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Metabolic activity of pelagic copepods from 5000-7000 m depth of the western subarctic Pacific, as inferred from electron-transfer-system (ETS) activity

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Running head: ETS activity of pelagic copepods from 5000-7000 m depth

Abstract

This study demonstrates reduced electron transfer system (ETS) activity of mixed copepods collected from 5,000 to 7,000 m depths [3.21 ± 1.25 μl O_2 (mg protein)$^{-1}$ h^{-1} at 10^{o}C] as compared with mixed copepods from 0 to 200 m depths [5.93 ± 1.66 μl O_2 (mg protein)$^{-1}$ h^{-1} at 10^{o}C] of the western subarctic Pacific. At the in situ temperature of 1.5^{o}C, the 5,000–7,000 m ETS data, in terms of wet mass (WM)-specific respiration rates (R), is equivalent to [0.052 ± 0.021 μl O_2 (mg WM)$^{-1}$ h^{-1}] which is similar to or greater than those reported for selected copepods or mixed mesozooplankton from 5,000 m depth by previous workers.

1. Introduction

Copepods are the major component of marine mesozooplankton and may be the most numerous multicellular organisms on the earth (Longhurst 1985; Mauchline 1998). Because of their ubiquitous distribution, high abundance and trophic importance, vital rates of copepods and other mesozooplankton are of particular relevance to understanding oceanic biogeochemical cycles of carbon and other elements (Hernandez-Leon and Ikeda 2005; Aristegui et al. 2005; Buitenhuis et al. 2006). Copepod respiration (=oxygen consumption) is a direct measure of mineralization, and voluminous data has been accumulated on the epipelagic copepods of the world's oceans (Ivleva 1980; Ikeda et al. 2001). In contrast, from the mesopelagic and bathypelagic zones, little respiration is currently available for live copepods except for two studies; one off California (Thuesen et al. 1998) and the other in the western subarctic Pacific (Ikeda et al. 2006a, 2007a). This paucity of information about respiration in deep-sea copepods reflects the technical difficulty in retrieving live specimens from depth in good enough condition for incubation experiments. To date, the maximum depth from which copepods have been retrieved for incubation experiments is 3000-5000 m (Ikeda et al. 2007a). Copepods living below 5000 m are known to be characterized by smaller body size than those distributing above (Vinogradov 1962; Mauchline 1972) but their respiration rates have not been studied.

An enzyme assay of intermediary metabolism can be a measure of potential respiration. Its advantage over a physiological measurement on deep-sea copepods is its freedom from problems associated with recovery of live copepods from depth. This is because the cellular enzyme concentration in a specimen does not vary appreciably on a short time scale (cf. Ikeda et al. 2000). If the enzyme is constitutive, this is especially

true. The intermediary enzymes, citrate synthase (CS), lactate dehydrogenase (LDH) and pyruvate kinase (PK) have been used as indices of respiration as well as indicators of aerobic metabolism, anaerobic metabolism and glycolytic flux, respectively, in deep-sea pelagic animals (see review of Childress 1995). However, both LDH and CS activities were poorly correlated with respiration rates of the deep-sea copepods (Thuesen et al. 1998). Furthermore, as indicators of respiratory O_2 consumption or CO_2 production, they are poor choices because they are not associated closely with the reactions of either O_2 consumption or with CO_2 production. As an alternative, enzyme activities of electron-transfer-system (ETS), the enzyme system that is known from all biochemistry text-books to control the respiratiory O_2 consumption, have been shown to be closely associated with respiration rates of marine zooplankton (Packard et al. 1975; King and Packard 1975a; Packard and Gómez 2008). ETS activity has been used as a practical method to estimate community metabolism of deep-sea zooplankton (King et al. 1978; Koppelmann et al. 2004; Hernández-León and Ikeda 2005). Theoretically, according to enzyme kinetics, the ratio of ETS activity to respiration is 2 (Owens and King 1975; King and Packard 1975a) and is little affected by temperature and body mass of zooplankton tested (King and Packard 1975a).

As part of a deep-sea zooplankton metabolism project (Ikeda et al. 2006a and b, 2007a and b), I determined potential respiration rates (ETS activity) of copepods living below 5000 m of the subarctic Pacific to gain insight into their metabolic characteristics.

2. Materials and methods

97 2.1 Zooplankton sampling

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99 Copepods were collected from 5,000-7,000 m depth at station D (42^{o}40’N 147^{o}58’E) off
100 the east coast of Hokkaido during RS Tansei-Maru Cruise KT-05-12 to the western
101 subarctic Pacific on June 3, 2005 (Fig. 1). A vertical closing net [80 cm diameter, 0.3
102 mm mesh size; modified from Kawamura (1968)] equipped with a large cod-end (2 l
103 capacity) was used to retrieve zooplankton from depth. The closing net was towed from
104 7,000 to 5,000 m at a speed of 0.5 m sec^{-1}, closed and raised to the surface at 2 m sec^{-1}.
105 The depth the net reached was estimated by the length of winch wire paid out on the
106 basis of a linear relationship between the wire length and the depth of the net reached;
107 the linear relationship was confirmed repeatedly by using a RMD depth meter (Rigosha
108 Co. Ltd.) monitoring the net at depths above 5000 m [note: the maximum depth to
109 which the depth meter withstands was 5,000 m]. The distance towed of the open net
110 (2,000 m) was confirmed by a flow-meter (Rigosha Co. Ltd.) attached to the mouth-ring
111 of the net. The flow meter reading was 14,328 revolutions which compared well with
112 14,171 (±1,770, SD, N = 4) revolutions, the value obtained by calibrating the net in a
113 vertical haul from 5,000 m to 3,000 m. After closing the mouth of the net at 5,000 m,
114 the net reached the surface in 42 min. As a control, shallow-living copepods were
115 collected with a Norpac net (0.3 mm mesh) which was towed from 200 m to the surface
116 at station S (40^{o}29’N 142^{o}30’E, Fig. 1) off the northeast coast of Honshu on June 6
117 during the same cruise.
118 Upon retrieval of the net, zooplankton samples were passed through 1.4 mm mesh
119 netting to remove gelatinous material. Undamaged copepods in the filtrate were sorted
120 immediately into chilled seawater which had been collected from 6,000 m depth with 20

l Niskin bottles or from the surface with a bucket just prior to zooplankton collection. Temperature, salinity and dissolved oxygen profiles were determined by using a CTD system down to 5,000 m.

2.2 ETS activity

Batches of copepods (10-30 specimens each) were placed on 0.2 mm mesh nettings, blotted on filter paper, preserved immediately in liquid nitrogen on board the ship, and brought back to the land laboratory for ETS analysis. Within one month after the cruise, each batch of frozen copepods was homogenized with a small piece of glass fiber filter in a glass-teflon tissue homogenizer. The Owens and King (1975) ETS assay was used. However, the final reaction volume was reduced from 6 ml to 1.5 ml. One ml of each homogenized sample was centrifuged yielding a cell-free extract that was used for the ETS assay. Preliminary tests indicated that the ETS activities from 5,000-7,000 m depth were too low to measure at *in situ* temperature (1.5^{o}C). To overcome this problem, the reaction temperature of the assays was raised to 10^{o}C. ETS activities were determined on two 0.25 ml aliquots of cell-free extract from each subsample. The effect of hydrostatic pressure on ETS activities of crustacean plankton has been demonstrated to be not significant at least down to 265 atm (King and Packard 1975b). As an indicator of copepod biomass, protein concentrations were determined on each homogenate to standardize ETS activities. Protein was determined in duplicate by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.3 Taxonomy/biometry

Part of each fresh subsample was preserved in 10% formalin-seawater for microscopic observation. Part of each frozen subsample was used to establish mean wet mass (WM), dry mass (DM) and the protein of individual copepods. DM was obtained by freeze-drying the subsamples.

3. Results and discussion

3.1 ETS activity of copepods from 5000-7000 m versus those from 0-200 m

Copepods from 5,000-7,000 m depth (Fig. 2) were composed of *Spinocalanus* spp. (33%, the total N = 91), *Scaphocalanus* spp. (11%), *Microcalanus* spp. (9%), *Lucicutia* spp. (8%) and others (39%), and those from 0-200 m depth *Pseudocalanus* spp. (28%, the total N = 72), *Metridia pacifica* (24%), *Neocalanus plumchrus* (21%), *Eucalanus bungii* (18%) and others (9%).

Mean WM, DM and protein content were 0.328 mg individual^{-1}, 0.066 mg individual^{-1} and 6.71 % of WM, respectively, for copepods from the 5000-7000 m, and were 0.394 mg individual^{-1}, 0.058 mg individual^{-1} and 7.19% of WM, respectively, for those from the 0-200 m (Table 1).

ETS activity determined at 10^{o}C was 3.21 ± 1.25 μl O_2 (mg protein)$^{-1}h^{-1}$ (N = 16) for the 5,000-7,000 m copepods and 5.93 ± 1.66μl O_2 (mg protein)$^{-1}h^{-1}$ (N = 15) for the 0-200 m copepods (Fig. 3). On the basis that each dataset was normally distributed (Chi-square test, $p > 0.1$) and the variance of both datasets did not differ significantly (F-test, $p > 0.05$), Student t-test detected a significant difference between the two means

($p < 0.001$).

The body mass and temperature have been documented as two major parameters affecting the metabolic rates of pelagic copepods across diverse species (Ikeda et al. 2001). From this, combined with an intimate relationship between respiration rates and ETS activity (Packard et al. 1975; King and Packard 1975a), mixed copepods from 5,000-7,000 m and 0-200 m of this study were anticipated to yield similar ETS activities since the body mass of each component copepod was effectively the same (Table 1) and all the assays were made at the same temperature (10^{o}C, Fig. 3). However, the observed ETS activities of the copepods from 5,000-7,000 m were about one half that of those from 0-200 m (Fig. 3), implying the presence of important parameter(s) other than temperature and body mass. This negative effect of habitat depth on body mass specific Rs (standardized by body mass and temperature) has already been documented for deeper-living micronektonic fishes, crustaceans and cephalopods with functional eyes (Childress,1995). According to Childress (1995), the vision-aided reaction distance in prey-predator relationships decreases downward due to diminishing light; thus, progressively less energy expenditure is required for vision-aided prey/predator reactions with increasing depth ("visual interactions hypothesis"). For pelagic copepods with no functional eyes, there are conflicting reports of neutral (Thuesen et al. 1998) and negative effects of habitat depth (Ikeda et al. 2006a; Ikeda 2008). The results here favor the view of Ikeda et al. (2006a) and Ikeda (2008). Because pelagic copepods lack functional eyes, the negative effect of habitat depth on the metabolism of pelagic copepods cannot be explained by the "visual interactions hypothesis". Ikeda et al. (2006a) proposed a new "predation-mediated selection hypothesis" that explained reduced respiration rates of deeper-living copepods to be a result of lowered selective

193 pressure for high activity at these depths because of the decrease in visual predators in
194 the dark. Reduced metabolic rates of deep-sea copepods are in concert with their lower
195 protein synthesis activities (= growth rates) as evidenced by their lower RNA:DNA
196 ratios (Ikeda et al. 2007b).
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198 3.2 ETS-respiration of copepods/mesozooplankton
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200 Previously (Table 2), ETS activities have been determined on selected copepods from
201 0-5,000 m in the western subarctic Pacific (Ikeda et al. 2006a; Ikeda unpublished), on
202 mixed mesozooplankton from 0-3,000 m in the tropical North Pacific (King et al. 1978),
203 and on mixed mesozooplankton from 2,500-4,000 m in the eastern Mediterranean and
204 Arabian Sea (Koppelmann et al. 2004). ETS data (ETS_{assay}) derived from various assay
205 temperatures were corrected to 1.5^{o}C (= near *in situ* temperature at 6000 m of this study,
206 Table 1) by the Arrhenius equation; $ETS = ETS_{assay} \times \exp [E_a(T_{assay}^{-1} - T^{-1})/ R_g]$, where
207 E_a is an activation energy (16 and 13.2 kcal mole^{-1} for epipelagic and bathypelagic
208 zooplankton, respectively, Packard et al. 1975), R_g is the gas constant (1.987×10^{-3} kcal
209 mole^{-1}), T is the absolute temperature (K). Given the temperature range of this study
210 (1.5-10^{o}C), the E_a = 16 or 13.2 kcal mole^{-1} of the Arrhenius equation is equivalent to
211 Q_{10} = 2.82 or 2.35 of van't Hoff rule. Corrected ETS data were converted to respiration
212 rates (R) on the basis of R = 0.5 × ETS (Owens and King 1975; King and Packard
213 1975a). Rs expressed per mg protein were re-expressed per mg WM by multiplying by
214 the appropriate conversion factors (protein contents of 6.71 or 7.19% of WM for the
215 present data, Table 1). For comparing metabolic activities, N or protein is superior to
216 WM as body mass unit (Ikeda 2008), but WM is used in this comparison since little is

known about the conversion factor of WM to N or protein for mixed mesozooplankton from depth. The sensitivity of ETS assay differs due to the use of different components in the homogenate buffer, with or without the detergent (Triton X-100), dissimilar pH and/or substrate concentrations (Christensen and Packard 1979). In this regard, the data of Koppelman et al. (2004) derived from Kenner and Ahmed (1975)'s assay method are comparable to the present data (Christensen and Packard, 1979) but those of King et al. (1978) derived from Packard (1971)'s assay method need to be multiplied by 3.3 (Owens and King 1975). The ETS data for copepods and mixed mesozooplankton from the epipelagic layer from several regions of the world's oceans thus standardized to WM-specific R ($\mu l\ O_2\ (mg\ WM)^{-1}h^{-1}$) at 1.5^{o}C ranged from 0.084 to 0.229 (Table 2).

While selected copepods by Ikeda et al. (2007a) and Ikeda (unpublished) exhibited a wide range of data and were composed of much larger specimens than those of mixed copepods in this study, their mean WM-specific Rs at 1.5^{o}C were similar to the present results if the sampling depth is considered. Mesozooplankton Rs were compared with copepod Rs on the premise that the latter is the major component of the former in ocean interiors (Vinogradov 1970; Yamaguchi et al. 2004). Compared with the present results on 5,000-7,000 m copepods, mesozooplankton data from 2,000-3,000 m in the tropical North Pacific (King et al. 1978) are comparable, but those from 2,750-4,250 m in the eastern Mediterranean and the Arabian Sea (Koppelmann et al. 2004) are less than the present 5,000-7,000 m data by a factor of 0.16-0.52 (Table 2). Consistently lower Rs of Koppelmenn et al. (2004) may be due to the overestimation of mesozooplankton biomass by detritus. Russian workers have been defining "seston" mass (detritus plus organisms) to be the entire net samples, and plankton biomass to be a sum of individual organisms in the sample (Rudyakov and Tseitlin 1992; Kitain et al.

1995). The proportion of detritus in plankton samples has been known to vary with mesh size of net used, study region, season and depth (Rudyakov and Tseitlin 1992; Koppelman 1994). By using the Russian term mentioned above, it is noted that the prime objective of Koppelmann et al. (2004) is to calculate mesozooplankton community respiration by combining WM-specific seston respiration and seston mass, for which separation of organisms and detritus is of little importance. In addition to detritus, possible dissimilar regional physical, chemical and biological conditions of their deep habitats could also affect ETS activities of resident zooplankton, but our knowledge about deep-sea zooplankton metabolism at present is too limited to allow rigorous inter-comparison among the regional data.

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Table 1 Summary of sampling data, biomass (WM and DM) and potein contents of copepods from the western subarctic Pacific. Means ± SD, with the number of replicates in parentheses.

Mid-sampling depth (m)	*In situ* T (^{o}C)	WM (mg indiv^{-1})	DM (mg indiv^{-1})	Protein (% of WM)
100 (control)[a]	3.6	0.394 ± 0.045(4)	0.058 ± 0.006(4)	7.19 ± 1.11(4)
6,000[b]	1.5[c]	0.328 ± 0.074(3)	0.066 ± 0.023(3)	6.71 ± 1.86(3)

[a] 0-200 m

[b] 5,000-7,000 m

[c] substituted by the data at 5,000 m

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Table 2 ETS-respiration (R) data of zooplankton from various depths and regions of the world's oceans. ETS data reported by various units were re-calculated and expressed as WM-specific R at 1.5°C. Values are means or means ± SD. N denotes the number of replicates (gothic) or species/stage/sex structured data (italic). ND = No data. See text for details.

Sampling depth (m)	Region	Zooplankton	Mean body mass (mgWM indiv^{-1})	Assay T (°C)	Original unit	N	R at 1.5°C (μlO_2(mgWM)$^{-1}h^{-1}$)	Reference
euphotic zone	Tropical N Pacific	Mixed mesozooplankton	ND	20	μlO_2(mgDM)$^{-1}h^{-1}$	27	0.229 ± 0.073[b]	King et al. (1978)
0-250	W. subarctic Pacific	Selected calanoid copepods	4.45	10	μlO_2(mg protein)$^{-1}h^{-1}$	*17*	0.105 ± 0.042[a]	Ikeda et al. (2006a)
0-200	W. subarctic Pacific	Mixed copepods	0.394	10	μlO_2(mg protein)$^{-1}h^{-1}$	15	0.088 ± 0.025	This study
2,000-3,000	Tropical N Pacific	Mixed mesozooplankton	ND	20	μlO_2(mgDM)$^{-1}h^{-1}$	1	0.067[b]	King et al. (1978)
2,750	E. Mediterranean	Mixed mesozooplankton	ND	15	μgC(gWM)$^{-1}d^{-1}$	2	0.027	Koppelmann et al. (2004)
4,250	E. Mediterranean	Mixed mesozooplankton	ND	15	μgC(gWM)$^{-1}d^{-1}$	2	0.0084	Koppelmann et al. (2004)
2,500	Arabian Sea	Mixed mesozooplankton	ND	15	μgC(gWM)$^{-1}d^{-1}$	2	0.023	Koppelmann et al. (2004)
4,000	Arabian Sea	Mixed mesozooplankton	ND	15	μgC(gWM)$^{-1}d^{-1}$	2	0.014	Koppelmann et al. (2004)
2,000-3,000	W. subarctic Pacific	Selected calanoid copepods	4.64	10	μlO_2(mg protein)$^{-1}h^{-1}$	*31*	0.062 ± 0.043[a]	Ikeda et al. (2006a)
3,000-5,000	W. subarctic Pacific	Selected calanoid copepods	4.46	10	μlO_2(mg protein)$^{-1}h^{-1}$	*35*	0.062 (0.024-0.158)[c]	Ikeda unpublished
5,000-7,000	W. subarctic Pacific	Mixed copepods	0.328	10	μlO_2(mg protein)$^{-1}h^{-1}$	16	0.052 ± 0.021	This study

[a] Protein = 0.11× WM (Ikeda, unpublished)

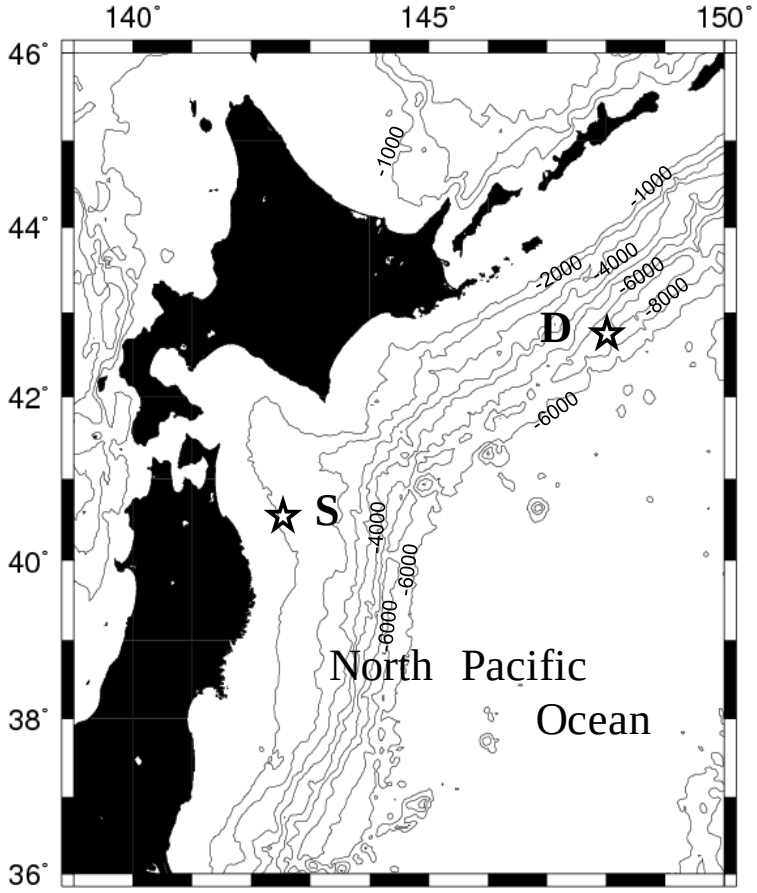
[b] DM = 0.24 × WM (Ikeda et al. 2006b)

[c] normalized by log-transformation (mean ± SD range)

Figure caption

Fig. 1 Sampling stations (star) at where zooplankton was collected from 5,000-7,000 m (D) and 0-200 m depth (S) in the subarctic western North Pacific Ocean. Depth contours (1,000, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000 and 8,000 m) are superimposed.

Fig. 2 Frequency distribution of ETS activities determined at 10^{o}C of mixed copepods from 0-200 m (top) and 5,000-7,000 m (bottom) in the western subarctic Pacific Ocean (histograms). The data fit to theoretical normal distribution curves, as was confirmed the Chi-square tests.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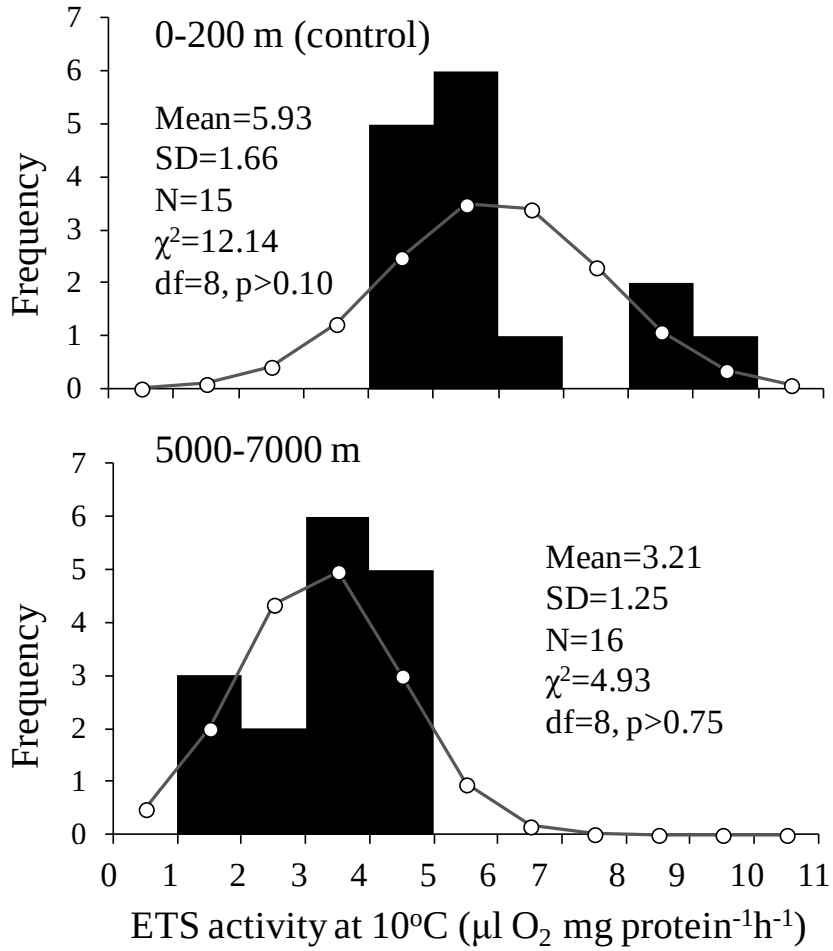
Ikeda Fig. 1


0-200 m (control)
Mean=5.93
SD=1.66
N=15
χ²=12.14
df=8, p>0.10
5000-7000 m
Mean=3.21
SD=1.25
N=16
χ²=4.93
df=8, p>0.75
Frequency
Frequency
ETS activity at 10°C (µl O2 mg protein-1h-1)

Ikeda Fig. 2