Metabolic adaptations of the pelagic octopod *Japetella diaphana* to oxygen minimum zones

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ABSTRACT

The pelagic octopod *Japetella diaphana* inhabits meso- and bathypelagic depths worldwide. Across its geographic and depth range, individuals encounter oxygen levels ranging from nearly air-saturated to nearly anoxic. In this study, we assessed the physiological adaptations of individuals from one of the largest and most extreme regions of ocean hypoxia, the Eastern Tropical North Pacific (ETNP) oxygen minimum zone (OMZ). Ship-board measurements of metabolic rate and hypoxia tolerance were conducted and a metabolic index was constructed to model suitable habitat for aerobic metabolism. We found that individuals from the ETNP had a low metabolic rate, yet higher than conspecifics from more oxygen-rich habitats. Despite their higher rates, hypoxia tolerance may be similar to or greater than in conspecifics from more oxygen-rich waters. Furthermore, hypoxia tolerance in *J. diaphana* has a reverse temperature-dependence from most marine ectotherms, a characteristic that uniquely suits the physical characteristics of the lower oxycline. Even with its high tolerance to hypoxia, *J. diaphana* is incapable of maintaining basal oxygen demand in the OMZ core, at a depth where populations are typically most abundant in more oxygenated regions. Despite the limited metabolically-suitable habitat, *J. diaphana* is abundant in the ETNP outside the OMZ core, suggesting that physiological and behavioral plasticity is sufficient to maintain species fitness in this region.

1. Introduction

The octopod *Japetella diaphana* (Octopoda: Bolitaeninae; Hoyle, 1885) inhabits tropical and subtropical meso- and bathypelagic waters worldwide (Norman and Finn, 2013). Bolitaenins are among the most abundant pelagic octopods (Judkins et al., 2017), yet little is known about them. They are gelatinous and can hide from predators by rapidly changing between being transparent or pigmented depending on the predatory search strategy (Zylinski and Johnsen, 2011). Their gelatinous musculature is less negatively buoyant than most cephalopod musculature, which allows them to have lower metabolic costs per unit mass (Seibel et al., 1997).

Throughout its geographic range, *J. diaphana* encounters a wide range of oxygen conditions ranging from the well-oxygenated North Atlantic to oceanic oxygen minimum zones (Norman and Finn, 2013). *Japetella diaphana* individuals living in oxygen minimum zones (OMZs)

have better tolerance to hypoxia than most shallow-water benthic octopods from which they likely evolved (Seibel and Childress, 2000). They are also more tolerant of hypoxia than conspecifics living in less pronounced OMZs despite similarly low metabolic rates (Seibel et al., 1997). This suggests that a low metabolic rate by itself is not enough to permit aerobic survival in this extreme hypoxia and that specific adaptations of oxygen uptake and transport systems are required for survival in OMZs. Despite respiratory and circulatory adaptations for hypoxia (Childress and Seibel, 1998) a variety of zooplankton and micronekton species distributions are still impacted by hypoxia in strong OMZs such as in the Eastern Tropical North Pacific (ETNP; Maas et al., 2014; Wishner et al., 2013, 2018).

In addition to its wide horizontal distribution, *J. diaphana* undergoes an ontogenetic vertical descent from 200 m down to at least 1250 m (Clarke and Lu, 1975; Roper and Young, 1975; Young, 1978). Sexual maturation and mating seems to occur near the lower end of their depth

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range. Gravid and brooding females have only been found deeper than 700 m (Young, 1978), and are often deeper than 1000 m. These females possess a large yellow photophore around their mouth, one of the few known bioluminescent structures in any octopod, presumably to attract males (Robison and Young, 1981; Herring et al., 1987). Unlike benthic octopods, female pelagic octopods brood their eggs and possibly paralarvae as well (Young, 1972). There is some suggestion that brooding females return to shallower depths (≈ 800 m) after mating occurs near the deepest part of their range (Young, 1978; Voight, 1995).

In the ETNP, the core of the OMZ (typically ≈300–600 m) occurs near the top of *J. diaphana's* range where juveniles begin their ontogenetic vertical descent. In the OMZ core, the partial pressure of oxygen (P_{O_2}) can fall as low as 0.14 kPa (1.8 $\mu mol \cdot kg^{-1}$, 10 °C; Wishner et al., 2013), lower than any cephalopod is known to be able to maintain basal metabolism (Seibel et al., 1997). Correspondingly, *J. diaphana* population density in the nearby Gulf of Panama is anomalously low around the OMZ (Thore, 1949).

Animals require oxygen to fuel metabolic production of adenosine triphosphate (ATP) for maintenance, prey capture and consumption, locomotion, and growth (Wells and Clarke, 1996). Populations are sustained in a given area when there is sufficient oxygen to support an "aerobic scope" for such activities. Deutsch et al. (2015) developed a "metabolic index" to quantify the suitability of a habitat for aerobic metabolism. They found that populations of a variety of marine organisms typically require ambient O_2 levels 2–5 times higher than the minimum required by an individual at rest.

The *J. diaphana* population that inhabits the ETNP should be sufficiently adapted to tolerate, or sufficiently mobile to move through the OMZ core. Construction of a metabolic index for *J. diaphana* in the ETNP OMZ should help us understand how this poorly understood species is adapted for this extreme environment. In this study, we investigated the effects of environmental P_{O_2} on hypoxia tolerance and oxygen consumption rate in *J. diaphana* in order to construct a metabolic index of its habitat and determine what adaptations allow it to survive in this region.

2. Materials and methods

2.1. Specimen collection

Japetella diaphana individuals were collected for physiological measurements with a 3 m^2 Tucker trawl with a 30-L insulated cod-end to protect animals from temperature shock (Childress et al., 1978). The volume of water filtered for each trawl was estimated from mouth area, ship speed, and open net duration. In total, 264,000 m^3 of water was filtered between 50 and 1500 m depth (Table 1). Additionally, a single individual was also collected from a 1 m^2 Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) but was not used in physiological experiments. The MOCNESS is probably not the appropriate device with which to sample this species, given that only one individual was captured from nearly 15 MOCNESS tows conducted, filtering over 100,000 m^3 of water in various tows with sequences of nets from the surface to 1000 m.

Trawls were conducted between 22 January and 11 February 2017 approximately 600 km off the Pacific coast of Mexico (21.2–21.9 °N, 117.0–118.1 °W) aboard the R/V *Sikuliaq* (Fig. 1A). The median depth range between opening and closing covered by the Tucker trawl was 131.5 m. Depth of capture was considered the average depth between opening and closing net depths. Upon capture, specimens were transferred to fresh chilled seawater for 2–48 h (mean = 20 h) prior to experiments to acclimate them to measurement temperature and to reduce the contribution of digestion to metabolic rate measurements.

Vertical profiles of temperature, salinity, and oxygen measurements were obtained from 19 CTD casts near where specimens were collected. Oxygen profiles were measured with a Seabird SBE 43 dissolved oxygen sensor.

2.2. Respirometry

Specimens were individually placed in darkened sealed chambers filled with 0.2 μm filtered seawater that had been treated with antibiotics (25 mg/L each of streptomycin and actinomycin) to minimize bacterial respiration. Chamber volume varied from 20 to 750 mL depending on individual size. Trials were conducted at surface pressure since hydrostatic pressure has little effect on metabolism in mesopelagic cephalopods (Belman, 1978). Upon placement in the chamber, individuals were allowed to consume the ambient oxygen until their O_2 consumption rate could no longer be sustained. Ambient P_{O_2} was measured optically with either a Loligo Systems Witrox 4 m or a PyroScience FireStingO2 meter. The trials lasted 12 h on average and ranged from 5 to 25 h. To assess the temperature-dependence of metabolism, 8 and 4 individuals were tested at 5 and 10 °C, respectively. Temperature was maintained with Lauda E100 and ThermoFisher NESLAB RTE-7 water baths. Oxygen meters were calibrated with air-saturated seawater and concentrated Na_2SO_3 solution ($P_{O_2} = 0$). Chamber water was mixed with magnetic stirrers (Cole-Parmer Immersible Stirrer EW-04636-50) to ensure homogenous oxygen levels throughout the chamber.

After experiments were completed, specimens were preserved in 10% formalin before wet mass and morphometrics were measured. Dorsal mantle length (DML) was measured as the linear distance from the midpoint between the eyes to the posterior end of the mantle. No corrections were made to account for potential shrinking from fixative.

2.3. Metabolic rate and P_crit analyses

Oxygen consumption rates (M_{O_2}) were calculated from the slope of P_{O_2} over time. Only linear regressions with an $R^2 > 0.8$ were included. The median M_{O_2} value from non-hypoxic oxygen levels (defined below) was calculated for each individual. Mass-specific oxygen consumption rate (the amount of O_2 consumed per gram of animal tissue per hour) is well known to decline with increasing animal size (Seibel, 2007). As such, all M_{O_2} values were mass-corrected to the median mass of 2.5 g according to Equation (1):

$$M_{O_{2_{mass-corrected}}} = b_0 M^b \tag{1}$$

where b_0 is the normalization constant specific for each individual, M is mass, and b is the metabolic scaling coefficient ($b = -0.19$ at 5 °C and was also applied to 10 °C individuals).

The P_{O_2} level below which an individual was unable to sustain its routine oxygen consumption rate was defined as the critical partial pressure of oxygen, or the P_{crit}. The P_{crit} for each individual was calculated using two metrics: first, we used the traditional "breakpoint" method, which fits a linear breakpoint function to the relationship between M_{O_2} and P_{O_2}. The "breakpoint" P_{crit} was defined as the P_{O_2} at the intersection between the relatively flat oxyregulating line and the steeply sloping oxyconforming line (Fig. 2, blue line). Due to natural variability in M_{O_2}, presumably caused by spontaneous changes in behavior and ventilation, M_{O_2} values that are well within the trend of the oxyregulating line are often observed well below the P_{crit} determined using the breakpoint method. For this analysis, however, we sought a metric that estimates the lowest reasonable threshold of oxygen tolerance.

To alleviate this issue, we developed the "sub-prediction interval (sub-PI)" metric of P_{crit} calculation. Using this method, a traditional breakpoint relationship was fit, as before, but a 95% prediction interval was added around the oxyregulating line to encapsulate a space in which all observed M_{O_2} values can reasonably be considered to be oxyregulating (Fig. 2, dashed lines). The "sub-PI" P_{crit} is defined as the P_{O_2} at which the oxyconforming line intersects the lower limit of the 95% prediction interval (Fig. 2, red line). Both P_{crit} metrics were calculated using the "calc_pcrit" function from the R package

Table 1
Sampling effort with a 3 m² Tucker trawl during mid-water sampling of the Eastern Tropical North Pacific (21.2–21.98 °N, 117.0–1187.18 °W).

| Cast #	Start time (local)	Latitude	Longitude	Ship speed (kt)	Trawl open depth (m)	Trawl closed depth (m)	Fishing duration (hr)	Volume filtered (m^3)	# of individuals caught	Nominal depth of capture (m)
2	2017-01-23 15:38	21.61	−117.07	2	764	620	1.35	15001		
3	2017-01-24 18:53	21.60	−117.02	2	635	680	1.20	13334		
4	2017-01-25 14:44	21.70	−117.18	1	477	402	0.77	4260	2	440
5	2017-01-25 15:46	21.75	−117.20	1.625[a]	810	682	1.10	9931		
6	2017-01-26 16:36	21.63	−117.65	1.25	621	580	1.18	8218		
7	2017-01-26 18:16	21.68	−117.67	1	779	1011	0.95	5278	2	895
9	2017-01-29 17:50	21.59	−117.61	2	912	850	1.02	11297		
10	2017-01-29 20:30	21.66	−117.60	2	150	55	0.42	4630		
11	2017-01-30 10:40	21.39	−117.72	1.3	436	205	1.30	9390		
12	2017-01-30 12:47	21.45	−117.61	1.5	422	170	0.98	8195		
13	2017-01-31 10:30	21.40	−117.59	1.3	463	200	1.25	9029		
14	2017-02-01 08:47	21.40	−117.59	1.625[a]	415	566	0.75	6771		
15	2017-02-02 08:55	21.71	−117.70	1.5	622	750	1.78	14862		
16	2017-02-02 12:29	21.60	−117.69	1.8	515	383	0.98	9834		
17	2017-02-03 18:16	21.61	−117.68	1.4	598	443	0.73	5704	2	521
18	2017-02-04 16:14	21.66	−117.58	1.625	570	362	1.07	9630		
19	2017-02-05 15:37	21.70	−117.60	2	929	784	1.00	11112		
20	2017-02-05 18:21	21.77	−117.62	2	98	68	0.48	5371		
21	2017-02-06 08:13	21.22	−117.73	1.4	751	620	1.08	8427		
22	2017-02-06 10:41	21.29	−117.77	1.5	418	363	1.05	8751	4	391
23	2017-02-07 15:37	21.64	−117.81	1.7	725	605	1.00	9445		
24	2017-02-08 16:31	21.81	−117.92	2	530	356	1.00	11112		
25	2017-02-08 18:29	21.87	−117.92	1	880	803	0.77	4260		
26	2017-02-09 16:52	21.75	−118.03	1.7	449	194	1.12	10547		
27	2017-02-09 18:46	21.69	−118.05	1.5	836	NR	0.68	5695		
28	2017-02-10 08:09	21.54	−118.00	1.7	1487	1076	1.53	14483		
29	2017-02-10 11:59	21.64	−117.99	1.8	382	345	0.87	8667		
30	2017-02-11 08:09	21.52	−117.96	1.8	810	682	1.02	10167	2	746
31	2017-02-11 10:39	21.56	−117.98	2.1	464	291	0.93	10890		
Total								**264,292**	**14**	

[a]: Ship speed was not recorded so the average from other casts was used.
[b]: Records maintained were unclear in which cast(s) these individuals were caught. Nominal depth of capture was taken as the average from all three casts.
NR: not recorded.
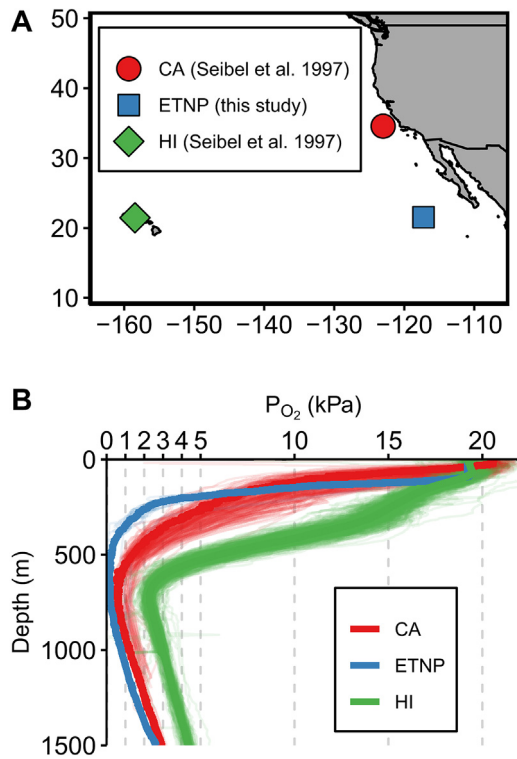
Fig. 1. Sampling sites of *Japetella diaphana* used in physiological experiments. **A**) Location of specimen collection sites. **B**) P_{O_2}-depth profiles at each collection site. Data are from the CTD casts from this study and the World Ocean Database 2013 using all OSD and CTD data from stations within 0.5° of collection sites. Thick lines are mean values.
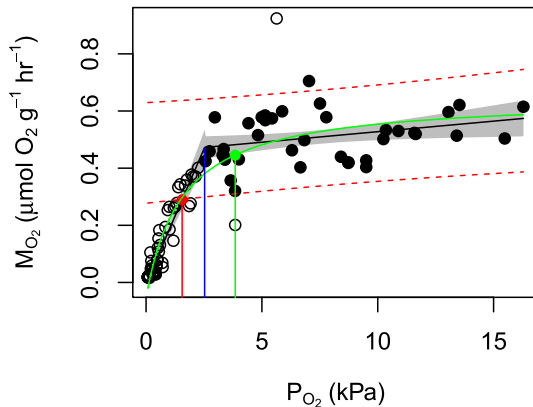
Fig. 2. A representative respirometry experiment showing the routine metabolic rate ($\mu mol\ O_2 \cdot g^{-1} \cdot hr^{-1}$) of *Japetella diaphana* as a function of oxygen partial pressure (kPa). At high P_{O_2}, oxygen consumption (M_{O_2}) is stable across a range of oxygen levels (oxyregulating region), while below the P_{crit} threshold, M_{O_2} is oxygen-dependent (oxyconforming region). The P_{crit} calculation methods are indicated by vertical colored lines. The blue line indicates the P_{crit} calculated using the breakpoint metric (2.53 kPa). The red line indicates the P_{crit} calculated using the sub-PI metric (1.56 kPa). The green line indicates the P_{crit} calculated using the nonlinear regression method developed by Marshall et al. (2013) with the threshold $dM_{O_2}/dP_{O_2} = 0.065$ as suggested by the authors (3.85 kPa). The gray shaded region represents the 95% confidence interval and the red dashed lines represent the 95% prediction interval for the regulated oxygen consumption rate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

"respirometry" (Birk, 2019). The nonlinear P_{crit} method developed by Marshall et al. (2013) was also considered but deemed not suitable for this study because the user-defined slope threshold recommended by the authors resulted in P_{crit} estimates (2.1–4.2 kPa) far higher than any of the oxygen levels at which the animals were found, and it is unclear how this threshold could be objectively adjusted to fit this study.

The sub-PI metric results in a systematically lower P_{crit} estimation than the traditional breakpoint metric. In this dataset, the sub-PI metric returned a 36% lower P_{crit}, on average. The sub-PI metric is also sensitive to variability in the data, such that trials with more variable M_{O_2} values will result in a lower estimated P_{crit}. This should be kept in mind when comparing trials with unequal variability. However, in this dataset, this sensitivity contributed little to variations in P_{crit} values between individuals, as evidenced by an R^2 of 0.916 from a linear regression of breakpoint versus sub-PI P_{crit}.

To assess geographic effects on physiology, metabolic rate and hypoxia tolerance data from the 5 °C trials were compared with data from *J. diaphana* collected off the coasts of California and Hawaii by Seibel et al. (1997) who used similar methods of collection and experimentation (Fig. 1). To our knowledge, these are the only metabolic data previously collected from *J. diaphana*. For an equivalent comparison to the analysis conducted by Seibel et al. (1997), only metabolic rates measured between 4 and 9.3 kPa were considered in this comparison, which eliminated observations from one individual that was only tested at $P_{O_2} > 9.3$ kPa. Due to the large size range of specimens captured, differences in M_{O_2} between populations were assessed by an ANCOVA with mass as a covariate. To assess tolerances to hypoxia in different populations, metabolic rates were normalized at multiple P_{O_2} levels to each individual's mean M_{O_2}. This eliminated the strong size-dependence of M_{O_2} between individuals. Then the mean normalized M_{O_2} for each population at multiple P_{O_2} levels was computed. This allowed population-level assessments of the oxygen-dependence of M_{O_2}.

The temperature-dependences (Q_{10}) of mass-corrected M_{O_2} and P_{crit} were estimated for the ETNP individuals using the "Q10" function of the R package "respirometry" (Birk, 2019) and were assessed using t-tests. For statistical comparisons, the breakpoint P_{crit} was used instead of the subPI metric because 1) a subPI P_{crit} could not be determined for two individuals with very low P_{crit} and modest noise in the data, 2) the breakpoint metric is sufficient for conducting relative comparisons between two temperatures, and 3) both metrics resulted in P_{crit} values that were highly correlated ($R^2 = 0.916$).

2.4. Metabolic index

The metabolic index, or the ratio of oxygen supply to demand, is a temperature-dependent metric to quantify suitable habitat for aerobic metabolism (Equation (2); Deutsch et al., 2015). E_0 and A_0 are physiological parameters defined as the slope and intercept, respectively, of the linear relationship between the natural logarithm of P_{crit} and $1/k_BT$, where k_B is the Boltzmann constant and T is temperature in Kelvin. It can also be parameterized as the ratio of ambient P_{O_2} to a temperature-dependent critical P_{O_2} (P_{crit_T}).

$$MI = A_0 \frac{P_{O_2}}{\exp(-E_0/k_B T)} = \frac{P_{O_2}}{P_{crit_T}} \tag{2}$$

An MI < 1 represents a region where oxygen demand exceeds supply (ambient P_{O_2} is below P_{crit}) and should not be able to support steady-state basal aerobic metabolism. An MI > 2–5 is typically required to sustain populations of marine animals (Deutsch et al., 2015). MI depth profiles were constructed using environmental data from CTD casts (temperature and P_{O_2}) and the physiologically-derived relationship between minimum sub-PI P_{crit} and temperature (Equation (2)). The minimum rather than mean P_{crit} from each temperature was chosen because the minimum P_{crit} (maximum hypoxia tolerance) of a net-caught mesopelagic animal inside a small respirometer is likely a more

accurate estimate of P_{crit} *in situ* than the mean value of multiple variably-stressed individuals.

2.5. Vertical distribution

To assess the likely impact of the metabolic index on *J. diaphana* vertical distribution, the metabolic index for the ETNP was compared to vertical distribution profiles of *J. diaphana* collected from the literature. Distribution data were collected from southern California (Isaacs-Kidd midwater trawl (IKMT); Roper and Young, 1975), the Eastern Tropical North Pacific (Tucker trawl; this study), the Gulf of California (ROV; Schlining and Stout, 2006), Hawaii (Tucker trawl and IKMT; Young, 1978), Monterey Bay (ROV; Schlining and Stout, 2006), and the North Atlantic (opening-closing rectangular midwater trawl; Clarke and Lu, 1975). All distributions were normalized for variable sampling efforts at each depth. To facilitate comparison between aerobic habitat and vertical distribution, environmental oxygen profiles were gathered from the World Ocean Database 2013 using all Ocean Station Data (OSD) and CTD data from stations within 0.5° of collection sites.

3. Results

3.1. Abundance

In total, 15 individual *Japetella diaphana* were collected in the ETNP between 170 and 1011 m depths, with a total abundance of 5.68 individuals per $10^5 \, m^3$ (Table 1, Table S1). Fourteen were captured in the Tucker Trawl and one was captured in the MOCNESS (650–675 m). Twelve were juveniles (11–37 mm DML), two were mature males, and one was a mature female. One of the mature males (52 mm DML) and the female (72 mm DML) were both caught in the same trawl between 779 and 1011 m depth.

3.2. Size- and region-specific physiology

Among *J. diaphana* in the ETNP, mass-specific M_{O_2} decreased with increasing size, with a scaling coefficient (b) of -0.19 at 5 °C (ETNP; n = 8). Despite the change in oxygen demand, no relationship was found between mass and P_{crit}. Raw M_{O_2} data for all individuals are provided in Table S2.

Although individuals from the ETNP were similar in size to those from other regions, mass-specific M_{O_2} of ETNP individuals was over twice as high as those found off California and Hawaii, even when tested at the same temperature (5 °C) over similar trial durations (p = 0.003; Fig. 3A; $M_{O_{2CA}} = 0.253M^{-0.151}$, n = 11; $M_{O_{2ETNP}} = 0.475M^{-0.131}$, n = 7; $M_{O_{2HI}} = 0.242M^{-0.252}$, n = 12). Despite their higher oxygen demand, individuals from the ETNP (n = 11) appeared to have similar hypoxia tolerance to an individual from California (n = 1) and seemed to be more tolerant than individuals from Hawaii (n = 3; Fig. 3B) although low sample sizes and differences in data collection methods between the studies prohibited statistical assessment.

3.3. Temperature-dependence of metabolism and hypoxia tolerance

Mass-corrected M_{O_2} (i.e. adjusted to a common size) of individuals from the ETNP increased significantly with increasing temperature, from $0.451 \pm 0.093 \, \mu mol \, O_2 \cdot g^{-1} \cdot hr^{-1}$ at 5 °C (n = 8) to 0.675 ± 0.234 $\mu mol \, O_2 \cdot g^{-1} \cdot hr^{-1}$ at 10 °C (n = 4; p = 0.035; Q_{10} = 2.24; Fig. 4A). Breakpoint P_{crit} decreased slightly but significantly over the same temperature range from 1.8 ± 0.73 kPa at 5 °C (n = 7) to 0.88 ± 0.34 kPa at 10 °C (n = 4; p = 0.043; Q_{10} = 0.24; Fig. 4B). The lowest sub-PI P_{crit} recorded was 0.63 and 0.31 kPa at 5 and 10 °C, respectively. These values were used in construction of the metabolic index (Fig. 4C).

3.4. Metabolic index and vertical distributions

We found a minimum ambient P_{O_2} of 0.143 kPa (5.6 °C; 2 $\mu mol \cdot kg^{-1}$) in the OMZ core. Ambient P_{O_2} levels were below P_{crit} at depths where temperature was between roughly 4.5 and 8 °C (Fig. 4C). This corresponded to a wide depth range (427–970 m) that had an MI < 1 (Fig. 5). Most of the individuals were captured near the oxyclines at depths where MI ≈ 1 (Fig. 5).

Japetella diaphana populations from various ocean regions that were examined displayed a peak in abundance between 600 and 800 m. In the ETNP, however, the metabolic index (MI) was < 0.5 in this depth range. Unfortunately, the low number of individuals captured in the ETNP precluded the construction of a reliable vertical distribution from which direct comparisons could be made to other distributions, but most of the individuals that were captured in the ETNP were found shallower, between 300 and 500 m where MI $\gtrsim$ 1 (Fig. 5; Fig. 6).

4. Discussion

4.1. Aerobic habitat and distribution

The metabolic index for *Japetella diaphana* in the ETNP was < 1 for a large depth range of the OMZ, suggesting that oxygen supply in this region is insufficient to meet the basal oxygen demand of *J. diaphana*. It is not surprising, therefore, that many of the individuals were captured in the upper and lower oxyclines where the MI was $\gtrsim$ 1, but few were captured where the hypoxia was most extreme. Although vertical distributions could not be reliably estimated from our low sample size (n = 15), the MI profile predicts an uninhabitable environment at depths where *J. diaphana* typically peak in density in more well-oxygenated regions. As predicted by this study, Thore (1949) reports that in the Gulf of Panama, where a strong OMZ also exists, *J. diaphana* density has a minimum between 200 and 1000 m, unlike more oxygenated stations from the same expedition. We expect that a similar drop in population density occurs in the ETNP between 500 and 1000 m, as predicted by the metabolic index.

In other marine animals, an MI of 2–5 is the lower limit for populations to be sustained (Deutsch et al., 2015). But this is higher than the MI levels where many of the octopods were found in this study. Therefore, it seems that *J. diaphana* populations are able to survive within a much narrower oxygen margin than many marine organisms, a trait shared by other OMZ-inhabiting zooplankton (Wishner et al., 2018). It should be kept in mind, however, that the P_{O_2} at any given depth may vary horizontally and over time, and this can influence the vertical and horizontal distribution of species (Wishner et al., 2018).

None of the individuals we examined were able to sustain aerobic metabolism below the most extreme hypoxia encountered in the OMZ core (0.14 kPa; 2 $\mu mol \cdot kg^{-1}$). Furthermore, *Japetella diaphana* does not seem to undergo diel vertical migration (Young, 1978), making it unlikely that the individuals we captured were temporarily metabolically suppressed in the OMZ core as part of their life history strategy, a behavior exhibited by more muscular cephalopods in the eastern Pacific OMZ (Hunt and Seibel, 2000; Seibel et al., 2014). Nevertheless, a few individuals were still found at depths where MI < 1. This may be due to methodological constraints on measuring hypoxia tolerance in mesopelagic animals. It is important to consider that net-caught and confined mesopelagic specimens may be stressed or injured in ways that, while not visible or detectable, may nonetheless impair tolerance to hypoxia compared to an individual *in situ* (Kassahn et al., 2009). Therefore, it is possible that the P_{crit} values reported here are overestimates of *in situ* values, thus underestimating the "true" metabolic index of this region for *J. diaphana*. We have sought to avoid this problem by utilizing a novel P_{crit} calculation method that results in lower values than the traditional method, yet the possibility remains. It is also important to consider that the MI is a ratio. Very small error in the denominator (P_{crit}) will result in large differences in the calculated
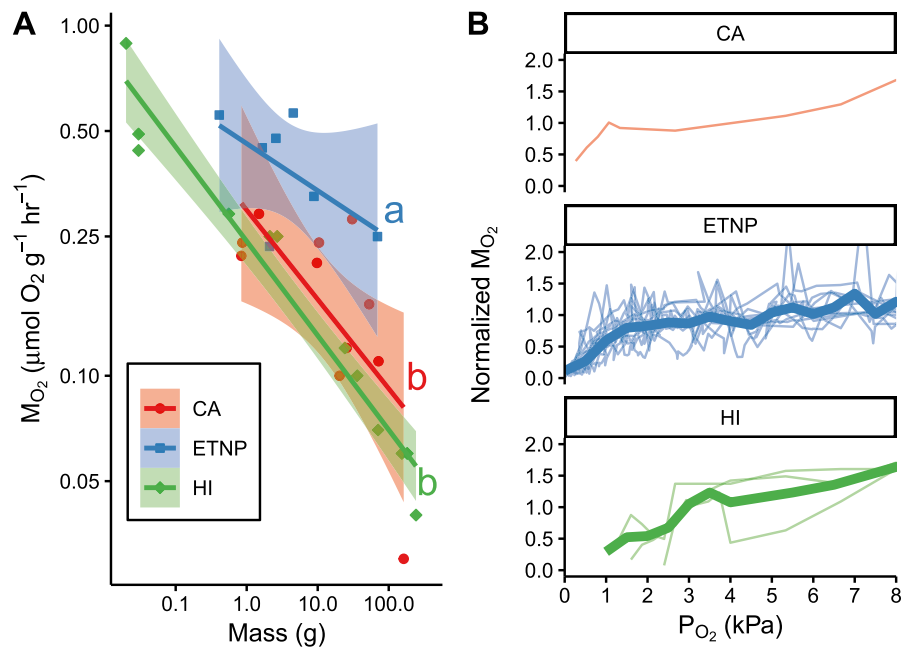
Fig. 3. Differences in **A**) oxygen consumption rates (M_{O_2}) at normoxia and **B**) the dependence of M_{O_2} on ambient P_{O_2} between *Japetella diaphana* from California (red circles), the Eastern Tropical North Pacific (blue squares), and Hawaii (green diamonds). All measurements were conducted at 5 °C. Letters indicate statistically significant differences between populations. Shaded regions are 95% confidence bands. Hypoxia tolerances could not be statistically compared due to differing methods and low sample size. Thin lines are normalized metabolic rates for each individual. Bold lines are population averages. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
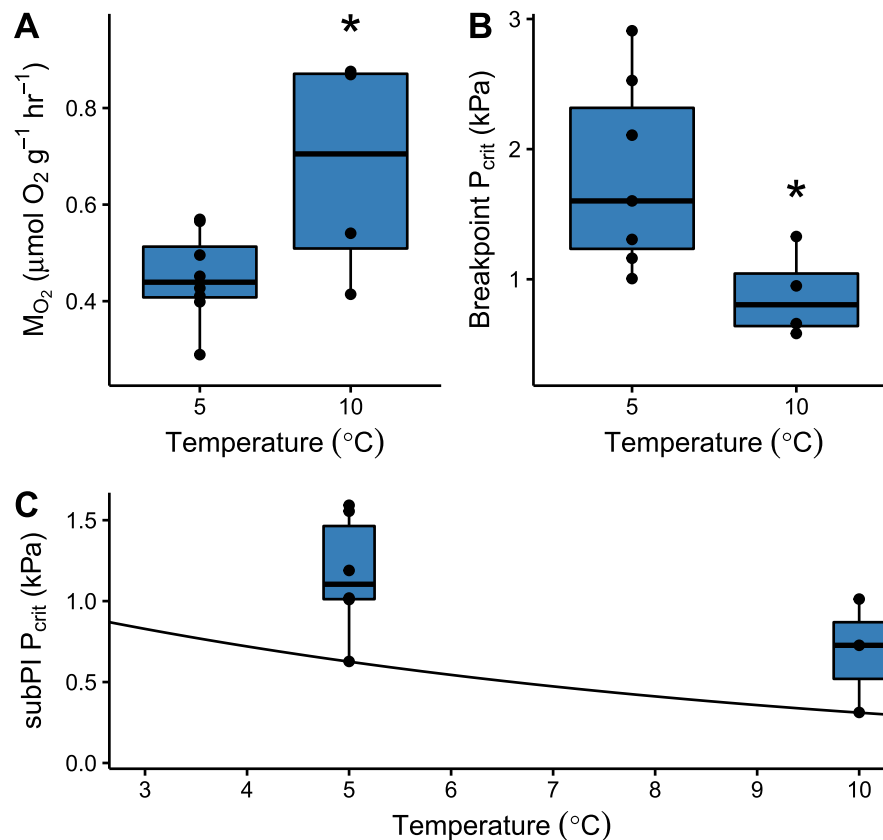
Fig. 4. Effect of temperature on **A**) mass-corrected oxygen consumption rate (M_{O_2}) (p = 0.035) and **B**) hypoxia tolerance (P_{crit}, breakpoint method) (p = 0.043) at 5 and 10 °C in *Japetella diaphana* from the Eastern Tropical North Pacific. **C**) Effect of temperature on hypoxia tolerance (P_{crit}, sub-PI method). Boxplots represent sub-PI P_{crit} values as in panels A and B. The black line represents the curve fit to the minimum values at each temperature used in construction of the metabolic index. In boxplots, the middle bar is the median, boxes span from 1st to 3rd quartiles, and whiskers extend to $1.5 \times IQR$.
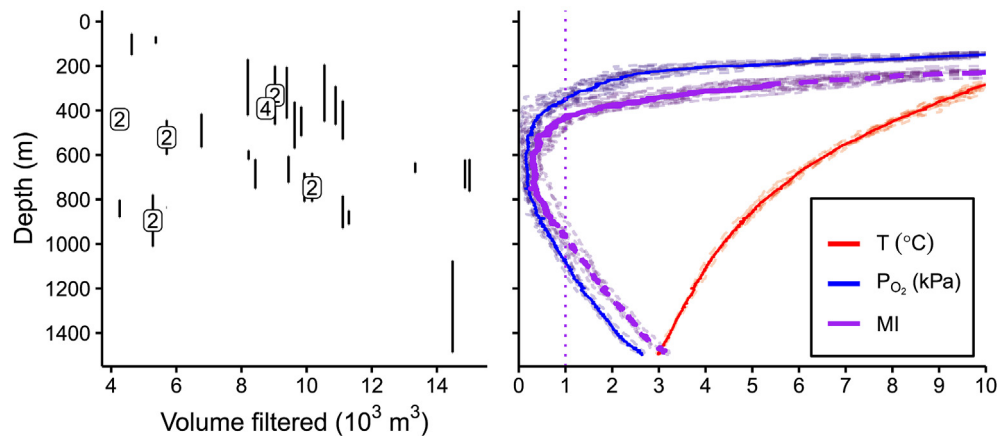
Fig. 5. Metabolic index of *Japetella diaphana* in the Eastern Tropical North Pacific (ETNP). Depth profiles are shown of sampling effort and individuals captured using a 3 m^2 Tucker trawl (left panel) and environmental conditions (right panel). In the left panel, vertical bars delimit the depth range sampled from each cast and numbers correspond to the number of individuals caught in each cast. In the right panel, thin lines represent values from individual CTD casts. Thick lines are mean values from all casts. Thick dashed purple lines (above 283 m and below 854 m depth) represent where the metabolic index was extrapolated beyond the 5–10 °C range from which physiological measurements were made. The vertical dotted purple line is placed at MI = 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
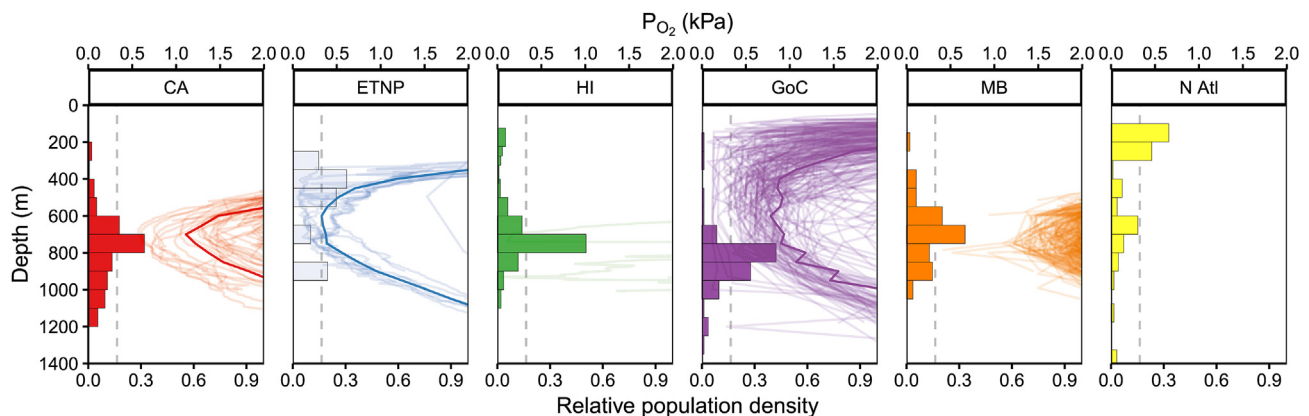
Fig. 6. Vertical distribution of *Japetella diaphana* abundance (bars) from southern California (red; Roper and Young, 1975), the Eastern Tropical North Pacific (blue; this study), Hawaii (green; Schlining and Stout, 2006), the Gulf of California (purple; Schlining and Stout, 2006), Monterey Bay (orange; Schlining and Stout, 2006), and the North Atlantic (yellow; Clarke and Lu, 1975). Within each region, the distribution was normalized for variable sampling efforts at each depth such that bar height indicates the relative population density at each depth range. Thin lines represent oxygen profiles from individual OSD and CTD casts in each region. Thick lines represent mean oxygen levels from all casts from each region. Paucity of lines in Hawaii and North Atlantic are indicative of high oxygen levels in these regions. Vertical dashed lines occur at the minimum P_{O_2} in the ETNP. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

metabolic index. Alternatively, it is possible that individuals captured in the OMZ core were passing through the core during ontogenetic vertical migration relying on anaerobic metabolism. More extensive sampling of this species in strong OMZs such as is found in the ETNP may help to clarify this matter.

We found that *J. diaphana* abundance in the ETNP (5.68 individuals per 10^5 m^3) was comparable to abundance estimates from the North Atlantic off the coast of Africa (2.2 individuals per 10^5 m^3; Clarke and Lu, 1975) and from the Gulf of Mexico (3.5 individuals per 10^5 m^3; H. Judkins, pers. comm). This is surprising given the apparent paucity of aerobic habitat, but this may be counteracted by the high productivity and prey biomass in this region (Fernández-Álamo and Färber-Lorda, 2006). Thore (1949) reported *J. diaphana* density in the Gulf of Panama to be comparable or larger than most other stations of the circumglobal *Dana* expeditions, and found that *J. diaphana* density often correlated with primary productivity. Admittedly, our sampling effort was small and comparisons between studies are complicated by differences in sampling gear, but it seems unlikely that the extreme hypoxia in this region has strong negative effects on the local abundance of *J. diaphana*.

It is common to find aggregations of zooplankton and micronekton in oxyclines around the OMZ where the individual *J. diaphana* in our study were found (Wishner et al., 2013). For predatory *J. diaphana*, this may be a feeding ground rich in fishes, calanoid copepods, decapod crustaceans, and other mollusks upon which it feeds (Passarella and Hopkins, 1991). As such, the foraging opportunities may outweigh the compressed aerobic habitat.

4.2. Metabolic adaptations to hypoxia

Individual *J. diaphana* from the ETNP had a higher M_{O_2} than individuals from California and Hawaii, however the physiological mechanism or adaptive significance of such differences is unclear. Despite their higher oxygen demand, individuals from the ETNP seem to have maintained a lower P_{crit} (improved hypoxia tolerance) compared to individuals from Hawaii (Seibel et al., 1997), but low sample sizes prohibited any definitive statistical analyses. In pelagic animals generally, P_{crit} correlates with the minimum encountered ambient P_{O_2} (Seibel, 2011). For example, in some mesopelagic crustacean species,

P_{crit} is higher in individuals from relatively well-oxygenated Hawaiian waters than from conspecifics in the OMZ off California (Cowles et al., 1991; Childress and Seibel, 1998). Crustacean and fish species are also known to adapt their hypoxia tolerance to match ambient conditions on much smaller spatial scales such as within the Monterey Canyon or southern California basins (Childress, 1975; Friedman et al., 2012).

Although oxygen demand increased under higher temperature, as is typical of ectotherms, mean P_{crit} decreased by 0.92 kPa (i.e. hypoxia tolerance improved) from 5 to 10 °C. In ectotherms, hypoxia tolerance typically decreases (i.e. P_{crit} rises) with rising temperature (Rogers et al., 2016) due to a combination of higher oxygen demand and reduced oxygen binding affinity of the respiratory proteins. However, this is not always the case. Freshwater darter fishes have been observed to lower P_{crit} despite higher M_{O_2} with rising temperature (Ultsch et al., 1978). Two crustaceans that inhabit the lower oxycline of the ETNP OMZ were also recently found to have an inverse temperature-dependence of P_{crit} (Wishner et al., 2018). The physiological underpinnings of this unique relationship may be an inverse temperature-dependence of blood-O_2 binding affinity as has been found in the respiratory protein of the vertically migrating krill, *Meganyctiphanes norvegica* (Brix et al., 1989). Whatever the mechanism, the functional benefit of this inverse temperature-dependence of P_{crit} is clear for a meso- and bathypelagic animal living in a strong OMZ. Within the depth range where *J. diaphana* are typically found (400–1200 m), warmer waters have lower P_{O_2} and thus require a lower P_{crit} to maintain aerobic metabolism. In this study, most individuals were caught in the upper rather than lower oxycline, but this is likely due to the low sample size attained here. When more extensive sampling efforts are made, most individuals are found in the lower oxycline (Fig. 6; Roper and Young, 1975; Schlining and Stout, 2006).

Recent growth in OMZ size and intensity worldwide has been predicted to impact the vertical distributions of many marine animals (Wishner et al., 2013; Breitburg et al., 2018). Metabolic indices of a region provide a useful quantitative metric to infer the likely impact of these shoaling oxyclines on resident fauna (Wishner et al., 2018). *Japetella diaphana* is unable to support aerobic metabolism in the core of the strong OMZ in the ETNP. As the OMZ shoals into shallower waters in the coming decades, the vertical distribution of *J. diaphana* in this region is likely to shift as well. Expansion of such unsuitable habitat in the middle of *J. diaphana* depth range has unknown consequences on ontogenetic vertical migration or abundance in this cosmopolitan yet enigmatic species.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2019.04.017.

Conflicts of interest

The authors have no competing interests to declare.

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