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Chironomids (Diptera) and oxy-regulatory capacity: An experimental approach to paleolimnological interpretation

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Abstract

We measured the ability to regulate oxygen uptake of 16 chironomid taxa from lakes in low-arctic West Greenland by means of oxygen microelectrodes in custom-made respiration chambers. The respiration patterns were modeled using piecewise linear regression with break-point and simple hyperbolic functions. The mathematical constants obtained from the controlled laboratory experiments were good ecophysiological indicators of species-specific ''oxy-regulatory capacity.'' The oxy-regulatory capacity of different chironomid communities was calculated for subfossil assemblages collected from 52 lakes in West Greenland. The overall assemblage structure was expressed using detrended correspondence analysis (DCA). The oxy-regulatory capacity was as strongly correlated to DCA axis 1 ($r = 0.72$–0.86, $p < 0.001$), as were surface water temperature ($r = 0.82$, $p < 0.001$) and nutrients ($r = 0.47$–0.86, $p < 0.001$). Warm-water chironomid assemblages characterized by taxa such as *Chironomus, Dicrotendipes, Ablabesmyia,* and *Procladius* had a high oxy-regulatory capacity. Cold-water assemblages were dominated by oxy-conformers such as *Heterotrissocladius, Micropsectra, Hydrobaenus,* and *Diamesa.* An expression of the oxy-regulatory capacity of a given chironomid assemblage can be directly inferred from a simple model using weighted averaging of the ecophysiological mathematical constants. The autecological information from controlled experiments provides important additional information for interpretations in chironomid paleolimnology. The results can also be used to identify secondary changes or mismatches in multiproxy down-core paleoclimate studies.

The immature stages of most chironomids (nonbiting midges) develop in freshwater, and the species composition closely reflects the freshwater environment in which they live (Lindegaard 1995). Because their larval head capsules preserve well as subfossils in lake sediments, nonbiting midges are also excellent paleoindicators of past environmental conditions, and quantitative inference models have been developed for morphometric (depth), physical (temperature and climate), chemical (salinity, total phosphorus, and oxygen), and biological (chlorophyll and macrophytes) variables (Walker 2001). These quantitative transfer functions are all based on the ''surface-sediment calibration'' or ''training set'' approach (Birks 1995), where assemblages of subfossil biota from surface sediments are correlated to the corresponding contemporary environmental variables from a wide range of lakes. The effects of these environmental variables, however, are often difficult to separate using the traditional training set methods because of strong intercorrelations among variables (Brodersen and Anderson 2002).

Subfossil chironomids have been recognized among the best late-glacial temperature/climate proxies (e.g., Walker et al. 1991). In mature systems, however, changes in water temperature caused by altered climate conditions can cause cascading effects on many lake ecosystem and catchment processes (Schindler 2001). Oxygen availability in particular is one of those variables that has a direct effect on the distribution of bottom-living chironomid larvae (e.g., Jónasson 1984; Heinis and Davids 1993). The oxygen conditions in the hypolimnion, however, are determined by a series of factors including lake morphometry, depth, temperature, ice cover, stratification, lake productivity, dissolved organic carbon, respiration, and mixing between layers. The character of the paleolimnological signal reflected by biological proxies, such as chironomids, might therefore not be a direct (primary) response to a single parameter but a response to complex cascading ecosystem dynamics (Battarbee 2000).

Models have been developed to quantitatively infer past hypolimnetic oxygen conditions from subfossil chironomid assemblages (Quinlan et al. 1998; Little and Smol 2001; Quinlan and Smol 2001). These models are based on the training set approach using either modeled or measured oxygen values. Although these models have a fairly good predictability, some limitations have been mentioned (Clerk et al. 2000), and because of the multiple limnological interrelations mentioned above, it is difficult to partial out the effects of other factors from oxygen availability.

The purpose of the present study was to overcome this problem by measuring species-specific oxy-regulatory capacity directly under controlled laboratory experiments. By measuring respiration rates at decreasing levels of oxygen saturation, it is possible to describe the respiration strategy and the ability of species to regulate and maintain sufficient oxygen uptake at low oxygen conditions (Berg et al. 1962; Jones 1972). The hypothesis was that taxa with optima in warm and nutrient-rich lakes also have a high oxy-regulatory capacity—that is, the ability to maintain a high and sufficient respiration rate at low-oxygen conditions (oxy-regulators), whereas taxa with optima in cold and nutrient-poor lakes

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Table 1. Study lakes used for sampling of chironomid larvae.

Lake	Latitude, N (°)	Longitude, W (°)	Area (ha)	Max depth (m)	Temp. (°C)	Conductivity (μS cm^{-1})
Glacier lake 1	67.13521	50.12833	40	22	4–7	2
Tasersuatsiaq (Lake Ferguson)	66.97898	50.69489	750	>80	11.8*	55
Aajuitsup Tasia	67.09253	50.30568	1,350	>32	6.5†	72
SS-2 (Lake Jean)	66.99667	50.97000	37	11	15.3‡	293
Sugar Loaf Pond North	67.04782	50.52082	6	—	—	423
Skaelverdam	67.00037	50.80578	0.5	8	—	2,552
Store Saltsø	66.99000	50.59833	31	11	14.0‡	2,798

* Surface water temperature recorded on 17 July 2002.
† Surface water temperature recorded on 9 July 2002.
‡ Mean July data from Brodersen and Anderson (2002).

cannot regulate (oxy-conformers). By running the experiments on chironomid taxa from an existing surface-sediment calibration set, it was possible to independently explore the relationship between the measured oxy-regulatory capacity and the assemblage structure of subfossil chironomids.

Study sites

The lakes in the Kangerlussuaq area of West Greenland have a mean July surface water temperature of 12.9°C (range, 7.3–16.5°C) and are frozen from mid-September to mid-June (Brodersen and Anderson 2002). A number of oligosaline lakes (conductivity, >1000 μS cm^{-1}) are found near the head of Søndre Strømfjord. The primary cause of the enhanced salinity in these closed-basin lakes is a negative precipitation–evaporation balance (Anderson et al. 2001). Most lakes stratify during the summer, and many of the more saline lakes are meromictic, with dense mats of purple sulfur bacteria on the lake bottom (Anderson et al. 2000; Brodersen and Anderson 2000). The biological structure in the lakes is relatively simple; only two fish species are present—Arctic char (*Salvelinus alpinus* L.) and three-spined stickleback (*Gasterosteus aculeatus* L.)—and many lakes are fishless.

Living chironomid larvae were collected from seven different lakes and ponds near Kangerlussuaq (Table 1). In 2002, the oligosaline Store Saltsø was strongly stratified; by 3 July, it had a thermocline, chemocline, and oxycline, with 17% oxygen at 11 m. Lake SS-2 also stratified and had 62% oxygen in the bottom water on 7 July. The two very large and oligotrophic lakes (Tasaersuatsiaq and Aajuitsup Tasia) never developed oxygen stratification during the field period. Some cold-water taxa were collected from the upper surf zone (0.5 m. depth) of Glacier Lake 1 close to the inland ice. Some warm-water and littoral taxa were collected from two shallow ponds (Sugar Loaf Pond North and Skaelverdam). The latter three sites were not accessed by boat; therefore, stratification data do not exist.

Materials and methods

From 30 June to 14 July, samples were obtained using Ekman- and Kajak bottom samplers from 1-29-m water depth and subsequently sieved through 200-500-μm mesh sizes. The sieved samples were stored in 12-liter buckets with in situ water and immediately transferred to the laboratory. Chironomid larvae and oligochaetes were sorted and identified under a dissection microscope, transferred to dishes for emptying gut contents, and acclimated to experimental temperature for 24 h. Depending on body size, one to five individuals were transferred via pipette to respiration chambers varying in size from 0.2 to 0.5 ml. Respiration chambers were constructed from glass tubes (Ø = 4 mm) fitted with butyl rubber stoppers in both ends. One stopper was fitted with an oxygen microelectrode (membrane opening <5 μm, tip diameter 500 μm; OX500, Unisense; Revsbech 1989) and another with a 27-gauge syringe functioning as an overpressure vent during closure of the chamber. In addition, the chamber was fitted with a magnet (Ø = 1.5 mm), to ensure sufficient stirring after movements of the animals had ceased. The chambers were flushed with sterile oxygen-saturated in situ water initially filtered through a 0.45-μm membrane filter (Millipore). Five to eight replicates, including a control chamber, were run simultaneously at 10°C (a few series were run at 15°C, to evaluate temperature aspects), and the signal from the multichannel pA meter (PA8000; Unisense) was logged using PicoLog (Picotech) on a laptop computer every 60 s by means of an ADC-16 A/D converter (Picotech). After each experiment, the volume (±0.01 ml) of the individual respiration chambers was determined by extracting the water with a syringe, which was subsequently weighed on a precision scale and converted to volume at 10°C under the assumption of a constant atmospheric pressure of 101.3 kPa. This procedure was found to be necessary; the volume of each respiration chamber varied from experiment to experiment because the flexible rubber stoppers could not be fitted in exactly the same way. The experiments were run for 6–18 h, depending on how quickly the chironomids depleted the oxygen present in the respiration chamber; alternatively, the experiments were stopped when the animals were unable to extract more oxygen from the water. Finally, the ash-free dry weights of the larvae were determined after drying at 60°C for 2 h and combustion at 550°C for 1 h.

Modeling the respiration curves—The original data were smoothed using a moving average over five points. The rate of oxygen consumption was calculated over 25 oxygen sat-

uration levels centrally fixed around nine data points ($t_1 - t_2 = 8$ min).

To express the oxy-regulatory capacity of individual taxa by a single or few constants or coefficients, we fitted a number of simple models to the relationship between the weight-specific respiration rate (R_w) and the oxygen saturation (Fig. 2D below). The typical oxy-conformer is not able to regulate (i.e., sustain a sufficient oxygen uptake with decreasing oxygen availability), and the respiration curve can be described as a straight line (linear regression [LR]) with intercept ≤ 0:

$$y = a + bx \tag{1}$$

Assuming that some species are able to regulate their oxygen uptake down to a certain critical level (Berg et al. 1962), one regression line can be fitted for values less than or equal to this level and another for values greater than this level. We used piecewise LR (PWLR) with a break point to optimally and objectively define this point using the iterative Quasi-Newton method. The break point was defined as the critical level (critical pressure; P_c), and the initial decrease (I) was calculated as the percentage reduction in respiration rate from the start of the experiment (mean 70–90%) to the break point (Fig. 2D below):

$$y_1 = a_1 + b_1x; \qquad y < \text{critical level } (P_c) \tag{2.1}$$

$$y_2 = a_2 + b_2x; \qquad y > \text{critical level } (P_c) \tag{2.2}$$

The ratio (''angle'') between the two slopes (b_1/b_2), (P_c), and (I) all express the degree of oxy-regulatory capacity in different terms.

The perfect oxy-regulator will fit a hyperbolic function asymptotically, approaching the maximum respiration rate as shown for the Phantom midge *Chaoborus* by Jäger and Walz (2002). In that study, the respiration curve was described as a hyperbolic function (J-W):

$$y = a + \frac{b}{x} \tag{3}$$

A hyperbolic function like Michaelis-Menten kinetics (M-M) or a similar function (Prosser and Brown 1961; Mangum and van Winkle 1973) will fit a smoothed curve somewhat in between the piecewise regression and the hyperbolic function:

$$y = y_{\max}\frac{x}{x + K_{\mathrm{M}}} \tag{4}$$

Physiologically, the two hyperbolic equations should assume a maximum respiration rate close to $x = 100\%$ and K_M is equal to oxygen saturation (x %), at which the respiration rate is half of its maximal value ($y_{\max}$). However, measures >90% oxygen saturation were discarded because of serious flicker in the beginning of the experiments resulting in unreliable $y_{\max}$ and K_M values. The respiration rate at 25% oxygen saturation ($R_{25\%}$; expressed as a percentage of maximum R_w at 90%) was interpolated from the resulting equations.

The results of the respiration experiments were merged with chironomid assemblage structures from 52 West Greenland lakes presented in Brodersen and Anderson (2002). The percentage of chironomid species composition is given, and, for each lake (assemblage; k), we calculated the weighted average (U) of the modeled respiratory constants. The weighted average of, for example, critical limits (U_{Pc}) for site k was calculated as

$$U_{Pck} = \sum_{i=1}^{n} y_{ik}P_{ci} \Big/ \sum_{i=1}^{n} y_{ik} \tag{5}$$

where y_{ik} is the percentage abundance of taxon i in sample k; ($i = 1, \ldots n$ chironomid taxa); P_{ci} is the value of the coefficient of interest (critical limit) for taxon i; ($k = 1, \ldots m = 52$ assemblages). The weighted averages (U_{Pc}, $U_{b1/b2}$, $U_{\mathrm{R25\%}}$) were correlated to the overall variation in chironomid assemblage structure extracted from detrended correspondence analysis (DCA; Birks 1995).

Notes on chironomid taxonomy—The taxonomic status of the Greenlandic chironomids is fairly poorly known. Therefore, in many cases, it was possible to identify larvae only to genus level. Additional species identification was made on pupal exuviae collected from the same sites during the experimental period and during summer 2000 (Brodersen et al. 2001). The experimental taxa are listed in Table 2. Two species of *Procladius* occurred in the area, but only *P. paragretis* was found in Store Saltsø and Lake SS-2. Larvae of the *Heterotrissocladius marcidus* group possessed a brownish black submentum, but the pupal exuviae did not fit the description of the European *H. marcidus* (Walker). Larvae of *Chironomus hyperboreus* were without ventral tubuli and, therefore, easily separated from the *C. riparius* type. In Store Saltsø all pupal exuviae of *Tanytarsus* belonged to *T. gracilentus.* Consequently also the larvae were considered to be exclusively *T. gracilentus.*

The careful handling and transfer of living *Micropsectra* spp. to respiration chambers did not allow separation into species groups before experiments. When merging and comparing physiological data to subfossil surface data sets (Eq. 5; Brodersen and Anderson 2002; Quinlan and Smol 2001), taxonomy was harmonized to genus levels (as used on subfossils), and average values were generated.

Results

PWLR (Eq. 2) and the M-M hyperbolic function (Eq. 4) could model all experiments with a median explained variance of 97% and 96%, respectively (Fig. 1). The J-W hyperbolic function (Eq. 3) performed poorly, with a median r^2 of 0.48, and only in three cases (*Tanytarsus gracilentus* and *C. hyperboreus*) did it capture >70% of variation (Fig. 1).

In Fig. 2, six examples of respiration curves are shown. The left panel (Fig. 2A–C) represents true oxy-conformers performing an almost linear relationship between respiration rate and oxygen tension. The critical limits (P_c) are high (>35%), and the $b_1 : b_2$ ratios are low (<2). The right panel (Fig. 2D–F) represents true oxy-regulators, with low P_c values (<20%) and high $b_1 : b_2$ ratios (>3). Five groups of respiration patterns were defined (Fig. 2A–E) using clustering of Euclidean distances among constants (Table 2). There was a significant difference among groups (Kruskal-Wallis one-

Table 2. Species-specific respiratory constants. Con. (oxygen consumption, μg O_2 mg^{-1} h^{-1}); P_e (critical limit, %); slope ratio in piecewise linear regression (b_1/b_2); initial decrease (l, %); respiration at 25% saturation ($R_{25\%}$, %); rank number according to $R_{25\%}$; and respiration group (A–E).

Taxon	Site	Con.	P_c (% sat)	PWLR b_1/b_2	l (%)	$R_{25\%}$ (%)	Rank	Group
Experimental temperature = 10°C								
Ablabesmyla pulchripennis (Lundbeck 1898)	Skealverdam	3.59	30.9	1.8	49.6	43.8	19	C
Procladius paragretis Roback 1971	Store Saltsø	2.61	32.4	1.9	46.8	43.5	20	C
Procladius paragretis	Lake SS2	2.43	31.2	1.3	52.0	37.5	25	C
Diamesa spp.	Glacier Lake 1	6.08	26.2	1.8	49.0	41.9	22	D
Diamesa spp.	Glacier Lake 1	9.23	43.0	0.9	51.5	29.1	33	B
Heterotrissocladius cf. *oliveri* Saether 1975	Tasersuatsiaq	3.15	23.6	3.1	45.9	53.1	11	D
Heterotrissocladius cf. *oliveri*	Tasersuatsiaq	2.32	31.2	2.5	45.1	46.2	17	C
Heterotrissocladius marcidus group	Tasersuatsiaq	4.19	65.3	0.9	44.5	36.8	26	A
Heterotrissocladius marcidus group	Tasersuatsiaq	4.31	43.8	0.6	58.3	27.8	39	B
Hydrobaenus fusistylus (Goetghebuer 1933)	Glacier Lake 1	6.02	38.4	1.2	49.3	33.2	29	B
Hydrobaenus fusistylus	Glacier Lake 1	5.60	35.0	1.3	47.3	33.0	30	B
Psectrocladius sp.	Tasersuatsiaq	5.94	34.4	2.1	44.0	43.2	21	C
Psectrocladius barbimanus Edwards 1929	Skaelverdam	8.17	28.7	1.2	55.7	31.0	31	B
Psectrocladius barbimanus	Skaelverdam	8.70	36.6	1.0	54.6	27.8	36	B
Psectrocladius sordidellus/limbatellus group	Aajuitsup Tasia	6.72	52.0	0.8	53.1	27.8	35	A
Psectrocladius sordidellus/limbatellus group	Aajuitsup Tasia	6.40	44.8	0.5	62.3	27.8	37	B
Chironomus hyperboreus Staeger 1845	Store Saltsø	1.96	23.2	4.5	34.7	66.4	6	E
Chironomus hyperboreus	Lake SS2	1.47	28.3	3.8	40.4	61.3	9	E
Chironomus hyperboreus	Tasersuatsiaq	1.39	37.5	3.2	37.2	69.9	1	E
Chironomus hyperboreus	Tasersuatsiaq	1.48	15.6	18.0	28.3	69.5	2	E
Chironomus hyperboreus	Tasersuatsiaq	1.87	28.0	3.5	38.0	63.0	8	E
Chironomus cf. *riparius* Meigen 1804	Lake SS2	2.27	19.8	3.8	42.7	67.1	5	E
Chironomus cf. *riparius*	Lake SS2	2.60	22.3	3.0	45.9	64.5	7	E
Dircrotendipes cf. *modestus* (Say 1823)	Store Saltsø	2.02	17.9	7.2	34.3	67.7	4	E
Dircrotendipes cf. *modestus*	Store Saltsø	2.52	26.3	2.6	44.1	49.0	14	D
Paracladopelma sp.	Tasersuatsiaq	6.92	23.2	3.2	45.9	47.9	15	D
Paracladopelma sp.	Tasersuatsiaq	4.53	35.4	1.4	49.7	40.2	23	C
Micropsectra sp.	Tasersuatsiaq	1.98	64.5	0.7	49.1	35.3	27	A
Micropsectra sp.	Tasersuatsiaq	2.25	55.0	0.6	50.3	27.8	38	A
Tanytarsus sp.	Tasersuatsiaq	3.38	33.6	1.1	50.5	28.8	34	B
Tanytarsus gracilentus (Holmgren 1883)	Store Saltsø	8.48	15.1	9.4	37.3	68.4	3	E
Tanytarsus gracilentus	Store Saltsø	6.92	18.3	6.4	35.6	57.4	10	E
Experimental temperature = 15°C								
Hydrobaenus fusistylus	Glacier Lake 1	8.03	42.3	1.3	44.4	34.3	28	B
Hydrobaenus fusistylus	Glacier Lake 1	6.21	37.1	1.1	53.9	30.2	32	B
Chironomus hyperboreus	Lake SS2	2.77	19.4	3.5	45.4	49.1	13	D
Chironomus hyperboreus	Lake SS2	2.56	21.6	2.6	48.2	43.8	18	D
Chironomus cf. *riparius*	Lake SS2	3.46	21.2	2.7	47.7	49.6	12	D
Chironomus cf. *riparius*	Lake SS2	4.68	25.0	2.2	47.9	47.7	16	D
Dircrotendipes cf. *modestus*	Suger Loaf Pond N.	6.38	31.3	1.2	54.3	38.0	24	C
Oligochaetes at experimental temperature = 10°C								
Lumbriculus variegatus (Müller 1774)	Lake SS2	1.35	44.2	2.1	39.6	72.7	B	E
Uncinais uncinata (Ørstedt 1842)	Tasersuatsiaq	3.58	98.1	0.6	44.5	78.0	A	E
	Min (10°C)	1.39	15.1	0.5	28.3	27.8	1	
	Mean (10°C)	4.30	33.2	3.0	46.0	45.9	20	
	Max (10°C)	9.23	65.3	18.0	62.3	69.9	39	
	Min (15°C)	2.56	19.4	1.1	44.4	30.2	12	
	Max (15°C)	8.03	42.3	3.5	54.3	49.6	32	

way-ANOVA; $p < 0.03$), and the overall differences are shown in Fig. 3. Group A–B taxa were oxy-conformers with high P_c values, low $b_1 : b_2$ ratios, and markedly reduced respiration rates at 25% oxygen saturation ($R_{25\%} < 37\%$). Taxa in this group were *Micropsectra* sp., *H. marcidus*, *Hydrobaenus fusistylus*, *Tanytarsus* sp., *Psectrocladius sordidellus/limbatellus* group, *P. barbimanus*, and small *Diamesa* spp. Group C taxa had intermediate oxy-regulatory capacity with $R_{25\%} > 37\%$, a $b_1 : b_2$ ratio between 1.2 and 2.5, and P_c values between 30% and 36%. Taxa in this group were *Abla-*

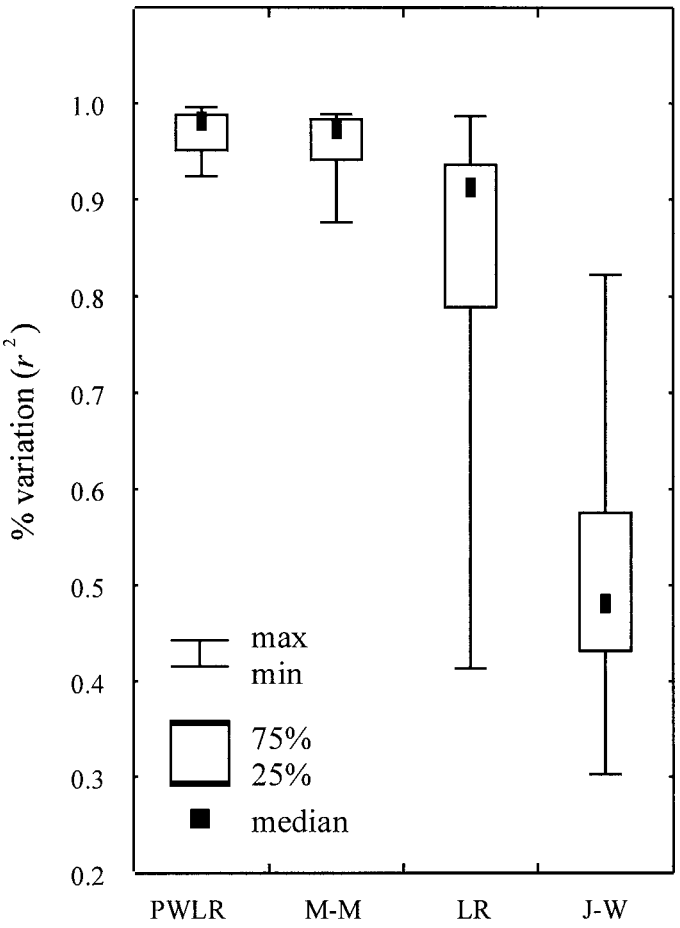

Fig. 1. Coefficients of determination (r^2) after fitting respiration curves to PWLR, M-M, LR, and the hyperbolic J-W function (Jäger and Walz 2002); $n = 32$ experiments (10°C).

besmyia pulchripennis, Procladius paragretis, Psectrocladius sp., *Paracladopelma* sp., and *Heterotrissocladius* cf. *oliveri*. Group E taxa were strong oxy-regulators such as *C. hyperboreus, Chironomus riparius* type, *T. gracilentus*, and the oligochaetes (*Lumbriculus variegatus* and *Uncinais uncinata*). The mean P_c for the group E chironomids was 22.6%, the mean $R_{25\%}$ was 65.5%, and the $b_1 : b_2$ ratios were >3.0 (mean = 6.3). The oligochaetes had the highest $R_{25\%}$ values for all experiments (>72%). The taxa belonging to group D had constants between group C and E values. These include experiments on *Chironomus* spp. run at 15°C (Table 2), *Dicrotendipes, Paracladopelma, Heterotrissocladius oliveri* sp., and *Diamesa* spp. The M-M hyperbolic fitted respiration curves are summarized in Fig. 4. The rank numbers on the curves correspond to numbers in Table 2.

The respiration rates at 70–90% saturation increased at the 15°C experimental temperature. Respiration rates for *C. hyperboreus* and *C. riparius* type increased by 63% and 67% respectively, which indicated a mean Q_{10} (e.g., Jones 1972) of 2.7 and 2.8, respectively. Respiration rates for *H. fusistylus* increased 23% ($Q_{10} = 1.5$). *Dicrotendipes* showed a substantial increase of 181% ($Q_{10} = 7.9$). The increased respiration rates were primarily found at high oxygen tensions. The critical limits were not changed markedly, but the $b_1 : b_2$ ratios were decreased for all taxa, (i.e., toward a more oxy-conform curve shape). *Chironomus* spp. fitted group D at 15°C and group E at 10°C, whereas *Hydrobaenus* did not change group (B) with increased temperature. *Dicrotendipes* appeared in group D–E at 10°C and in group C at 15°C.

The chironomid assemblages presented in Brodersen and Anderson (2002) were analyzed by DCA, and the results are summarized in Table 3. The weighted average of the respiratory constants for the 52 assemblages were highly correlated to the DCA axis 1 sample scores (Fig. 5A–C), which indicates a strong relationship between the experimentally measured oxy-regulatory capacity and the chironomid assemblage structure in the West Greenland lakes. As was shown by Brodersen and Anderson (2002), the chironomid communities were also strongly correlated with mean July surface water temperatures and with the trophic variables total nitrogen (TN) and total phosphorus (TP; Fig. 5D–F). Chironomid assemblages with low oxy-regulatory capacity (high U_{Pc}, low $U_{b1/b2}$, and low $U_{R25\%}$) had low DCA axis 1 scores and occurred in cold and oligotrophic lakes, whereas assemblages with good oxy-regulatory capacity (low U_{Pc}, high $U_{b1/b2}$, and high $U_{R25\%}$) occurred in warm and meso- and eutrophic lakes (Fig. 5).

Discussion

Aside from oxygen availability, the rate of oxygen consumption is influenced by a number of factors, such as activity, temperature, body size, nutrition, season, previous oxygen experience, and life-cycle stage (Prosser and Brown 1961; Nagell 1973). Therefore, respiration experiments should be conducted that are as close to natural conditions as possible. However, because of the natural variation in all these variables, this is difficult to accomplish; hence, controlled laboratory experiments where these factors are kept constant are preferred, to facilitate comparison from species to species. Thus, the methods chosen in these kinds of studies should be dependent on the facilities available and the overall aim of the study (Lampert 1984). The aim of the present study was to produce respiration curves and numeric constants with high interspecific comparability. In statistical terms, precision (curve shapes with high confidence) was weighted higher than accuracy (the actual metabolism in terms of energy turnover). The respiration rates recorded in the closed microchambers with physical movement of the animals and constant magnetic stirring cannot be considered to be ''basal'' metabolic rates. The relatively high R_w values in this study are reflecting a metabolism between standard and forced activity, and, as was mentioned by Prosser and Brown (1961), the metabolic levels recorded are meaningful only for the particular conditions of measurements. For further discussion and thorough reviews on respiration physiology and experimental methods, we refer to Prosser and Brown (1961), Jones (1972), and Lampert (1984).

The curves and constants obtained from PWLR and the M-M hyperbolic function describe the experimental data well and with consistency within taxa. It is difficult to develop a mathematical model to describe the degree of oxy-conformity and oxy-regulation strictly on the basis of bio-

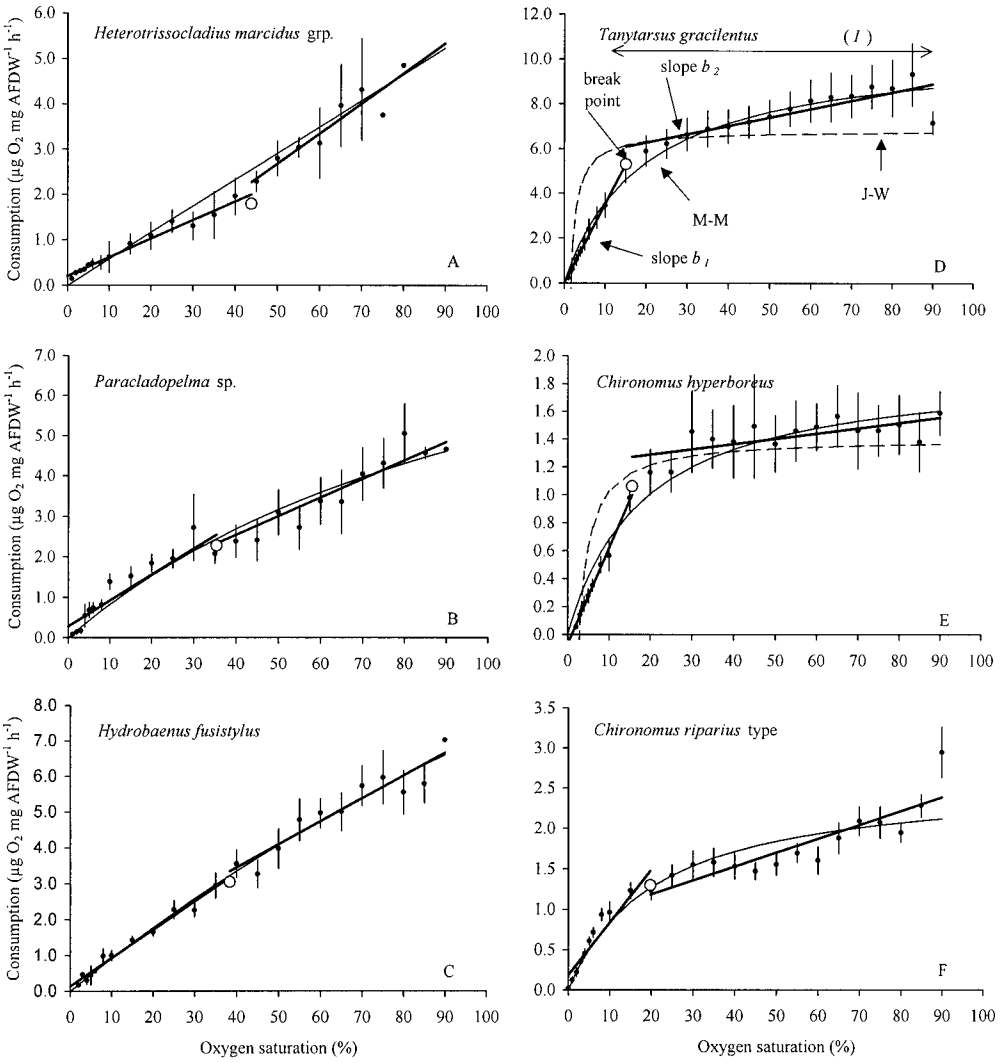

Fig. 2. Examples of respiration curves for six different chironomid taxa. (A–C) Typical oxy-conformers. (D–F) Typical oxy-regulators. Data points with SEs are shown with piecewise linear regressions (thick lines) and break points (P_c, open circles), M-M hyperbolic functions (thin lines), and J-W hyperbolic functions (stippled lines), where possible. *I* is the initial decrease. See text for further explanation.

logical reasoning (Mangum and van Winkle 1973). The empirical models presented here, however, succeeded in classifying the taxa into significantly different respiratory groups, and the attained constants convincingly matched the distributional patterns in the West Greenland chironomid assemblages. The results might be improved if the taxonomic resolution in merged subfossil and experimental data are further harmonized. For example, *Psectrocladius* is commonly identified only to the genus level in subfossil data but is divided into two (three) groups in the experimental data. *Micropsectra* type *insignilobus* and *M.* type *radialis* showed slightly different habitat preference in Greenland lakes (Brodersen and Anderson 2002), but the two types could not be separated during the experiments. However, both temperature and trophic optima were not significantly different for the two *Micropsectra* types (Brodersen and Anderson 2002), which also indicates that a respiratory resemblance is likely.

The ecophysiological importance of bottom-water oxygen for the distribution of chironomids has been recognized for many years (Lindegaard 1995), and our results are in clear correspondence with the findings of earlier experimental studies (e.g., Konstantinov 1971; Hamburger et al. 1998) and observations on species distributional patterns (e.g., Heinis and Davids 1993; Johnson 1995; Pinder 1995). Species that are commonly recognized as cold-adapted and living in oligotrophic lakes (e.g., *H. marcidus, Micropsectra, Hydrobaenus,* and *Diamesa;* Sæther 1979), and members of the littoral warm-water genus *Psectrocladius* were identified as oxy-conformers. *Heterotrissocladius* cf. *oliveri, Procladius,* and *Paracladopelma* were moderate oxy-regulators, and the most significant oxy-regulators were the warm-water and eutrophic-adapted *Dicrotendipes, Chironomus,* and *T. gracilentus.*

Critical limits (P_c) ranging 15–65% oxygen saturation (Ta-

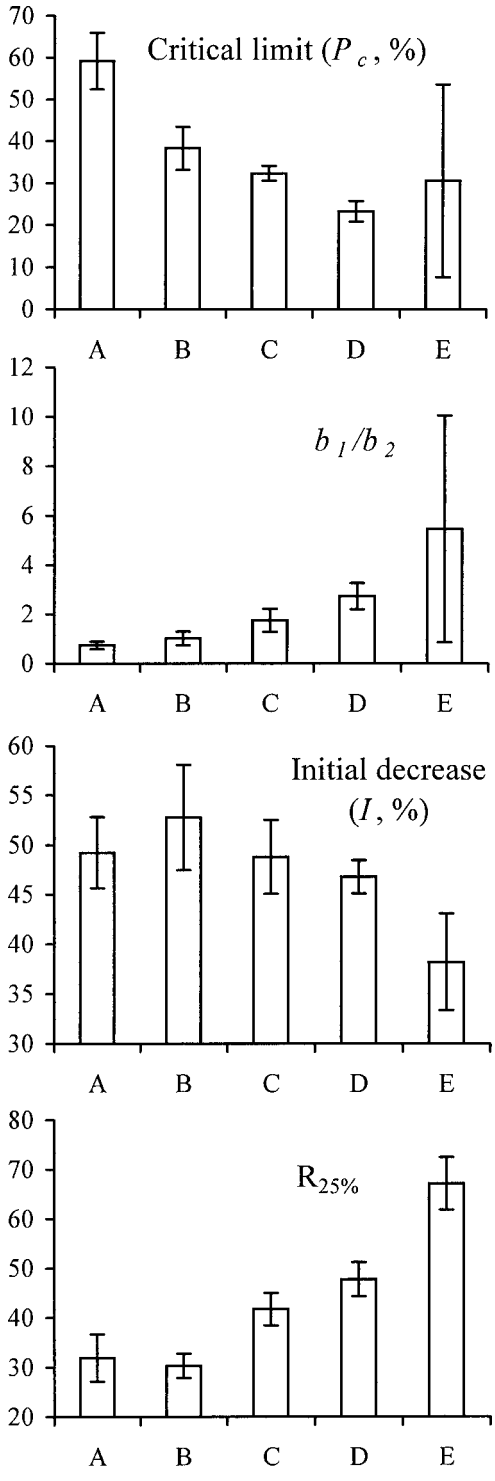

Fig. 3. Summary of modeled constants expressing oxy-regulatory capacity for the five defined respiratory groups, A–E. Values are means ±1 SD for the critical limit (P_c; %), the ratio of the slopes in piecewise regression ($b_1:b_2$), the initial decrease (I), and $R_{25\%}$. See text and Table 2 for explanations.

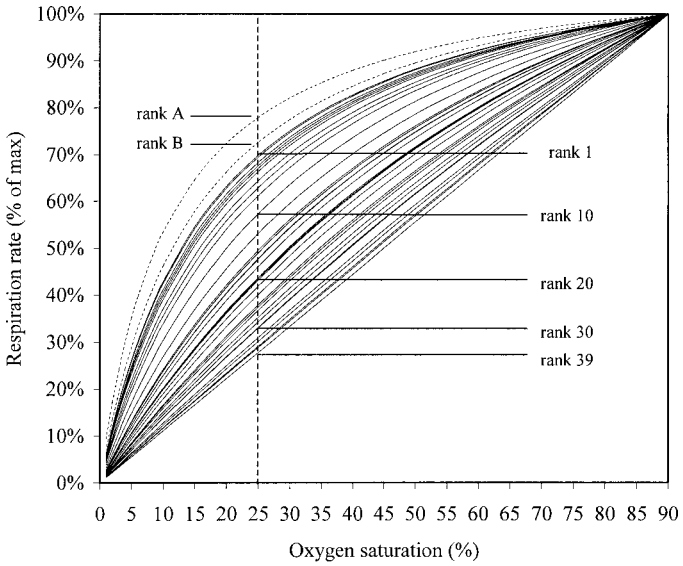

Fig. 4. M-M hyperbolic fitted respiration curves for 39 chironomid experiments (10°C and 15°C) and two oligocheate experiments. Rank numbers, vertically with corresponding taxa, are listed in Table 2.

ble 2) support the hypothesis that respiratory adaptation has an important ecological significance. Adaptations that allow some chironomid species to spend a proportion of their life-cycles under hypoxic or anoxic conditions can be of behavioral, physiological, or morphological character (Eriksen et al. 1996). Exposure to microxic or anoxic conditions induces migration, reduced metabolic rate (inactivity), and ventilation movements. Some chironomid larvae from the subfamily Chironominae (e.g., *Chironomus* and *Tanytarsus gracilentus*) are able to synthesize the high-affinity respiratory pigment hemoglobin, which enhances the transport and storage of oxygen. In the present study, we also found the highly pigmented red larvae to be the best oxy-regulators. *T. gracilentus* is an indicator of strongly stratified and meromictic West Greenland lakes (Brodersen and Anderson 2002). These lakes are practically anoxic every year and support mats of purple sulfur bacteria on the hypolimnetic sediment surfaces (Anderson et al. 2000). The adaptive and/or competitive advantages of *T. gracilentus* in these lakes do not, however, have to be related to oxygen regimes. Consumer-resource interactions has recently been shown to be important as well (Einarsson et al. 2002). The two pigmented *Chi-*

Table 3. Results of DCA analysis of subfossil chironomid assemblages from lakes in West Greenland. Percentage data were ln-transformed before analysis. Data are from Brodersen and Anderson (2002; n = 42 lakes) plus additional lakes (n = 10) and contain 28 active taxa.

	Axis			
Measurement	1	2	3	4
Eigenvalues	0.35	0.12	0.08	0.05
Length of gradient	2.93	2.04	1.36	1.36
Variance of species data (%)	22.1	7.7	4.8	3.3

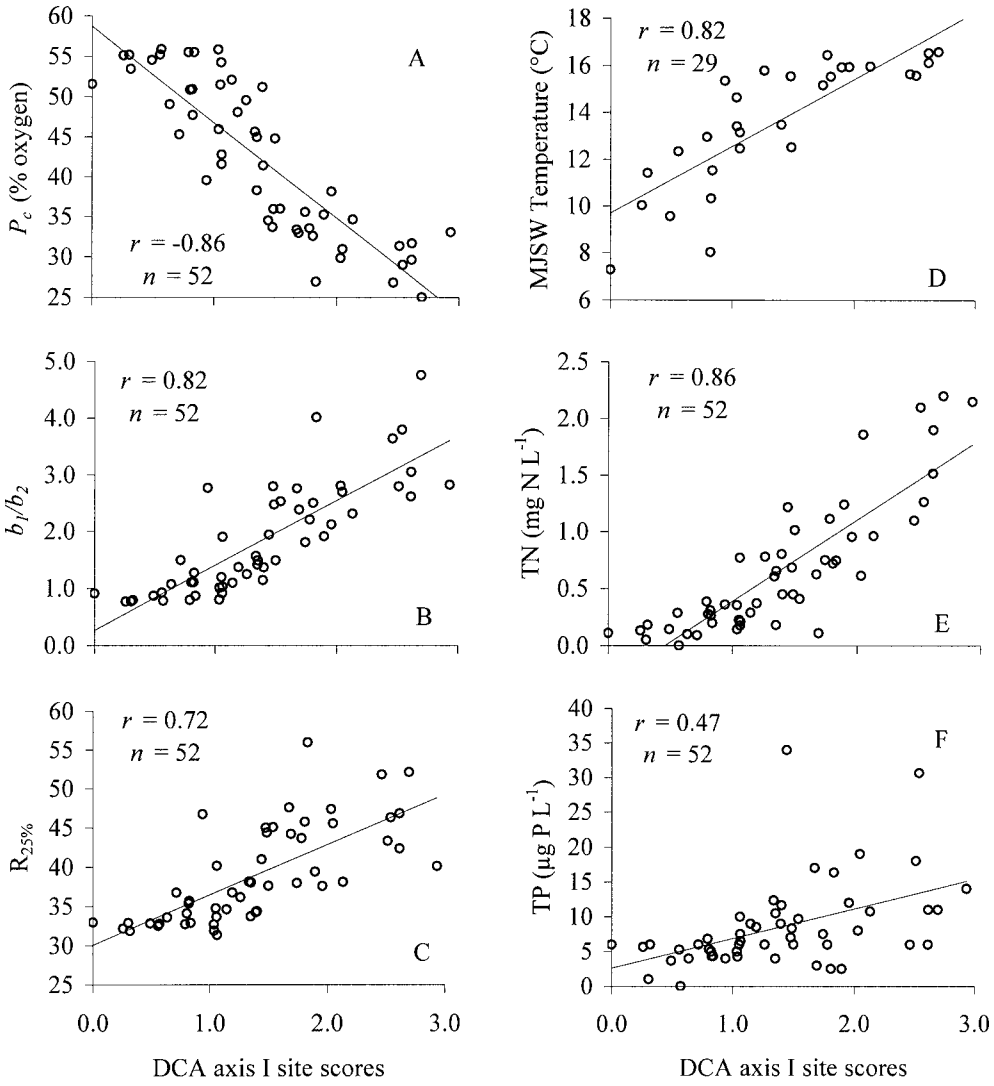

Fig. 5. (A–C) Correlations between the weighted averages of the modeled respiratory constants and the overall variation in chironomid assemblage structure in West Greenland lakes. (A) Critical limit (P_c, %), (B) ratio between slopes in piecewise regression ($b_1 : b_2$), and (C) $R_{25\%}$ (% of max respiration). (D–F) Correlations between environmental variables and chironomid assemblage structure. (D) Mean July surface water temperature (°C), (E) TN (mg N L^{-1}), and (F) TP (μg P L^{-1}). All correlations are significant ($p < 0.001$). Results of DCA analysis (Table 3) and environmental variables are from Brodersen and Anderson (2002).

ronomus larvae were also good oxy-regulators and came out as group E species at 10°C and as group D species at 15°C (Fig. 3). *C. hyperboreus* without ventral tubuli on the penultimate segment had on average a slightly higher P_c (26.5%) than *C. riparius* type with ventral tubuli (21%). Nevertheless, the respiratory role of ventral tubuli still remains unclear (Nagell and Orrhage 1981).

Hemoglobin, however, cannot provide a solution to long-term total anoxia, and the ability to switch to anaerobic metabolism is important. An anaerobic metabolism has been shown in both oligochaetes and chironomids, and both groups include species that are able to withstand anoxic conditions for shorter or longer periods (e.g., Hamburger et al. 1998). Environmental oxygen shortage, as distinct from physiological anoxia (e.g., muscular work), induces anaerobic pathways yielding two- to three-fold higher energy gain per unit of glycogen degraded than the classic Embden-Meyerhof pathway. The metabolism of the animals decreases gradually down to 5–10% during the first days of anoxia and further down to 1–2% during prolonged anaerobiosis, eventually entering a kind of dormancy (quiescence) after a few weeks (Hand 1991).

The P_c was the best constant derived to describe the overall patterns in the 52 chironomid assemblages. The P_c values, however, are dependent on metabolic activity and temperature, and an increase in temperature is expected to increase the level of P_c (Jones 1972). We found a slight increase in P_c for *C. riparius* and *Hydrobaenus* (9%), a substantial in-

crease for *Dicrotendipes* (42%), and a decrease for *C. hyperboreus* (22%). A reason for the diverging results is probably that the relatively high experimental temperatures influence the basal respiration rate but not necessarily the activity, which, for some species, can either be depressed or increased under critical experimental conditions. Respiration rates for *Diamesa* and *Hydrobaenus* collected from Glacier Lake 1 (~4–7°C) might be somewhat overestimated when run at 10°C experimental temperature, despite the 24-h acclimatization period. However, the results suggest that (globally) increased water temperatures theoretically can have a direct physiological influence on the habitat range for some low arctic species that are already close to their limits of existence.

Paleolimnological perspective—The strong relationship between chironomid distribution and the oxygen environment has been applied in paleolimnological inference models. By using the surface-sediment calibration techniques in Canada, weighted-average (WA) species optima have been calculated for an anoxic factor (Quinlan et al. 1998), for average end-of-summer hypolimnetic dissolved oxygen (Little and Smol 2001), and for volume-weighted hypolimnetic oxygen concentration (VWHO; Quinlan and Smol 2001). The ecological distributions and the WA optima agree well with our results and with those of other studies. The critical limits (average P_c) found in the present study were closely related to the VWHO optima calculated by Quinlan and Smol (2001) for seven genera in common (*Heterotrissocladius, Micropsectra, Psectrocladius, Tanytarsus, Dicrotendipes, Procladius,* and *Chironomus;* VWHO = 0.08, P_c + 1.72; $r^2 = 0.81$, $p < 0.006$).

However, although statistically robust, the predictive power of the WA inference models is moderate, probably because of an overestimation of oxygen concentrations in the low end of the gradient. Subfossil assemblages integrate taxa from the upper and lower littoral (Brodersen and Lindegaard 1999), and it is likely that head capsules of oxy-conformer taxa, such as *Heterotrissocladius,* can appear in profundal sediments (and assemblages), where they in fact never lived (Quinlan and Smol 2001). On the other hand, our results have shown that taxonomically complex taxa, such as *Heterotrissocladius,* can show both high degree of oxy-conformity and some degree of oxy-regulation within the same genus (Table 2, Fig 4.). Thus, a proportion of biases and errors shall probably be found not only in our methods of exploring respiratory preference but also in the difficulties with taxonomy and with subfossil taxonomy in particular.

The oxy-regulatory capacity was correlated as well, or even better, to the overall assemblage structure, as did temperature and trophic variables (Fig. 5). Hypolimnetic anoxia is strongly related to lake morphometry and trophic conditions (Nürnberg 1995). The water temperature is also partly determined by lake morphometry, and trophic variables are (in most training sets) often positively correlated to temperature (Brodersen and Anderson 2002). It is therefore difficult to point out a single variable as the causal factor determining the distributional patterns when using the traditional surface-sediment calibration methods. This problem is inherent when interpreting the results of inference models. The present new experimental results, which were produced under controlled laboratory conditions, show that the oxy-regulatory capacity can independently explain most of the species variation in lake data sets with more than one strong environmental gradient. For paleolimnological interpretation, it is therefore important to note that increases in temperature, nutrients, or anoxia will produce nearly identical chironomid responses. This is not a problem if, for example, increased productivity and subsequent oxygen depletion are a result of increased temperature (i.e., the three variables always interrelate homogeneously). If, however, in-lake processes are changed because of lake ontogeny (e.g., catchment development, colonization, habitat alteration, or infilling), this will interfere with chironomid-inferred environmental and climate reconstruction. The experimental and distributional results indicate that even moderately cold low arctic lakes will lack or underrepresent cold-water indicators (oxy-conformers) if poor oxygen conditions eliminate their preferred habitat, which results in an overestimation of chironomid-inferred temperatures. Conversely, subfossil assemblages from lakes that lack profundal taxa because of severe anoxia might be dominated by littoral and sublittoral oxy-conformers (e.g., *Psectrocladius, Paracladopelma*), which would result in an overestimation of oxy-regulatory capacity. In that case, it is important to differentiate between littoral and profundal taxa in the subfossil assemblage.

The constants presented in Table 2 can be used directly to infer an expression of oxy-regulatory capacity for a chironomid community (Eq. 5) or to identify secondary changes or inconsistencies in multiproxy down-core studies. Mismatches in climate trends or in accuracy of inferred temperatures (Velle 2003) might, in part, be explained from basic or altered oxygen conditions. Likewise, inconsistencies between pelagically (diatom) and zoobenthic (chironomid) inferred trophic trends are common (e.g., Little et al. 2000); in such situations, knowledge of species-specific oxy-regulatory capacity might assist the interpretations. Even though we used averaged values for taxa at the ''type'' or generic level, the 16 taxa make up 88% of the average West Greenland chironomid assemblage structures (Brodersen and Anderson 2002) and therefore make inferences realistic. It is reasonable to assume that the model can be used outside Greenland as well, partly because of the good consistency with earlier findings (up. cit.) and partly because of the commonly good agreement in species autecology among regions (Lotter et al. 1999).

It can be concluded that large differences in the ability to regulate oxygen uptake were shown for the 16 West Greenland chironomid taxa, and we believe that autecological information from controlled experiments can provide important additional information for interpretations of chironomid transfer functions. The ecophysiological constants obtained from the controlled laboratory experiments were reliable indicators of species-specific oxy-regulatory capacity. Warm-water chironomid assemblages have a high oxy-regulatory capacity, whereas cold-water assemblages are dominated by oxy-conformers. As a result, poor oxygen conditions will theoretically infer warm water (warm climate) when chironomid-temperature transfer functions are used. An expression of oxy-regulatory capacity for a chironomid community can

directly be inferred from a simple model using weighted averages of the ecophysiological constants. The results can also be used to identify secondary changes or mismatches in multiproxy down-core paleoclimate studies. Studies like this are building the important bridge between neolimnology and paleolimnology (Smol 1990) and will potentially enhance the quality of both qualitative and quantitative paleolimnological reconstructions.

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