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# Respiration of fragile planktonic zooplankton: Extending the possibilities with a single method

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ABSTRACT

Gelatinous zooplankton are increasingly being included within ecosystem models. However, for the majority of species, their respiratory and excretory processes are poorly understood, making accurate model predictions difficult. Fragility and a broad size range have resulted in a number of methods being used for different species, some *in situ* and others under laboratory conditions. This makes it difficult to compare studies and incorporate the data into models. Oxygen optodes have been used here to obtain respiration rates of seven species ($n = 65$ individuals), utilising the same method across a large range of incubator sizes (252 mL to 31.25 L) and specimen masses (<1–2560 g wet mass). These data add respiration rates over a wider mass range to five gelatinous genera — *Cestum*, *Geryonia*, *Rhizostoma*, *Mnemiopsis*, *Solmissus* and provide the first respiration rate of the fragile ctenophore *Leucothea multicornis*. *In situ* data are compared with laboratory rates and trends developed for several species by adding to previously published work. Finally these data do not significantly elevate the allometric slope of a gelatinous zooplankton carbon mass: respiration rate ($b = 0.795$) relationship, despite increasing both the mass range and sample size.



## 1. Introduction

Gelatinous zooplankton are prolific within the marine environment, although their impact on the ecosystem is often poorly understood, with a wide diversity of species within the cnidarian taxa. Higher gelatinous biomasses have been recorded from coastal regions, with highly-productive or well-oxygenated waters favouring many species (Lilley et al., 2011; Lucas et al., 2014). Contrastingly, many smaller genera and larval forms can easily go unobserved, or may disintegrate in plankton nets (Purcell, 2009). This has contributed to an incomplete understanding of the role of gelatinous zooplankton and their poor representation within ecosystem models (Pauly et al., 2009). Interspecific predation between species (*e.g.* Grondahl, 1988) further complicates their impact. In the absence of observations it is difficult to speculate on the role of other poorly-observed species.

Biochemical exports by gelatinous zooplankton mainly come from the metabolic processes of respiration, excretion and mucus production (Condon et al., 2011). Respiration has been well studied because it provides an estimate of the metabolic rate of an individual, within the limits of the common species and size ranges. Gelatinous taxa, however, are widely diverse in size, mass and body structure (Haddock, 2004), and range from larvae of <1 mm length to small hydromedusae, long chains of salps or 200 cm diameter adult medusae (Mills, 2001). Given that the mass significantly alters the metabolic demands and outputs of an individual (Purcell, 2009; Purcell et al., 2010), it is important to be able to study a range of sizes of each species in a comparable manner without incurring methodological biases.

Respiration rate measurements have typically used chemical titrations or oxygen electrodes (reviewed by Gatti et al., 2002), with oxygen optodes gaining favour recently; optodes are easier to use, their calibrations drift slower and they can provide continuous measurements. In essence polarographic oxygen electrodes also provide continuous data, but are subject to steady changes in the chemical composition of the electrode electrolyte and require regular calibration. The consumption of oxygen by the electrodes affects measurements, but importantly requires stirred samples, potentially damaging fragile planktonic individuals. Winkler titrations of water samples have the best accuracy of all the methods for measuring oxygen, at 0.2% of oxygen saturation or 0.4 μmol $O_2$ $L^{-1}$, but require the incubator to be opened to obtain samples and have usually been used as initial and final measurements. In the absence of intermediate measurements, a linear decline in oxygen saturation between the data points must be assumed, thereby ignoring any variations within the incubation caused by handling stress, adaption of the individual to the conditions within the incubator or temperatures changes.

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Advances in the development of oxygen optodes (Klimant et al., 1995) have allowed accurate intermediate measurements without experiencing drift (Gatti et al., 2002). Non-invasive optical oxygen sensors (hereafter oxygen 'spots') are glued to the inside of transparent incubators and allow intermediate optical measurements without disturbing the internal conditions. Furthermore, the second generation of optodes does not rely on the intensity of the fluorescence measurement, but on the delay between excitation and the fluorescence peaks (the phase difference, Tengberg et al., 2006). This phase difference is not impacted by the thickness or nature of the incubator walls on which the oxygen 'spot' is glued (as long as they are transparent and not fluorescent); nor by the incidence angle or the specific optical fibre used.

Methodologically, both incubator volume and capture of organisms are known to affect physiological measurement experiments. Small incubators may reduce the movement of organisms and thus their metabolic rates (Purcell, 2009; Purcell et al., 2010), while overly large incubators risk losing the metabolic signal in background respiration unless finely-filtered water is used. Gelatinous zooplankton frequently sustain damage or stress during capture (Purcell, 2009) and laboratory conditions, thereby casting doubt on the validity of the results obtained. Gentle *in situ* methods of capture and incubation (reviewed by Raskoff et al., 2003) may reduce the stress to the individuals, but have limitations with regard to cost or flexibility. Alternatively, laboratory incubations may also adversely affect the results obtained, with sub-ideal conditions or the requirement to starve individuals prior to incubations.

Meta-analyses have previously addressed a variety of correlations between respiration in gelatinous zooplankton and carbon mass (Acuña et al., 2011), equivalent spherical diameter (Pitt et al., 2013) or dry mass and temperature (Ikeda, 2014). However these only encompass a small proportion of the known species and further empirical data on poorly-studied species are required to confirm whether all species and taxonomic groups follow these trends. Here opportunistic observations of respiration have been obtained for some lesser-known species and a wider mass range in some well-known species, using oxygen optodes.

## 2. Materials and methods

All gelatinous zooplankton were caught by snorkelling or using a bucket from a small inshore research vessel in the bay of Villefranche-sur-Mer (43°40.81′N; 7°18.55′E), or in the Berre lagoon (Etang de Berre, Marseille 43° 26.75′N; 5° 6.83′E), France in the case of the 11 smaller *Mnemiopsis leidyi* A. Agassiz 1865.

Incubations were carried out in screw-topped plastic incubators of 252 mL, closed underwater to remove bubbles, or larger custom-made incubators of 5.24 and 31.25 L (PSP Industrie, Marseille, France; Fig. 1). A silicon O-ring and twist-lock lids allowed the insertion of gelatinous zooplankton into the incubators. Light-sensitive foils 'oxygen spots' were glued to the inside walls of the transparent incubators, or on a small glass window in the case of the largest incubators. All spots were calibrated at 0 and 100% oxygen saturation. A Fibox-3 oxygen meter (PreSens Precision Sensing GmbH, Germany) was used for all experiments, with measurements obtained by holding a 2 mm diameter optical-fibre against the oxygen spot until the phase stabilised (typically 5–10 s). A custom-made waterproof housing for the Fibox-3 was constructed (PSP Industrie, Marseille, France) to allow *in situ* measurement of oxygen saturations.

Repeated calibrations indicated that calibration drift was slow, and it was not necessary to take reference measurements after every incubation; the 100% oxygen saturation calibration drifted faster than at 0% saturation. Drift was around 0.9% ±0.46 per month, equivalent to 2.17 μmol $O_2$ $month^{-1}$ ($n = 26$, range 1–4.9 μmol $O_2$ $month^{-1}$, duration 9–22 months) for the 100% oxygen saturation state. The oxygen spots in the largest incubators drifted fastest and recalibration every two months was found to be sufficient to avoid significantly different data between two calibration points. Measurement precision was typically 0.19% of the oxygen concentration.

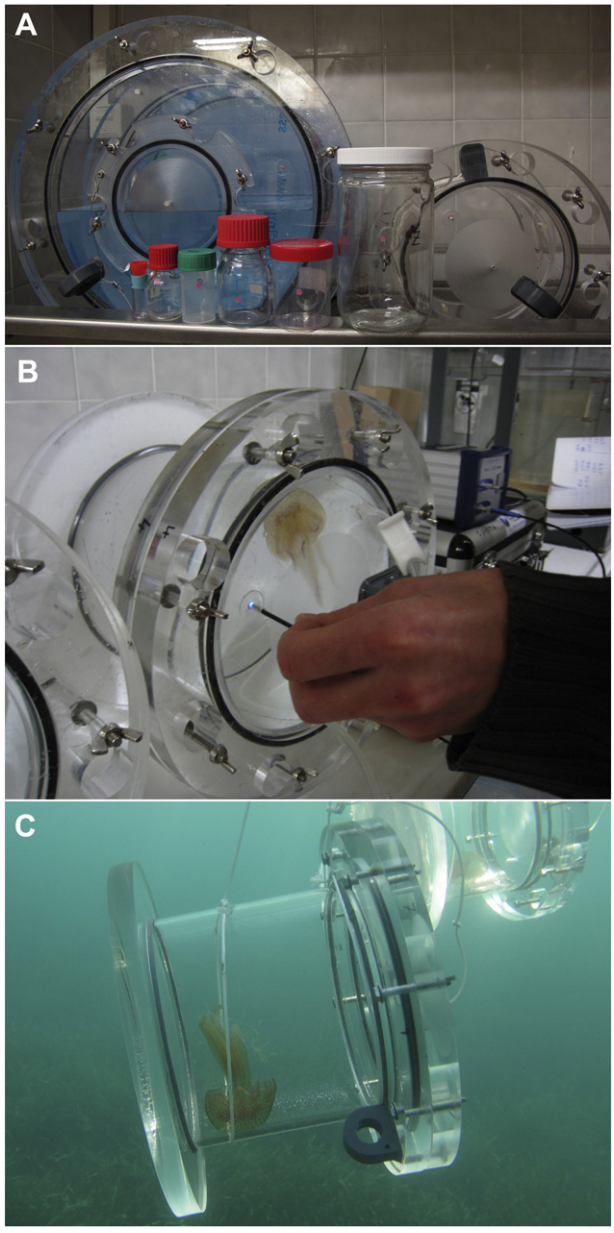


**Fig. 1.** Incubations with oxygen optodes: A) 7–3100 mL incubators to suit animal size; B) recording an intermediate measurement on a 5 L incubator; C) *in situ* incubation of a *Pelagia noctiluca* in a 5 L incubator.

*In situ* incubations were performed on 18 individuals ($n = 5$ species) in unfiltered ambient-temperature seawater from the location of animal capture (12.3–17.6 °C). Incubators were hung from a 2 m windsurfboard at 1 m sea depth during calm conditions (<Beaufort 1) for the duration of the *in situ* measurements. All incubators, control and experimental, were subjected to ambient temperature and equal levels of light, shade and internal waves. Light-sensitive spots were shaded during measurements to prevent over-excitation by sunlight. Randomised positioning of control incubators among the experimental ones allowed accurate quantification of background metabolic changes in the unfiltered water. The incubators were not close enough to each other or the bottom to allow collisions during incubations, which might disturb the incubated gelatinous species.

Laboratory incubations were performed in a temperature-controlled room, or water bath controlled by a cryo-thermostat. Temperature was recorded at every oxygen measurement by a probe in the water bath or equivalent additional unused incubator. All laboratory experiments