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The use of trichloroacetic acid fixation and propylene phenoxetol conservation in quantitative sampling of ctenophores

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Abstract

The fixation and preservation method for individual ctenophores developed by Adams, Flerchinger and Steedman (1976) was modified and successfully used for quantitative sampling of ctenophores, including Mnemiopsis leiydi.

Keywords: Mnemiopsis leidyi, Pleurobrachia pileus, Beroe gracilis, fixation, preservation, Trichloroacetic acid, Propylene glycol, Propylene phenoxetol

Introduction

Most gelatinous zooplankton species are so fragile, that collection is hampered making research difficult. In addition, most species, especially lobate ctenophores such as Mnemiopsis leidyi are impossible or difficult to preserve using traditional methods such as formalin preservation or as Haddock (Haddock, 2004) stated: “If an organism could not be fixed in formalin, it was not studied”. In recent decades there has been a renewed interest in gelatinous zooplankton, mostly caused by an increase in the frequency and size of blooms and the effects of the introduction of Mnemiopsis leidyi on populations of commercial fished fish stocks (reviewed in Richardson et al. 2009).

Because of the difficulty of fixing and preserving Mnemiopsis leidyi, most studies used a combination of total ctenophore live volume or weight in a catch to estimate biomass, coupled with the measuring

and counting of a live subsample of the catch on board (most recent examples Riisgård et al. 2007; Viitasaloi et al. 2008; Javidpour et al. 2009; Mutlu 2009; McNamara et al. 2010).

The disadvantages of measuring on board are that live ctenophores are often fragmented, and without an inspection under a dissection microscope, fragments of large individuals could be mistaken for small animals. Another issue is the difficulty of identifying juvenile ctenophores that are in their cydippid stage (Gorokhova and Lehtiniemi 2010). Furthermore, these methods can be very time consuming often requiring more shipping time. A method allowing fixation and preservation of samples for later examination in the lab would therefore be welcome.

Over time, different methods have been applied to preserve ctenophores for later examination and estimation of parameters such as densities, biomass and size distribution. Van der Veer and Sadée (1984), studying seasonal density and biomass of Pleurobrachia pileus, used a 4% formaldehyde solution. Yip (Yip, 1982) found that while Pleurobrachia pileus could be preserved in 4% formaldehyde solution, Bolinopsis infundibulum and Beroe gracilis either disintegrated or became too fragile to handle. Both authors used a fixed factor of 20% shrinkage to estimate the diameter of fresh animals. This however is probably inaccurate especially for small ctenophores (Thibault-Botha and Bowen 2004). A method to estimate wet mass of formaldehyde preserved Mnemiopsis leiydi and other ctenophores was developed by Purcell (Purcell, 1988) based on the correlation of length of preserved tentacle bulbs and wet weight.

Recently, a method of preserving ctenophores in a solution of acidic Lugol's solution was proposed (Engell-Sorensen et al. 2009). However, this method has some downsides, such as discoloration and a wide variation in success of preservation and amount of shrinkage for different concentrations of Lugol's solution. After 105 days precise measurement was only possible for 53 – 80% of the fixed ctenophores. A method which has largely been unnoticed or possibly considered as too labor intensive (Purcell 1988) is a method developed for preservation of individual specimens from samples by Adams et al. (1976). This method consists of fixation using a trichloroacetic acid (TCA) solution and subsequent preservation in a seawater solution containing propylene phenoxetol, propylene glycol and formaldehyde. In 2009, we tested this method for preservation and fixation of field samples and subsequently applied it with some modifications successfully for a year-round quantitative sampling programme of the ctenophores Mnemiopsis leiydi, Pleurobrachia pileus and Beroe gracilis in the western Wadden Sea. This paper describes the use of this modified fixation method and its advantages and disadvantages.

Results

This study shows that the method of Adams et al. (1976) also worked well for preservation of field samples of ctenophores, allowing detailed quantitative measurements in the lab. Heavily damaged specimens could often still be measured because the mouth-stomodaeum-statocyst complex would remain intact (Fig. 2-d). This method, while being slightly more labor intensive than the method using Lugol's solution proposed by Engell-Sorensen et al (2009), could potentially be more effective as it does not have some of the disadvantages of that method such as the high variation in shrinkage and discoloration. The percentages of ctenophores that are still measurable are also higher using this method. The Lugol's solution method has been shown to work also for larval ctenophores (Sullivan and Gifford 2009), whether that is also the case for this method is still unknown. The fixed oral – statocyst length – fresh mass relationship for Mnemiopsis leiydi (Fig. 1) and the other species can be used to accurately estimate the wet mass or bio-volume of live ctenophores. It would have been better

to sample all ctenophores for the length–volume relationship at the same time, but this was not possible due to time restrictions and differences in size structure of the population at the different sampling times. However, length decrease due to shrinkage after preservation did not appear to continue significantly between the last two measuring moments, which were 5 months apart. Overall, this method is useful for quantitative sampling of ctenophore populations efficiently and accurately, decreasing for example the probability of misidentification and increasing the accuracy of length distribution- and bio-volume estimates.

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