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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.

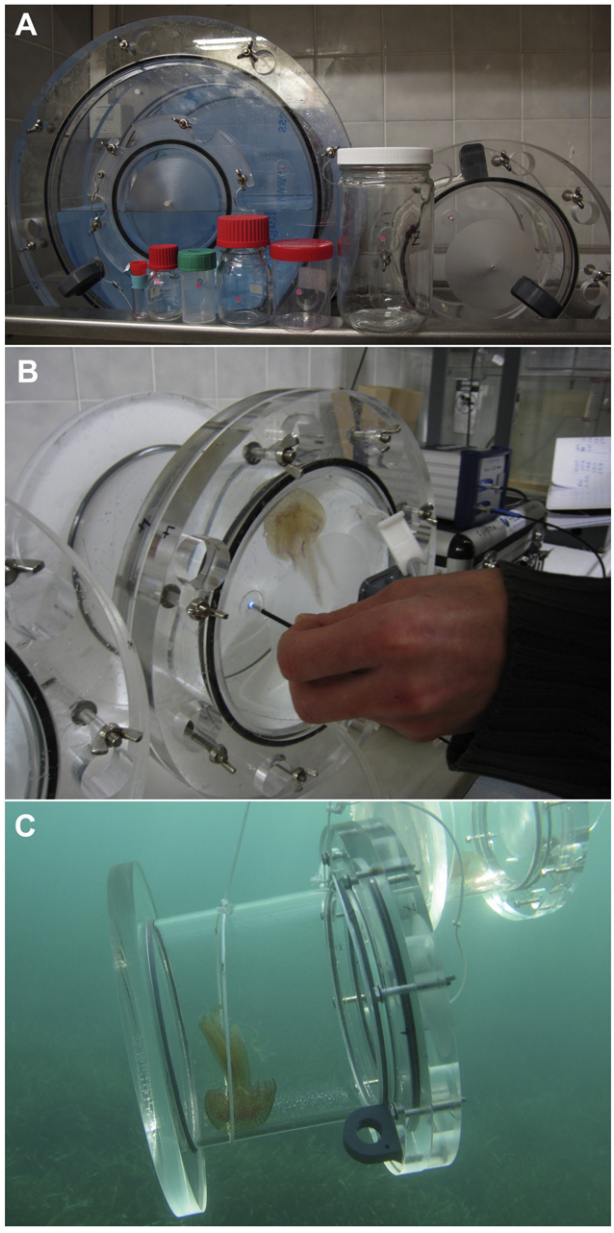

Fig. 1. Incubations with oxygen optodes: A) 7–3100 mL incubators to suit animal size; B) recording an intermediate measurement on a 5 L incubator; C) *in situ* incubation of a *Pelagia noctiluca* in a 5 L incubator.

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

were performed in pre-filtered 1 μm seawater acclimatised to the laboratory temperature. Salinity of the seawater was 38, except for some of the *M. leidyi* (Table 1) where salinity at the capture location was 24 on 12 July 2011 (Lilley et al., 2014c). Individuals were rinsed in a bath of filtered water to limit bacterial or mucus contamination before introducing to pre-filled incubators using a large kitchen ladle. To close the incubators, filtered water was slowly injected into the incubator, removing air bubbles, and the holes plugged with a plastic stopper. Preliminary trials showed that oxygen spots in control incubators stabilised to changes in pressure on closure within 15 min. Thereafter, in every experiment, oxygen measurements started at least 15 min after closure, also giving the individuals a chance to acclimatise within the incubator.

Oxygen measurements were taken at the beginning, end and roughly hourly during incubations, except overnight, until 10–15% of the initial oxygen saturation had been consumed by the individual. Animal size, incubator size and ambient temperature dictated the rate of oxygen decrease and duration of the experiment (range — 2.1-28.7 h). Individual spot calibration, using the temperature and salinity at the time of measurement, allowed correction of the phase delay of the returning light pulse to obtain an oxygen concentration (μmol O_2 L^{-1}). For each incubation a linear regression was fitted through all the data points, plotting oxygen concentration against time (mean 6, range 3–9 measurements per individual), with the slope of the line giving the rate of oxygen consumption (μmol O_2 L^{-1} day^{-1}). Average background respiration from control measurements was subtracted from experimental measurements. Only data with an r^2 greater than 0.9 were deemed to have given linear declines in oxygen saturation, with the majority greater than 0.98. In two cases an irregular reading was removed (most likely a result of the individual being close to the oxygen spot), leaving 6 or 7 data points for that dataset, to fulfil the criteria. All respiration equations were defined as Ordinary Least Squares regressions in the form $Y = aX^b$, where Y represented the respiration rate over time, and X the wet mass (*WM*) of an individual.

3. Results

Seven species of diverse gelatinous zooplankton sporadically present in the NW Mediterranean were individually incubated to obtain mass-specific oxygen consumption rates (Table 1), with the mass range spanning nearly four orders of magnitude (0.9–2560 g *WM*, $n = 65$). Across the two taxonomic phyla, the mean decline in respiration was 13% at the end of the experiment. Most species showed a strong allometric trend (Fig. 2A, Table 1) of increasing individual respiration with mass, although a single juvenile *Leucothea multicornis* Quoy & Gaimard 1824 had a relatively low respiration rate compared to the other species studied of comparable mass.

While most of the data were obtained under laboratory conditions, incubations were conducted *in situ* on eighteen individuals — *Cestum veneris* (2), *Geryonia proboscidalis* (2), *L. multicornis* (1), *Pelagia noctiluca* (6) and *M. leidyi* (7). Control incubators, during these incubations, were filled with unfiltered seawater containing a natural plankton assemblage;

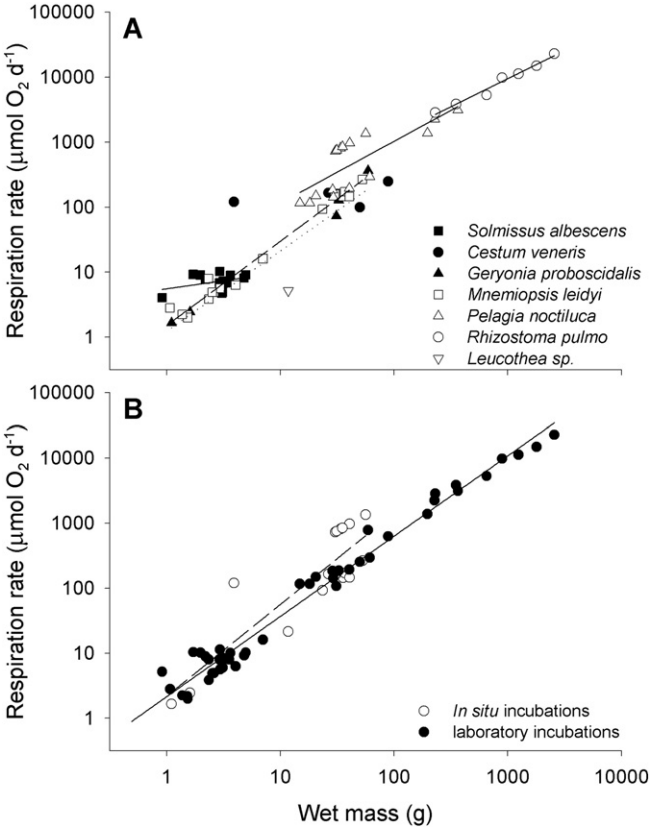

Fig. 2. Respiration rates as a function of wet mass for A) seven species of gelatinous zooplankton; B) between laboratory and *in situ* incubations. See Table 1 for species-specific regression fits and incubation details. Fitted regressions in part b were $R_{lab} = 0.332\ WM^{1.23}$ (solid line) and $R_{in\ situ} = 0.339\ WM^{1.41}$ (dashed line). Note log scales.

these controls had higher variability than laboratory controls with filtered seawater. Despite the change in background respiration, rates of oxygen consumption were similar between the two methods (Fig. 2B). No significant deviation was observed between lab and *in situ* data (ANCOVA, $F_{1,61} = 1.70$, $p = 0.197$). Higher *in situ* respiration rates than would otherwise be expected were observed from six *P. noctiluca* Forsskål 1775 and a small *C. veneris* Lesueur 1813.

3.1. Results combined with previous data

Several of the species studied have been incubated previously by other authors. Similar respiration rates were obtained to previously available data on *C. veneris* and *G. proboscidalis* Forsskål 1775 (synonyms: *Cestus veneris* and *Carmarina hastata* respectively (Vernon, 1895)) (Fig. 3A, B), although over a wider mass range. This study also doubled the mass range of the large scyphozoan *Rhizostoma* sp. Cuvier 1800 over which

Table 1
Respiration rates of the gelatinous species incubated in this study. All animals were obtained from Villefranche-sur-Mer, France except *M. leidyi** from Berre Lagoon, Marseille, France. Raw data is shown in Fig. 2. All respiration fits are calculated from a power relationship where Respiration (μmol $O_2 \cdot day^{-1}$) = a. $WM(g)^b$, ±1 standard error, except **where a linear fit of $R = a.\ WM + b$ is shown and is not significant ($p = 0.293$). †may have included a *Velamen sp.* individual among the *Cestum sp.*

Species	*n*	Wet mass (g)	Temp. (°C)	Incubator volume (*L*)	C:V ratio	Mean respiration ±1SD (μmol $O_2 \cdot d^{-1}$)	Respiration fit (±1SE)		r^2
							a	*b*	
Cestum veneris†	4	3.9–88.8	12.9–17.6	5.24	426	158.39 ± 57.0	104.1 ± 47.8**	1.28 ± 0.91**	0.50**
Geryonia proboscidalis	5	1.1–59.1	12.3–15.2	0.252/5.24	177	113.7 ± 127.2	1.35 ± 1.29	1.29 ± 0.09	0.99
Leucothea multicornis	1	11.7	17.6	0.252	22	5.21	–	–	–
Mnemiopsis leidyi	7	23.5–52.7	17.3	0.252/1.02	15	139.32 ± 43.19	1.84 ± 1.70	1.21 ± 0.15	0.93
*M. leidyi**	11	1.07–7.03	16.5	0.252	122	5.35 ± 3.85	1.78 ± 1.19	1.07 ± 0.17	0.81
Pelagia noctiluca	16	14.8–364	16.1–17.3	5.24	240	837.66 ± 828.2	12.9 ± 2.13	0.95 ± 0.19	0.63
Rhizostoma pulmo	7	230–2560 g	16.1	5.24/31.35	19	10097.68 ± 6547.6	24.5 ± 1.48	0.86 ± 0.06	0.98
Solmissus albescens	14	0.9–5.0 g	12.8	0.252	101	7.40 ± 1.8	5.50 ± 1.20	0.24 ± 0.17	0.15

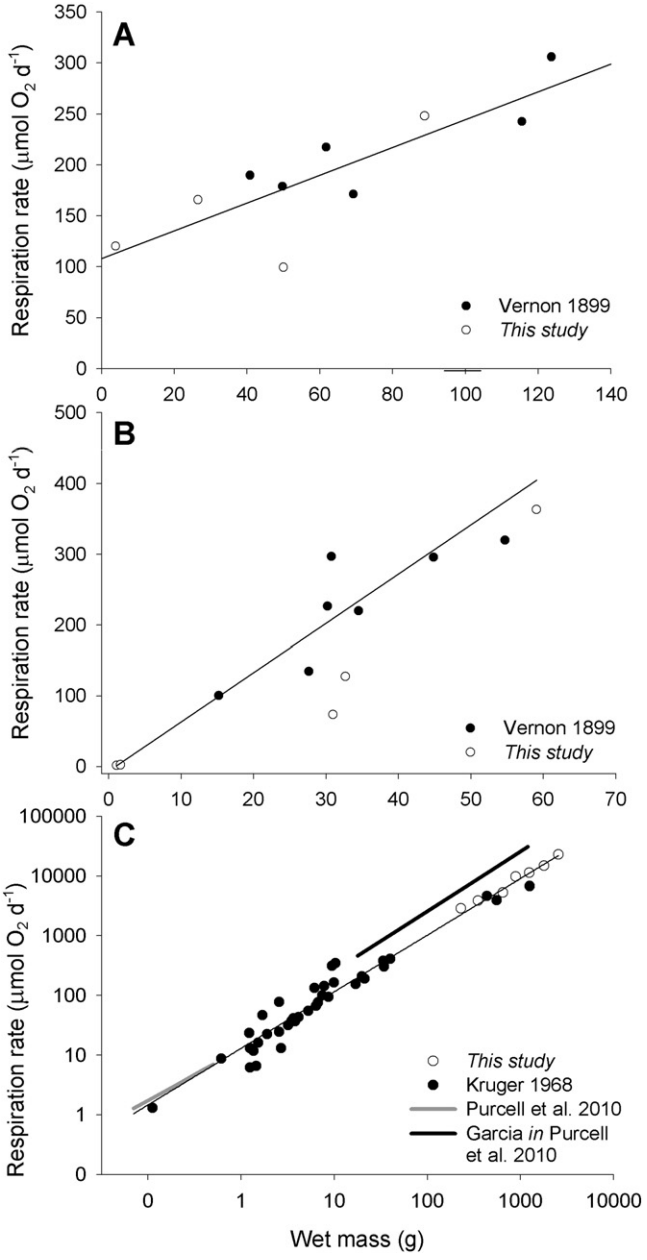

Fig. 3. Data comparisons between published data (filled circles) and this study (open circles) — A) *Cestum veneris* (*Cestus veneris* in Vernon, 1895); B) *Geryonia proboscidalis* (*Carmarina hastata* in Vernon, 1895); C) *Rhizostoma spp.*, where individual fits are presented in Supplementary Table 1.

metabolic rates have been measured, providing a broader understanding of the mass-scaling of its respiration rate (Fig. 3C). Two of the three previous studies on *Rhizostoma* also provided data at similar temperatures to this study — juveniles in Barcelona (Purcell et al., 2010), and the observations of Krüger (1968), (Fig. 3C, Table S1). Combining these datasets together allowed a combined regression equation for the *Rhizostoma* genus to be quantified, for studies between 15 and 20 °C:

$$R_{Rsp.} = 13.05\ WM^{0.947} \quad (1)$$

where the *Rhizostoma* sp. respiration rate (R_{Rsp}) was converted to µmol O_2 individual^{-1} day^{-1} for all studies and *WM* (range 0.9–2560 g) given in grams ($r^2 = 0.974$, $F_{1,49} = 1818.63$, $p < 0.001$). By contrast the data of Garcia (in Purcell et al., 2010) and Vernon (1895)(Table S1) were not comparable because of a temperature of 24.5–27 °C and a small mass range respectively.

4. Discussion

Oxygen optodes have been shown to be a practical option for measuring respiration rates of individual organisms (Gatti et al., 2002; Jacoby et al., 2009; Warkentin et al., 2007) and are suitable for gelatinous zooplankton, despite the wide diversity of individual sizes and variable incubator volumes. Optodes have an accuracy approaching Winkler titrations (± 0.4 µmol·L^{-1}), but also allow intermediate measurements to be obtained without sacrificing replicates (Ikeda et al., 2000); therefore they provide an accessible method to measure fragile aquatic species. Modern optodes use a phase shift, or 'lifetime' of the returning signal to establish the oxygen concentration (Tengberg et al., 2006), which effectively removes any issues previously encountered with variable materials, such as the angle of the optical fibre and signal intensity (Klimant et al., 1995). For fragile gelatinous zooplankton, such as the Cnidaria, damage occurs frequently if magnetically-stirred incubators are used, which are essential for prolonged incubations using oxygen-consuming electrodes. Both electrodes and optodes allow for any changes in temperature or conditions during incubations to be quantified using intermediate measurements. This allows some understanding of the variability in oxygen consumption rates within an incubation to be observed (*e.g.* Jacoby et al., 2009) and naturally variable *in situ* conditions to be accounted for (*e.g.* Lilley et al., 2014c). Importantly, the ability to calibrate the optodes after the experiment has significant advantages over the quick calibration drift of electrodes because of their electrolyte consumption (Klimant et al., 1995; Tengberg et al., 2006), particularly for studying sporadically-occurring species.

In this study, oxygen optodes allowed data to be collected from a wide size range of gelatinous zooplankton individuals, in both the laboratory and field. Previous empirical studies have, at times, been limited in the size of incubators or their assumptions of linear respiration rates. Here the size of the incubator was adjusted to the individual, allowing a clear understanding of how mass affected individual respiration rates, rather than measuring a combined metabolic rate for several individuals and averaging after the incubation. Incubator volumes (Table 1) were a compromise between minimising the incubator effect and observing sufficiently measureable changes in oxygen saturation. Potentially some of the largest individuals may have had their movement, and therefore their metabolic demands, reduced as a result, causing within-species variation (Purcell et al., 2010). For instance the smaller *Rhizostoma* individuals incubated in both the 5 L and 31 L incubators appeared to have slightly higher respiration rates per unit mass than the other individuals in the same experiment (Fig. 3C).

Successful deployment of oxygen optodes *in situ* combined with comparable results in the laboratory should give confidence that optodes can be applied widely across the gelatinous zooplankton to explore species previously difficult to study. For instance, the similarity between respiration rates in the laboratory and *in situ* suggests that handling and laboratory stress to the individual zooplankton was minimised during these experiments. Similarly observations of fragile species, or those liable to unpredictable behaviour in the laboratory, can now be confidently examined without significant disturbance in a more natural situation.

At a species level, *P. noctiluca* studied *in situ* had respiration rates above average compared to laboratory-studied individuals at comparable temperature and salinity (Fig. 2A, open triangles). Contrastingly, seven large *M. leidyi* (Fig. 2A, open squares) measured during the same *in situ* experiment had respiration rates close to those obtained in the laboratory. Given the ideal conditions (calm sea, individuals with no obvious damage, high plankton abundance, little handling) under which these data were obtained, *P. noctiluca* may change its behaviour in the laboratory more than the ctenophore *M. leidyi*. Reduced pulsation rates and metabolic rates of *P. noctiluca* may be a result of low or artificial illumination (Axiak, 1984) or starvation in filtered water, compared to unfiltered *in situ* experiments in bright conditions. *M. leidyi*, by contrast, is known to feed voraciously and could have exhausted any plankton available in the *in situ* incubators much quicker than the *P. noctiluca*, thereby

mimicking laboratory conditions with no prey available (see also Lilley et al., 2014c) despite the varying salinity and productivity between the two field locations studied. Another species to show an unusual metabolic rate was a single juvenile ctenophore (*L. multicornis*), incubated for the first time. The *in situ* respiration rate obtained for this species was relatively low compared the other species of a comparable size studied. Further work will show whether this species has a particularly low metabolic rates, or this data point was the result of an exterior factor such as handing or containment (Raskoff et al., 2003).

Previous observations of the narcomedusae *Solmissus albescens* Gegenbaur 1856, obtained at a higher temperature, showed higher rates of respiration than those observed in this study (Table 1); equivalent to 4.75 μmol g WM^{-1} d^{-1} at 8 °C measured immediately after capture (Bailey et al., 1994). These medusae were subjected to a three hour transfer to the laboratory from offshore, which may have inhibited the activity level of this deep-water species. By contrast, two other cnidarian classes and a ctenophore species all showed rates of respiration comparable to previously recorded data (Fig. 3) despite different methods of measuring respiration. Of these only one *C. veneris* (50 g *WM*) appeared to show considerably lower respiration than would otherwise be expected and may have been a misidentified as the very similar *Velamen parallelum* Fol 1869 (L. Madin *pers. comm.*, Mills and Haddock, 2007). Large incubators and the ability to measure *in situ* facilitated the data collection from both larger and smaller individuals than had previously been obtained. These comparisons had allometric exponents (b) for mass of 1.049 ± 0.260 (*G. proboscidalis*) and 0.947 ± 0.022 (*Rhizostoma* sp.) emphasising the effect of different body structures on the rate of respiration, with a linear relationship for *C. veneris* across all the available data ($R = a\ WM + b$, where $a = 108.1 \pm 23.1$ and $b = 1.37 \pm 0.32$). The allometric exponents obtained conform with metabolic theory, where the b-exponent is typically between 0.6 and 1, depending on the proportion of surface area or tissue mass used to regulate the exchange surfaces for respiration respectively (Glazier, 2005). Higher exponents are not uncommon in some cnidarians, such as *G. proboscidalis*, and chordates, with exponents up to 1.6 inversely related to buoyancy and often coinciding with high growth rates (Glazier, 2006). Recent work has also shown that variation in metabolic scaling may be related to whether a species tends to enlarge in 1, 2 or 3 dimensions during growth; where three dimensional or isomorphically growing species have a lower mass-respiration exponent than species that tend to elongate (Hirst et al., 2014).

The scyphozoan *Rhizostoma* sp. appeared to have a similar respiration rate over the same temperature ranges (Eq. (1), Fig. 3C, Table S1), although increasing proportionately at higher temperatures (Garcia data, in Purcell et al., 2010). There was no difference between the respiration rates of the northern species of *Rhizostoma octopus* Linnaeus 1758 and the southern *Rhizostoma pulmo* Macri 1778 (Lilley et al., 2009; Russell, 1970). This finding was surprising because most zooplankton have greater mass-specific respiration rates at lower latitudes, resulting from acclimation to higher water temperatures (Ikeda, 1985). On the assumption that both European species of *Rhizostoma* behave comparably it was possible to calculate the effect of temperature on metabolic functioning, comparing the change in respiration rate over a 10 degree temperature range (corresponding to the Q_{10} parameter). For *Rhizostoma* sp. the rate of respiration increased by 2.64 times from 16.2 to 25.75 °C (Mar Menor and Barcelona in Purcell et al., 2010, this study; Table S1), equivalent to a Q_{10} of 2.77 ($r^2 = 0.92$). Although a Q_{10} for *Rhizostoma* sp. had not previously been proposed, this value is in agreement with other scyphozoan species (*Aurelia aurita* Linnaeus 1758, 2.9; *Cyanea capillata* Linnaeus 1758, 2.6–3.4 (Larson, 1987)). Finally, while containment may change the metabolic rates obtained in larger individuals (Table 2; Purcell et al., 2010), these are the first respiration estimates for *Rhizostoma* sp. up to 2.5 kg *WM* and it will be interesting to see if larger individuals continue the trends observed here.

Trends in data can also be used to give a generalised picture, for instance whether gelatinous zooplankton abundances are increasing globally (Brotz et al., 2012; Condon et al., 2013) or if estimates of the expected biomass can be made from physical conditions in a region (Lilley et al., 2011; Lucas et al., 2014). Ecosystem models allow the extension of data to unexplored regions or to account for unmeasured size classes. In most models the gelatinous zooplankton are included as a single trophic level around 3, but this generalisation is unlikely to take account of the diverse range of prey consumed (Pauly et al., 2009) or predation by one species on another (Grondahl, 1988; Lilley et al., 2014b). Ongoing research may highlight where species-specific knowledge is required, both among gelatinous species and broadly within the zooplankton, while in other situations a taxa-level assumption is possible. For instance, mass-specific respiration rates can be used to describe the overall metabolic and energetic requirements of an organism. Allometric exponents between mass and respiration quantify the dependence of an organism on surface area to exchange soluble gases such as oxygen (Glazier, 2006). In the plankton, multi-dimensional growth trends are closely tied with the exponent of respiration rate to body mass (b_R) at a taxonomic, rather than species, level (Hirst et al., 2014).

To date, in the gelatinous zooplankton, the broadest analyses of respiration rates to mass have been published by Acuña et al. (2011), and provide an estimate of the mass-respiration scaling for incorporation into biochemical ocean models. Data from this study have been added to that of Acuña et al. (2011, Table S2) to improve the robustness of their data by increasing the mass range and diversity of species studied. In addition to the 65 data points in the current study, two recently published datasets for the ctenophore *M. leidyi* (Lilley et al., 2014c, $n = 154$) and scyphozoan *P. noctiluca* (Lilley et al., 2014a, $n = 77$) were also included. All data were converted to carbon masses using published equations and adjusted for temperature (see Supplementary information S2). An Ordinary Least Squares regression of the combined data confirmed that respiration rates were proportional to carbon mass to an exponent of 0.795 (±0.010) over a carbon mass range of 1.25×10^{-5}–9.47 g ind^{-1}:

$$\mathrm{Log_{10}\ Respiration\ rate} = \log_{10} 11.50(\pm 0.025) + 0.795(\pm 0.010) \log_{10} \text{body carbon (g)}$$

where the units of the respiration rate were: $\log_{10}$ (mmol O_2 ind^{-1} d^{-1} / $\exp(-E_a / kT)$).

These data extended the upper limits of the data presented by Acuña et al. (2011) without significantly increasing the slope of the regression fit (ANCOVA $F_{1,1436} = 0.118$, $p = 0.731$). It must be noted that this broadscale trend covers all the available data across the Cnidaria and Ctenophora; however it might be expected that individual species and taxonomic groups would deviate significantly from the overall mean (*e.g.* Fig. 2A, Table 1) in either slope or intercept (Kiørboe and Hirst, 2014; Pitt et al., 2013).

Further empirical work on the more fragile or rare and data-poor species, and larger individuals of the well-known species, using new methods of measuring metabolic rates should help to confirm both the broadscale and taxa-specific trends in metabolic scaling. The extent to which a species grows in 1, 2, or 3 dimensions may also account for much of the variability between species as their metabolic rates are known to vary in proportion to their surface area or the distance over which a resource must pass to reach the relevant tissue (Hirst et al., 2014). Finally it must be borne in mind that changes in body structure through life-history stages may have variable metabolic scaling rates with mass if the life stages are considerably different in morphology (Glazier et al., 2015). For instance ephyrae of scyphozoans are likely to have considerably different metabolic scaling rates to the adult form.

Further study should confirm or refute estimates of allometric scaling between mass and respiration. To expand these data, incubations at both ends of the size spectra are required, with size and fragility both having imposed limitations on the methods available for incubations to date; however micro-optodes can now provide the accuracy required to study very small or larval individuals, and there is the potential for *in situ* incubations of very large individuals using flexible transparent

plastic sacks and optodes if mass and 'incubator' volume could be calculated.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2015.06.013.

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