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SHORT COMMUNICATION

Preservation of the invasive ctenophore *Mnemiopsis leidyi* using acidic Lugol's solution

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During baseline studies of ichthyoplankton for environmental impact assessment in the Western Baltic Sea, we developed a simple method for preservation of the abundant ctenophore, Mnemiopsis leidyi, using acidic Lugol's solution.

The ctenophore, *Mnemiopsis leidyi*, is a widely distributed species in North American and European coastal waters. The species is a significant planktonic predator feeding on prey such as copepods, fish eggs and fish larvae. *Mnemiopsis leidyi* are self-fertilizing hermaphrodites with each individual producing several thousand offspring and blooms of this ctenophore occur at regular intervals (Purcell *et al.*, 2001). In such situations *M. leidyi* may seriously influence zooplankton communities and cause long-lasting perturbations of the ecosystem; the most prominent example is perhaps the recent rapid changes that took place after its introduction into the Black Sea causing a complete collapse of the pelagic ecosystem and serious declines in anchovy and other fish populations (Shiganova, 1998). Because of the high feeding rates of the species (Purcell and Arai, 2001; Purcell *et al.*, 2001; Waggett and Sullivan, 2006), the spread of *M. leidyi* to waters outside its historical range is currently of great concern due to the possible impacts on pelagic ecosystems in general and fish populations in particular (Purcell and Arai, 2001). In 2006, *M. leidyi* was observed in the North Sea, Kattegat and western parts of the Baltic Sea (Hansson, 2006; Javidpour *et al.*, 2006) and by 2007 it was widely distributed in inner Danish waters and the Baltic Sea (Tendall *et al.*, 2007; Kube *et al.*, 2007).

The potential threat of this invasive ctenophore makes close monitoring of its patterns of distribution and abundance imperative. However, routine sampling and monitoring of *M. leidyi* abundance has been seriously hampered because no method for preservation has been available. Biomass estimates of ctenophores have therefore commonly been restricted to measuring the total live volumes from plankton tows. Estimates of density and size distribution, which are essential for calculation of clearance rate and predation impact, have required immediate and labour intensive examination of fresh material. The commonly used fixatives, buffered formaldehyde and ethanol, both cause gelatinous plankton such as ctenophores to disintegrate making species identification, counting and measuring practically impossible (e.g. Hosia and Båmstedt, 2007; Kube *et al.*, 2007). Only one study describes a method in which the size distribution and density of preserved

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(5% formalin), and therefore deteriorated, specimens of *M. leidyi* were estimated using the size of tentacle bulbs of preserved animals (Purcell, 1988). Preservation of larval stages of *M. leidyi* with 5% acid Lugol's solution was reported by Costello *et al.* (2006), but the efficiency of this preservative was not discussed in detail.

Here we describe a simple but efficient method to preserve ctenophores using acidic Lugol's solution. Lugol's solution is a recommended fixative for phytoplankton, acidic Lugol's solution being the most gentle fixative for delicate organisms with the exception of organisms having calcium carbonate in their cell wall structures (Edler, 1979). Acidic Lugol's solution may also be used for fixation of microzooplankton (Stoecker *et al.*, 1994).

Adult *M. leidyi* for this study were collected in Femern Belt (Western Baltic Sea) in the beginning of December 2008, using a standard Bongo net with a mesh size of 500 μm. Salinity was 14 psu and water temperature was 6°C at the time of collection. Live specimens were kept cold and aerated during transport to the laboratory. Subsamples were collected for analysis of the effect of different fixation methods on the average length of the animals. The following acidic Lugol fixation strengths (final concentrations) were tested: 0% (reference), 1, 2, 5 and 10%. Acid Lugol's solution was made by dissolving 100 g KI in 1 L of distilled water. Fifty grams of crystalline iodine (I_2) were dissolved in 100 mL glacial acetic acid, and the two solutions were mixed (Throndsen, 1978). Precipitates were later removed and the solution was stored in a dark bottle. Following fixation for approximately 24 h, a number of *M. leidyi* were measured to quantify shrinkage in different concentrations of fixative. Ctenophore body length was expressed as oral–aboral length (Sullivan and Gifford, 2007). Reference specimens were measured prior to fixation.

When preserved with 1% acidic Lugol's solution, the animals disintegrated very quickly. Shaking of the fixed sample resulted in a "soup" of slime with no trace of *M. leidyi* other than fragments like comb-plates and parts of the digestive system. However, with increasing strength of the acidic Lugol's solution, preservation of the animals was markedly improved. Thus, at a final concentration of 2%, the animals stayed intact and were quite stable even after preservation for 105 days. After this time, about 80% of the specimens remained intact and identification was possible. With further increase in the strength of the Lugol's solution, the individuals became increasingly tanned, hardened and the mechanical strength of the gelatinous bodies of the animals improved (Fig. 1). At 5% solution, it was possible to manipulate the animals with a pair of tweezers without

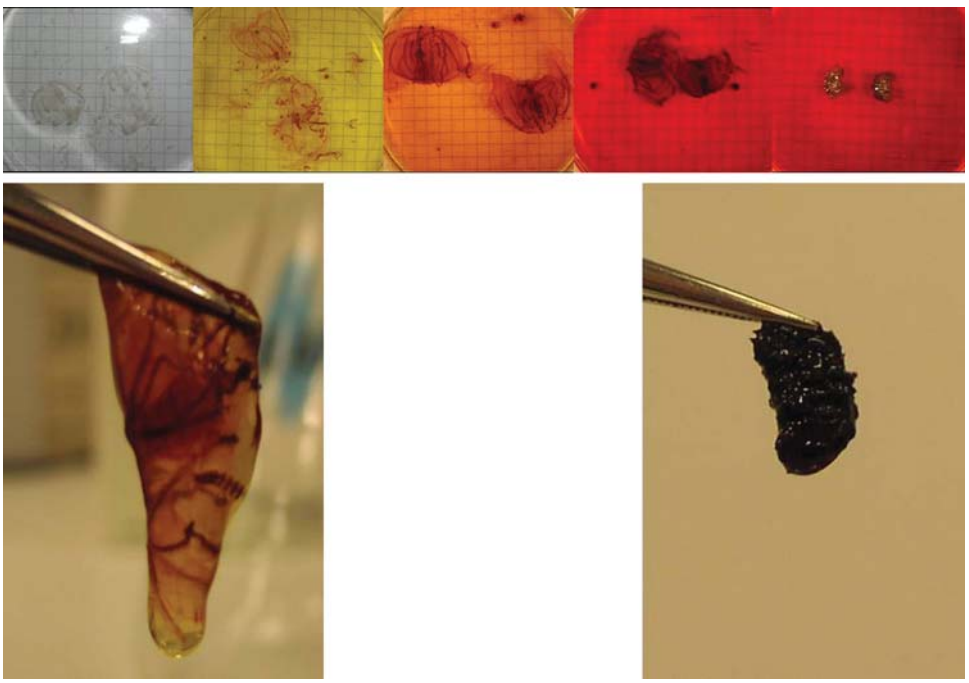

Fig. 1. Photographs of fixed *Mnemiopsis leidyi* after 24 h of fixation in petri dishes, as well as specimens manipulated with a pair of tweezers. Upper panel, from left to right: unfixed, 1, 2, 5 and 10% acidic Lugol's solution; a 5 by 5 mm grid is shown to indicate size of specimens. Lower panel, from left to right: 2 and 10% acidic Lugol's solution.

Table I: Mean oral–aboral length (mean ± SD) of Mnemiopsis leidyi after fixation for 24 h and 105 days, respectively, in 14 psu sea water with different concentrations of acidic Lugol's solutions

Final concentration (%)	Length after 24 h (mm)	Length after 105 days (mm)
Un-preserved sample	6 ± 6 (48)	–
1	Not determined[a]	Not determined[a]
2	4.6 ± 3.6 (88)	2.6 ± 0.9 (71)
5	3.3 ± 2.2 (89)	2.0 ± 0.8 (47)
10	2.7 ± 1.5 (91)	1.5 ± 0.6 (73)

A subsample of 100 mL was analysed at each concentration except for un-preserved animals where only a 50 mL subsample was used. Figures in brackets indicate the number of observations.
[a]Length and number of animals could not be determined because animals disintegrated at this concentration.

any damage (Fig. 1). After 24 h at 10% Lugol's solution, the shrinkage effect was significant with preserved animals resembling a raisin-like structure.

Size distributions of the samples fixed at strengths of acidic Lugol's solutions from 2 to 10% were easy to obtain, and the number of individuals per subsample was remarkably constant between the different subsamples indicating no loss of animals as a result of the fixation during the first 24 h (Table I). After 105 days, some specimens deteriorated, and precise measurement of length was possible for only 53–80% of the preserved animals. The average measured length of *M. leidyi* decreased with increasing strength of the acidic Lugol's solution. For example, in 10% acid Lugol's solution, individuals shrank to 45% of their original size, indicating that a correction factor of approximately 2.2 must be used to estimate the length of live specimens from specimens preserved in 10% solution. The degree of shrinkage increased with time and after 105 days the length of preserved specimens was only about half that of specimens preserved for 24 h. This is largely consistent with the results reported by Sullivan and Gifford (2009) for larval stages of *M. leidyi* preserved with acidic Lugol's solution. We used the data shown in Table I to generate a relationship between the fixative strength and the conversion factor (Fig. 2). The shrinking observed for *M. leidyi* is similar to shrinking rates found for phytoplankton preserved in acidic Lugol's solutions (Montagnes *et al.*, 1994; Stoecker *et al.*, 1994).

This study and the accompanying paper by Sullivan and Gifford (2009) demonstrate that, contrary to what has often been stated, it indeed is possible to preserve *M. leidyi*. Depending on the desired stability of preserved specimens, there will be a trade-off between the increasing stability of the fixed animals and a reduced possibility of observing details in/on the animal. For quantitative studies with ctenophore populations dominated by *M. leidyi*, strong fixation can be used routinely. In more complex situations with mixed populations of different species of ctenophores, the relative abundance

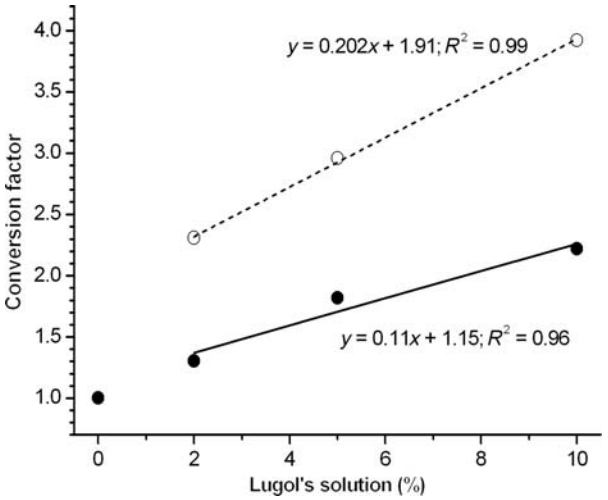

Fig. 2. Linear relationships between final concentration of acidic Lugol's solution in 14 psu sea water and the conversion factor necessary to estimate oral–aboral length of *Mnemiopsis leidyi* prior to preservation from oral–aboral length of preserved specimens. Closed circles are preservation for 24 h. Open circles are preservation for 105 days. Each point is the mean of at least 47 observations. See text for further details.

of each species may be obtained from lightly preserved (2%) and/or live samples, provided that preservation of other ctenophore species is possible with this technique; more research is needed to answer this question. However, the shrinkage observed may depend on salinity (Sullivan and Gifford, 2009) and probably is species specific. More studies are needed to elucidate these issues before specific recommendations for preservation can be given. However, for most purposes, it appears that preservation in 2% acidic Lugol's solution gives good results with the quality of specimens remaining stable for up to 3 months (Table I; Sullivan and Gifford, 2009). Specimens kept for 3 months could easily be identified with morphological characters remaining distinct. If tanning makes identification difficult, the colour can be removed by adding small amounts of sodium thiosulphate. The amount of

sodium thiosulphate can be varied to achieve the desired bleaching effect.

A simple method for preservation of ctenophores, as we describe here, has several advantages apart from those that are obvious. For example, when performing ichthyoplankton surveys, various medusae, including lobate ctenophores, are often much more numerous in the samples than fish eggs and fish larvae. If the ctenophores disintegrate during fixation of samples, this will result in potential drawbacks such as failure to estimate the predation impact that ctenophores might have on ichthyoplankton and making it difficult to find ichthyoplankton in the sample. Lastly, it should be mentioned that acidic Lugol's solution is less harmful to humans when compared with aldehyde-based fixatives.

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