Metabolic performance and survival of medusae in estuarine hypoxia

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ABSTRACT: Increasing eutrophication and hypoxia in marine environments appear to differentially promote the survival of some medusae species and the disappearance of others. To understand the physiological basis for this phenomenon, respiration rates and critical oxygen tensions (P_c) were measured for 12 species of medusae from Puget Sound (Washington State, USA). Mean mass-specific respiration rates ranged between 0.064 µmol O_2 g^{-1} h^{-1} for *Aequorea victoria* to 0.78 µmol O_2 g^{-1} h^{-1} for *Cyanea capillata*. Six of 12 species studied were oxyregulators; including the scyphomedusae *Aurelia labiata*, *C. capillata*, and *Phacellophora camtschatica* and the hydromedusae *A. victoria*, *Polyorchis penicillatus*, and *Proboscidactyla flavicirrata*. Mean P_cs ranged from 5.5 hPa in *Muggiaea atlantica* to 39.5 hPa in *Euphysa flammea*. The relationship between mass-specific metabolic rate and P_c was significant for oxyregulators but not oxyconformers. An apparent metabolic depression occurred variably within all oxyregulating species and 2 oxyconforming species, *Clytia gregaria* and *Sarsia* sp., whereby sub-P_c oxygen uptake decreased by 77 to 99% relative to standard aerobic metabolic rate (SMR). Anoxia survival varied from less than 2 h for *E. flammea* and *Eutonina indicans* to more than 10 h for *A. victoria*. The poor low oxygen tolerance of several Puget Sound species in our study was consistent with the historical disappearance of related species in the Adriatic Sea following increased frequency of dysaerobic events. Interspecies variation in aerobic metabolic characteristics and hypoxia and anoxia tolerance may explain why some medusae thrive in low-oxygen conditions, while others disappear.

KEY WORDS: Medusae · Oxyregulation · Hypoxia tolerance · Critical PO_2 · Hydromedusae · Scyphomedusae

INTRODUCTION

Changes in the composition and abundance of gelatinous marine zooplankton communities have occurred worldwide. Recent reviews of gelatinous zooplankton outbreaks and the factors that promote such outbreaks have emphasized the importance of these events ecologically and with respect to human endeavors (CIESM 2001, Graham et al. 2001, Mills 2001). While outbreaks of medusae have been increasingly frequent, medusan species richness has declined in some regions (Benović et al. 1987, 2000a, Mills 2001). It is becoming clear that hypoxia and eutrophication are important factors in the outbreaks of some medusae and the decline of others (Benović et. al 1987, 2000a,

Arai 2001, Mills 2001, Purcell et al. 2001). The effects of low dissolved oxygen have been shown to alter trophic interactions and food-web dynamics, increasing predation by medusae (Breitburg et al. 1994, 1997). The disappearance of certain species of medusae may act as an environmental indicator for dysaerobic and anoxic events (Benović et al. 1987, 2000a). However, many medusae are found in low-oxygen habitats ranging from the oceanic oxygen minimum layer (Thuesen & Childress 1994) to the 'Dead Zone' of the Gulf of Mexico (Purcell et al. 2001).

Nearshore and estuarine hypoxic areas are increasing worldwide and affecting coastal ecosystems (Diaz 2001). Many organisms become adversely affected by hypoxia when dissolved oxygen levels fall to 20 to 30%

of air saturation (Rabalais & Turner 2001). Historical records from 1911 to 1982 of medusae in the Adriatic Sea, where dysaerobic events have become increasingly frequent, have shown a dramatic decrease in the number of hydromedusae species present over time, a change especially apparent among the Anthomedusae and the Leptomedusae, which have benthic hydroid phases that could be adversely affected by bottom-layer hypoxia (Benović et al. 1987). A more recent study of the Adriatic Sea (Benović et al. 2000a) confirmed a paucity of hydromedusae, especially Anthomedusae, relative to similar environments that are not affected by episodic hypoxia and anoxia. In contrast, certain species of medusae are known to occur in areas of episodic or even permanent hypoxia. The moon jelly *Aurelia aurita* has been found to be abundant just above the anoxic layer in the southern Adriatic Sea (Benović et al. 2000b) and within the uppermost layer of the suboxic zone in the Black Sea (Kideys & Romanova 2001). In Saanich Inlet of British Columbia, the hydromedusae *Aequorea aequorea* and *Clytia gregaria* have been found within the oxygen minima (<10% O_2 saturation) (Davis 1975, Mackie & Mills 1983). Another study revealed the scyphomedusa *Mastigias* sp. was present in the nearly anoxic oxygen minima of marine lakes of Palau during night-time (Dawson & Hamner 2003). Studies of the scyphomedusa *Chrysaora quinquecirrha*, which has been found within hypoxic (<30% O_2 saturation) waters of Chesapeake Bay, have shown both medusae and scyphistomae are relatively unaffected by low-oxygen concentrations (Keister et al. 2000, Condon et al. 2001, Breitburg et al. 2003).

Aerobic metabolic rates measured under declining oxygen tension can provide information about the physiology and hypoxia tolerance of zooplankters. Metazoa generally follow 1 of 2 broad respiration patterns when exposed to increasingly hypoxic conditions, oxyregulation or oxyconformation (Grieshaber et al. 1988, 1994, Pörtner & Grieshaber 1993). Animals that oxyregulate maintain a relatively constant rate of oxygen consumption during progressive hypoxia, whereas oxyconformers consume oxygen at a linearly decreasing rate. The partial pressure of oxygen (PO_2) at which an animal deviates from its respective oxygen consumption pattern is known as the critical oxygen tension (P_c). Occurring in both oxyregulators and oxyconformers, P_c is manifested by an abrupt change in oxygen consumption rate and usually the onset of anaerobiosis (Grieshaber et al. 1988, Pörtner & Grieshaber 1993). P_c is an estimator of the oxygen tension at or above which an animal's anaerobic metabolism is unaffected by O_2 availability, and in this sense P_c is an indicator of hypoxia tolerance. However, P_c is not necessarily a good indicator of the animal's metabolic

characteristics in hypoxia below the critical PO_2 or in anoxia. For example, in spite of a higher P_c than most other midwater copepods, *Gaussia princeps* can endure anoxia many times longer (Childress 1977a). Many midwater medusae from the oceanic oxygen minimum layer off of California are aerobically poised with regard to metabolic enzyme ratios, presumably surviving in the low-oxygen environment through aerobic adaptations (Thuesen & Childress 1994). Furthermore, it appears the glycolytic scaling patterns of *Periphylla periphylla* are related to environmental oxygen content (Thuesen et al. 2005a).

Budd Inlet, in southern Puget Sound (Washington State, USA), supports more than a dozen species of medusae (cf. Mills 1981) and is threatened by nutrient loading from regional population growth. Budd Inlet experiences ecologically significant bottom-layer hypoxia periodically during the late summer and early fall (Uhlenhopp & Devol 1998, P. L. Brommer & E. V. Thuesen unpubl.). Improved knowledge of the physiological effects of low oxygen on medusae could provide an important tool for predicting changes in zooplankton community structure as a result of hypoxia. Although a number of studies have measured the respiration rates of medusae (Vernon 1895, Arai 1986, Larson 1987a, Childress & Thuesen 1993, Thuesen & Childress 1994), no studies on the oxygen-consumption rates under declining PO_2 and critical oxygen tensions of medusae have been undertaken. The present study investigated a potential cause of changing medusae community structure in hypoxic waters by examining aerobic metabolism and low-oxygen tolerance characteristics of various Puget Sound hydromedusae and scyphomedusae.

MATERIALS AND METHODS

Collection of medusae. All specimens were hand-collected from the upper 2 m of the water column from various sites in southern Puget Sound, starting in summer of 2000 through spring of 2003. Specimens were transported to the laboratory in plastic containers. They were carefully inspected for damage and the presence of commensal or epizoic organisms. Damaged specimens and those that had commensal organisms that could not be easily removed were not used in our study. All specimens were identified to species, with the exception of *Halitholus* sp. and *Sarsia* sp. (Wrobel &Mills 1998). Specimens were held in 0.5 to 2.0 l containers of filtered seawater with antibiotics (0.45 μm, 10°C, 30 psu, 50 mg l^{-1} each streptomycin and ampicillin, salinity adjusted using Instant Ocean®). During the study period, surface temperatures and salinities ranged from 3 to 21°C and 10 to 31 psu in

southern Puget Sound, and 10°C and 30 psu were chosen as the experimental temperature and salinity for this study. Specimens were maintained for a maximum of 2 wk. Containers were checked daily, and animals that appeared not to be thriving were removed. Specimens kept for more than several days were periodically fed with *Artemia fransicana* nauplii or with plankton tows and afterward transferred into clean filtered seawater and a clean container. Before metabolic rates were measured, specimens were kept unfed for a minimum of 24 h prior to use and given at least 24 h to acclimate to 30 psu seawater at 10°C.

Oxygen-consumption-rate measurements. Oxygen concentrations were measured using PreSens fiber optic oxygen meters (Precision Sensing). In the earliest experiments, a Microx 8, 8-channel meter was used to measure oxygen; however, more than 90% of measurements in this study were made using Microx TX and TX3 temperature-compensated fiber-optic oxygen meters with type B2 optically insulated microoptodes in insertable, needle-type housings. Meters were calibrated at 2 points using an aqueous 5% sodium sulfite solution for oxygen-free water and gently stirred filtered seawater (10°C, 30 psu) for oxygen-saturated seawater. Data were recorded on a personal computer through a serial connector.

Glass syringes (0.5 to 50 ml) were used as miniature respiration chambers for small specimens. Large specimens were placed in glass chambers (100 to 500 ml) fitted with custom-built acrylic lids sealed with silicone o-rings. Both types of chamber used Luer-type fittings and septa for insertion of oxygen optodes. Specimens were placed in chambers filled with 10°C, 30 psu filtered seawater with antibiotics (0.22 μm, with streptomycin and ampicillin, salinity adjusted with Instant Ocean®). Seawater in chambers of 1 ml or less contained 100 mg l^{-1} of each antibiotic and larger volumes contained 50 mg l^{-1} of each. Syringe chamber volumes ranged from 0.15 to 30 ml. Control experiments were performed in an identical fashion to respirometry experiments, except no specimens were used. Controls showed no significant bacterial respiration over the period of time in which experimental trials were conducted.

Prior to being placed in respirometry chambers, animals were carefully inspected for damage. Once an animal was placed in the chamber and the chamber sealed, a microoptode was pushed through the septum and the oxygen-sensing tip ejected into the chamber. All specimens were run individually in respirometry chambers. Respirometry with the small species *Muggiaea atlantica* and *Proboscidactyla flavicirrata* was often begun using partially deaerated (N$_2$-bubbled) seawater in order to shorten the duration of experiments. Chambers were kept at a constant 10°C atop an orbital shaker at 85 rpm to facilitate stirring within the chambers. To minimize phototaxis and other light-dependent behaviors of specimens, chambers were enclosed in a darkened box. Experiments were run until the specimen had exhausted all of the oxygen in the respirometry chamber or had ceased to consume oxygen.

In order to determine hypoxia and anoxia tolerance, medusae were opportunistically allowed to remain in the low-oxygen conditions of the chamber for various time periods. Once an experiment was completed, the specimen was observed for signs of life, its general condition was recorded, and normoxic seawater was introduced into the chamber. The specimen was allowed to remain in the chamber for 10 to 30 min following introduction of normoxic seawater and then observed again for signs of life. Before determining the wet weight of a specimen, excess water was very gently blotted from larger specimens and wicked away from smaller specimens using paper towels or bibulous paper. Specimens were weighed on an analytical balance. Following weighing, specimens that had shown no signs of life were immediately placed in a holding container and checked after an hour and periodically thereafter. Specimens showing no sign of life after 24 h were considered dead.

Data analysis. Respirometry data were analyzed using Graphical Analysis (Vernier Software & Technology 2002), StatView II (Abacus Concepts 1995), and Kaleidagraph (Synergy Software 2003). Oxygen-consumption rates were determined by performing a linear regression on the slope of the oxygen concentration against time curve and converting the slope into a consumption rate. Linear regressions were used to determine the partial pressure at which a specimen's aerobic metabolic rate began to deviate from oxyregulation or oxyconformation and recorded as the P_c (Grieshaber et al. 1988). Instead of subtracting the specimen volume from the chamber volume when calculating the volume of water contained in the respirometry chamber, specimens were considered to contain the same amount of oxygen as an equal volume of air saturated seawater at 10°C and 30 psu (Thuesen et al. 2005b). Throughout this paper, O$_2$ saturation refers to the amount of dissolved oxygen in air-saturated seawater at 10°C and 30 psu. Under these conditions, PO$_2$ = 212.3 hPa and [O$_2$] = 9.32 mg l^{-1}; therefore the PO$_2$ is 22.8 hPa at an [O$_2$] of 1.0 mg l^{-1}.

Wet weight was chosen as a measure of size rather than dry weight or protein content, because wet weight is the mass parameter of physiological significance determining constraints on swimming, predation, etc. Utilizing other parameters of body size can lead to misinterpretation concerning the biology and

ecology of the whole organism. The interpretation of ecophysiological information based on different parameters of body size has been explored thoroughly by Childress (1977b) and Childress & Somero (1979).

RESULTS

Respiration and critical-oxygen-tension measurements were made on 106 specimens from 12 species of pelagic Cnidaria (Table 1). Mean mass-specific respiration rates ranged from 0.064 µmol O_2 g^{-1} h^{-1} for *Aequorea victoria* to 0.78 µmol O_2 g^{-1} h^{-1} for *Cyanea capillata*. When initially placed in a respirometry chamber, most medusae reacted with rapid swimming behavior, the duration of which was unquantified. During the remainder of respirometry, specimens usually appeared quiescent, with occasional bell pulsations. Respiration rates during the initial 0.2 to 1.5 h of each respirometry experiment were appreciably higher than the remainder of the experiment. This phenomenon occurred irrespective of initial oxygen concentration and in nearly all runs. Therefore, rates at 80% oxygen saturation are presented in Table 1.

A significant relationship was found between mass and mass-specific metabolic rate of the 12 species studied (linear regression, $y = 0.23x^{-0.22}$, $R^2 = 0.24$, n = 93, p < 0.0001), with smaller specimens having higher metabolic rates in proportion to their mass (Fig. 1). A significant relationship between mass and mass-specific oxygen consumption rate was found for hydromedusae (linear regression, $y = 0.154x^{-0.46}$, $R^2 = 0.32$, n = 74, p < 0.0001). A similar relationship for only the scyphomedusae was not significant.

Oxygen-consumption rate as a function of oxygen tension varied widely between species. Six species were found to oxyregulate: the scyphomedusae *Aurelia labiata*, *Cyanea capillata*, and *Phacellophora camtschatica* (Fig. 2) and the hydromedusae *Aequorea victoria*, *Polyorchis penicillatus*, and *Proboscidactyla flavicirrata* (Fig. 3). Conversely, the 5 other species of hydromedusae studied (*Clytia gregaria*, *Euphysa flammea*, *Eutonina indicans*, *Halitholus* sp. and *Sarsia* sp.), as well as the siphonophore *Muggiaea atlantica*, were oxyconformers (Figs. 4 to 6a). Seven of 8 specimens of *M. atlantica* showed an abrupt increase in O_2-consumption rate at ~10% O_2 saturation, followed by an abrupt decline in respiration rate at lower PO_2s

Table 1. Oxygen-consumption rates and critical oxygen tensions at 10°C and 30 psu of medusae collected in southern Puget Sound. SMR: specific metabolic rate; na: not applicable

Class Order Genus and species	Mean wet weight (g) ± SE (n)	Wet weight (range, g)	Mean specific oxygen-consumption rate at 80% O_2 saturation (µmol g wet weight^{-1} h^{-1}) ± SE (n)	Oxy-regulator/ oxycon-former	Mean critical PO_2 (hPa) ± SE (n)	Mean depressed metabolic rate, % SMR (n)
Hydrozoa						
Anthomedusae						
Euphysa flammea	0.225 ± 0.026 (12)	0.067–0.331	0.400 ± 0.051 (12)	Oxyconformer	39.5 ± 6.7 (12)	na
Halitholus sp.	0.297 ± 0.078 (6)	0.121–0.644	0.672 ± 0.032 (6)	Oxyconformer	30.6 ± 4.1 (6)	na
Polyorchis penicillatus	0.731 ± 0.144 (11)	0.179–1.479	0.347 ± 0.051 (11)	Oxyregulator	9.7 ± 2.7 (11)	4.8 (8)
Sarsia sp.	0.082 ± 0.012 (8)	0.053–0.160	0.544 ± 0.081 (8)	Oxyconformer	12.1 ± 3.3 (8)	1.4 (1)
Leptomedusae						
Aequorea victoria	2.194 ± 0.484 (9)	0.974–5.240	0.064 ± 0.007 (9)	Oxyregulator	8.7 ± 2.67 (7)	5.3 (4)
Clytia gregaria	0.245 ± 0.028 (14)	0.094–0.394	0.279 ± 0.067 (12)	Oxyconformer	14.4 ± 2.2 (14)	2.3 (4)
Eutonina indicans	0.515 ± 0.033 (8)	0.384–0.616	0.190 ± 0.021 (8)	Oxyconformer	22.8 ± 3.0 (3)	na
Limnomedusae						
Proboscidactyla flavicirrata	0.120 ± 0.011 (6) 0.110 ± 0.010 (11)	0.093–0.162 0.057–0.162	0.327 ± 0.029 (6) 0.287 ± 0.034 (11)[a]	Oxyregulator	12.6 ± 1.6 (6) 9.7 ± 1.4 (11)	1.3 (4)
Calycophora						
Muggiaea atlantica[a]	0.021 ± 0.003 (2) 0.018 ± 0.002 (8)	0.018–0.025 0.011–0.025	0.730 ± 0.061 (2) 0.770 ± 0.160 (8)[a]	Oxyconformer	4.4 ± 0.5 (2) 5.5 ± 0.8 (8)	na
Scyphozoa						
Sematostomae						
Aurelia labiata	11.218 ± 2.722 (10)	1.832–26.646	0.297 ± 0.033 (10)	Oxyregulator	9.3 ± 1.6 (10)	3.3 (3)
Cyanea capillata	0.714 ± 0.452 (2)	0.262–1.166	0.780 ± 0.218 (2)	Oxyregulator	13.0 ± 1.5 (2)	3.0 (1)
Phacellophora camtschatica	0.873 ± 0.175 (7)	0.535–1.852	0.180 ± 0.025 (7)	Oxyregulator	9.1 ± 1.4 (7)	1.9 (3)

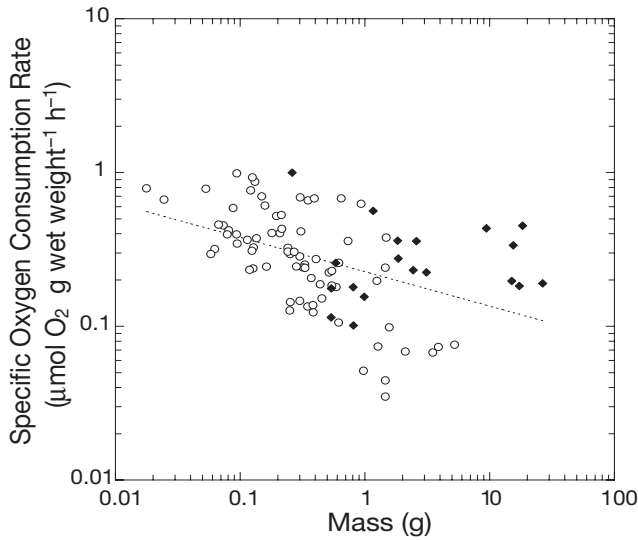[a]Mean mass-specific respiration rate at 35% O_2 saturation

Fig. 1. Mass-specific oxygen-consumption rates for medusae of southern Puget Sound at 80% O_2 saturation, 10°C and 30 psu, as a function of mass (n = 93 individuals). The slope of the regression line is $y = 0.23x^{-0.22}$, $R^2 = 0.24$, p < 0.0001. (○) hydromedusae; (◆) scyphomedusae

(Fig. 6b). Mean P_cs for the medusae ranged from 5.5 hPa for *M. atlantica* to 39.5 hPa for *E. flammea* (Table 1). One species, *E. indicans*, presented difficulties with regard to determination of P_c, and only 3 of 8 individuals showed the abrupt change in O_2-consumption rate that indicated P_c. Oxyregulating species had lower P_cs than oxyconforming species, with the exception of the oxyregulator *C. capillata* having a slightly higher P_c than the oxyconformer *Sarsia* sp., and *M. atlantica* having the lowest P_c. Among the oxyregulating medusae, there was a significant relationship between metabolic rate and P_c ($R^2 = 0.20$, p = 0.001). The relationship between metabolic rate and P_c was not significant for oxyconforming medusae. There was no significant relationship between mass and P_c.

Individuals of each species were allowed to remain in anoxia or severe hypoxia, defined as PO_2 below a given specimen's P_c (Grieshaber et al. 1994) for an unspecified time following respirometry (Fig. 7). Many specimens were clearly alive after spending time in anoxia, and many others showed signs of life shortly after normoxic seawater was introduced to the chamber. Overall mortality in species varied from none for *Aequorea victoria*, *Cyanea capillata* and *Polyorchis penicillatus* to more than 70% for *Euphysa flammea*, *Muggiaea atlantica*, and *Sarsia* sp. (Fig. 8). Both *A. victoria* and *P. penicillatus* were able to survive severe hypoxia for more than 12 h and anoxia for more than 10 and 4 h, respectively. In contrast, all specimens of *E. flammea* and *Eutonina indicans* exposed to more than 2 h of anoxia died.
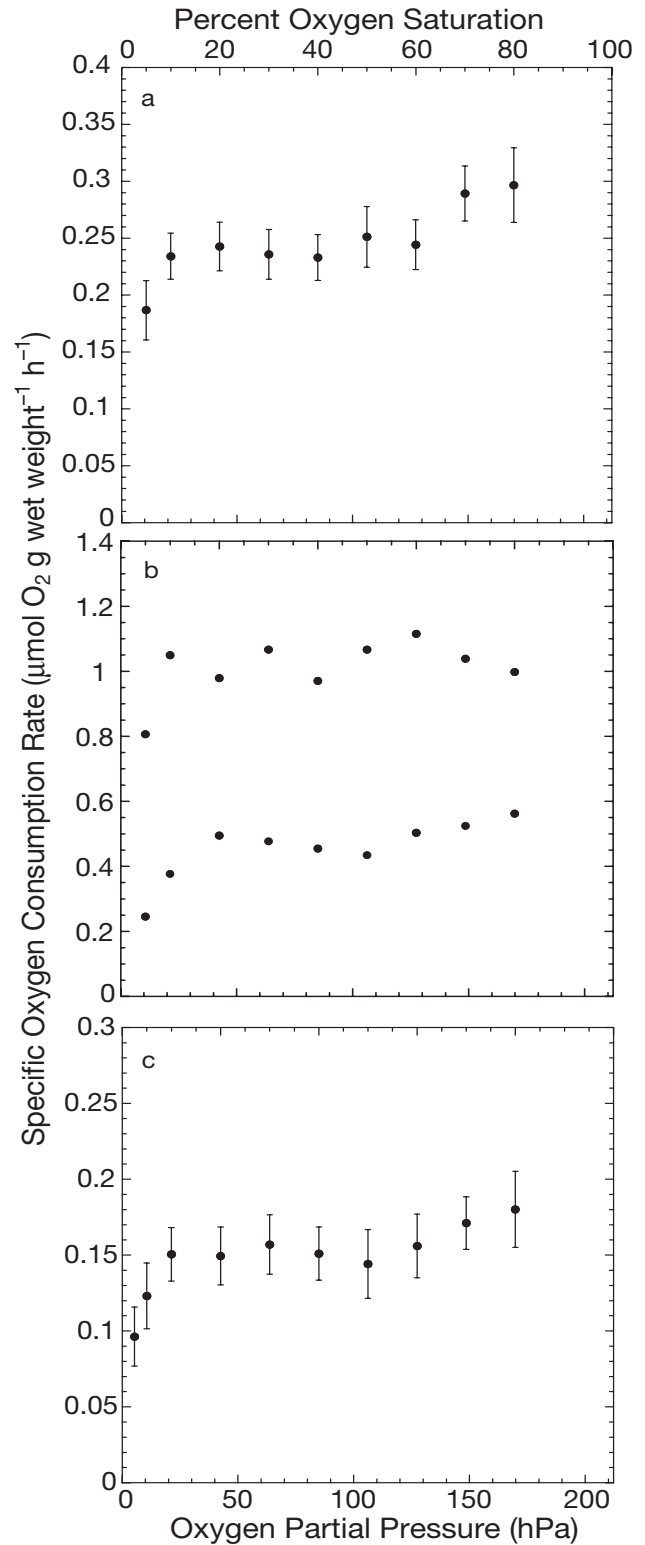

Fig. 2. Mean mass-specific oxygen-consumption rates of 3 species of oxyregulating scyphomedusae as a function of seawater oxygen content. Experiments were done at 10°C and 30 psu. Error bars represent standard error. (a) *Aurelia labiata* (n = 10), (b) *Cyanea capillata* (n = 2), and (c) *Phacellophora camtschatica* (n = 7)
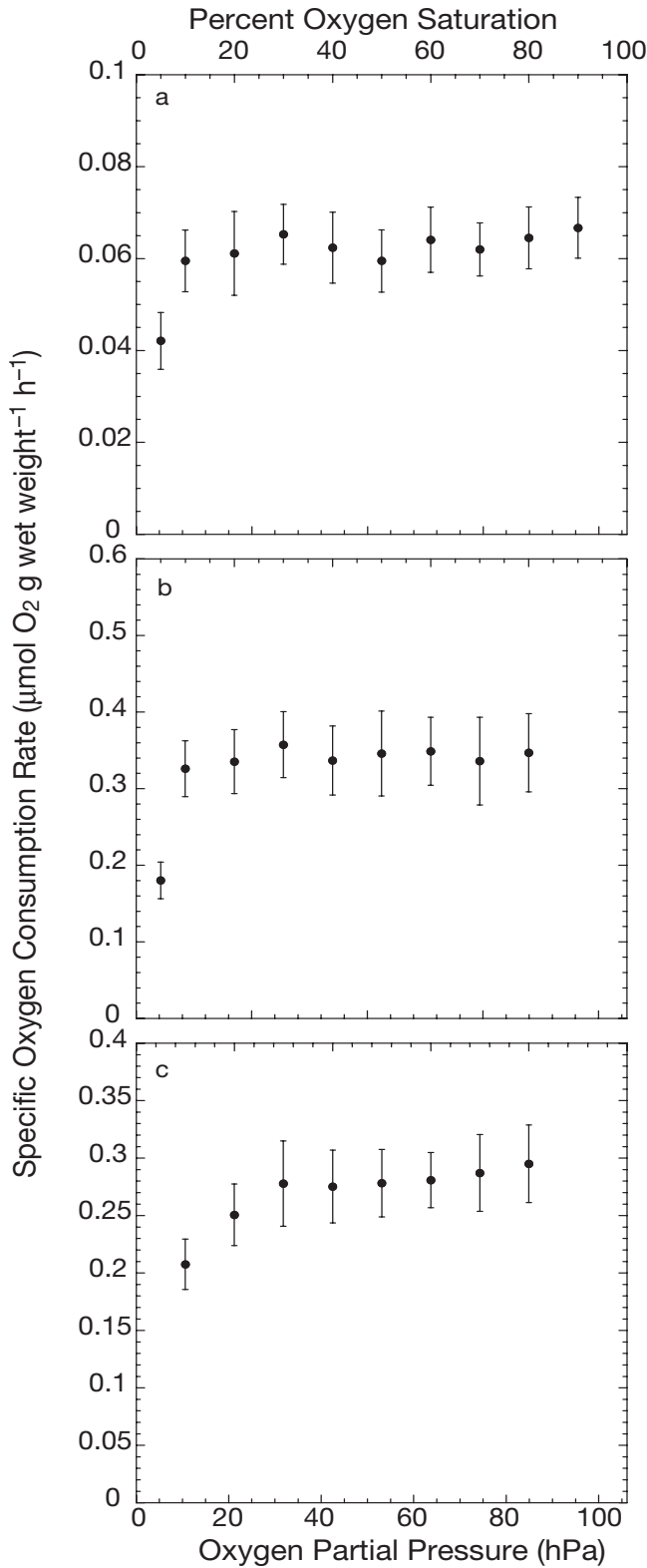
Fig. 3. Mean mass-specific oxygen-consumption rates of 3 species of oxyregulating hydromedusae as a function of seawater oxygen content. Experiments were done at 10°C and 30 psu. Error bars represent standard error. (a) *Aequorea victoria* (n = 9), (b) *Polyorchis penicillatus* (n = 11), and (c) *Proboscidactyla flavicirrata* (n = 11)
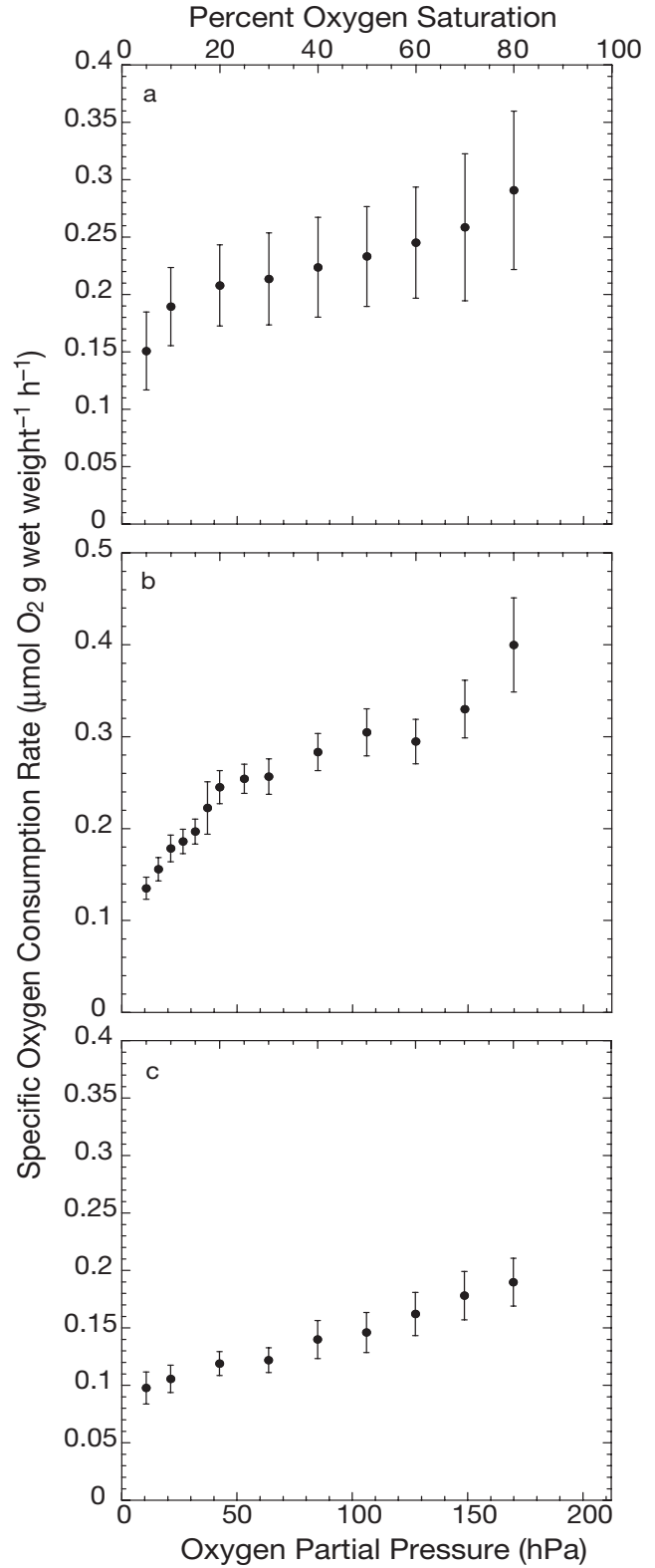
Fig. 4. Mean mass-specific oxygen-consumption rates of 3 species of oxyconforming hydromedusae as a function of seawater oxygen content. Experiments were done at 10°C and 30 psu. Error bars represent standard error. (a) *Clytia gregaria* (n = 12), (b) *Euphysa flammea* (n = 12), and (c) *Eutonina indicans* (n = 8)
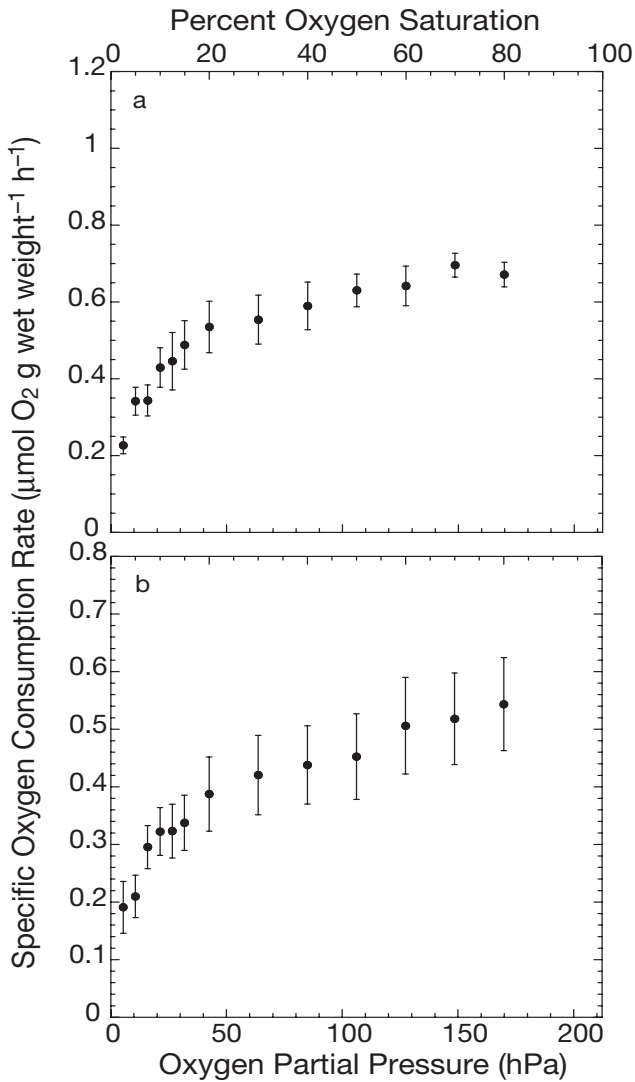
Fig. 5. Mean mass-specific oxygen-consumption rates of 2 species of oxyconforming hydromedusae as a function of sea-water oxygen content. Experiments were done at 10°C and 30 psu. Error bars represent standard error. (a) *Halitholus* sp. (n = 6), and (b) *Sarsia* sp. (n = 8)
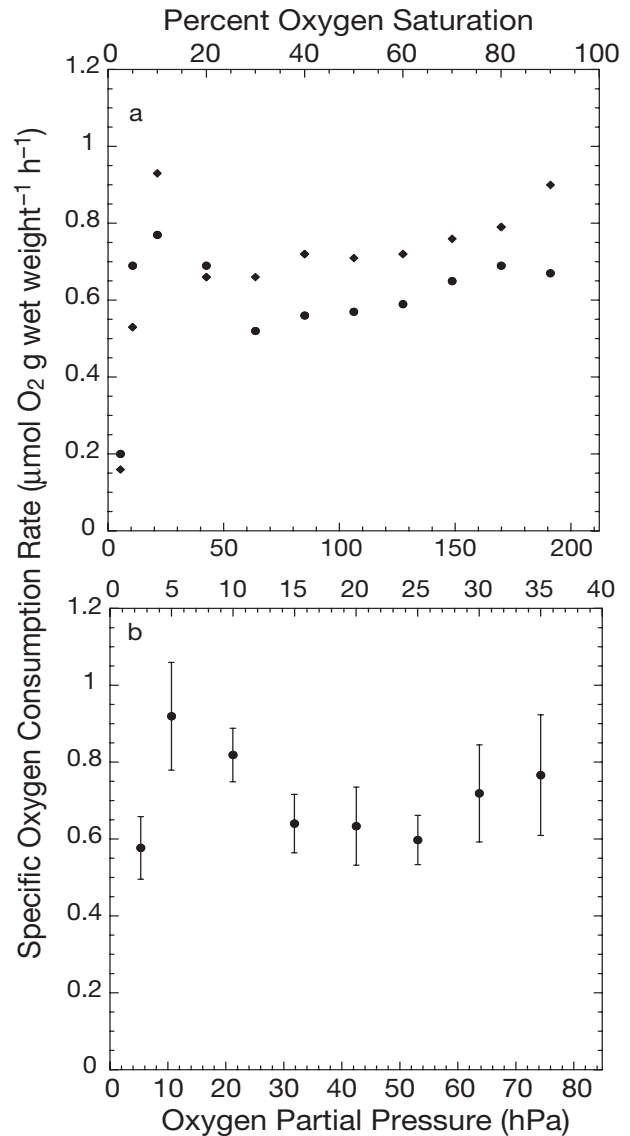

Fig. 6. Mass-specific oxygen-consumption rates of the oxyconforming siphonophore *Muggiaea atlantica* as a function of seawater oxygen content. (a) Mass-specific oxygen consumption rates of 2 specimens: (♦) Specimen 1 and (●) Specimen 2, demonstrating oxyconformation; (b) mean mass-specific oxygen-consumption rates (n = 8) showing the marked increase in O_2 uptake at 10% saturation. Experiments were done at 10°C and 30 psu. Error bars represent standard error

In a number of instances during respirometry, a specimen would almost completely cease oxygen consumption coincident with, or shortly after, the specimen reached P_c. This was noted with varying frequency in all 6 oxyregulating species and 2 of the oxyconforming species, *Sarsia* sp. and *Clytia gregaria* (Table 1). This phenomenon, apparent metabolic depression, was most pronounced in *Polyorchis penicillatus*, and 8 of 11 specimens displayed depressed respiration rates. During apparent metabolic depression, aerobic metabolic rates of medusae decreased by 77% to more than 99% of a specimen's standard oxygen-consumption rate, with most rates decreasing by 95% or more.

DISCUSSION

Aerobic metabolism

Many factors affect oxygen-consumption rate, and animal activity is one of the most important. Metabolic scope, the relative change in aerobic metabolic rate from rest to maximal exertion (Schmidt-Nielsen 1984), has been little studied in medusae (Daniel 1985, Larson 1987b). The scyphomedusa *Stomolophus melagris* had a
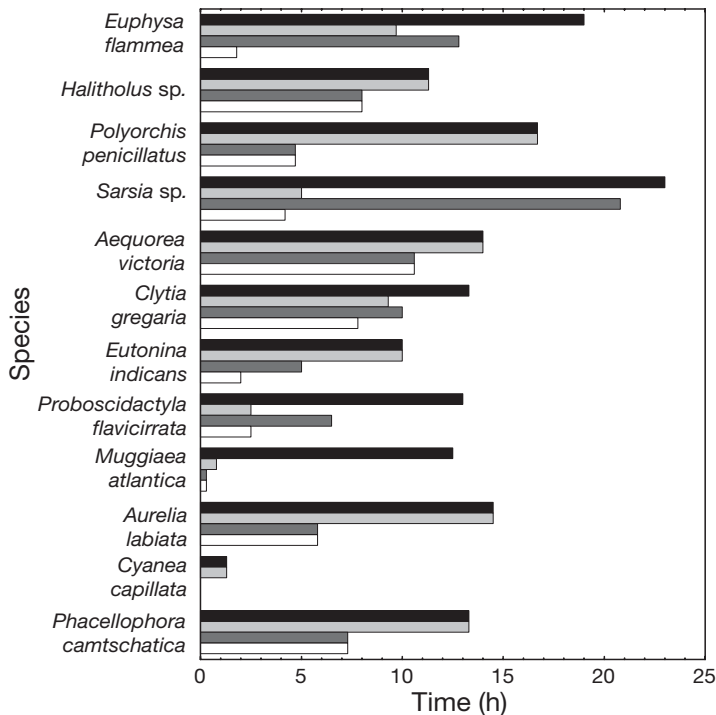
Fig. 7. Maximum exposure and survival times in severe (sub-P_c) hypoxia and anoxia for 12 species of medusae. (■) Maximum severe hypoxia exposure time; (▨) maximum severe hypoxia survival time; (■) maximum anoxia exposure time; (□) maximum anoxia survival time
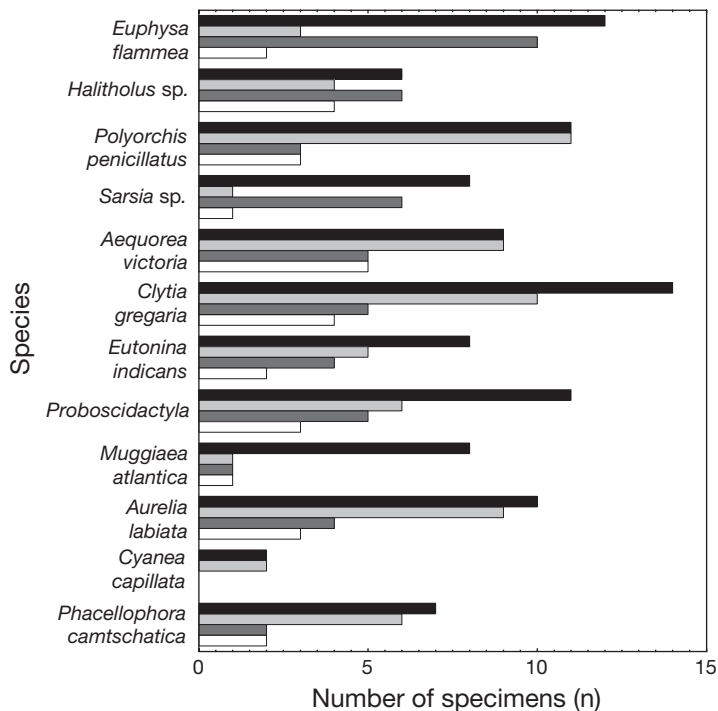

Fig. 8. Number of individuals of 12 species of medusae that were exposed to severe (sub-P_c) hypoxia and anoxia and number of individuals that survived such exposure. (■) Number of specimens exposed to severe hypoxia; (▨) number of specimens that survived severe hypoxia exposure; (■) number of specimens exposed to anoxia; (□) number of specimens that survived anoxia exposure

standard metabolic rate (SMR) about half that measured during forceful swimming and thus a metabolic scope of ~2 (Larson 1987b). The initial high respiration rates observed in our study are not unusual in respirometry (Childress 1977b) and can be explained by animal activity. After the initial portion of respirometry medusae typically appeared quiescent, or occasionally pulsed, and the respiration rates we obtained probably represent a close approximation of SMR. The effect of spatial confinement on the activity of medusae is unknown and probably variable. Hydromedusae reportedly exhibit a crumple effect and briefly cease activity when contacting a solid object (Leonard 1983), but no crumple effect was observed in the present study.

Aerobic metabolic rates for a number of species in this study were substantially lower than previously published rates collected under similar environmental conditions, and probably stem from differences in animal activity (Table 2). Although neither Arai (1986) nor Larson (1987a) mentioned any particular activity levels observed during their experiments, Larson (1987a) noted that his results were likely affected by activity and that the rates obtained likely represented the active metabolic rate of the medusae rather than the SMR. Thus, with the exception of *Sarsia* sp., most of the differences between metabolic rates of hydromedusae in the present study and those of Larson (1987a) can be explained by a metabolic scope of less than 3. The differences between the mean respiration values of *Sarsia* sp. are not easily explained. The specific rates found for the scyphozoans *Aurelia labiata* and *Cyanea capillata* were similar in this study and Larson (1987a). The metabolic scope of ~2 reported by Larson (1987b) can account for many of the differences between this and previous studies in the oxygen-consumption rates of hydromedusae.

Oxyregulation and oxyconformation

The species in this study can be divided into oxyregulators and oxyconformers. Unlike osmoconformation and thermoconformation, which show a directly proportional relationship to osmolarity and temperature (Schmidt-Nielsen 1997, Willmer et al. 2004), oxyconformation, although linearly related to PO_2, is not directly proportional to PO_2. In fact, the P_c of

Table 2. Oxygen-consumption-rate comparisons for 6 species of medusae. Specific metabolic rates and weights from Arai (1986) and Larson (1987a) have been converted to grams wet weight using the factors published in Larson (1986)

Genus and species	Mean specific O_2 consumption (μmol g wet weight^{-1} h^{-1}) ± SE	Mean mass (g)	n	Source
Hydrozoa				
Anthomedusae				
Sarsia princeps	0.27 ± 0.034	0.39	8	Larson (1987a)
Sarsia sp.	0.54 ± 0.081	0.08	8	This paper
Leptomedusae				
Aequorea victoria	0.23 ± N/A	N/A	13	Arai (1986)
	0.17 ± 0.016	8.3	45	Larson (1987a)
	0.064 ± 0.007	2.19	9	This paper
Clytia gregaria	0.45 ± 0.035	0.41	24	Larson (1987a)
	0.28 ± 0.067	0.25	12	This paper
Eutonina indicans	0.48 ± 0.034	1.13	25	Larson (1987a)
	0.19 ± 0.021	0.52	8	This paper
Scyphozoa				
Aurelia labiata[a]	0.24 ± 0.017	12.89	18	Larson (1987a)
	0.30 ± 0.033	11.22	10	This paper
Cyanea capillata	0.90 ± 0.075	5.71	16	Larson (1987a)
	0.78 ± 0.22	0.71	2	This paper

[a]The native west coast species of *Aurelia*, *A. labiata*, has been historically identified in the literature as *A. aurita* (Dawson & Jacobs 2001). The species collected from Saanich Inlet, BC (Larson 1987a) was almost certainly *A. labiata*, the same species used in the present study

an organism precludes such a relationship. Oxygen consumption as a function of oxygen-concentration curves (Figs. 2 & 3) clearly demonstrate that *Aurelia labiata*, *Cyanea capillata*, *Phacellophora camtschatica*, *Aequorea victoria*, *Polyorchis penicillatus*, and *Proboscidactyla flavicirrata* are oxyregulators and maintain a nearly constant oxygen-consumption rate over a range of PO_2s down to P_c. In contrast, the relationship between oxygen consumption and PO_2 for *Clytia gregaria*, *Euphysa flammea*, *Eutonina indicans*, *Halitholus* sp. and *Sarsia* sp. demonstrate oxyconformation, with oxygen consumption decreasing more or less with PO_2 down to P_c (Figs. 4 & 5). *Muggiaea atlantica* shows a similar relationship, but oxygen uptake increases abruptly just before P_c (Fig. 6). Oxyconformation can be achieved through 2 means, oxyconformation with anaerobiosis and oxyconformation without anaerobiosis. Oxyconformation with anaerobiosis is done by otherwise oxyregulating animals that are unable to effectively distribute O_2 to all respiring tissues, due to limitations of ventilation or circulation, so that some tissues may switch to anaerobic energy production while other tissues continue aerobically (Pörtner & Grieshaber 1993). Aerobic oxyconformers (true oxy-

conformers) decrease energy usage at the cellular level; thus a tissue or animal will consume less oxygen as PO_2 decreases without necessarily having any tissues use anaerobic energy production (Pörtner & Grieshaber 1993).

The oxyconforming hydromedusae in this study all have relatively narrow velar apertures that restrict seawater exchange between their subumbrellar cavities and the surrounding seawater. When these animals become quiescent, the subumbrellar tissues draw oxygen from a very small volume of water that will become depleted of oxygen. Oxygen supply to the tissues of the subumbrellar cavity would be limited to that which diffused through the velar tissue, the velar aperture, and from the mesogleal oxygen reserve (Thuesen et al. 2005b). Prolonged cessation of swimming by hydromedusae with narrow velar apertures would lead to increasing hypoxia within the subumbrellar cavity, and swimming would serve to ventilate subumbrellar tissues. The oxyconforming hydromedusae in this study may actually oxyregulate, but because of protracted quiescence during respiration runs, they appear to oxyconform and fit the model of oxyconformation with anaerobiosis. These medusae may be more susceptible to environmental hypoxia, because they need to expend more energy to ventilate subumbrellar tissues when environmental oxygen levels begin to approach severe hypoxia.

Critical oxygen tensions

Critical oxygen tension and a consequent transition to anaerobiosis indicate that the O_2 gradient between the environment and tissue is inadequate to allow sufficient oxygen to diffuse into the tissue to meet aerobic metabolic demand (Grieshaber et al. 1988). The P_cs observed in this study show that both oxyregulating and oxyconforming species could endure hypoxia of less than 20% O_2 saturation without undergoing any major metabolic transition.

In general, all medusae have a very similar basic bauplan that consists of the ectoderm, which covers the entire exterior of the animal, the gastroderm, which lines the gastrovascular cavity, and a largely acellular mesoglea (Brusca & Brusca 1990). Most of the respiring tissues of medusae (i.e. the ectodermis

and gastrodermis) are either in or near O_2-containing seawater, and diffusion distances are generally short. Given the similarities of gross morphology and diffusion-dependent oxygen delivery, critical oxygen tensions of medusae could be expected to fall within a relatively narrow range of values. For the oxyregulating species of medusae, mean P_cs fell into a narrow range of oxygen tensions, from 8.7 hPa for *Aequorea victoria* to 13.1 hPa for *Cyanea capillata*. In contrast, oxyconforming medusae displayed generally higher P_cs over a wider range of PO_2s, from a mean of 5.5 hPa for *Muggiaea atlantica* to 39.5 hPa of *Euphysa flammea*. Ventilatory activity can effectively lower P_c (Childress 1971, Grieshaber et al. 1994, Wohlgemuth et al. 2000) and give rise to the differences in P_c noted between oxyregulating and oxyconforming medusae.

In 5 out of 8 respirometry experiments, a P_c could not be determined for the oxyconformer *Eutonina indicans*, as no deviation in the declining oxygen-consumption rate was observed. In contrast, *Muggiaea atlantica*, at very low PO_2s, demonstrated a marked increase in metabolic rate, followed by a sharp decline (Fig. 6b). The PO_2 associated with increased O_2 consumption may represent the P_c (Pörtner & Grieshaber 1993), but the increase could also be ascribed to increased activity due to an escape response to hypoxia. The increased metabolic rate is similar to the high respiration rate noted early in the respirometry of this species and is probably related to swimming activity.

Mortality and exposure to hypoxia and anoxia

There was a great deal of interspecies variation in mortality due to severe hypoxia and anoxia. The survival times in this study represent minimum survival times, since specimens were observed for life following, but not during, respirometry and anoxia. Some mortality of small hydrozoans like *Muggiaea atlantica*, *Proboscidactyla flavicirrata* and *Sarsia* sp. could have been an experimental artifact, because very small medusae could be more negatively affected by post-respirometry handling and weighing due to their delicate nature and low heat capacity. Mortality of very small species aside, it is clear that oxyregulating medusae species experienced low mortality. No mortality was observed in *Aequorea victoria*, *Cyanea capillata*, and *Polyorchis penicillatus*, while *Aurelia labiata* and *Phacellophora camtschatica* each had a single individual expire. The hypoxia tolerance of some medusae is remarkable because of their low anaerobic potential (Thuesen & Childress 1994). Hydromedusae and scyphomedusae lack detectable

levels of the -opine dehydrogenase enzymes (Thuesen & Childress 1994), which are common to many invertebrates that are exposed to episodic hypoxia (Grieshaber et al. 1994), including benthic cnidarians such as sea anemones (Shick 1991).

Down regulation of metabolic demand during hypoxia and anoxia, termed metabolic depression, is well known among invertebrates (Grieshaber et al. 1994). Hochachka et al. (1996) proposed a theory of hypoxia tolerance involving both defensive and rescue components. The defensive component was shown to down-regulate energy usage in a number of cellular processes by 90% or more (Hochachka et al. 1996). The rescue phase occurs after the defense phase and includes the activation of genes that allow for the production of proteins used to protect and stabilize cell components, augment glycolytic pathways, and inhibit the Kreb's cycle (Hochachka et al. 1996). Although other cnidarians (sea anemones) are known to exhibit metabolic depression under hypoxic conditions (Shick 1991), our study is the first to demonstrate metabolic depression in medusae. The extreme decline in respiration rates seen in some medusae may be a result of such down-regulation of metabolic requirements as well as some component of the rescue phase. In most cases, there was still ongoing consumption of oxygen by medusae, but at a small percentage (0.1 to 13%) of SMR. The abrupt near cessation of oxygen uptake implies that aerobic metabolism is being limited by some factor other than oxygen-diffusion constraints. *Polyorchis penicillatus*, in which the majority of specimens displayed apparent metabolic depression, had no mortality in up to 16 h of severe hypoxia.

Ecological implications

This study's examination of the ecophysiological characteristics of southern Puget Sound medusae with respect to hypoxia and anoxia revealed a number of important differences between species. The results of this study support the observation that the composition of gelatinous zooplankton communities reflects sensitivity to hypoxic and anoxic events. Benović et al. (1987, 2000a) showed a correlation between increased bottom-layer hypoxia and declines in species abundance of hydromedusae in the Adriatic Sea, hypothesizing the cause to be mortality among benthic polyp stages. This Puget Sound study showed high mortality in 2 of 4 anthomedusan species and 1 of 3 leptomedusan species after exposure to low oxygen. The Leptomedusae *Aequorea victoria* and *Clytia gregaria* have been noted to inhabit the oxygen minima of Saanich Inlet (Davis 1975). Interestingly, in the eutrophic Adriatic Sea, the related *Clytia hemisphaerica* is still pres-

ent, while *Aequorea aequorea* has disappeared (Benović et al. 1987). The present study indicates that both *A. victoria* and *C. gregaria* are tolerant to low oxygen, and *A. victoria* can tolerate many hours of anoxia. Two species that were unable to tolerate more than 2 h of anoxia, *Euphysa flammea* and *Eutonina indicans*, were once represented in the Adriatic Sea by similar species, *Euphysa aurata* and *Eutonina scintillans*, that have since disappeared (Benović et al. 1987). Except for data on the genus *Aequorea*, our results support those of Benović et al. (1987) and indicate that hypoxia sensitivity is not necessarily limited to the polyp phases of hydrozoans.

Reviews by Mills (2001) and Arai (2001) cited evidence that a number of medusae species were increasing in abundance in eutrophic waters. Prominent among these were scyphomedusae of the genus *Aurelia* and *Chrysaora quinquecirrha*. The 3 scyphomedusae species in the present report had P_cs that would not require adjustment of metabolic regime except in very hypoxic waters (<10% O_2 saturation) and had low mortality after many hours of severe hypoxia and anoxia. Puget Sound experiences increased hypoxia during the late summer. The severity of oxygen depletion varies depending on location and conditions, but it is typically mild (>30% O_2 saturation) (Newton et al. 1998). This level of hypoxia is unlikely to have a serious impact on any of the medusae studied, although it may affect other pelagic zooplankters. If conditions in Puget Sound worsen as predicted (Mumford 2002), changes in distribution and abundance of medusae seem likely to follow the trend seen in the Adriatic Sea. The scyphomedusae *Aurelia labiata*, *Cyanea capillata*, and *Phacellophora camtschatica* would not be directly affected by a modest increase in hypoxia in southern Puget Sound, since all are tolerant of very low oxygen concentrations.

The effect of hypoxia on the polyp stages of medusae may be a critical factor in predicting their survival in declining oxygen levels. Because many estuaries are characterized by seasonal hypoxia or anoxia, it is possible that the polyps of certain species are absent or dormant during hypoxic events, and thus may not suffer high mortality in the early phases of eutrophication. This may be especially true of species with shallow-living polyp stages. The polyp of *Chrysaora quinquecirrha* has been shown to be resistant to long-term exposure to moderate hypoxia and up to 5 d of very low oxygen (<10% O_2 saturation) (Condon et al. 2001). The development of more persistent hypoxia or anoxia, or a change in the seasonality of low-oxygen events in a system could cause major habitat loss for polyps intolerant of such conditions. In order to predict the overall effect of increasing hypoxia on medusae, more information is needed on habitat requirements necessary to sustain populations, low-oxygen tolerance of polyp stages, and effects of low oxygen on recruitment levels.

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