Influence of temperature changes on oxygen uptake and ammonia and phosphate excretion, in relation to body size and weight, in *Oikopleura dioica* (Appendicularia)

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Abstract

The relationship between the rates of oxygen consumption, ammonia and phosphate excretion of a pelagic tunicate, the larvacean *Oikopleura dioica* Fol, 1872 were assessed as a function of size, dry weight and ash-free dry weight at 15°, 20° and 24°C. *O. dioica* has higher respiration and excretion rates than copepods of similar weight, but the weight exponent of the allometric power function: $Y = aX^b$ is similar to that of other poikilotherms. Temperatures above 20°C have a depressing effect on respiration and ammonia excretion. 90% of the variance in metabolic rates is explainable by body mass and temperatures Q_{10} values for oxygen consumption, ammonia and phosphate excretion, respectively, are 2.45, 1.86 and 1.75 between 15° and 20°C, and 3.75, 2.90 and 3.60 between 20° and 24°C. Metabolic quotients (O:N, O:P, N:P) indicate a protein-oriented diet. The results of this study suggest weak metabolic regulation in *O. dioica*, an energetic strategy which allows an immediate response to favourable changes in feeding conditions.

Introduction

Appendicularians are filter-feeding tunicates common in all pelagic ecosystems (Fenaux, 1967). They are a link in the marine food chain between nanoplankton and small detritic material (King *et al.*, 1980; Alldredge, 1981) and higher trophic levels such as fish larvae (Shelbourne, 1962; Ryland, 1964). They feed using a gelatinous structure called the "house", which is secreted by a specialised epithelium of the trunk (Lohmann, 1899; Fenaux, 1971). In the family Oikopleuridae, the house generally has a spherical form and the animal occupies its interior (Alldredge, 1976). Larvaceans can become seasonally very abundant

(Seki, 1963; Paffenhöfer, 1976; Taguchi, 1982), but their collection and culture in the laboratory is a delicate process.

Despite the recent increase of publications on the subject, knowledge of the biology of larvaceans is still limited. Fenaux and Hirel (1972) described the expansion of a newly secreted house, and Fenaux (1976) described the early development and hatching of *Oikopleura dioica*, as well as the rhythm of house secretion (Fenaux, 1985). Paffenhöfer (1976) observed their feeding, growth and reproduction in the North Sea; Alldredge (1981) measured grazing on natural food assemblages; Alldredge (1976), Taguchi (1982), and Gorsky *et al.* (1984a) detailed the importance of larvaceans as a source of suspended organic matter. There is no information on appendicularian metabolism, except for preliminary studies on the respiration of *O. dioica* (Ikeda, 1974) and *O. longicauda* (Gorsky *et al.*, 1984b). It is difficult to extrapolate experimental data on oxygen consumption and excretion rates of zooplankton to those obtained in field studies. Furthermore, the metabolic rates of captured zooplankton vary according to experimental protocol. The unknown physiological condition of collected animals introduces some additional errors. It is therefore important to obtain data on unstressed, healthy animals, observed under conditions similar to those encountered in nature. We consider appendicularians which successfully secrete houses and occupy them, when incubated for short periods, to fulfil the conditions mentioned above.

Appendicularians are too fragile to be collected by nets, and are not easily preserved in the laboratory without damage. The first report on their successful culture was made by Paffenhöfer (1973). Recently, Fenaux and Gorsky (1979, 1985) described the methodology of capture and permanent culture in the laboratory. These methods allowed us to study several appendicularian species in excellent physiological condition. The present paper is the first of a series dealing with metabolism, feeding and growth of appendicularians. Its chief purpose is to investi-

gate the effect of body mass and temperature on the metabolism of *Oikopleura dioica*.

Materials and methods

Oxygen consumption, ammonia and phosphate excretion at 15°, 20° and 24°C were determined for various size-classes of the appendicularian *Oikopleura dioica* Fol, 1872 cultured in our laboratory. For measurements of oxygen consumption rate, larvaceans were taken out of culture jars and introduced into beakers containing filtered (30 μm net) seawater. This water contained enough naturally occurring particles to allow the larvaceans to feed during the incubation period ($3.5 \pm 1.5 \times 10^5 \mu m^3 ml^{-1}$ of phytoplankton and organic particles in 1.5 to 25 μm equivalent spherical diameter size-range, counted by a TA II Coulter counter); 250 ml glass bottles were used as incubation vessels. Similar bottles containing 30 μm filtrate of natural seawater, without larvaceans, were used as controls. The number of individuals incubated in the experimental bottles depended on the larvaceans' size-class. During the incubation, bottles were wrapped with aluminium foil to exclude light, and submerged in plastic tanks which served as a constant (± 0.2 C°) temperature bath. The hermetically-closed bottles were gently shaken during incubation (4 to 8 h) to maintain the larvaceans in suspension. Oxygen uptake was measured with a polarographic analyser IL 125 S with a Clark electrode, at the beginning and the end of the incubation period. Experiments where mortality was detected were discarded.

Larvaceans were counted, and divided into two aliquots. The first aliquot was transferred to beakers containing a seawater filtrate (Sartorius cellulose-acetate filter; 0.2 μm pore-size), until the digestive system became empty. The appendicularians were then forced to abandon their houses by drawing them carefully into a pipette with an opening diameter smaller than the diameter of the house. Individuals without a house were measured to the nearest 20 μm, fixed for a few seconds in Bouin's solution, and washed in three successive baths of distilled water. They were then transferred with a tungsten needle to a preashed and preweighed aluminium-foil square and dried at 60°C for more than 72 h. After weighing on a Cahn electronic balance to the nearest microgramme, the appendicularians were ashed at 520°C (Paffenhöfer, 1976) for 4 h. The ash was weighed after being maintained at 80°C for 1 h. Specimens of the second aliquot were fed for 4 h in fresh 30 μm-filtered seawater. They were then forced to abandon their houses, washed three times in 0.2 μm-filtered seawater and gently transferred (when a new house expanded) to 100 ml acid-rinsed incubation bottles filled with 0.2 μm-filtered seawater, to prevent ammonia and phosphate uptake by phytoplankton. Incubation time varied from 2 to 8 h and the sampling frequency varied from 30 min to 1 h for ammonia and from 2 to 3 h for phosphate. Sampling frequency depended on experimental temperature and size-class. Minimum incubation

periods of 30 min for ammonia and of 2 h for phosphate were necessary to obtain sufficient amounts of excretory products for quantitative measurements. Incubation bottles were kept in a dark, temperature-controlled waterbath, and were gently shaken. Ammonia in the air was trapped in 50% H_2SO_4 before entering the incubation bottle. For ammonia and phosphate determination, we used a Technicon II automatic analyser and the methodology described by Slawyk and McIsaac (1972).

During their larval development, larvaceans do not feed. At the end of this stage, the tail shifts toward the mouth (Lohman, 1899; Fenaux, 1977), the appendicularian secretes its first house, and begins to filter. At this stage, trunk length varies from 140 to 180 μm. Our first observation was made on individuals with trunk lengths of 160 $\pm$ 10 μm. Considering that this stage still belongs to the unfed period of appendicularian development, we later decided to exclude the data corresponding to this size-class from the calculation of predicting functions.

The best fit of predicted and measured values of the size-weight relationship of *Oikopleura dioica* was obtained by the equation:

$$(W - W_0) = a TL^b, \tag{1}$$

where weight (W = dry weight or ash-free dry weight, W_0 = ordinate intersect, and a and b are constants) was expressed as a power function of the trunk length (TL).

We employed a nonlinear iterative least-squares method (Glass, 1967, 1969) for fitting the power function:

$$M = a TL^b \tag{2}$$

to metabolic rates (M) versus trunk length (TL) data. Dry weight (DW) and ash-free dry weight (AFDW) in μg were calculated from Eq. (1). The equation:

$$M = a b^T \tag{3}$$

expresses the temperature (T)-dependence of the metabolic rates. The simultaneous influence of body mass and temperature on metabolism was described as follows:

$$M = b_0 b_1^T W^{b_2}, \tag{4}$$

where b_0, b_1 and b_2 are constants.

We tested the significance in regression by the F-test and the homogeneity of slopes by the analysis of covariance (Sokal and Rohlf, 1981).

Results

Size-weight relationship

Dry weight (DW) and ash-free dry weight (AFDW) of *Oikopleura dioica* with empty digestive tracts were obtained for a wide size range (trunk length, TL, 150 to 1200 μm: Table 1). DW and AFDW increased with increasing size of larvaceans, and ranged over two orders of magnitude, from 0.17 to 18 μg DW and from 0.16 to 13.71 AFDW per individual (Fig. 1, Table 1). The parameters
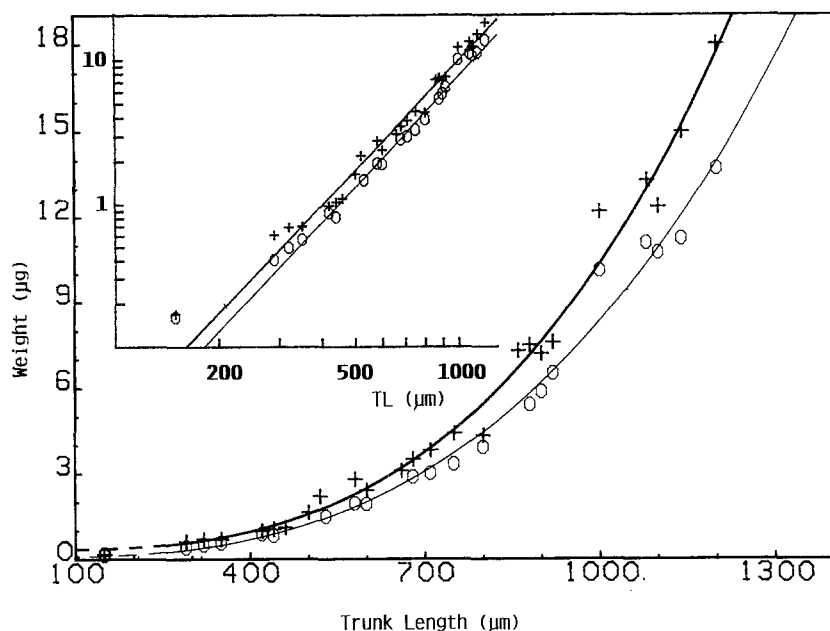
Fig. 1. *Oikopleura dioica.* Nonlinear curve-fitting of equation $Y = Y_0 + aX^b$. +: trunk length (TL) vs dry weight (DW); o: TL vs ash-free dry weight (AFDW). Inset: GM linear regression on log-transformed data

Table 1. *Oikopleura dioica.* Mean dry weight (DW) and ash-free dry weight (AFDW) of twelve size-classes with empty digestive tracts. TL: trunk length; n: no. of individuals per sample; (N): no. of replicates

n	TL $\pm$ SD (μm)	DW $\pm$ SD (μg)	(N)	AFDW $\pm$ SD (μg)	(N)	AFDW/ DW$\times$100
150	150 $\pm$ 20	0.17	(1)	0.16	(1)	94.1
100	190 $\pm$ 20	0.61	(1)	0.41	(1)	67.2
80	340 $\pm$ 60	0.70 $\pm$ 0.02	(2)	0.54 $\pm$ 0.05	(2)	77.1
60	440 $\pm$ 80	1.04 $\pm$ 0.06	(3)	0.85 $\pm$ 0.04	(2)	81.7
40	530 $\pm$ 80	2.21 $\pm$ 0.59	(3)	1.72 $\pm$ 0.33	(2)	77.8
30	650 $\pm$ 100	3.00 $\pm$ 0.55	(3)	2.42 $\pm$ 0.66	(2)	80.7
30	730 $\pm$ 60	4.12 $\pm$ 0.42	(2)	3.17 $\pm$ 0.23	(2)	76.9
30	850 $\pm$ 120	6.38 $\pm$ 1.78	(3)	3.92 $\pm$ 0.92	(2)	61.4
20	910 $\pm$ 40	7.40 $\pm$ 0.28	(2)	4.67 $\pm$ 0.72	(2)	63.3
15	1 040 $\pm$ 120	12.75 $\pm$ 0.78	(2)	10.61 $\pm$ 0.69	(2)	83.2
10	1 120 $\pm$ 80	13.69 $\pm$ 1.85	(2)	11.01 $\pm$ 0.35	(2)	80.4
7	1 200 $\pm$ 60	18.00		13.71	(1)	76.2

of Eq. (1) are compared (Table 2) to the parameters of the equation $W = aTL^b$ in its logarithmic-transformed form: $\log W = \log a + b \log TL$.

Respiration and excretion

Individual oxygen-consumption rates of appendicularians increased both with increasing size and increasing temperature. Oxygen-uptake rates ranged from 0.021 to 0.184 μl O_2 individual^{-1} h^{-1} at 15 °C, from 0.032 to 0.297 μl O_2 individual^{-1} h^{-1} at 20 °C and from 0.039 to 0.348 μl O_2 individual^{-1} h^{-1} at 24 °C (Table 3, Fig. 2).

Time-series measurements indicated that increase of excretion rates with time was linear for all incubation temperatures, and seemed to be a direct function of size (Table 4).

Ammonia excretion ranged from 0.92×10^{-4} to 16×10^{-4} μM N$-$NH$_4$ individual^{-1} h^{-1} at 15 °C, from 1×10^{-4} to 18×10^{-4} μM N$-$NH$_4$ individual^{-1} h^{-1} at 20 °C, and from 1.9×10^{-4} to 29×10^{-4} μM N$-$NH$_4$ individual^{-1} h^{-1} at 24 °C. Phosphate excretion ranged from 0.4×10^{-5} to 7×10^{-5} μM P$-$PO$_4$ individual^{-1} h^{-1} at 15 °C, from 0.33 $\times 10^{-5}$ to 7.5×10^{-5} μM P$-$PO$_4$ individual^{-1} h^{-1} at 20 °C, and from 1.1×10^{-5} to 15×10^{-5} μM P$-$PO$_4$ individual^{-1} h^{-1} at 24 °C. Ammonia and phosphate excretion rates and their weight-specific excretion rates are expressed in micrograms in Table 5.

The nonlinear iterative curve fitting of the power function (Eq. 2) to the data on ammonia and phosphate excretion are shown in Figs. 3 and 4. Relationships between these metabolic rates and the dry weight are described by a GM[1] functional regression (Ricker, 1973) on the log-transformed data (Insets in Figs. 3 and 4). Table 6 compares values for the relationships between metabolic rates and dry weight obtained by nonlinear fitting to untransformed data with those obtained by GM regression on the log-transformed data.

Weight-specific metabolic rates decreased with increasing body mass. Table 7 presents Model II regressions relating weight-specific oxygen uptake and weight-specific ammonia and phosphate excretion to dry weight at 15°, 20° and 24 °C. Slopes for each weight-specific metabolic rate at 15°, 20° and 24 °C, were not significantly different at the 0.05 probability level (Fig. 5), i.e., were not affected by temperature.

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[1] GM regression approach is also called "reduced major axis method". It yields a regression slope which is geometric mean of Model I regression slopes of Y on X and X on Y, effectively minimizing the absolute value of the sum of the products of the deviations of the observations from the line in the X-direction and the Y-direction
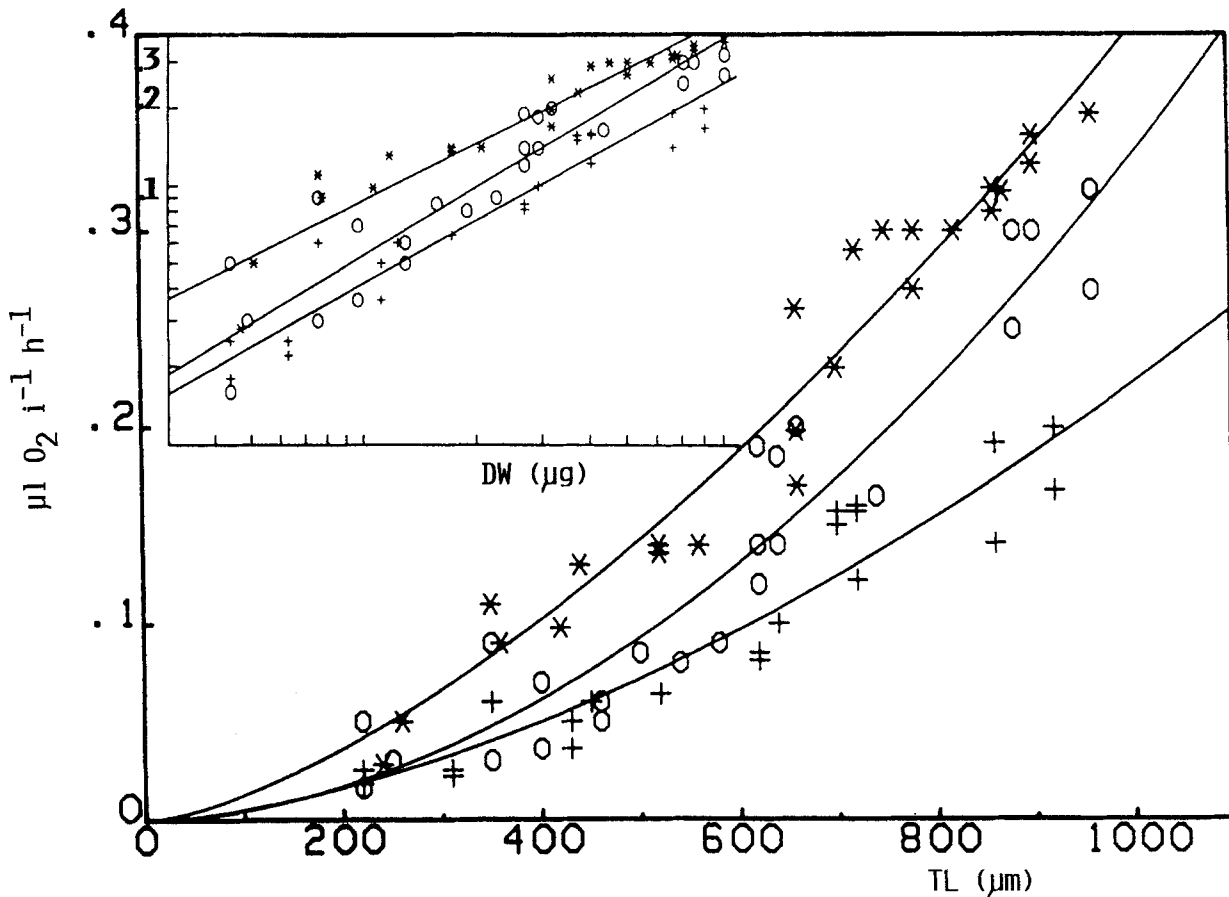
Fig. 2. *Oikopleura dioica.* Relationship between oxygen consumption and trunk length (TL) at 15° (+), 20° (o) and 24°C (*); non-linear curve fitting of equation $Y = a X^b$. Inset: Relationship between oxygen uptake and dry weight (DW) at 15°, 20° and 24°C. i: individual

Table 2. *Oikopleura dioica.* Relationship between body mass (W) and trunk length (TL). Nonlinear curve-fitting: $W = W_0 + a TL^b$; linear regression; $\log W = \log a + b \log TL$. $W =$ ordinate intersect. $F =$ regression significance; $r =$ correlation coefficient; a and $b =$ constants

			$W = W_0 + a TL^b$				
	Y_0	(SE)	a	(SE)	b	(SE)	F
DW	0.3363	(0.322)	1×10^{-8}	1×10^{-8}	3.01	(0.20)	1523.5**
AFDW	0.0798	(0.328)	2×10^{-8}	4×10^{-8}	2.85	(0.23)	1117.9**
			$\log W = \log a + b \log TL$				
	r		$\log a$	(SE)	b	(SE)	F
DW	0.9901		-6.5385	(0.2147)	2.51	(0.076)	1091**
AFDW	0.9949		-6.7360	(0.1718)	2.54	(0.061)	1763**

** Significant $p < 0.001$

Metabolic quotients

Metabolic quotients (O:N, O:P and N:P) are expressed as ratios of the number of oxygen atoms consumed to the number of atoms of nitrogen excreted as ammonia, and to the number of phosphorus atoms excreted as phosphate.

O:N and O:P ratios at three experimental temperatures were negatively correlated with dry weight. The N:P ratio was positively correlated with dry weight at 20° and 24°C, and negatively at 15°C (data calculated from Table 6). The mean O:N ratio of 15 remained constant at 15° and 20°C, but rose to 20 at 24°C. The mean O:P ratio decreased with increasing temperatures from 490 at 15°C to 433 at 24°C. The mean N:P ratio was 20 at 24°C and 32 at 15° and 20°C.

Discussion

The best predictive accuracy of the size-weight relationship in *Oikopleura dioica* was obtained using Eq. (1) and the nonlinear method (Table 2) for fitting this function to our data (Fig. 1).

Table 3. *Oikopleura dioica.* Mean respiration (R) and dry weight-specific respiration (Rw) at 15°, 20° and 24°C. TL: trunk length; DW: dry weight; AFDW: ash-free dry weight; (N): no of replicates; i: individuals.

TL ± SD (μm)	DW (μg)	AFDW (μg)	$R \pm$ SD (μl O$_2$ i^{-1} h^{-1})	$Rw \pm$ SD (μl O$_2$ mg^{-1} h^{-1})	(N)
15°C					
220 ± 40	0.44	0.19	0.021 ± 0.005	48.9 ± 11.1	(2)
320 ± 40	0.66	0.40	0.036 ± 0.021	54.1 ± 31.9	(3)
440 ± 70	1.18	0.88	0.049 ± 0.012	41.3 ± 10.3	(3)
520 ± 50	1.73	1.36	0.064	37.0	(1)
630 ± 60	2.83	2.29	0.089 ± 0.010	31.3 ± 3.5	(3)
710 ± 110	3.91	3.19	0.149 ± 0.016	38.2 ± 4.0	(5)
860 ± 90	6.70	5.45	0.166 ± 0.036	24.9 ± 5.4	(2)
920 ± 80	8.14	6.58	0.184 ± 0.023	22.6 ± 2.8	(2)
20°C					
230 ± 40	0.46	0.21	0.032 ± 0.017	69.6 ± 37.2	(3)
350 ± 60	0.76	0.49	0.060 ± 0.042	79.1 ± 55.7	(2)
430 ± 70	1.13	0.83	0.054 ± 0.014	47.9 ± 12.8	(4)
540 ± 80	1.90	1.51	0.085 ± 0.005	44.7 ± 2.6	(3)
630 ± 100	2.83	2.29	0.162 ± 0.033	57.4 ± 11.7	(6)
740 ± 80	4.38	3.58	0.165	37.7	(1)
880 ± 90	7.16	5.81	0.275 ± 0.035	38.4 ± 4.9	(2)
940 ± 80	8.66	6.99	0.297 ± 0.025	34.3 ± 2.9	(3)
24°C					
250 ± 40	0.49	0.24	0.039 ± 0.016	79.6 ± 31.8	(2)
360 ± 60	0.80	0.53	0.104 ± 0.014	130.0 ± 17.6	(2)
430 ± 40	1.13	0.83	0.114 ± 0.023	100.9 ± 20.0	(2)
530 ± 80	1.18	1.43	0.139 ± 0.002	76.2 ± 1.3	(3)
660 ± 90	3.20	2.60	0.209 ± 0.046	80.5 ± 17.7	(3)
750 ± 110	4.72	3.71	0.278 ± 0.029	58.9 ± 6.3	(5)
850 ± 80	6.70	5.27	0.314 ± 0.010	46.8 ± 1.5	(3)
920 ± 100	8.14	6.58	0.348 ± 0.013	42.7 ± 1.6	(3)

Table 4. *Oikopleura dioica.* Time-series measurements of ammonia and phosphate excretion of different size-classes at 15°, 20° and 24°C. a and b are the constants of the linear regression $Y = aX + b$. r = correlation coefficient. TL: trunk length

TL ± SD (μm)	Ammonia excretion			Phosphate excretion		
	a (10^{-4})	b (10^{-5})	r	a (10^{-5})	b (10^{-6})	r
15°C						
320 ± 40	1.87	4.12	0.9931	0.50	0.05	0.9967
460 ± 60	2.70	4.05	0.9967	0.88	1.30	0.9963
580 ± 60	5.70	− 2.47	0.9972	1.90	− 0.10	0.9963
660 ± 60	6.98	8.10	0.9975	1.80	− 0.22	0.9991
740 ± 100	7.13	14.00	0.9974	2.50	4.80	0.9879
960 ± 80	14.80	7.04	0.9996	5.40	4.40	0.9979
20°C						
260 ± 20	1.37	− 1.18	0.9979	0.57	− 0.35	0.9836
400 ± 40	2.85	− 5.62	0.9961	1.10	− 1.70	0.9919
550 ± 80	7.04	− 2.63	0.9997	1.90	− 2.40	0.9922
700 ± 60	10.30	3.20	0.9986	3.36	− 3.00	0.9949
900 ± 100	16.80	4.27	0.9998	5.80	− 1.40	0.9975
24°C						
320 ± 60	2.14	1.19	0.9996	1.58	1.50	0.9933
460 ± 60				2.20	0.76	0.9981
550 ± 80	6.50	− 5.64	0.9974	2.50	− 1.00	0.9975
660 ± 60	8.75	4.76	0.9991	4.30	−17.00	0.9752
720 ± 80	12.10	24.00	0.9972	4.85	− 4.80	0.9854
850 ± 100	19.60	− 2.19	0.9998	9.60	− 6.60	0.9976
960 ± 120	26.00	11.60	0.9993	12.70	5.80	0.9988

Table 5. *Oikopleura dioica*. Mean ammonia and phosphate excretion rates and dry weight-specific excretion rates of different size-classes at 15°, 20° and 24°C. TL: trunk length; DW: dry weight; AFDW: ash-free dry weight; (*N*): no. of replicates

| TL ± SD (μm)	Weight		Ammonia excretion (± SD)			Phosphate excretion (± SD)		
	DW(μg)	AFDW (μg)	(μg Ni⁻¹ h⁻¹)	(μg N mg⁻¹ h⁻¹)	(*N*)	(μg Pi⁻¹ h⁻¹)	(μg P mg⁻¹ h⁻¹)	(*N*)
15°C								
320 ± 40	0.66	0.40	0.0026 ± 0.0009	3.99 ± 1.38	(7)	0.00015 ± 0.00003	0.23 ± 0.05	(3)
460 ± 60	1.30	0.98	0.0039 ± 0.0005	3.02 ± 0.37	(7)	0.00028 ± 0.00008	0.21 ± 0.06	(3)
580 ± 60	2.28	1.83	0.0077 ± 0.0016	3.38 ± 0.71	(5)	0.00058 ± 0.00015	0.25 ± 0.06	(3)
660 ± 60	3.20	2.60	0.0099 ± 0.0020	3.12 ± 0.61	(5)	0.00057 ± 0.00006	0.18 ± 0.02	(3)
740 ± 100	4.38	3.58	0.0102 ± 0.0020	2.33 ± 0.45	(5)	0.00093 ± 0.00028	0.21 ± 0.06	(3)
960 ± 80	9.20	7.42	0.0207 ± 0.0018	2.25 ± 0.16	(6)	0.00167 ± 0.00037	0.18 ± 0.04	(4)
20°C								
260 ± 20	0.51	0.26	0.0019 ± 0.0004	3.71 ± 0.80	(4)	0.00019 ± 0.00009	0.36 ± 0.17	(3)
400 ± 40	0.97	0.69	0.0036 ± 0.0011	3.75 ± 1.12	(5)	0.00034 ± 0.00011	0.35 ± 0.12	(3)
550 ± 80	1.99	1.58	0.0098 ± 0.0005	4.94 ± 0.27	(4)	0.00059 ± 0.00017	0.29 ± 0.08	(3)
700 ± 60	3.76	3.07	0.0146 ± 0.0018	3.87 ± 0.48	(5)	0.00105 ± 0.00024	0.28 ± 0.07	(3)
900 ± 100	7.63	6.19	0.0237 ± 0.0017	3.10 ± 0.22	(5)	0.00180 ± 0.00040	0.24 ± 0.05	(4)
24°C								
320 ± 60	0.66	0.40	0.0030 ± 0.0003	4.56 ± 0.51	(7)	0.00046 ± 0.00017	0.69 ± 0.25	(3)
460 ± 60	1.30	0.98				0.00066 ± 0.00011	0.51 ± 0.09	(3)
550 ± 80	1.99	1.58	0.0087 ± 0.0018	4.36 ± 0.91	(4)	0.00078 ± 0.00013	0.39 ± 0.07	(3)
660 ± 60	3.20	2.60	0.0125 ± 0.0015	3.92 ± 0.48	(6)	0.00120 ± 0.00056	0.34 ± 0.17	(4)
720 ± 80	4.06	3.32	0.0171 ± 0.0033	4.21 ± 0.83	(6)	0.00150 ± 0.00064	0.37 ± 0.16	(2)
850 ± 100	6.48	5.27	0.0276 ± 0.0015	4.26 ± 0.23	(3)	0.00300 ± 0.00067	0.47 ± 0.10	(4)
960 ± 120	9.20	7.42	0.0364 ± 0.0032	3.96 ± 0.35	(3)	0.00390 ± 0.00048	0.43 ± 0.05	(4)
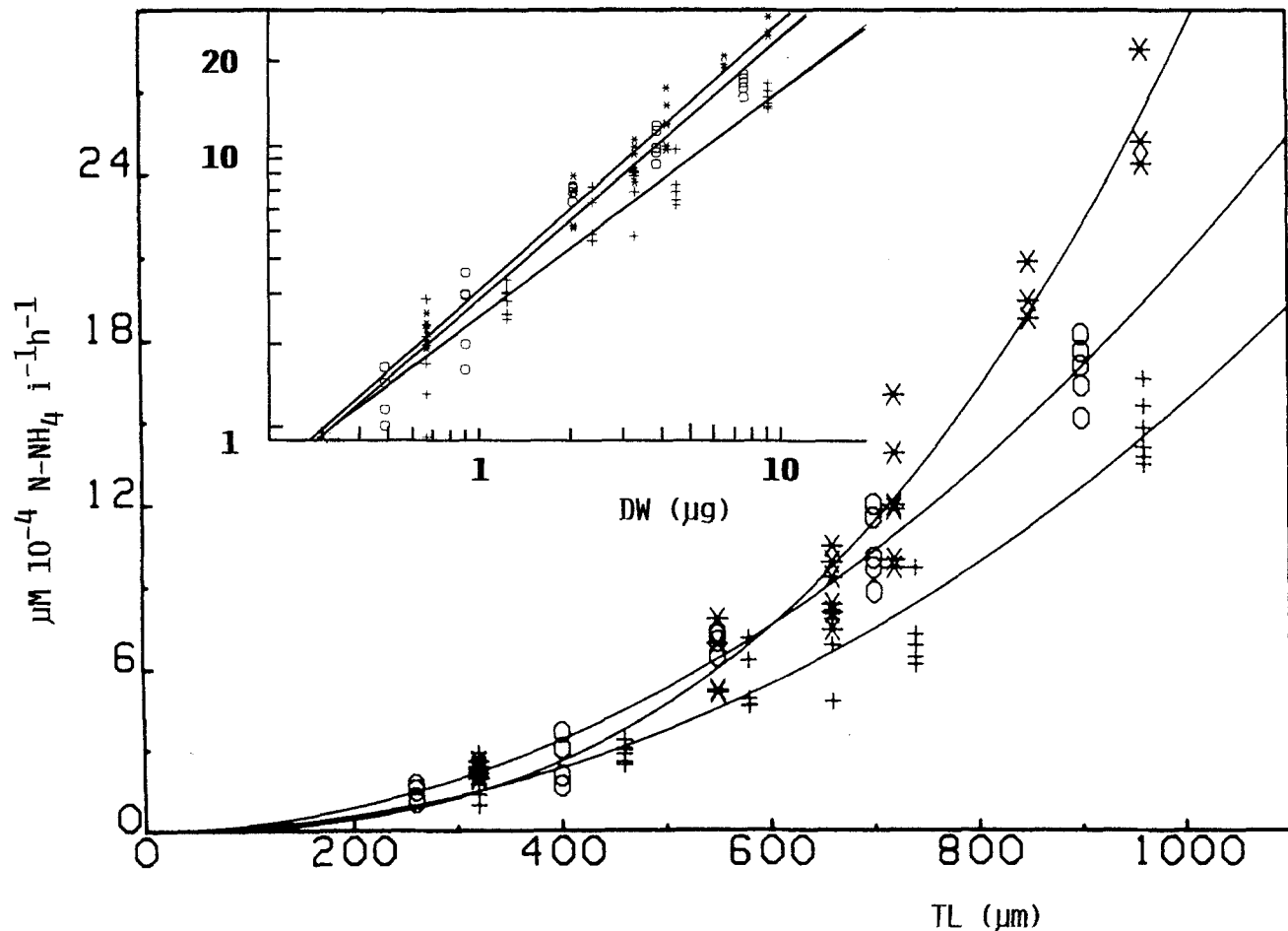
Fig. 3. *Oikopleura dioica*. Relationship between ammonia excretion and trunk length (TL) at 15° (+), 20° (o) and 24°C (*); nonlinear curve fitting of equation $Y = a X^b$. Inset: Relationship between ammonia excretion and dry weight (DW) at 15°, 20° and 24°C

Table 6. *Oikopleura dioica.* Oxygen uptake and ammonia and phosphate excretion as a function of body mass (DW, AFDW) and size (TL). *a* and *b* obtained by nonlinear curve-fitting and by GM linear regression are given for 15°, 20° and 24°C. *m*: multiplier of *a*; F = variance ratio; R^2 = coefficient of determination. (*N*): no. of measurements

Metabolic rates	*m*	$Y = aX^b$			$\log Y = \log a + b \log X$				(*N*)
		a	*b*	*F*	$\log a$	*b*	R^2	*F*	
15°C									
Oxygen uptake									
DW	10^0	0.05 ± 0.01	0.52 ± 0.07	145**	− 01.38	0.81	0.916	207	(21)
AFDW	10^0	0.06 ± 0.01	0.60 ± 0.06	162**	− 01.24	0.69	0.922	224	(21)
TL	10^{-6}	2.90 ± 3.30	1.63 ± 0.17	166**	− 05.86	1.74	0.914	202	(21)
Ammonia excretion									
DW	10^{-5}	26.0 ± 2.00	0.79 ± 0.04	525**	− 03.62	0.82	0.912	342	(35)
AFDW	10^{-5}	32.0 ± 2.20	0.76 ± 0.04	515**	− 03.50	0.76	0.908	326	(35)
TL	10^{-10}	08.4 ± 6.50	2.10 ± 0.10	505**	− 08.90	2.03	0.904	311	(35)
Phosphate excretion									
DW	10^{-6}	07.1 ± 1.50	0.91 ± 0.11	126**	− 05.16	0.93	0.923	204	(19)
AFDW	10^{-6}	08.8 ± 1.60	0.90 ± 0.10	124**	− 05.03	0.85	0.918	190	(19)
TL	10^{-12}	02.0 ± 4.10	2.49 ± 0.30	123**	− 11.04	2.26	0.923	204	(19)
20°C									
Oxygen uptake									
DW	10^0	0.06 ± 0.01	0.71 ± 0.01	224**	− 01.27	0.88	0.863	138	(24)
AFDW	10^0	0.08 ± 0.01	0.67 ± 0.05	239**	− 01.13	0.74	0.856	130	(24)
TL	10^{-7}	9.21 ± 9.00	1.86 ± 0.15	230**	− 06.16	1.90	0.843	118	(24)
Ammonia excretion									
DW	10^{-5}	43.0 ± 5.60	0.78 ± 0.04	671**	− 03.53	1.01	0.880	154	(23)
AFDW	10^{-5}	45.0 ± 2.80	0.73 ± 0.39	710**	− 03.69	1.24	0.837	107	(23)
TL	10^{-9}	02.2 ± 1.50	1.99 ± 0.10	766**	− 09.21	2.19	0.953	426	(23)
Phosphate excretion									
DW	10^{-6}	11.0 ± 2.20	0.82 ± 0.11	122**	− 05.00	0.91	0.821	64	(16)
AFDW	10^{-6}	01.0 ± 0.10	0.79 ± 0.10	121**	− 04.85	0.78	0.906	135	(16)
TL	10^{-11}	02.5 ± 5.00	2.16 ± 0.29	119**	− 09.07	1.54	0.854	82	(16)
24°C									
Oxygen uptake									
DW	10^0	0.10 ± 0.01	0.60 ± 0.04	370**	− 01.06	0.73	0.910	224	(24)
AFDW	10^0	0.12 ± 0.01	0.56 ± 0.39	382**	− 00.96	0.67	0.920	253	(24)
TL	10^{-6}	13.0 ± 8.50	1.50 ± 0.12	446**	− 05.35	1.66	0.947	393	(24)
Ammonia excretion									
DW	10^{-5}	27.0 ± 2.80	0.99 ± 0.05	550**	− 03.54	0.99	0.974	1 011	(29)
AFDW	10^{-5}	40.0 ± 3.00	0.95 ± 0.05	701**	− 03.36	0.85	0.968	816	(29)
TL	10^{-11}	03.4 ± 3.02	2.65 ± 0.59	678**	− 09.33	2.25	0.964	723	(29)
Phosphate excretion									
DW	10^{-6}	14.0 ± 2.90	1.02 ± 0.11	178**	− 04.82	0.93	0.839	109	(23)
AFDW	10^{-6}	17.0 ± 3.30	1.00 ± 0.10	172**	− 04.68	0.86	0.815	92	(23)
TL	10^{-13}	06.0 ± 0.10	2.80 ± 0.30	169**	− 10.78	2.29	0.803	85	(23)

** Significant at $p < 0.01$

Paffenhöfer (1976) described the TL-AFDW relationships of *Oikopleura dioica* at two different food concentrations: (i) from 1.878 to $3.514 \times 10^5 \, \mu m^3 \, ml^{-1}$, and (ii) from 1.050 to $1.224 \times 10^5 \, \mu m^3 \, ml^{-1}$. The parameters *a* and *b* of the log-transformed Model I linear regression are: − 6.222 and 2.491 for the higher food concentration and − 6.659 and 2.598 for the lower one. We found the parameters *a* = − 6.736 ± 0.17 and *b* = 2.54 ± 0.06 for the TL-AFDW relationship for *O. dioica* with empty digestive tracts. Slopes were not significantly different, but intercept values decreased with decreasing food concentration. Thus, weight-specific metabolism can be affected by feeding conditions. To avoid this source of error, all of our

results were calculated for *O. dioica* with empty digestive tracts.

The only previous data on oxygen consumption of larvaceans were reported by Ikeda (1974), who measured respiration in two size-classes (10.9 and 41.9 μg DW) of *Oikopleura dioica* in Oshoro Bay, Japan, at approximately 15°C. *O. dioica* weighing more than 40 μg DW were not found in the Ligurian Sea, because maximum size corresponds to the spawning male size during the bloom period, and the slightest perturbation (e.g. contact with a plankton net) triggers spawning, after which the larvacean dies (Fenaux and Palazzoli, 1983). Ikeda's first size-class values were about three times lower than our results for
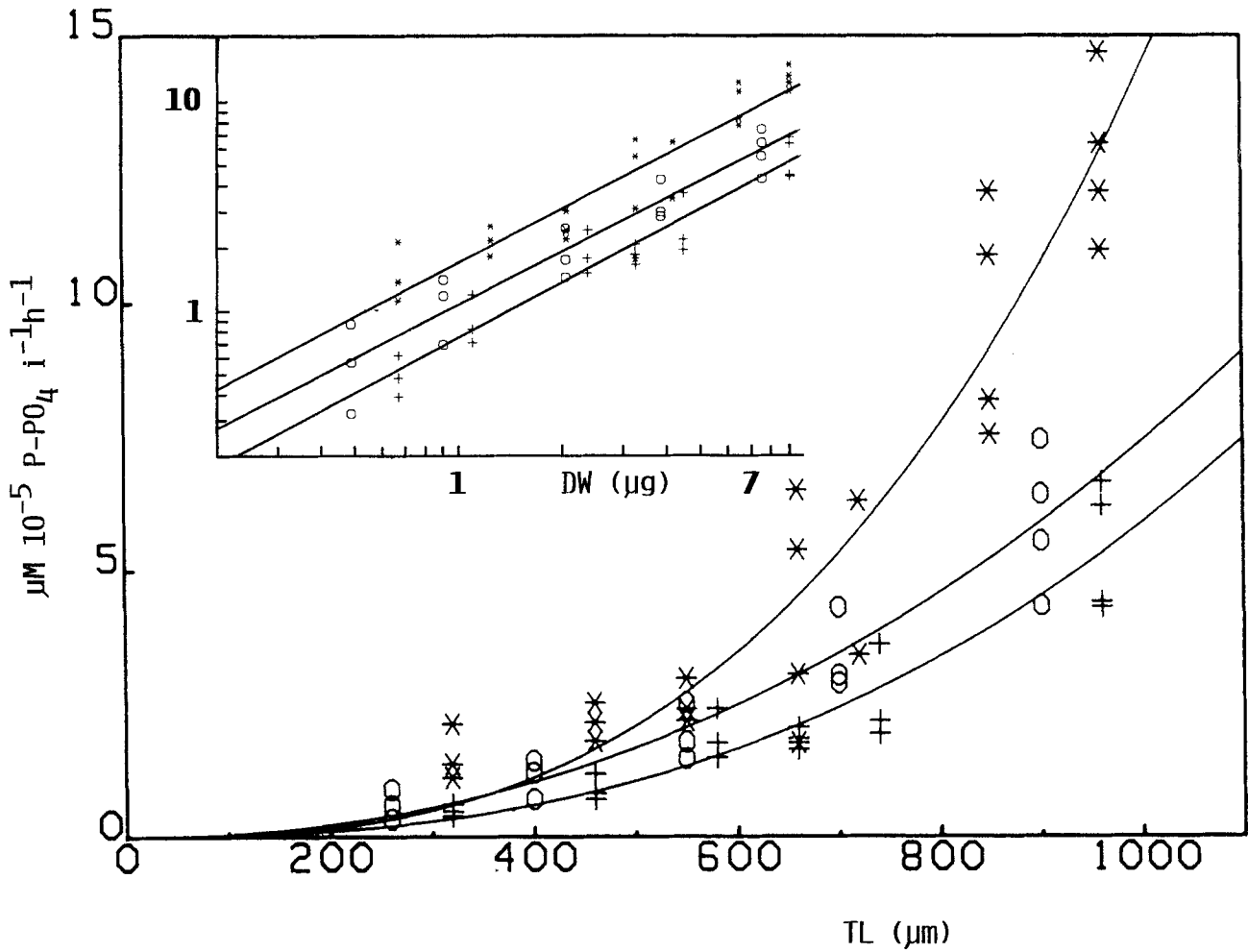
Fig. 4. *Oikopleura dioica.* Relationship between phosphate excretion and trunk length (TL) at 15° (+), 20° (o) and 24 °C (*); nonlinear curve fitting of equation $Y = a\,X^b$. Inset: Relationship between phosphate excretion and dry weight at 15°, 20° and 24 °C

Table 7. *Oikopleura dioica.* GM linear regression of weight-specific metabolic rates on dry weight at 15°, 20° and 24 °C; $\log Y = \log a + b \log X$, where a and b are constants. CI = confidence interval of b; r = correlation coefficient; (N): no of observations

Metabolic rate	$\log a$	b	CI	r	(N)
15 °C					
Oxygen uptake	1.8195	− 0.3234	− 0.2301 − 0.4544	− 0.6918	(21)
Nitrogen release	0.5832	− 0.3167	− 0.2398 − 0.4181	− 0.6105	(35)
Phosphorus release	− 0.5818	− 0.2784	− 0.1863 − 0.4396	− 0.3820	(19)
20 °C					
Oxygen uptake	1.9634	− 0.3739	− 0.2570 − 0.5439	− 0.4977	(24)
Nitrogen release	0.1370	− 0.3549	− 0.2295 − 0.5487	− 0.1340	(23)
Phosphorus release	− 0.4385	− 0.3085	− 0.1883 − 0.5052	− 0.4438	(16)
24 °C					
Oxygen uptake	2.1418	− 0.3750	− 0.2912 − 0.4829	− 0.8161	(24)
Nitrogen release	0.7195	− 0.2805	− 0.1920 − 0.4097	− 0.1852	(29)
Phosphorus release	− 0.1630	− 0.4008	− 0.6051 − 0.6051	− 0.3580	(23)

the same size-class. Taking into consideration that the lar-vacean digestive systems from Oshora Bay were not empty, we recalculated Ikeda's results converted to AFDW (DW×0.767; see below) using the TL-AFDW relation-ship proposed by Paffenhöfer (1976). This resulted in our data being only twice as high as Ikeda's. Such results are difficult to compare, because one must consider not only the several different protocols used by different investi-gators (e.g. in extraction of salt for dry weight measure-ments, in the degree of gut fullness and in the physiologi-cal conditions of the experiment animals), but also the scarcity of data and the different geographical locations of populations.

Oxygen uptake of *Oikopleura longicauda* at 20 °C was reported by Gorsky *et al.* (1984b). The intercept (a) and the weight exponent (b) of the relationship between re-spiration and body weight, expressed by the log-trans-formed GM (Ricker, 1973) linear regression are: $a = -1.56$ and $b = 0.968$. Parameters of weight-specific oxygen uptake as a function of dry weight of *O. longicauda* are: $a = 2.36$ and $b = -0.5965$. Whereas the weight-exponent (b) values of the respiration rates of *O. longicauda* and *O. dioica* at 20 °C are not significantly different from each other, the intercepts are. This indicates that although the metabolic rates are different, the body-size dependence of the metabolic rates are similar.

Curl (1962) found that AFDW accounts for 23% of the DW in the pelagic tunicate *Salpa fusiformis*. Madin *et al.* (1981) obtained AFDW values of 30% in *Pegea confede-rata*, 31% in *Salpa maxima* (aggregated forms) and 22% in *S. cylindrica* (solitary form). In our study, AFDW ex-pressed as a percentage of DW in *Oikopleura dioica* (with-out its house) was 76.7 ± 9%, which is much higher than that of tunicates but similar to that of crustaceans as reported by Curl.

We found the oxygen consumption of *Oikopleura dioica* to be 2.5 times higher than that reported for *Cala-nus pacificus* by Vidal (1980) and 3 times higher than the respiration rate of *Acartia clausi* measured by Nival *et al.* (1974). Our methodology was similar to that used by the above authors, i.e., appendicularians were feeding during the incubation period. In comparison to starved copepods, the oxygen consumption rate of *O. dioica* is 2.5 times higher than the respiration rate of *Temora stylifera* (Conover, 1960), and 3 to 8.5 times higher than of *A. clausi* (Vacelet, 1961); 6.6 times higher than of *Coricaeus typicus* and 10 times higher than of *Euterpina acutifrons* in winter (Gaudy, 1970); about 7 times higher than of *A. clausi* (Ikeda, 1974). All these results were obtained at 15 °C. (For additional results on oxygen uptake of zooplankton see Ikeda, 1974 and Ivleva, 1980). High metabolic activity during starvation suggests a weak metabolic regulation, which may be related to the specific ecological strategy of appendicularians, e.g. short generation time and semel-parity.

The values of the weight exponent (b) of the log-transformed linear regressions (Table 6) expressing the re-lationship between respiration and dry weight of *Oiko-*

pleura dioica did not differ significantly at the three ex-perimental temperatures from the coefficient recorded for poikilotherms by Hemmingsen (1960). Weight exponents calculated by the nonlinear iterative method displayed slightly lower values. In *O. dioica*, the metabolic rate in-creased with increasing temperature and weight-specific oxygen uptake decreased with increasing body weight (Fig. 5). As expected, smaller size-classes displayed higher respiration rates per unit biomass than larger ones.

Ammonia is the main nitrogenous excretory product of aquatic invertebrates. Numerous studies deal with ex-cretion rates of zooplankton, but data on appendicularian excretion rates are lacking. Ammonia excretion recorded for *Oikopleura dioica* at 15 °C in the present study is about four times higher than that reported by Ikeda (1974) and Nival *et al.* (1974) for the copepod *Acartia clausi*, and about ten times higher than reported for *Oncaea venusta* at 20 °C by Gaudy and Boucher (1983). Duration of star-vation did not alter the rates of ammonia and phosphate excretion by *O. dioica*, which increased with increasing
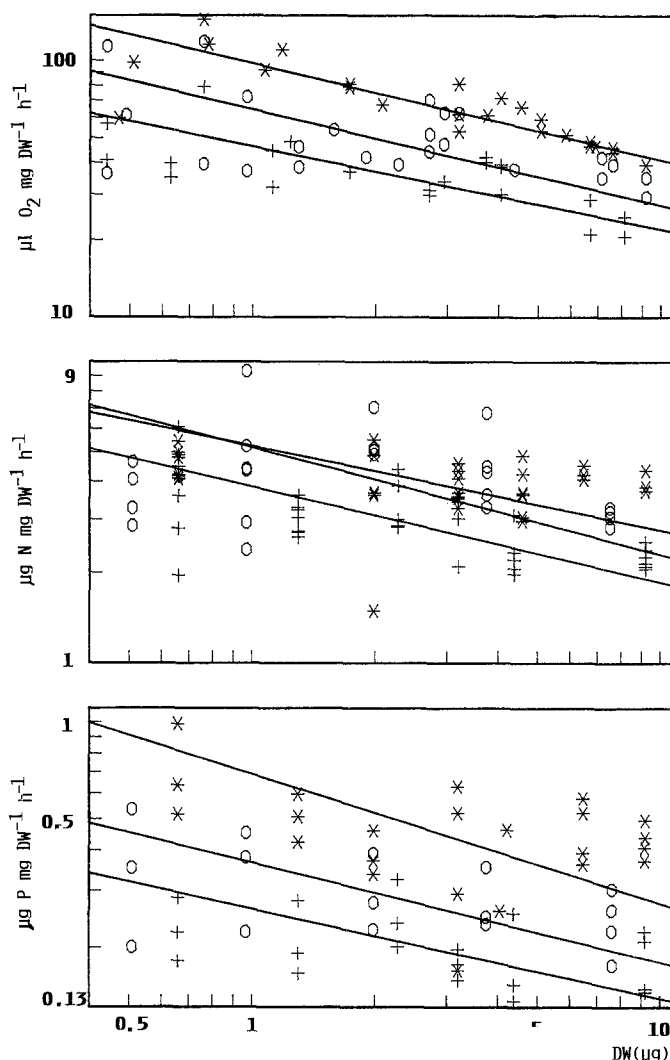

Fig. 5. *Oikopleura dioica.* Weight-specific metabolic rates related to body dry weight at 15° (+), 20° (o) and 24 °C (*). GM linear regressions of log-transformed data

Table 8. *Oikopleura dioica.* Influence of body mass and temperature on metabolic rates. a, b, b_1 and b_2 are constants; M: metabolic rates; T: temperature; W: dry weight. r = correlation coefficient; R^2 = coefficient of determination; t = Student's t-test for b_1 and b_2. Eqs. (3) and (4) are defined in "Materials and methods")

| | $M = a\,b^T$ (Eq. 3) | | | |
	log a ($\pm$ SD)	log b ($\pm$ SD)	r	F(dd1)
Oxygen uptake	1.1184 ± 0.0979	0.0289 ± 0.0048	0.5892	35.6 (1 67)**
Ammonia excretion	0.2536 ± 0.0700	0.0153 ± 0.0035	0.4234	18.6 (1 85)**
Phosphate excretion	-1.2857 ± 0.0943	0.0384 ± 0.0046	0.7416	68.4 (1 56)**

| | $M = b_0\,b_1^T\,W^{b_2}$ (Eq. 4) | | | | | | | |
	b_0	b_1	b_2	R^2	F	(dd1)	$t\,(b_1)$	$t\,(b_2)$
Oxygen uptake	-1.7630	0.0258	0.7963	0.8890	168	(2 44)**	3.588**	18.04**
Ammonia excretion	-4.0421	0.0274	0.8675	0.8902	223	(2 57)**	4.141**	21.05**
Phosphate excretion	-5.5889	0.0299	0.8779	0.9154	173	(2 34)**	3.887**	18.54**

** Significant at $p < 0.001$

body mass and temperature (Table 4). The weight-specific excretion rates as a function of dry weight displayed inverse trends, but the intercepts were positively correlated to temperature (Fig. 5, Table 7).

Temperatures above 20 °C seem to have an inhibitory effect on oxygen consumption and ammonia excretion of *Oikopleura dioica* (Tables 3 and 5). Nival *et al.* (1974) observed the same phenomenon for a number of species of Mediterranean copepods. High Q_{10} values between 20° and 24 °C (Table 8) are associated in *O. dioica* with a weak thermal tolerance. In our cultures a temperature of 27 °C was the upper limit for survival of *O. dioica* (unpublished data). Metabolic rate is exponentially related to temperature. If we assume a constant Q_{10} between 15° and 20 °C, we can describe the simultaneous influence of body mass and temperature on the metabolic rates by the multiple regression equation (Eq. 4), with which 89% of the variance in oxygen consumption, 89% in ammonia release and 92% in phosphate release can be explained by dry weight and temperature (Table 8). Ammonia release was more depressed at high temperatures than oxygen consumption, a result reflected in high O:N and low N:P ratios at 24 °C. Le Borgne (1973) proposed a mean O:N ratio of 13.48 for mixed zooplankton. This value is close to our results for *O. dioica* at 15° and 20 °C, and suggests a protein-oriented metabolism.

High O:P and N:P ratios indicate the presence of carbohydrates (Ikeda, 1977) in the appendicularian diet. *Oikopleura dioica* seems to catabolise proportionally more protein in relation to carbohydrates as weight increases (Ikeda, 1977).

According to Biggs (1977), most gelatinous herbivores, and especially small forms such as the doliolids, have high rates of respiration and excretion. Torres and Childress (1983) found high "active" metabolism in agile zooplankters. Appendicularian filtering and house-secreting activity begins at the end of larval development with the secretion of the first house, and continues without interruption until shortly before spawning. This continuous activity, which goes on in the absence of food (Fenaux,

1985) until exhaustion and death, is in contradiction to the hypothesis that appendicularians discard the house when this ceases to collect particles efficiently (Lohmann, 1909; Alldredge, 1976).

In conclusion, oxygen consumption, and ammonia and phosphate excretion all depend on temperature as expressed by Eq. (3) (Table 8), and on body mass, and fit the allometric equation $M = a\,W^b$. The weight exponent (b) in Eq. (3) is temperature-independent and close to the weight exponent for poikilotherms reported by Hemmingsen (1960).

According to Vidal (1980) and Miller and Landry (1984), the metabolism of *Calanus pacificus* can be ingestion-independent for a "relatively short period". This energetic strategy allows for an immediate response to favourable changes in feeding conditions. Moreover, the short exposure time to predators due to extremely rapid generation time, also observed for other tunicates such as *Thalia democratica* (Heron, 1972), may be an efficient method for the rapid colonisation of the environment.

Thus, in favourable conditions appendicularians might contribute significantly to the rapid recycling of nitrogen and so to the maintenance of phytoplankton populations in nitrogen-limited environments. Having regard to the many differences in the biology and in the nutritional strategies of the various members of the zooplankton, we agree with Calder (1985) that "a physiological or metabolic time scale is a concept needed in marine ecology".

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