readily apparent in living and preserved specimens of *Mnemiopsis leidyi*. It was heart-shaped in small ctenophores, and very elongate with the tentacle at one end in large ctenophores. In living ctenophores, tentacle bulb length in millimeters *(x)* was related to wet weight in grams *(y)* according to the equation $y = 0.284x^{2.108}$ (n = 55; r = 0.98) (Fig. 1). Measurements of the 2 tentacle bulbs were usually the same in a living ctenophore (Fig. 1); however, in preserved specimens, bulbs differed by a mean of 10.3 % ± 9.3 SD.

Preservation in 5 % formalin caused the ctenophores to break apart, but the tentacle apparatus always remained intact. The tentacle bulbs did not lose their orange coloration during 9 mo of storage in 5 % formalin. Tentacle bulb length decreased a mean of 42.8 % ± 9.8 SD during 5 mo in storage. At 5 mo, preserved tentacle bulb length *(x)* was related to live wet weight *(y)* according to the equation $y = 0.810x^{1.913}$ (n = 67; r = 0.99) (Fig. 1). After 9 mo storage, bulb lengths had decreased a mean of 47.9 % ± 9.6 SD from live bulb lengths, indicating that an additional 5 % shrinkage occurred between 5 and 9 mo.

Tentacle bulbs were used to quantify ctenophore abundance from formalin-preserved plankton tows made on 2 dates in 1987. On 4 June, water temperature was 23.0 °C and salinity was 12.8 ‰ at the surface. Hydromedusae *Nemopsis bachei* in the sample (425 ml volume) could be separated only by hand from the ctenophores (90 ml volume). Ctenophore live volume

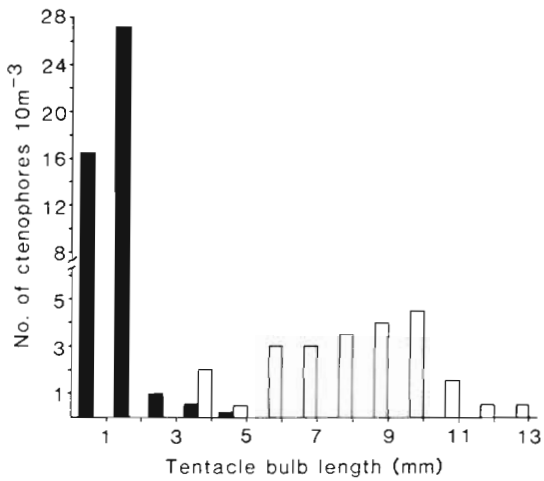

Fig. 2. *Mnemiopsis leidyi*. Abundances and size distributions based on tentacle bulbs in formalin-preserved plankton tows. Black bars: 4 June 1987; open bars: 13 August 1987. Note that scale changes above 6 ctenophores m^{-3}

was about 0.6 ml m^{-3}. Preserved in the sample were 257 hydromedusae and 756 ctenophores (1512 tentacle bulbs), equivalent to 4.6 ctenophores m^{-3}. The size distribution of tentacle bulbs showed that very small ctenophores predominated on that date (Fig. 2).

On 13 August, water temperature was 26.3°C and salinity was 15.1 ‰ at the surface. The sample contained 10 ml of scyphomedusae *Chrysaora quinquecirrha*, and 6365 ml of ctenophores. Ctenophore live volume was 71.6 ml m^{-3}. Preserved in the sample were 4 scyphomedusae and 205 ctenophores, equivalent to 2.3 ctenophores m^{-3}. Ctenophores collected in August were much larger than in June (Fig. 2). Live volume calculated from preserved tentacle bulb lengths was 2.3 % greater than that measured directly.

Discussion. Tentacle bulbs of the lobate ctenophore *Mnemiopsis leidyi* provide a direct measure of number and size of the ctenophores in formalin-preserved plankton samples. This method is more accurate and more convenient than previous methods, which all require immediate sorting and handling of live specimens, and are especially difficult for small ctenophores. Data on tentacle bulb size can be converted to ctenophore biomass in wet weight (herein) or to other units. Kremer & Nixon (1976) give formulas to convert from volume to wet weight and from wet weight to dry weight for *M. leidyi*.

I suggest the following protocol for the quantitative sampling of *Mnemiopsis* spp. ctenophores. (1) In order to minimize destruction of the ctenophores during collection, a plankton net with a large cod end is preferred (Reeve 1981), and the net tows should be of short duration. (2) The total live displacement volume of ctenophores in each plankton tow should be measured. This eliminates the need to calculate from preserved tentacle bulb lengths back to live volume, and provides straightforward comparisons with earlier studies (Table 1). (3) The entire sample of ctenophores should be preserved (final concentration of 5 % formaldehyde). The number and sizes of the preserved ctenophore tentacle bulbs are measured using a dissecting microscope fitted with an ocular micrometer. (4) A few ctenophores over a range of sizes should be measured (volume or wet weight) and individually preserved for each sampling effort, to ensure accurate calculation from preserved tentacle bulb lengths to live volumes. These specimens provide calibration for possible differences in shrinkage of the tentacle bulbs depending on salinity, formalin concentration, temperature, or duration of sample storage. Hay (1982) found salinity and formalin concentrations to have the greatest effects on fixation shrinkage of herring larvae. Shrinkage of some preserved gelatinous zooplankters increased with the length of time in storage (Möller 1980, Yip 1982, Larson 1985).

Tentacle bulbs were used to quantify abundance and biomass of the lobate ctenophore *Mnemiopsis leidyi* from preserved plankton samples. The bulbs also can be recognized in the preserved gut contents of predators of *M. leidyi* (Purcell pers. obs.). Such methods may be applicable to other ctenophore species that retain tentacle bulbs in the adult form, and that were considered to be unquantifiable from plankton samples.

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