The comparative biology of diving in two genera of European Dytiscidae (Coleoptera)

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Abstract

Surfacing behaviour is fundamental in the ecology of aquatic air-breathing organisms; however, it is only in vertebrates that the evolutionary ecology of diving has been well characterized. Here, we explore the diving behaviour of dytiscid beetles, a key group of surface-exchanging freshwater invertebrates, by comparing the dive responses of 25 taxa (*Deronectes* and *Ilybius* spp.) acclimated at two temperatures. The allometric slopes of dive responses in these dytiscids appear similar to those of vertebrate ectotherms, supporting the notion that metabolic mode shapes the evolution of diving performance. In both genera, beetles spend more time submerged than on the surface, and surface time does not vary with the temperature of acclimation. However, presumably in order to meet increased oxygen demand at higher temperatures, *Deronectes* species increase surfacing frequency, whereas *Ilybius* species decrease dive time, an example of 'multiple solutions.' Finally, widespread northern species appear to possess higher diving performances than their geographically restricted southern relatives, something which may have contributed to their range expansion ability.

Introduction

Most aquatic animals carry out gas exchange either across the integument or using some form of specialized gill or respiratory plastron (Jones, 1972; Kooyman, 1973; Mill, 1974; Chapman, 1982; Schmidt-Nielsen, 1997). However, several aquatic groups (including many insects, amphibians, reptiles and mammals) obtain oxygen by exchanging respiratory gases with air, during surfacing (Mill, 1974; Chapman, 1982; Boyd, 1997; Schreer & Kovacs, 1997; Šamajová & Gvoždík, 2009; Brischoux et al., 2011). Aquatic Coleoptera possess a variety of exhaustible air stores, all of which include a subelytral reservoir into which the majority of the insect's functional spiracles open (Crowson, 1981; Chapman, 1982; Jach, 1998; Wichard et al., 2002). In the majority of diving beetles (Dytiscidae), the air store is wholly subelytral (Heberdey, 1938; Balke, 2005), acting only to some extent as a physical gill (Brocher, 1916; Ege, 1915;

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Madsen, 1967). Consequently, most adult dytiscids are highly dependent on atmospheric oxygen and must surface periodically to renew their air supply (Calosi et al., 2007a). Whereas most species appear to spend the majority of their time underwater at rest, the diving behaviour of dytiscids (and indeed of other surface-exchanging aquatic insects) reflects the proportion of time individuals can spend in essential activities that require them to be submerged, such as foraging and reproduction, thus being fundamental to their physiology and behavioural ecology.

In diving beetles, and other surface-exchanging animals, an individual dive cycle can be divided into two components: the time spent underwater, or dive time, and the time spent exchanging the air store at the water surface, or surface time (see Calosi et al., 2007a for further details). Surface time and dive time have been used to explore the biology of diving across groups of aquatic mammals, birds and reptiles (Boyd, 1997; Schreer & Kovacs, 1997; Halsey et al., 2006a,b; Brischoux et al., 2008; and references therein), but quantitative studies of diving responses and performance (e.g. dive length) are almost nonexistent for Dytiscidae, limiting our

understanding of the evolutionary ecology of diving in this group of insect and in air-breathing invertebrates in general. Diving responses are thought to be function of the quantity of air stored and the rate at which oxygen is consumed. Direct measurement of oxygen consumption by aquatic air-breathing insects is technically challenging, and physiological techniques employed so far do not allow experiments to be conducted under seminatural conditions in the laboratory (Ege, 1915; Matthews & Seymour, 2006). Consideration of metabolic rates (Krogh, 1914) and previous studies on other organisms (De Ruiter *et al.*, 1952; Eddy & Mcdonald, 1978) suggest that changes in surfacing frequency (the number of surfacing events divided by the observation time) underlie an increase in oxygen demand and more frequent surfacing allowing for a more frequent renewal of the insect's subelytral air reservoir (Gilbert, 1986). Thus, surfacing frequency may provide an integrative measure of oxygen demand that in turn relates to the activity of diving beetles (Calosi *et al.*, 2007a). Additionally, acknowledging variation in the duration of individual dive cycles, a measure of the voluntary time submerged (VTS), can be calculated by multiplying surfacing frequency by average dive duration, thus representing an integrative measure of dive time and frequency. Here, components of the dive cycle, including surfacing frequency and VTS, are used as proxies to explore activity levels, interspecific variation in responses to temperature and the presence of broad phylogenetic and physiographic/ecographic patterns in the diving response of diving beetles.

Based on theoretical considerations, a number of predictions can be made regarding the diving behaviour of diving beetles and other surface exchangers. Given the intimate link between surfacing and both predation risk (Kramer *et al.*, 1983) and risk of parasitism by water mites (Gerecke, 2006), most species may be expected to first maximize their submergence time, with the rate of oxygen depletion being a major constraining factor, and second to minimize the time spent at the surface, this being dictated by mechanical restrictions in replenishing subelytral air stores. Third, according to the *oxygen store–usage hypothesis* (proposed in homoeotherms see Butler & Jones, 1982; Boyd, 1997; Halsey *et al.*, 2006a; b, but see also Brischoux *et al.*, 2008), species with larger bodies are expected to have on average longer dives and longer surface intervals, following the assumption that oxygen stores scale isometrically with mass, whereas oxygen demand scales allometrically (see Halsey *et al.*, 2006a,b; Brischoux *et al.*, 2008). Fourth, given that elevated temperatures increase the ratio between oxygen demand and oxygen supply in aquatic ectotherms (Verberk *et al.*, 2011), diving beetles will incur oxygen deficits more rapidly at higher temperatures, leading to a reduction in dive duration and/or an increase in surfacing frequency to enable more frequent renewal of the insect's air reservoir (Gilbert, 1986). Finally, given that rare and common diving beetles have been shown to differ dramatically in thermal tolerance ranges

(Calosi *et al.*, 2010), we expect different diving responses among species with differing geographical range sizes. In particular, we expect widespread species to show higher levels of activity (based on Bernardo *et al.*, 2007 observations in salamander) and possibly greater physiological performances (i.e. diving performances, including the ability to maintain unvaried mean VTS at higher temperatures) when compared with their geographically restricted relatives (based on Calosi *et al.*, 2007b observations in amphipods, Calosi *et al.*, 2010 observations in diving beetles).

Here, we present quantitative data on the diving response of 25 taxa of European dytiscids, belonging to two separate genera (*Deronectes* and *Ilybius*) inhabiting lotic and lentic environments, respectively. We use these data to (i) characterize the structure of the relative length of dive and surface time and their allometric relationships, (ii) investigate how diving responses change in response to temperature between genera and across species and (iii) explore the relationship between species diving responses and the size and position of their geographical range. Our study is the first to quantify and compare the diving response of a large group of related aquatic air-breathing insects within a phylogenetically informed context, characterizing the evolutionary ecology of diving in these invertebrate ectotherms. We show that dytiscid beetles spend more time submerged than on the surface at both temperatures tested and that in both genera, mean surface time does not vary between acclimation temperatures. Nonetheless, in order to meet increased oxygen demand at the higher temperature, species of *Deronectes* show an increase in surfacing frequency, whereas species of *Ilybius* show a decrease in dive time, suggesting fundamentally different respiratory responses ('multiple solutions') in these genera. In addition, our results corroborate the idea that metabolic mode has shaped the evolution of diving performances, because diving beetle responses resemble those of vertebrate ectotherms more than those of vertebrate endotherms. Finally, despite clade (genus) being the strongest predictor of species variation, widespread northern species show a higher mean surface time and VTS, but lower mean surfacing frequency and smaller shifts in VTS with increased temperature, when compared with restricted southern species. This seems to indicate that widespread species possess higher diving performances and that these may have played an important role in defining their post-glacial colonization success.

Materials and methods

Species ecology, distribution and phylogenetic relationship

Species of *Deronectes* occur in fast-flowing streams at intermediate elevations across the Palaearctic, with greatest diversity in the Mediterranean region (Balfour-Browne, 1950; Franciscolo, 1979; Millán & Ribera, 2001;

Ribera & Vogler, 2004). *Ilybius* is a widespread Holarctic genus, with highest species richness in Europe and the Mediterranean, with most taxa being found in small, often temporary lentic water bodies (Nilsson & Holmen, 1995). The phylogenetic relationships of both *Deronectes* and *Ilybius* species are documented, based largely on mitochondrial cytochrome oxidase (COI) and 16s ribosomal DNA sequences (Ribera *et al.*, 2001; Ribera & Vogler, 2004). Species of both genera are generalist predators, feeding benthically on a range of small aquatic invertebrates, particularly dipteran larvae. Within a genus, species have broadly similar general ecologies and occupy similar habitats across their geographical ranges, although taxa differ markedly in latitudinal range size and position and thermal tolerance windows (Calosi *et al.*, 2010; P. Calosi, D.T. Bilton & J.I. Spicer personal communications.).

Specimens' collection, maintenance and preparation

Adult *Deronectes* and *Ilybius* were collected during spring and summer 2006 (see Tables S1 and S2) by working a D-Framed pond net (1 mm mesh, dimensions 20 × 25 cm) along stream or pond banks. All species were collected as close as possible to the central point of their known latitudinal ranges, to avoid the possible confounding effects of local adaptation in range-edge populations and to ensure that data were comparable for each species (Thompson *et al.*, 1999; Calosi *et al.*, 2008a,b, 2010). Given the largely allopatric occurrence of many species, and differences in the latitudinal position of their ranges, it is not possible to sample all taxa from the same latitude. Data on species' geographical distributions were taken from Fery & Nilsson (1993), Fery & Brancucci (1997) and Fery & Hosseinie (1998). Latitudinal range extents (LREs) were calculated as the difference (in degrees latitude) between northern and southern distributional limits (following Gaston, 1991, 1994), and latitudinal range central points (LRCPs) were the mid-point of each species' LRE (see Tables S1 and S2).

After collection, individuals were transported to the laboratory in plastic containers (vol. = 1 L) filled with damp, aquatic vegetation, kept within thermally insulated bags (Thermos[©], Rolling Meadows, IL, USA). In the laboratory, specimens were maintained in aerated artificial pond water [APW, pH 7.5, acidified using HCl (ASTM, 1980)], distributed between a number of aquaria (vol. = 5 L, max. 20 individuals per aquarium) on a 12 : 12 h L/D regime and fed chironomid larvae *ad libitum*. No food was provided during experimental trials. Aquaria were sealed with cling-film to reduce evaporation and prevent escape. The maximum temperature fluctuation of water among all aquaria over the acclimation period was of 0.6 °C, measured with a maximum–minimum thermometer (*Jumbo Thermometer* Oregon Scientific[©] model EM899 ± 0.1 °C; Oregon Scientific[©], Portland, OR, USA). In an attempt to avoid possible confounding effects of individuals' recent environmental thermal history, specimens were maintained under identical, constant conditions in the laboratory prior to experiments (e.g. Sokolova & Pörtner, 2001; Calosi *et al.*, 2008a,b, 2010). Each species was divided haphazardly into two equal groups, acclimated to a temperature of either 14.5 or 20.5 °C for 7 days (Calosi *et al.*, 2008a,b, 2010). Temperatures were chosen to be within the range experienced by *Deronectes* and *Ilybius* adults in the field (D.T. Bilton, S. Fenoglio, A. Millan, P. Abellan & D. Sanchez, personal observations) and also to avoid extreme temperatures that could have acted (at least for certain species) as *pejus* (worsening) temperatures (see Pörtner, 2001, 2002; Woods & Harrison, 2002).

For individual beetles, we measured mean surfacing frequency, mean surface bout length, mean dive bout length and voluntary time submerged (VTS – surfacing frequency × mean dive bout length) for both groups acclimated and tested at the two different temperatures. Further details on the quantification of the diving response are given in Appendix S1. Mean surfacing frequency, mean surface bout length, mean dive bout length and mean VTS were determined for each species at both acclimation temperatures (see Tables S3 and S4).

Data analysis

In order to characterize the effects of acclimation temperature (14.5 and 20.5 °C), together with species and genus membership, on diving behaviours, data were analysed using two-way ANCOVAS of individual values (with body mass as a covariate; species nested within genus), using $\log_{10}$ adjusted surfacing frequency, $\log_{10}$ mean surface bout length, $\log_{10}$ mean dive bout length and mean VTS. Models were analysed both with and without genus × temperature and species × temperature interactions. Similar analyses were also conducted separately for each genus. All analyses were performed using SPSS version 15.0 (IBM, New York, NY, USA).

Secondly, employing mean values for each species, we tested for phylogenetic signal in $\log_{10}$ surfacing frequency, $\log_{10}$ surface bout length, $\log_{10}$ dive bout length, VTS and $\log_{10}$ body mass using the randomization test described in the study by Blomberg *et al.* (2003). We also report their K statistic as a measure of the strength of phylogenetic signal. A *K* value of unity would indicate that, averaged across the entire phylogeny, species tend to resemble their relatives as much as would be expected under a Brownian-motion model of trait evolution. Values of *K* lower than unity can occur because of adaptation of particular species (or groups of species) to particular environmental conditions, sexual selection or measurement error (Ives *et al.*, 2007), including errors in the phylogenetic topology and/or branch lengths. Values of *K* greater than unity can occur when phylogenetic clumping exists, e.g. if a particular clade (or clades) has substantially larger or smaller values of a measured trait

than other species in the analysis (see also Garland *et al.*, 2005).

Finally, in order to examine the relationships between diving responses and biogeography, we used multiple regression models to explore possible relationships between dive characteristics (surfacing frequency, surface bout length, dive bout length and VTS) and latitudinal range extent and position. We computed these regressions in three ways (see Garland *et al.*, 2005; Lavin *et al.*, 2008; Gartner *et al.*, 2010; Brischoux *et al.*, 2011; Oufiero *et al.*, 2011): conventional (i.e. nonphylogenetic) ordinary least squares (OLS); phylogenetic generalized least squares (PGLS); and regression in which the residuals of the model are modelled as having evolved *via* an Ornstein–Uhlenbeck process (RegOU), intended to mimic stabilizing selection. These three models form a continuum between assuming a star phylogeny with no hierarchical structure (OLS), a phylogeny as specified by the user (PGLS) and something that can take on values intermediate between the star and the specified hierarchical phylogeny (RegOU). The RegOU model contains an additional parameter, *d*, that estimates the transformation of the phylogenetic tree (Lavin *et al.*, 2008), and hence, its fit can be compared with the OLS or GLS models by a ln maximum-likelihood ratio test, where twice the difference in the ln maximum likelihood is assumed to be distributed asymptotically as a χ^2 with 1 degree of freedom (e.g. Oufiero *et al.*, 2011). Similar tests were used to compare the fit of models within the OLS, PGLS or RegOU classes when they contained nested subsets of independent variables. As a heuristic indicator of model support, we report the Akaike Information Criterion (AIC), using the smaller-is-better formulation [AIC = (−2*ln maximum likelihood) + (2*No. of parameters)]. When comparing a set of models, nested or not, the one with the lowest AIC is considered to be the best. As a rule of thumb, models whose AIC is <2 units larger can also be said to have substantial support (Burnham & Anderson, 2002). Maximum likelihoods are used for computing AIC and likelihood ratio tests, whereas REML is used for estimating coefficients in the model, such as the allometric scaling exponent. REML estimates of the OU transformation parameter, *d*, are also reported. All of the regression models were computed using the Matlab Regressionv2.m program of Lavin *et al.* (2008). The OLS models were also checked against output from spss version 15.0. The same procedure described earlier was also repeated substituting the factor 'clade' (genus) with the factor 'habitat' (discriminating between lotic and lentic species), but as models containing this factor showed lower predictive power, only the set of analyses including 'clade' as factor is reported.

The topology of the phylogenetic tree used for comparative analyses (Fig. S1) was modified from Ribera & Vogler (2004), branch lengths being set using the arbitrary method of Grafen (1989), in which each node is at a depth from the tips of the tree that is one less than the number of tips descending from it. Thus, for example, the root of the tree is set at a depth of 24 for a set of 25 tip species.

Finally, no significant correlation was found between the number of individuals of each species examined and the parameters examined here (all *P* values nonsignificant; Bonferroni correction applied), indicating that interspecific differences in sample size did not influence results.

Results

Individual data analysis

Deronectes *and* Ilybius *combined*
Surface time, dive time and adjusted surfacing frequency differed greatly both between genera and among species (minimum $F_{1,39} = 8.772$; $P = 0.005$), whereas VTS differed only marginally between genera and among species (maximum $F_{23,654} = 1.461$; $P = 0.076$). At the higher temperature, a significant decrease in dive time and a significant increase in surfacing frequency were observed (minimum $F_{1,654} = 4.457$; $P = 0.035$), whereas surface time and VTS were not significantly affected (maximum $F_{1,654} = 0.137$; $P = 0.711$). The response to temperature was broadly similar in both genera (see also Fig. 1); no statistically significant interactions between genus and temperature were found for any measured trait (maximum $F_{1,653} = 1.176$; $P = 0.279$). Finally, body mass had no significant effect on diving responses (maximum $F_{1,654} = 1.772$; $P = 0.184$).

Within-genus analyses
When examined separately, the diving responses of *Deronectes* and *Ilybius* responded differently to acclimation temperature. Whereas species of *Deronectes* significantly increased their surfacing frequency at the higher temperature ($F_{1,464} = 11.142$; $P = 0.001$; Fig. 1), this trait appeared almost temperature independent *in Ilybus* species ($F_{1,217} = 3.570$; $P = 0.060$). Conversely, *Ilybius* species showed a significant decrease in their dive time at the higher temperature ($F_{1,217} = 4.895$; $P = 0.028$; Fig. 1). In addition, in *Deronectes*, individual species differed in the way their dive time, surfacing frequency and VTS responded to temperature, as significant interactions between species and temperature were found for these traits (minimum $F_{15,464} = 2.435$; $P = 0.002$). In contrast, in *Ilybius*, species only differed in the way their surface time responded to temperature ($F_{8,217} = 2.716$; $P = 0.007$). Again, no effect of body mass on any of the four diving traits considered was found for *Deronectes* or *Ilybius* (maximum $F_{1,217} = 2.112$; $P = 0.148$). In terms of absolute values, *Deronectes* species differed significantly in their surfacing frequency, dive and surface time and VTS (minimum $F_{15,464} = 3.803$; $P < 0.0001$), whereas *Ilybius* species differed only in dive time and surfacing frequency (minimum $F_{8,217} = 3.384$; $P = 0.001$). Finally, interspe-
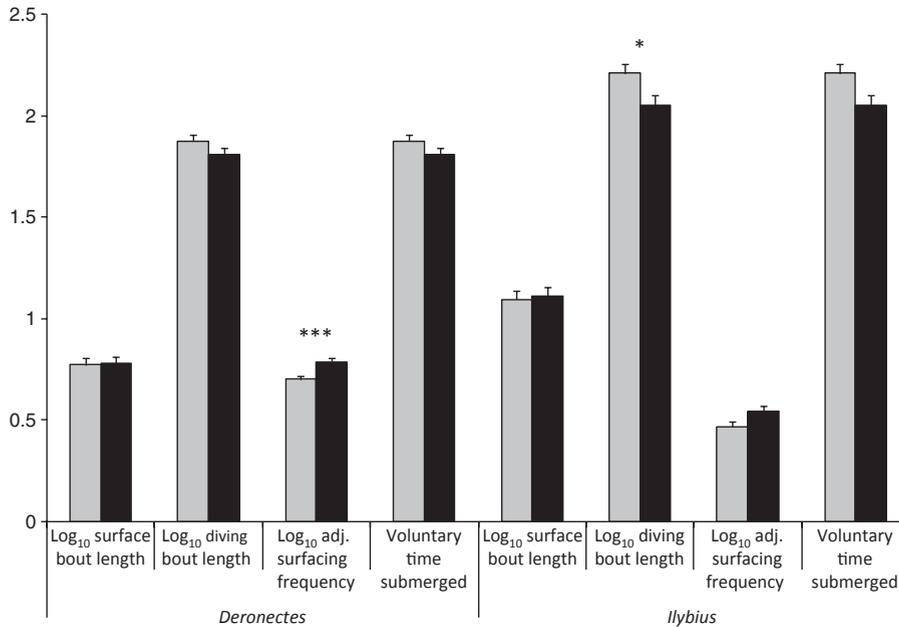
Fig. 1 Response to temperature in each of the diving responses separately for genus *Deronectes* and *Ilybius*: Log_{10} mean surface bout length (s); log_{10} mean diving bout length (s); log_{10} mean adjusted surfacing frequency (No. of surfacing events per time); mean voluntary time submerged (diving bout length*adjusted surfacing frequency; dimensionless). Note the log scale of the *y*-axis. Asterisks denote significant differences between 14.5 °C (grey) and 20.5 °C (black) ($P < 0.05$; 1-way ANOVA, using a within-clade analysis on values for individual beetles). Data points represent mean values for genus ± SE.

cific variability for all dive traits appeared to be generally higher in *Deronectes* than in *Ilybius* (see Fig. 1, Tables S3 and S4).

Phylogenetic signal

Adjusted surfacing frequency and VTS had significant phylogenetic signal across the examined taxa (surfacing frequency – $P = 0.032$ with $K = 0.26$ and $P = 0.027$ with $K = 0.28$, at 14.5 and 20.5 °C, respectively; VTS – $P < 0.0001$ with $K = 0.55$ and $P = 0.002$ with $K = 0.39$, at 14.5 and 20.5 °C, respectively). In contrast, mean surface time and mean dive time did not exhibit significant phylogenetic signal (surface time – $P = 0.326$ with $K = 0.16$ and $P =$ and 0.315 with $K = 0.16$, at 14.5 and 20.5 °C, respectively; dive time – $P = 0.504$ with $K = 0.014$ and $P = 0.345$ with $K = 0.16$, at 14.5 and 20.5 °C, respectively). A highly significant phylogenetic signal was found for body mass ($P < 0.001$), with a K statistic of 2.66 and 2.50 at 14.5 and 20.5 °C, respectively. This very large K value reflects the large difference in body size (complete lack of overlap) between the two genera (see Fig. 4, Tables S1 and S2).

Mean species data analysis

At 20.5 °C, surfacing frequency (negatively) and surface time (positively) were significantly related to latitudinal range extent and latitudinal range central point (Table 1

and Fig. 2). Also, VTS was significantly positively related to latitudinal range extent at both 14.5 and 20.5 °C (Table 1). However, strong differences between both clades were found for surfacing frequency (generally higher in *Deronectes*) and surface time, dive time and VTS (generally higher in *Ilybius*) (see Table 1 and Fig. 3). Likelihoods were higher for models including clade as an independent variable for all diving responses considered here (Table 1). Likelihood ratio tests indicated that adding other candidate independent variables (log_{10} body mass, range size and range position) to a model that included clade never resulted in a significantly improved model for explaining the diving responses examined at either temperature, with the exception of mean dive time measured at 20.5 °C. The model for mean dive time at 20.5 °C including log_{10} body mass, clade and their interaction, was a significant improvement compared with all other models (minimum $\chi^2 = 6.19$, d.f. = 2, $P = 0.045$; Table 1f, see also Fig. 4a). Despite the strong effect of clade and its close association with body mass, body mass was significantly related to surface frequency, surface time and VTS at both temperatures (Table 1). At 14.5 °C, body mass was significantly (negatively) related to dive time, but such relationship was not statistically significant at 20.5 °C (Table 1 and Fig. 4). For all models considered, the phylogenetic PGLS and RegOU models never fit the data better than the nonphylogenetic (OLS) model (Table 1). At 14.5 °C, surfacing frequency, surface time and dive time were

Table 1 Model selection to estimate factors influencing diving beetles diving responses (a–b $\log_{10}$ adjusted frequency, c–d $\log_{10}$ mean surface bout length, e–f $\log_{10}$ mean dive bout length, g–h voluntary time submerged) separately for acclimation temperature 14.5 and 20.5 °C in European diving beetles. Body mass (BM), clade (genus), latitudinal range extent (LRE) and latitudinal range position (LCRP) were included as independent variables. Probability of models' candidate variables (P var 1, P var 2, P var 3), ln maximum-likelihood and Akaike's Information Criteria (AIC) values for models (OLS, PGS and RegOU) are reported.

Variables	Probability values for model variables			ln max likelihood for model			d for OU	AIC for model		
	P variable 1	P variable 2	P variable 3	OLS	PGLS	RegOU		OLS	PGLS	RegOU
a) $\log_{10}$ adjusted frequency at 14.5 °C										
$\log_{10}$ mass	0.011			10.171	4.173	10.612	0.056	−14.343	−2.346	−13.223
Clade	0.002			11.693	3.562	12.125	0.051	−17.386	−1.123	−16.250
Range size	0.096			8.050	3.488	8.652	0.232	−10.100	−0.976	−9.304
Range position	0.078			8.244	3.426	8.967	0.186	−10.488	−0.851	−9.935
$\log_{10}$ mass + clade	0.217	0.042		12.577	4.995	13.143	0.081	−17.154	−1.989	−16.285
Clade + range size	0.012	0.973		11.742	3.618	12.171	0.049	−15.484	0.765	−14.342
Clade + range position	0.012	0.553		11.946	3.549	12.317	0.046	−15.892	0.902	−14.634
$\log_{10}$ mass + clade + interaction	0.242	0.312	0.729	12.650	5.026	13.220	0.084	−15.300	−0.052	−14.439
Clade + range size + range position	0.014	0.641	0.458	12.079	3.634	12.476	0.052	−14.157	2.733	−12.953
b) $\log_{10}$ adjusted frequency at 20.5 °C										
$\log_{10}$ mass	0.021			5.015	0.024	6.191	0.162	−4.031	5.952	−4.382
Clade	0.006			6.222	0.040	7.252	0.115	−6.445	5.919	−6.504
Range size	0.037			4.440	−0.135	5.290	0.175	−2.880	6.270	−2.581
Range position	0.009			5.834	−0.095	6.420	0.078	−5.668	6.190	−4.839
$\log_{10}$ mass + clade	0.285	0.073		6.886	0.188	7.849	0.096	−5.771	7.625	−5.699
Clade + range size	0.055	0.451		6.580	−0.072	7.424	0.082	−5.160	8.145	−4.848
Clade + range position	0.057	0.088		7.940	−0.035	8.493	0.045	−7.880	8.070	−6.985
$\log_{10}$ mass + clade + interaction	0.847	0.781	0.097	6.702	0.226	7.856	0.098	−3.405	9.548	−3.711
Clade + range size + range position	0.058	0.638	0.117	8.075	−0.013	8.652	0.048	−6.150	10.027	−5.305
c) $\log_{10}$ mean surface bout length at 14.5 °C										
$\log_{10}$ mass	0.026			−4.363	−16.216	−4.364	2.38E-10	14.727	38.432	16.728
Clade	0.020			−4.164	−16.233	−4.165	2.38E-10	14.328	38.466	16.329
Range size	0.184			−6.176	−15.709	−6.176	9.54E-10	18.351	37.417	20.353
Range position	0.216			−6.305	−16.017	−6.306	1.19E-10	18.611	38.034	20.612
$\log_{10}$ mass + clade	0.949	0.556		−4.162	−16.203	−4.162	4.77E-10	16.323	40.406	18.325
Clade + range size	0.063	0.949		−4.162	−15.668	−4.162	4.77E-10	16.323	39.337	18.325
Clade + range position	0.053	0.790		−4.123	−15.972	−4.124	4.77E-10	16.246	39.944	18.247
$\log_{10}$ mass + clade + interaction	0.516	0.460	0.324	−3.568	−16.018	−3.569	1.91E-09	17.136	42.036	19.138
Clade + range size + range position	0.071	0.886	0.772	−4.110	−15.668	−4.111	9.54E-10	18.220	41.337	20.222
d) $\log_{10}$ mean surface bout length at 20.5 °C										
$\log_{10}$ mass	0.002			−1.670	−16.146	−1.670	3.73E-12	9.339	38.292	11.340
Clade	0.001			−0.519	−16.303	−0.519	7.45E-12	7.038	38.607	9.039
Range size	0.004			−2.426	−14.996	−2.427	1.19E-10	10.852	35.992	12.853
Range position	0.006			−2.836	−15.028	−2.836	1.49E-11	11.671	36.057	13.672
$\log_{10}$ mass + clade	0.631	0.137		−0.384	−15.906	−0.385	1.49E-11	8.769	39.811	10.770
Clade + range size	0.021	0.151		0.679	−14.964	0.679	1.49E-11	6.641	37.927	8.642
Clade + range position	0.009	0.088		1.172	−14.993	1.172	7.45E-12	5.656	37.985	7.657
$\log_{10}$ mass + clade + interaction	0.553	0.396	0.717	−0.304	−15.725	−0.305	1.49E-11	10.609	41.450	12.610
Clade + range size + range position	0.024	0.661	0.317	1.290	−14.715	1.289	7.45E-12	7.421	39.430	9.422

Table 1 (*Continued*)

Variables	Probability values for model variables			ln max likelihood for model			d for OU	AIC for model		
	P variable 1	P variable 2	P variable 3	OLS	PGLS	RegOU		OLS	PGLS	RegOU
e) Log_{10} mean dive bout lenght at 14.5 °C										
Log_{10} mass	0.0032			3.06904	−12.2559	3.0687	9.31E−13	−0.13808	30.5118	1.8626
Clade	0.0011			4.15156	−12.8081	4.15114	1.86E−12	−2.30311	31.6161	−0.30228
Range size	0.0804			−0.05686	−12.8562	−0.05728	7.45E−12	6.11372	31.7123	8.11455
Range position	0.138			−0.53197	−11.9642	−0.53244	5.96E−11	8.20679	29.9284	−0.53244
Log_{10} mass + clade	0.5876	0.1419		4.32247	−11.7249	4.32209	1.86E−12	1.35506	31.4497	1.35582
Clade + range size	0.0071	0.9737		4.15219	−12.8004	4.15177	1.86E−12	−0.30438	33.6008	1.69646
Clade + range position	0.0045	0.8718		4.16668	−11.9175	4.16628	1.86E−12	−0.33336	31.835	1.66744
Log_{10} mass + clade + interaction	0.4432	0.3075	0.5688	4.52037	−11.5883	4.51995	1.86E−12	0.959266	33.1766	2.96011
Clade + range size + range position	0.0088	0.9242	0.8562	4.1722	−11.2915	4.17169	3.73E−12	1.65561	32.583	3.65663
f) Log_{10} dive bout length at 20.5 °C										
Log_{10} mass	0.1053			−1.10093	−11.6311	−1.05745	7.28E−03	8.20187	29.2623	10.1149
Clade	0.0745			−0.79145	−11.8391	−0.75292	4.79E−03	7.5829	29.6782	9.50585
Range size	0.9129			−2.5508	−9.09337	−2.31122	1.40E−01	11.1016	24.1867	12.6224
Range position	0.7986			−2.52126	−11.2933	−2.35463	3.56E−02	11.0425	28.5866	12.7093
Log_{10} mass + clade	0.753	0.4268		−0.73389	−11.6056	−0.73389	5.59E−02	9.46777	31.211	11.4678
Clade + range size	0.0375	0.254		−0.0353	−9.06881	0.083233	2.30E−02	8.07061	26.1376	9.83353
Clade + range position	0.0662	0.5277		−0.55965	−11.2827	−0.54214	5.18E−03	9.11931	30.5654	11.0843
Log_{10} mass + clade + interaction	0.0605	0.0089	0.0124	3.07561	−5.38218	3.41088	4.74E−02	3.84878	20.7644	5.17824
Clade + range size + range position	0.0432	0.3475	0.9881	−0.02176	−8.78704	0.152567	4.79E−02	10.0435	27.5741	11.6949
g) Voluntary time submerged at 14.5 °C										
Log_{10} mass	0.0001			5.57707	−2.38553	5.57815	2.38E−03	−5.15415	10.7711	−3.15629
Clade	0.0001			9.36612	−2.2171	9.39351	5.50E−03	−12.7322	10.4342	−10.787
Range size	0.0096			−5.60598	−2.03489	−0.25845	7.13E−01	17.212	10.0698	8.51691
Range position	0.0805			−7.63438	−2.25684	−0.61611	7.30E−01	21.2688	10.5137	9.23222
Log_{10} mass + clade	0.6278	0.0093		9.50274	−0.5568	9.56723	1.32E−02	−11.0055	9.11361	−9.13445
Clade + range size	0.0001	0.9738		9.36675	−1.41508	9.4026	7.96E−03	−10.7335	10.8302	−8.80521
Clade + range position	0.0001	0.605		9.52165	−1.63075	9.52224	3.57E−03	−11.0433	11.2615	−9.04447
Log_{10} mass + clade + interaction	0.5818	0.6055	0.1249	10.9378	1.33573	11.2468	3.43E−02	−11.8755	7.32853	−10.4936
Clade + range size + range position	0.0001	0.6257	0.483	9.6667	−1.36635	9.68786	7.67E−03	−9.3334	12.7367	−7.37571
h) Voluntary time submerged at 20.5 °C										
Log_{10} mass	0.0001			5.42482	−5.22539	5.42482	−2.60E−17	−4.84965	16.4508	−2.84965
Clade	0.0001			5.67642	−7.06425	5.67614	3.71E−06	−5.35285	20.1285	−3.35228
Range size	0.0306			−6.78663	−7.01615	−3.83887	5.80E−01	19.5733	20.0323	15.6777
Range position	0.1522			−8.24445	−6.09985	−3.4514	6.31E−01	22.48890	18.1997	14.9028
Log_{10} mass + clade	0.2688	0.3742		6.13541	−5.20039	6.13511	6.52E−05	−4.27082	18.4008	−2.27022
Clade + range size	0.0001	0.6296		5.81164	−6.7011	5.81129	3.97E−05	−3.62327	21.4022	−1.62258
Clade + range position	0.0001	0.399		6.0898	−5.74367	6.0898	−2.60E−17	−4.1796	19.4873	−2.1796
Log_{10} mass + clade + interaction	0.6646	0.0379	0.0686	8.15636	−3.24126	8.15636	−2.60E−17	−6.31271	16.4825	−4.31271
Clade + range size + range position	0.0001	0.9265	0.4954	6.09498	−5.68302	6.09498	−2.60E−17	−2.18997	21.366	−0.18997

PGLS, phylogenetic generalized least squares; OLS, ordinary least squares.
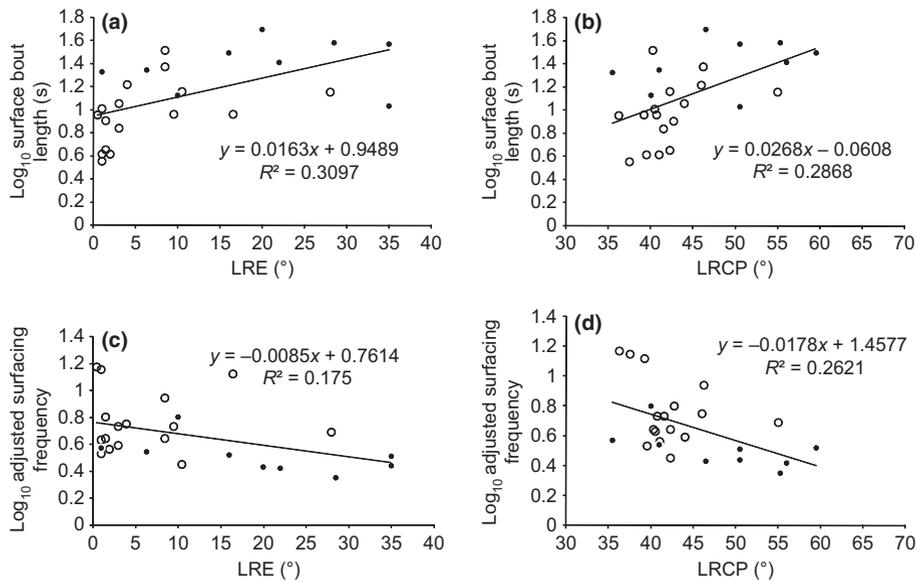
Fig. 2 Relationships between (a–b) $\log_{10}$ mean surface bout length (s) and (c–d) $\log_{10}$ mean adjusted surfacing frequency (bouts $h^{-1}/(h^{-1})^{-1.124}$) with latitudinal range extension (LRE – ° latitude) and latitudinal range central position (LRCP – ° latitude) at 20.5 °C. Dotted lines show nonphylogenetic ordinary least-squares linear regressions for all data. Open and closed circles represent *Deronectes* and *Ilybius* species, respectively. Data points represent mean values for species.

not significantly related to latitudinal range extent and latitudinal range central point, and the main predictor of these diving components was still clade (Table 1). Conversely, at 20.5 °C, including latitudinal range extent and latitudinal range central point resulted in better models (lower AIC) for dive time, although the relationships between dive time and latitudinal range were nonsignificant (Table 1).

Discussion

Dive responses: the effects of temperature

Diving is a fundamental behaviour of dytiscid beetles, which we explored here, using a convenient and repeatable observational method and within a phylogenetically informed analytical context. Unsurprisingly, we show that dytiscid beetles spend more time submerged than on the surface. This pattern was found at both acclimation temperatures tested here, with beetles spending on average 84% of their time submerged and only 16% on the surface-exchanging air. Consistent with the notion that oxygen demand rises with increasing temperatures (Ege, 1915; Kramer, 1988; Calosi *et al.*, 2007a), we show that individuals spend on average less time submerged (−21.1%) at higher temperatures. However, they do not significantly modify the time they spend on the surface (+1.9%). Exposure to higher temperatures therefore leads to a shortening of the average duration of a complete dive cycle (−19.5%), and a shift in the average ratio of time submerged/time surfacing (from 44.4 to 27.6), causing a substantial

change in the temporal structure of a dive cycle and a significant increase in surfacing frequency.

Dive responses: allometric relationships

The ability of an organism to mount a thermal response is primarily governed by its physiology and constrained by the boundaries set by its morphology, life history and phylogenetic history (Gould & Lewontin, 1979; Poff *et al.*, 2006). The two dytiscid genera that we studied are (broadly speaking) similar in body shape and respiratory mode, but differ greatly in body mass. However, body mass does not consistently explain the differences in thermal response observed between genera, neither for phylogenetically controlled nor for nonphylogenetic analyses. Halsey *et al.* (2006a) reported an overall slope of 0.33 for the relationships between $\log_{10}$ mean body mass (in Kg) and $\log_{10}$ mean dive or surface duration (in min) in endothermic birds and mammals. In the beetles studied here, the effect of body mass was weaker, slopes being 0.20 for $\log_{10}$ mean dive duration and 0.26 for $\log_{10}$ mean surface duration. Such results appear to support the notion that the evolution of diving performances and behaviour are in part determined by differences in metabolic rate or mode (i.e. ectothermy vs. endothermy), as proposed by Brischoux *et al.* (2008), although caution is necessary here given the different phylogenetic scale at which these and other studies (e.g. Schreer & Kovacs, 1997; Halsey *et al.*, 2006a; Brischoux *et al.*, 2008) were conducted when compared with ours. Nonetheless, given that diving responses were not singularly governed by body mass, the oxygen store–
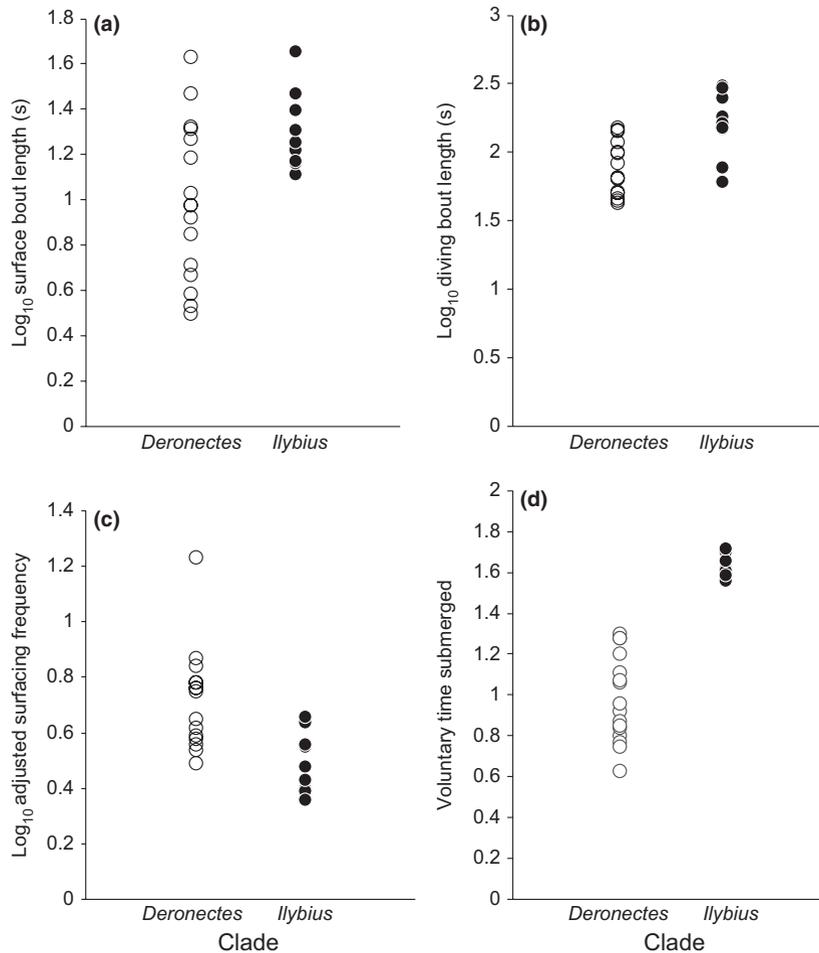
Fig. 3 Between genera differences at 14.5 °C for (a) $\log_{10}$ surface bout length (s); (b) $\log_{10}$ diving bout length (s); (c) $\log_{10}$ adjusted surfacing frequency (No. of surfacing events per time); (d) voluntary time submerged ($\log_{10}$ diving bout length*$\log_{10}$ surface bout length; dimensionless). Open and closed circles represent *Deronectes* and *Ilybius* species, respectively. Data points represent mean values for species.

usage hypothesis does not appear to apply to diving beetles. Such a result contrasts with what is observed in endothermic vertebrate divers (Halsey *et al.*, 2006b), but is in line with observations on vertebrate ectotherms (Brischoux *et al.*, 2008).

Meeting increased oxygen demand at increasing temperature

We have shown that the relative length of different parts of a dive cycle, and surfacing frequency, shifts with temperature in these insects. Differences among individual species notwithstanding, *Deronectes* and *Ilybius* were shown to differ in the way they respond to changes in temperature in their diving. In general, *Deronectes* species increase access to surface air by increasing the frequency of surfacing at higher temperatures, whereas the time individuals spend on the surface remains unchanged. In contrast, in *Ilybius*, the average time an individual spends

submerged decreases with temperature, whereas surfacing frequency and surface time remain unchanged. This suggests that these two genera differ in their respiratory physiology, exhibiting what may be termed 'multiple solutions' (*sensu* Bergmann & Irschick, 2010; Garland *et al.*, 2011). The fact that surfacing time does not apparently change in response to temperature in these insects could be due to strong selection to minimize the time spent at the surface, thus also minimizing the risk of fish predation (Kramer *et al.*, 1983) and mite larval attachment (Gerecke, 2006). It is interesting therefore that mean surface time is one of the traits with the highest level of interspecific variability. This most likely results from differences in the size of the air stores of individual taxa, as the surface time is most strongly related to VTS, which is an integrative measure of dive time and frequency. In addition, the degree to which air stores function as physical gills, the species mass-corrected metabolic rates and/or the degree to which species are
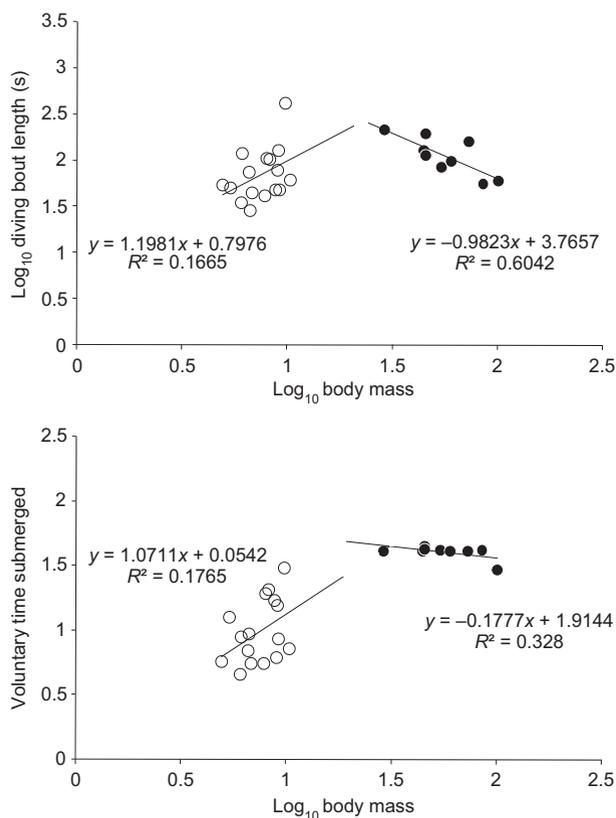
Fig. 4 Between genera differences in allometric relationships with $\log_{10}$ diving bout length (upper) and voluntary time submerged (lower) at 20.5 °C. Full lines show nonphylogenetic ordinary least-squares (ordinary least squares) linear regressions for all data. Open and closed circles represent *Deronectes* and *Ilybius* species, respectively. Data points represent mean values for species.

chemically defended against vertebrate predators may also play a role.

Our results also reveal for the first time that closely related species of diving beetles differ in the way fundamental components of the diving response are affected by temperature, with *Deronectes* showing a higher level of interspecific variation for these traits than *Ilybius*. *Deronectes* taxa also show much greater variation in the way their dive cycle changes with temperature. For example, at higher temperatures, surfacing frequency increased in *Deronectes algibensis*, but decreased in *Deronectes fairmairei*. Recent ultrastructural work on *Deronectes* and related genera (Madsen, 2008; Kehl & Dettner, 2009) has revealed the presence of tracheated setae in these insects, which apparently function as tracheal gills, allowing the insects to remain submerged longer than if they solely relied on their subelytral air store. These tracheated setae are only fully functional in flowing water (Madsen, 2008), and their contribution to an individual's gas exchange budget in standing water (as in our experiments) is unclear. Despite this, interspecific

variation in the size and density of such structures, and therefore the insect's reliance upon them in standing water, may account for some of the results observed. Tracheated setae are absent in *Ilybius* species, which may partly account for the lower level of interspecific variability of dive components in this genus.

Genus vs. body mass

The model selection approach (Table 1) shows that genus is the main predictor of the observed interspecific variation in surfacing frequency and mean surface time, thus reinforcing the idea that relatively discrete differences exist in the fundamental components of the diving behaviour at a phylogenetic level. Genera differ in body mass, and body mass showed the strongest phylogenetic signal. When examined using mean species values, we show that body mass and all diving responses examined, except mean dive time at 20.5 °C, are significantly related (Table 1 and Fig. 4). This contrasts with the results of the analyses based on individual values, where body mass was never found to be a good predictor of diving components and performance. This discrepancy may be explained by differences in the sign of the relationship between body size and diving responses. For example, intraspecific allometric relationships in the water strider *Gerris buenoi* were found to differ from those expected from interspecific studies (Klingenberg & Spence, 1997). Furthermore, we demonstrate that relationships between body size and mean dive time and VTS differ substantially for *Deronectes* (positive) and *Ilybius* (negative). Given these variable correlations with body size at different hierarchical levels, and that genera differ in more respects than body size (e.g. thermal physiology and presence/absence of specialized tracheated setae), it is perhaps not surprisingly that genus (clade) is a better predictor of diving behaviour than is body mass. More generally, various previous phylogenetic comparative studies have found that clade is often a good predictor of variation in physiological traits, even when simultaneously accounting for statistical associations with body size and ecological predictors (e.g. see Gartner *et al.*, 2010; Brischoux *et al.*, 2011).

The diving response of species with differing geographies

Our biogeographical analyses show that widespread taxa, which are also more northern (Pearson's correlation $r_{25} = 0.742$, $P < 0.0001$; after phylogenetic transformation minimum $r_{25} = 0.686$, $P < 0.0001$), possess higher mean surface time and VTS, and lower mean surfacing frequency, as compared with their southern and more narrowly distributed relatives. The fact that northern/widespread species spend more time on the surface-exchanging air may indicate that they exchange their subelytral and tracheal air more thoroughly and/or

they possess larger air stores (which take longer to fill). The positive relationship between body mass and either range size or position (minimum Pearson's correlation $r_{25} = 0.425$, $P = 0.034$) may support the idea of larger air stores, as air stores are expected to vary isometrically with body size (see Butler & Jones, 1982; but also above). The fact that northern/widespread species also show higher mean VTS (and a greater ability to maintain constant VTS levels at the higher temperature) than their southern/narrow distributed relatives is consistent with the idea that widespread species may possess greater diving performances, although the possibility that these species have lower oxygen requirements and/or a greater ability to rely on physical gills cannot be ruled out. Different abilities to remain submerged in these aquatic insects are likely to contribute to individual species abilities to allocate energy to growth and reproduction across a broader range of abiotic conditions and thus directly influence ecological success of species (see in Calow & Forbes, 1998; Spicer & Gaston, 1999) and ultimately range expansion. In addition, respiratory performance may underlie heat tolerance in aquatic insects (Verberk & Bilton, 2011). In *Deronectes*, we have already shown that thermal tolerance range is a strong predictor of geographical range extent (Calosi *et al.*, 2010), and southern endemic species appear to be less tolerant to heat and this trait to be less plastic when compared with their northern widespread relatives (Calosi *et al.*, 2008a). The data presented here on *Deronectes* and *Ilybius* suggest that aspects of their diving behaviour may have also played an important role in determining differences in post-glacial colonization success among closely related species, perhaps acting together with their basal thermal tolerance level and its plasticity.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 *Deronectes* species' collection localities, geographical parameters and phylogenetic coding.

Table S2 *Ilybius* species' collection localities, geographical parameters and phylogenetic coding.

Table S3 *Deronectes* species' number of individuals tested, and mean ± SD body mass, surface bout length, dive bout length, adjusted surfacing frequency, surface bout length:dive bout length ratio at both temperatures of exposure.

Table S4 *Ilybius* species' number of individuals tested, and mean ± SD body mass, surface bout length, dive bout length, adjusted surfacing frequency, surface bout length: dive bout length ratio at both temperatures of exposure.

Figure S1 Relationships between surface bout length and adjusted surfacing frequency with latitudinal range extension and central position at 14.5 °C in species of *Deronectes* and *Ilybius*.

Appendix S1 Details on the quantification of the diving response.

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