

Morphology of dragonfly larvae along a habitat gradient: interactions with feeding behaviour and growth (Odonata: Libellulidae)

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ABSTRACT

It has been shown that life history, behavioural as well as morphological traits vary with the habitats occupied by odonate larvae. Here we ask the following questions: (1) Are the morphological traits, which are associated with perception and foraging, related to the larval habitat? (2) Do these traits influence foraging success and growth rate? We analysed the morphology of species pairs belonging to the genera *Crocothemis*, *Orthetrum* and *Trithemis*; one species in each pair occurring in perennial spring-fed streams, the other able to develop in temporary waters. A PCA reveals four principal components of morphological characters which may be expressed as PC1: prey handling, PC2: visual perception, and PC3 and PC4: density of long and short setae on the feet. The variances of PC1, PC2 and PC3 were affected by phylogeny. PC1, PC2 and PC4 differed between habitats. Species of perennial springs had larger values for visual perception. These waters are clear and larger eyes should be beneficial. But, a high PC2 value was associated with low growth rate and did not affect foraging success. We therefore conclude that investment in better sight made by perennial water species may reflect the need of avoiding predators. Development in temporary waters mainly requires rapid growth and species may not be capable to invest also in visual perception. PC1 was negatively correlated with foraging behaviour and PC3 was positively so. This indicates the importance of prey capture mode to foraging success, which may, however, not translate into a higher growth rate.

INTRODUCTION

Freshwater organisms may be classified along habitat gradients with respect to, e.g. drying out or the presence of predators (Wellborn et al. 1996; Stoks & McPeck 2003, 2006). Thus, the environmental variables range from one extreme where the distribution of organisms is limited by harsh abiotic conditions – e.g. short existence due to drying – to another where species presence is regulated mainly by biotic factors such as competition and predation (Stoks & McPeck 2003; Stoks et al. 2003)

although some abiotic factors such as low pH can naturally have a strong effect in some perennial waters. Several morphological, behavioural, and life history traits have been suggested to determine the success or failure of species, for instance in anuran tadpoles and dragonfly larvae, living in various types of aquatic habitats (e.g. Sih 1987; Wellborn et al. 1996; Relya & Werner 1999, 2000; Johansson 2000; Mikolajewski & Johansson 2004). In the following we will mainly focus on Odonata examples.

Rapid growth is a particularly crucial feature to develop successfully in temporary waters (Johansson & Suhling 2004). It has been demonstrated that the growth of species living in temporary waters is faster than that of other species of the same genus living in perennial waters. Living in predator-free habitats may also be associated with faster growth within the same genus (Suhling 2001; Stoks & McPeck 2003; Suhling et al. 2005). This implies that high growth rates might evolve as response to the habitat where a species is living rather than only being due to the phylogenetic background.

Several studies have demonstrated that the locomotory activity of dragonfly larvae differs between habitats with different types of predators (review in Johnson 1991). Species co-occurring with fish are typically sedentary whereas those occurring in lakes with only invertebrate predators are more active (Blois-Heulin et al. 1990; McPeck 1990). In species occurring in temporary waters, where large predators are normally absent, activity is high compared to perennial water species hosting large predators (Stoks & McPeck 2003; Johansson & Suhling 2004; but cf. Suhling et al. 2005).

Numerous authors have addressed the relationship between morphology and habitat. Corbet (1962: 65, 1999: 148) described that the general body shape and particularly that of the legs is associated with the larval microhabitat. Pritchard (1965) linked foraging-related morphology and behaviour, e.g. body shape of climbers and sprawlers and their prey handling, to the larval habitat. Some studies report on morphological traits influenced by the presence of predators, usually in perennial waters, such as shape of zygopteran lamellae used for swimming and length of abdominal spines operating as defence against fish attacks in anisopterans (McPeck 1995; Mikolajewski & Johansson 2004; Mikolajewski et al. 2006). In *Leucorrhinia dubia* (Vander Linden) it has been shown that an increased spine length can be induced during larval development if a fish predator is present (Arnqvist & Johansson 1998; Johansson 2002). By employing evolutionary contrast analysis Stoks et al. (2003) found that some species of different lineages in the genus *Enallagma* have evolved the ability to escape large attacking dragonfly predators by a quick swimming response using their caudal lamellae; large lamellae increase survival. This demonstrates evolution in response to predation pressure. It has also been shown that the phenotypes of species are constrained by the adaptations of their ancestors to the specific habitats in which they have evolved (McPeck 1995; Stoks et al. 2003). Richardson (2001) stated that if the ancestral phenotype has a large influence on how a lineage diversifies into new habitat types. Species from the same lineage can be expected to be more similar in phenotype than species originating from different lineages when using the same pond type. If, on the other hand, survival in a habitat requires the acquisition of a key trait, then species using that habitat type will have similar phenotypes regardless of their origin.

In this study we address the questions: (1) how are the morphological traits of dragonfly larvae, which are associated with perception and foraging, related to the larval habitat? (2) Do these traits influence foraging success and growth rate? We analysed morphological characters of pairs of species belonging to the genera *Croco-*

themis, *Orthetrum* and *Trithemis*. While *Crocothemis* species are visual/active, *Orthetrum* species are tactile/active and *Trithemis* species are visual/sedentary (Suhling et al. 2005). From each of the three genera we compared one species occurring exclusively in perennial spring-fed streams, where it had to adapt to coexistence with large invertebrate predators, with a species occurring in a wide range of different habitats, including temporary waters, which should therefore have traits that allow rapid development. Because the phenotype of a species is determined not only by adaptations to various environmental factors but also by its phylogenetic background (see above), we took the phylogenetic background into account by using the genus as a random variable. No complete phylogeny that covers our study species is available. Recent libellulid phylogenies do, however, indicate that the genera belong to three different branches of the phylogenetic tree, with *Orthetrum* and *Trithemis* being more closely related than *Crocothemis* (Ware et al. 2007; Pilgrim & von Dohlen 2008).

Brief review of the structures involved in feeding and perception

The structure and function of the body parts related to foraging and perception in dragonfly larvae are fairly well known (Tillyard 1917). Prey is detected either by vision, by tactile means or through a combination of these depending on way of life (Corbet 1999: 89). In visual predators, most ommatidia in the compound eyes are directed forward (Pritchard 1966; Sherk 1977) thus giving the most detailed field of vision directly in front of the larvae. When larvae moult new ommatidia are added from the front of the eye meaning that the smallest ommatidia are directed towards the front and the largest (and thus oldest) backwards (Sherk 1978). Sherk (1977) describes the relationship between eye specialisation (e.g. shape, size and the number of ommatidia; cf. Corbet 1999: 89 for a summary) and foraging mode in dragonflies. Four modes of foraging are distinguished, namely active, sedentary, visual and tactile, which have recently been combined to visual/active, visual/sedentary, tactile/active, and tactile/sedentary (Suhling et al. 2005). Larvae also use sensory setae, which occur on different parts of the larval body, e.g. the edge of the labium, the legs and the antennae (Tillyard 1917). The extendable labium increases the possibility for the larvae to reach prey at some distance (Tillyard 1917; Corbet 1953). The foraging mode of a species is therefore dependent on the length and the shape of its labium (Pritchard 1965). Mechanoreceptors for tactile prey detection appear as various types of setae on antennae, legs and feet, e.g. as described for *Gomphus vulgatissimus* (Linnaeus) by Müller (1995) and *Libellula depressa* Linnaeus by Rebera et al. (2004). Antennal setae are movable and will register movements in the surrounding water (Tillyard 1917). The long, thin setae on the antennae of young *Xanthocnemis zealandica* McLachlan larvae are similar to the long, thin ones on the legs, suggesting that they detect the same kind of stimuli (Rowe 1994). Winkelmann (1973) described different types of setae on the tarsi of *Sympetrum vulgatum* (Linnaeus): long, short as well as branched setae are present. But, he did not discuss different functions. Richard (1960) showed that the antennae as well as the tarsi are important for prey detection in the tactile species *Cordulia aenea* (Linnaeus), while only the eyes are of importance to the visual *Aeshna cyanea* (Müller). In *Hemicordulia tau* Selys prey contact with antennae resulted in 100% successful feeding strikes with the labium while contact with fore legs resulted in only 50% hit rate and contact with mid leg in a hit rate of only 17% (Richards & Bull 1990). Of the legs and feet the front tarsi are most sensitive, while the hind tarsi are least sensitive (Caillère 1974).

MATERIAL AND METHODS

Study species

Our study species belong to three libellulid genera that are widespread in the African tropics. Species developing in both temporary and perennial waters exist in all three genera. From each of the genera we chose one species that breeds successfully in temporary waters and another species that breeds only in perennial waters. *Crocothemis erythraea* (Brullé), *Orthetrum chrysostigma* (Burmeister), and *Trithemis kirbyi ardens* (Gerstäcker) breed in temporary waters but also to a certain extent in perennial waters (Suhling et al. 2005). Their larval development is rapid in temporary waters (Suhling et al. 2004), and we will refer to these species as temporary water species. In our study area in Namibia, *Crocothemis sanguinolenta* (Burmeister), *Orthetrum julia falsum* Longfield, and *Trithemis stictica* (Burmeister) breed exclusively in undisturbed perennial springs and streams (= perennial water species; Suhling et al. 2006), habitats strongly reminiscent of the perennial, fish free habitats defined by Wellborn et al. (1996) where large aeshnid larvae (Odonata) are the top predators; in our case mainly *Anax imperator* Leach and *A. speratus* Hagen.

Pre-experimental treatment

All six species were reared from eggs under similar conditions in the laboratory at Tsaobis (22°22'S, 15°44'E), which is situated in the Namibian semi-desert. Eggs were obtained from two to seven females per species by the method of Boehms (1971), catching females arriving at their oviposition habitat and dipping their abdomens into a jar of water. In the laboratory the egg clutches were transferred to larger containers (diameter 9 cm, height 7 cm). The larvae hatched 5-10 days after the oviposition. The temporary water species were caught at artificial ponds at Tsaobis while the perennial water species were caught at perennial springs in the Naukluft Mountains (24°16'S, 16°14'E) and the Damaraland (19°08'S, 13°49'E). Suhling et al. (2005) described the rearing methods in detail.

For all parts of this study we measured the larvae because the specimens used were not always similar in size; the average head width of the species varied between 1.46 and 1.93 mm. Larval size was measured by head width (distance between outer lateral eye margins), as this is the most reliable measurement of overall size in dragonfly larvae (Benke 1970), using an ocular-micrometer of a dissecting microscope (magnification 16x, precision 0.01 mm). We used eight larvae of all species except the two *Trithemis* (seven) and *C. sanguinolenta* (five).

Morphology

To analyse the morphology we used dragonfly larvae which were 30-36 days old but differed in size. After recording their foraging behaviour (see below), the larvae were killed and stored in 80% alcohol. Microscopic slides were made from antennae, eyes, and prementum with labial palpi and tarsi. We used a microscope fitted with a Sony digital video camera and the image analysis programme Easy Image 2000® by Bergström Instrument AB, Lund, Sweden, to obtain accurate measurements. This program gave a resolution of < 0.001 mm at a magnification of 100x and ca 0.0001 mm at 400x magnification. All size measurements are in µm unless otherwise stated. To account

for size differences in our material, all measurements were divided by the head width prior to analysis. We measured characters that may affect foraging behaviour.

Labium (100x): The major prey capture organ in odonate larvae is the labium, of which we measured the prementum and the palpi. Both were mounted flat between glasses. We measured the length of the prementum along the central line, from the tip back to the end of the cuticle at the membrane joining it to postmentum (Fig. 1a). The palpi were measured crosswise in a straight line at their widest point and the mean value of both palpi was used in the analyses (Fig. 1a). No palpus was missing or damaged.

Antennae and tarsi (200x): We took measurements from all three legs and the antennae, all of which are used for tactile perception (e.g. Pritchard 1965). We counted the number of long and short setae per tarsus. Numbers from individual specimens were given as the mean values of the left and right foot. If one foot was damaged, the measurements from the undamaged side were used in the analysis. Scapus and pedicellus of the antennae were often damaged, and as the larvae were not equal in size, the number of flagellomeres varied between specimens (and species). We therefore used the two basal flagellomeres (flagellomeres 1 and 2) for the comparison. We measured the maximum length of the individual flagellomeres, and counted the number of setae present (Fig. 1b). Size and numbers from each individual are given as the mean values of the left and right antenna. If one antenna was missing or damaged, the measurements from the undamaged side were used in the analysis.

Ommatidia (400x): When mounted on the slides, the eyes split open, often dividing into an anterior and a posterior section. We measured the diameter of the ommatidia as the mean value of between six and 25 individual lenses from three regions of the eye: close to the anterior (front) margin of the eye, at the centre of the eye (dorso-lateral) and at the posterior (rear) margin of the eye, respectively (Fig. 1c). At the three locations the number of ommatidia per square mm was estimated by counting the number present within an area of 2,500 to 10,000 μm^2 . Size and numbers from a single individual are given as the mean values of the left and right eye. If one eye was damaged, the measurements from the undamaged side were used in the analysis.

Foraging behaviour

Prior to the observations, the larvae had starved for 12 hours. The larvae were observed under a microscope (magnification 8x) in small plastic containers (diameter 3.7 cm, height 2 cm) filled with 5 ml of filtered subsurface water. Three min prior to the start of an observation, one single dragonfly larva was introduced into each container. The observation started immediately after adding 20 brine shrimp naupliae to each container. Each larva was observed for at least 3 min, or until it had captured 5 prey items. The maximum observation time was 10 min. We recorded the number of strikes and noted all successful strikes (captures). We discriminated between strikes directed in front of (at an angle of $\leq 45^\circ$ from a line between the eyes) and strikes directed to the side (46° - 120°) of the larva. We calculated the strike and capture rates per second (both lateral and frontal) the latter serving as an indicator of foraging success. We also calculated the fraction of captures per strike as a measurement of strike efficiency. Part of the raw data had been used in another paper (Suhling et al. 2005), but was re-analysed here.

Growth rates

The growth rates were derived from the study of Suhling et al. (2005), to which the reader is referred for a more detailed description. We estimated the growth rate of each species when raised together with con-specifics. The eggs were derived from egg clutches of three to seven females per species. Experiments were performed in small plastic containers (diameter 9 cm, height 7 cm), which contained fine sand as bottom substrate and several pieces of gauze as foraging supports and were filled with filtered subsurface water from the nearby ephemeral river up to 6.5 cm water level. Eight replicates were performed for each species, with the exception of *C. sanguinolenta*, in which we ran only five replicates due to shortage of eggs. Experiments started with the introduction of 10 measured individuals within 12 h after hatching, i.e. in stadium 2, into each container. We mixed for each replicate larvae of all available egg clutches (see above) to reduce potential maternal effects on growth data. Brine shrimp naupliae and protozoans were provided as food every day and we therefore assumed that food resources were ad libitum. After 11 and 22 days we counted and measured all larvae that were alive. Because cannibalism and other causes of mortality may affect the results the mortality was estimated as number of larvae missing. To obtain growth rates the head widths of the individual species from each experimental treatment were regressed against the number of days using exponential regression, $y = a(e^x)$, with a = intercept and e = the mathematical constant e (Euler's number). The exponent x is the slope and describes the daily growth rate.

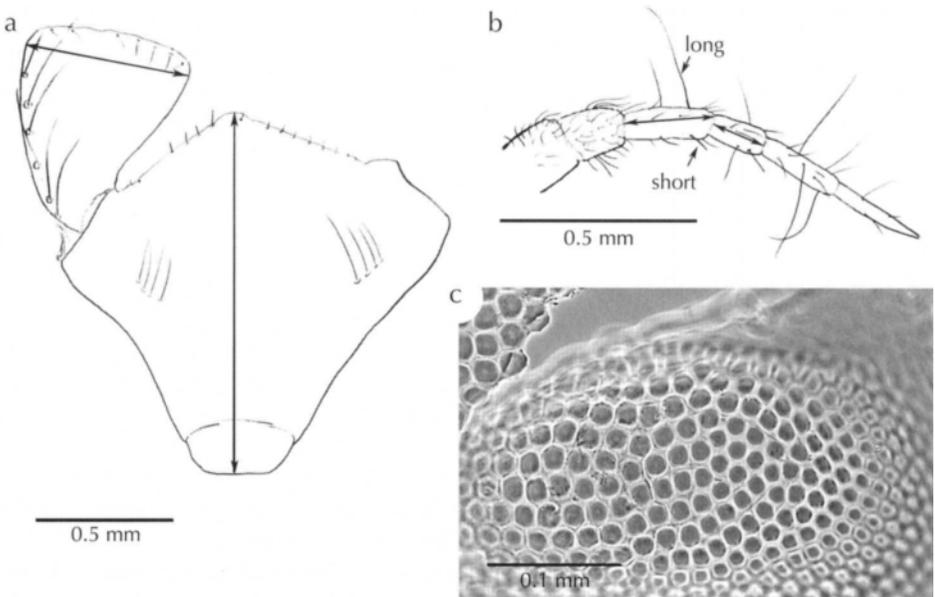


Figure 1: Explanation of morphological variables measured illustrated by *Orthetrum chryso stigma* — (a) length of prementum and width of labial palpus measured after flattening the labium between two slide glasses; (b) length of flagellomere 1 and 2 on antenna and difference between long and short setae; (c) central region of eye; note the larger ommatidia to the left (posterior side of eye) and smaller to the right (anterior side of eye).

Statistical analyses

We analysed a total of 18 morphological variables, namely prementum length, palpus width, number of long and short setae on tarsus of fore, mid and hind legs, flagellomere 1 length, number of setae on flagellomere 1, flagellomere 2 length, number of setae on flagellomere 2, anterior ommatidia size and density, dorsolateral ommatidia size and density, and posterior ommatidia size and density. All variables per individual were entered into a PCA (varimax method) to determine interrelationships between the set of variables and to reduce variables for further analyses (see below). In order to decide which factors to retain we used the scree method.

We performed ANOVAs to determine the impact of the habitat on the morphological characters with habitat type – i.e. the species occurring in perennial or temporary habitat – as fixed factor. Because no complete phylogeny is available for our study species the genus to which the species belong (*Crocothemis*, *Orthetrum*, *Trithemis*) served as a random factor to estimate the amount of variance caused by the generic origin (cf. Sokal & Rohlf 1995). The PCA scores of the factors defined by PCA served as response variables (principal components = PC).

We then used the PCs as variables to determine whether these were correlated with the other traits, namely the foraging behaviour variables recorded for each individual and the growth rate per species. As we expected that the morphological variables might also be influenced by the behavioural or growth variables we applied simple correlation analyses with Fisher's r to z test on significance. We are aware of likely collinearity between the behavioural variables. However, we were mainly interested in the correlations between the single components of behaviour as different potential measurements for foraging success and the morphological variables derived from PCA. Whereas we had data on the behavioural variables from each individual, growth data was only available per species. Thus, the replicates for the correlations between the morphological PCs and the growth rates were six in all cases.

RESULTS

Principal component analysis

The means and standard deviations of all measurements taken are shown in the appendix. The PCA revealed four major factors explaining 70.9% of the total variance (Table 1). Principal component 1 (PC1) accounted for 33.8% of the variance. It had high positive coefficients of the eigenvectors for measurements associated with the size of the labium, with size and number of setae on flagellomere 2 and for the number of ommatidia in the anterior region of the eye. High negative coefficients appeared for the size of the ommatidia of all three parts of the eye measured. Species with high values for PC1 thus had small ommatidia compared to the size of their labium. It may therefore reflect traits that are mainly interacting to promote the immediate prey capture process (i.e. PC1 = prey handling).

Principal component 2 accounted for 18.4% of the variance and had high positive coefficients for all measures of the eyes, ommatidia size as well as density. It also had high coefficients for size and setae on flagellomere 1 of the antenna, which is the part of the flagellum closest to the head. PC2 mainly reflects size and number of the ommatidia and thus a component of visual perception (PC2 = visual perception).

Principal components 3 and 4 accounted for 10.4% and 8.3% of the variance, respectively. Whereas PC3 had high positive coefficients only for the eigenvectors of the variables describing numbers of long setae on all three tarsi, PC4 had high values for the short setae on the tarsi. PC3 also had high negative eigenvector coefficients for posterior ommatidia density and anterior ommatidia size. Thus, PC3 (= density of long setae) and PC4 (= density of short setae) reflected different aspects of tactile reception, particularly towards the substrate and lateral to the larva.

Effect of habitat on morphological characteristics

We used the principal components derived from the morphological variables for further analyses. ANOVAs revealed that PC1 (prey handling), PC2 (visual perception) and PC4 (short setae) differed significantly between the habitat types (Fig. 2). Particularly in PC2 the difference between habitat types was striking, with all perennial water species having similar positive values and all temporary water species similar negative values. This derived mainly from the larger ommatidia of the perennial water species in all parts of the eye, whereas the density of ommatidia was not always greater in the perennial water species.

The genus contributed significantly to the variance in PC1, PC3 (long setae) and PC4 (Fig. 2). The prey handling values (PC1) differed distinctly between the genera, being negative in *Crocothemis*, about 0 in *Orthetrum* and positive in *Trithemis*. PC3 values were negative in *Crocothemis* and *Trithemis*, and positive in *Orthetrum*, with much higher number of long setae on all three legs in *Orthetrum*.

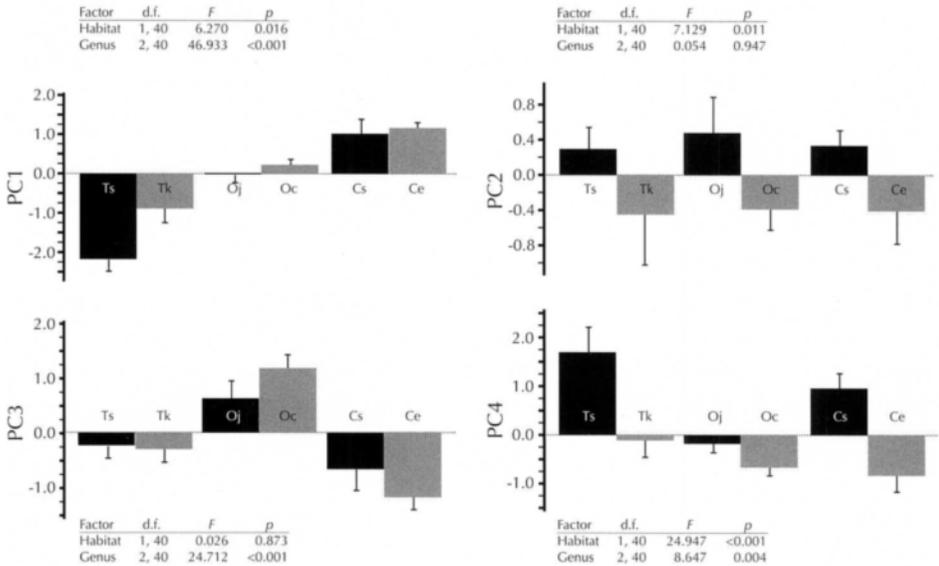


Figure 2: Principal components of morphological features per genus and habitat type. The mean values ± s.e. are depicted. Perennial water species are black bars, temporary water species are shaded bars. Please note the different scales for PC1-PC4. Ce: *Crocothemis erythraea*, Cs: *C. sanguinolenta*, Oc: *Orthetrum chrysostigma*, Oj: *O. julia*, Tk: *Trithemis kirbyi*, Ts: *T. stictica*; n = 8 except the two *Trithemis* (n = 7) and *C. sanguinolenta* (n = 5).

Table 1. Results of principal component analysis on morphological characters of the larvae of the six libellulid species. Bold digits indicate high eigenvector values.

		PC1	PC2	PC3	PC4
Eigenvalue		6.092	3.307	1.871	1.488
Variance		0.338	0.184	0.104	0.083
Cum. variance		0.338	0.522	0.626	0.709
Eigenvectors					
Labium					
Prementum	length	0.858	0.066	0.025	-0.192
Palpus	width	0.800	0.126	0.129	0.022
Antenna					
Flagellomere 1	length	-0.027	0.763	0.109	-0.188
	# setae	-0.114	0.765	-0.228	0.000
Flagellomere 2	length	0.589	0.107	0.109	0.303
	# setae	0.798	0.219	0.224	-0.240
Eye					
Anterior	# ommatidia	0.537	0.477	-0.585	0.252
	ommatidia size	-0.855	0.472	0.096	-0.099
Dorsolateral	# ommatidia	0.121	0.697	-0.131	0.406
	ommatidia size	-0.958	0.365	0.075	0.031
Posterior	# ommatidia	-0.160	0.771	0.099	0.298
	ommatidia size	-0.441	0.527	-0.486	0.056
Tarsus					
Fore	# long setae	0.503	0.000	0.505	-0.161
Mid	# long setae	-0.002	-0.008	0.829	0.070
Hind	# long setae	0.029	-0.076	0.805	0.027
Fore	# short setae	-0.302	0.033	0.421	0.669
Mid	# short setae	0.052	0.083	-0.317	0.768
Hind	# short setae	-0.239	-0.045	0.155	0.947

Table 2. Descriptive statistics of the behavioural recordings of six libellulid dragonfly species. Ce: *Crocothemis erythraea*, Cs: *C. sanguinolenta*, Oc: *Orthetrum chrysostigma*, Oj: *O. julia*, Tk: *Trithemis kirbyi*, Ts: *T. stictica*.

Behavioural trait	n	<i>Crocothemis</i>		<i>Orthetrum</i>		<i>Trithemis</i>	
		Ce	Cs	Oc	Oj	Tk	Ts
		8	7	8	8	8	7
Frontal							
Strikes min ⁻¹	Mean	1.98	1.56	2.58	1.44	1.14	0.78
	s.d.	0.72	0.66	1.02	0.66	1.50	0.60
Captures min ⁻¹	Mean	1.50	1.26	1.80	1.08	1.02	0.54
	s.d.	0.48	0.54	0.84	0.48	1.26	0.36
Captures strike ⁻¹	Mean	0.79	0.83	0.71	0.80	0.83	0.64
	s.d.	0.19	0.19	0.16	0.19	0.35	0.32
Lateral							
Strikes min ⁻¹	Mean	1.68	0.96	0.72	1.08	0	0
	s.d.	1.98	0.9	0.36	0.78	0	0
Captures min ⁻¹	Mean	0.96	0.78	0.54	0.96	0	0
	s.d.	1.02	0.78	0.42	0.72	0	0
Captures strike ⁻¹	Mean	0.69	0.64	0.74	0.85	0	0
	s.d.	0.22	0.44	0.40	0.19	0	0

Correlation between morphology and behaviour

The means \pm s.d. for the behavioural traits are presented in Table 2. PC1 and PC3 were significantly correlated with some of the behavioural traits (Table 3). Whereas PC1 was negatively correlated with the efficiency of prey capture (captures per strike) regardless of whether the strikes were frontal or lateral, PC3 correlated positively with frontal captures per minute, lateral strikes per minute and capture efficiency of frontal strikes. PC2 and PC4, which both varied clearly with habitat, did not correlate with any of the recorded behavioural traits.

Correlations between morphology and growth rate

Growth rates were only available per species, not for every larva used in our other experiments (see above). Thus, we plotted means of the morphology PCs against the growth rates per species (Fig. 3). A particularly clear pattern appeared when plotting PC2 (visual perception) against growth rate, showing that all species with a high growth rate – i.e. the temporary water species – had clearly negative PC2 values, whereas those with a lower growth rate – i.e. the perennial water species – had clearly positive PC2 values. We did not perform a correlation for this pattern since the data were obviously not distributed along a linear function. PC4 was negatively correlated with growth rate, i.e. the number of short tarsal setae was higher in species that grew slower ($r = -0.974$, $p < 0.001$). The plot of PC1 (prey handling) vs growth rate depicted that in all three genera the species with the higher PC1 value – i.e. the temporary water species – also had a higher growth rate.

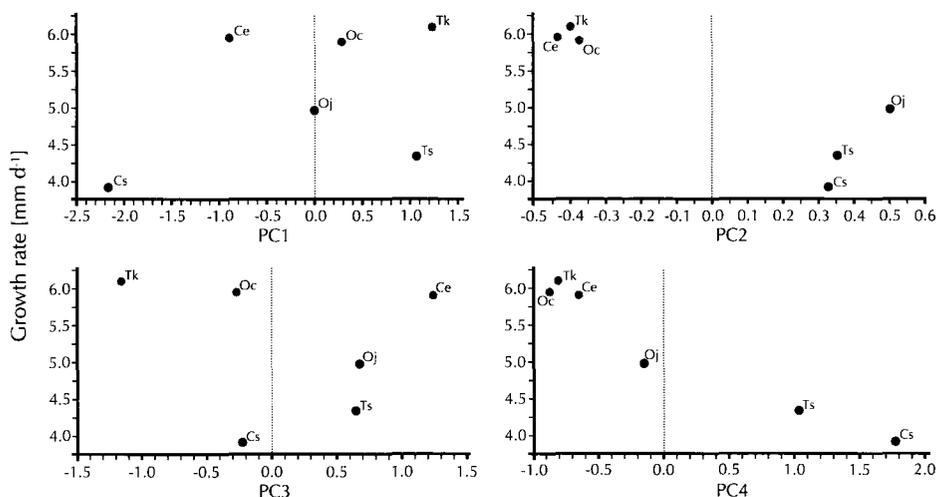


Figure 3: Scatterplots of the four principal components of morphological features vs growth rates per species (derived from Suhling et al. 2005). Black dots represent perennial water species, shaded dots temporary water species. Ce: *Crocothemis erythraea*, Cs: *C. sanguinolenta*, Oc: *Orthetrum chrysostigma*, Oj: *O. julia*, Tk: *Trithemis kirbyi*, Ts: *T. stictica*; $n = 8$ except the two *Trithemis* ($n = 7$) and *C. sanguinolenta* ($n = 5$).

Table 3. Correlations (r -values) between the principal components derived from morphological characters and behavioural traits. Significant ($p < 0.05$) correlations are in bold type.

Behavioural trait	PC1	PC2	PC3	PC4
Frontal				
Strikes min ⁻¹	-0.258	-0.241	0.250	0.033
Captures min ⁻¹	-0.275	-0.162	0.361	0.013
Captures strike ⁻¹	-0.394	0.107	0.451	-0.146
Lateral				
Strikes min ⁻¹	-0.117	-0.256	0.306	-0.056
Captures min ⁻¹	-0.148	-0.235	0.263	-0.047
Captures strike ⁻¹	-0.324	0.125	0.159	-0.056

DISCUSSION

Our results reveal that the morphological characters summarised under the principal components PC1 (prey handling), PC2 (visual perception) and PC4 (short tarsal setae) vary with the type of habitat the larvae of the species occupy. Phylogenetically, these morphological traits have probably evolved independently because they can be found in each of three different clades of the Libellulidae (cf. Ware et al. 2007; Pilgrim & von Dohlen 2008). We cannot say which ones are the derived and which are the more ancestral characters because the phylogenies available do not allow any such analyses. Our results do, however, suggest a kind of standard scenario for evolutionary changes in response to temporary versus permanent waters.

We found that the morphological characters associated with visual perception (PC2) were especially closely linked to the type of habitat a species occupies. At least in the three libellulid genera studied, they were independent of the phylogenetic relationship. We deduce that species with high PC2 values have good vision and those with small values have reduced visual perception, in line with the characteristics for visual vs. tactile species defined by Sherk (1977). Species living in perennial habitats had high visual perception values, whereas those living in temporary waters had low values. The fact that vision seems less important than tactile sensory organs in temporary waters is easily explained, since visibility tends to be rather low in the turbid conditions of temporary waters (cf. Sherk 1977; Suhling et al. 2005). But how do we explain the good vision of the perennial water species? Initially, we inferred that good vision might be associated with foraging. The perennial waters are clear and larger eyes should be beneficial to prey detection, but our data show to the contrary: high values of PC2 (visual perception) had no positive impact on foraging success and were even associated with a slower growth rate. Thus, we conclude that another factor associated with perennial waters may have caused the investment in better visual perception. Good visual perception in perennial water species may be beneficial to the detection of large and mobile predators (e.g. fish). It has been shown that also other sensory organs in odonate larvae have developed to recognise predators, i.e. the perception of chemical cues (Chivers et al. 1996; Koperski 1997; Wisenden et al. 1997). Thus, good vision and resulting morphological alteration in the eyes may

mainly reflect the need of avoiding large predators, since the costs of lethal and even sublethal damages caused by predators are higher than that of slow growth (McPeck 2004; Stoks et al. 2005). Development in temporary waters, on the other hand, requires rapid growth, and since the energy available is limited, species may not be able to invest also in good visual perception. Due to the rarity of larger predators in their habitat the need of good vision may also be limited.

We also found that the morphology of organs used in the immediate foraging process vary with the type of habitat, but the differences are not so pronounced as they are probably somewhat obscured by the high variability caused by the phylogenetic background. The temporary water species within each genus did, however, have higher PC1 values than their respective perennial water con-generics (Fig. 3) and thus invested more in the direct foraging organs, e.g. the labium. This observation corroborates our conclusion that temporary water species may have a greater need, or ability, to invest in their foraging organs. Although we did not find a direct hint that this translates into greater foraging success, we found that all temporary water species had higher growth rates than their perennial water con-generics (see also Suhling et al. 2005).

PC4 (short tarsal setae) is also clearly associated with habitat type, with values being higher in perennial water species. As for long setae (below), multiple uses are possible, ranging from providing attachment surfaces for mud, detritus and periphyte algae to cleaning, grooming and increasing the grip as suggested in some tropical species (Sahlén & Hedström 2005). The presence of a row of setae does, of course, increase the stability of the cuticle, and the presence or absence of setae might also be due both to evolutionary history and the behaviour of the adult dragonfly, where tarsal setae are involved in prey capture and resting (cf. Corbet 1999: 357). We deem camouflage to be the most likely cause behind the high number of short setae in perennial water species, but the pattern is far from clear; cf. below.

The number of long setae on the tarsi (PC3) is obviously genus-specific and does not vary with the type of habitat occupied by the species. Thus, it may have derived earlier in the evolution of each genus, particularly in *Orthetrum* which are tactile predators (Suhling et al. 2005) and hence by definition should have long setae. *Orthetrum* larvae are typical bottom-dwellers, often covering themselves with mud in order to camouflage (Corbet 1999: 153-154). The long setae may help in keeping mud and debris attached to the body (cf. above). This character may therefore be an antipredation trait associated with the bottom dwelling life style of the larvae developed at an earlier evolutionary stage. *Trithemis* larvae, which are also bottom dwellers, seem to use other tactics, viz. a sedentary life style (Suhling et al. 2005) and dark dorsal markings on the abdomen (e.g. Carchini et al. 1992) to avoid predation. Naturally, long setae are also better than short ones for detecting movements in water, as shown by Rowe (1994); see also Gaino & Reborá (2001) and Reborá et al. (2004). Improved camouflage may therefore not be the major function of these structures, although they will naturally be used also for that purpose. Evolutionary history might have established these long, prey detecting setae on the antennae of bottom dwelling *Orthetrum* larvae living in a dark and perhaps muddy environment.

Growth rate is determined by a variety of variables including foraging activity (cf. review by Johnson 1991). Physiological processes where enzyme systems and other physiological pathways in different lineages have been optimised for faster growth

and development may, however, play a more crucial role (Arendt 1997; Stoks et al. 2005). It has been shown that differences in growth rate derive from different rates of ingestion of food into own biomass (McPeck 2004). Our results on growth rate versus morphology imply that investments in good vision and/or other sensory organs do not necessarily translate into rapid growth. As shown in Table 3, it may also not translate into a higher prey capture rate. Investments in sensory organs, which may be necessary to spot predators, may in fact reduce the growth rate. Because the resources and the energy available are limited, all traits associated with a certain part of the permanence continuum cannot be optimised simultaneously, which may lead to trade-offs. Particularly the eyes are relatively large organs in Odonata larvae, the development and metabolism of which may require a large part of the energy available. It would be inspiring to investigate whether or not the eyes of a given species grow larger in the presence of predators, as demonstrated in the case of defence organs (i.e. spines; Arnqvist & Johansson 1998).

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Appendix. Descriptive statistics of the morphological measurements of six libellulid dragonfly species. All measurements are divided by head width. Ce: *Crocothemis erythraea*, Cs: *C. sanguinolenta*, Oc: *Orthetrum chrysostigma*, Oj: *O. julia*, Tk: *Trithemis kirbyi*, Ts: *T. stictica*.

Morphological character		n	Crocothemis		Orthetrum		Trithemis	
			Ce	Cs	Oc	Oj	Tk	Ts
Labium								
Prementum length	[µm]	Mean	0.577	0.581	0.762	0.719	0.813	0.806
		s.d.	0.158	0.084	0.075	0.115	0.048	0.062
Palpus width	[µm]	Mean	0.306	0.294	0.358	0.345	0.345	0.394
		s.d.	0.024	0.014	0.02	0.023	0.031	0.027
Antenna								
Flagellomere 1 length	[µm]	Mean	0.123	0.116	0.124	0.134	0.109	0.146
		s.d.	0.048	0.017	0.011	0.012	0.008	0.009
Flagellomere 1 setae	#	Mean	0.009	0.011	0.008	0.01	0.009	0.012
		s.d.	0.007	0.002	0.001	0.002	0.002	0.002
Flagellomere 2 length	[µm]	Mean	0.079	0.078	0.101	0.104	0.088	0.130
		s.d.	0.019	0.029	0.009	0.006	0.006	0.011
Flagellomere 2 setae	#	Mean	0.007	0.004	0.014	0.015	0.012	0.016
		s.d.	0.002	0.002	0.002	0.004	0.003	0.002
Eye								
Anterior ommatidia	[# mm ²]	Mean	4.044	4.214	3.128	3.217	2.756	1.866
		s.d.	1.190	0.334	0.703	1.141	0.733	0.434
Anterior ommatidia size	[µm]	Mean	0.005	0.005	0.005	0.006	0.006	0.008
		s.d.	0.001	0.002	0.001	0.002	0.001	0.001
Dorsolateral ommatidia	[# mm ²]	Mean	2.896	3.121	2.178	2.171	1.951	1.579
		s.d.	0.763	0.287	0.528	0.182	0.614	0.279
Dorsolateral ommatidia size	[µm]	Mean	0.007	0.009	0.007	0.009	0.007	0.009
		s.d.	0.001	0.001	0.001	0.001	0.001	0.001
Posterior ommatidia	[# mm ²]	Mean	1.650	1.967	1.171	1.394	1.707	1.420
		s.d.	0.351	0.145	0.307	0.329	0.438	0.226
Posterior ommatidia size	[µm]	Mean	0.008	0.011	0.009	0.011	0.008	0.010
		s.d.	0.001	0.001	0.001	0.002	0.001	0.001
Tarsus								
Fore long setae	#	Mean	0.014	0.021	0.033	0.025	0.021	0.026
		s.d.	0.004	0.006	0.003	0.004	0.003	0.005
Mid long setae	#	Mean	0.004	0.003	0.011	0.009	0.003	0.006
		s.d.	0.002	0.001	0.005	0.004	0.003	0.004
Hind long setae	#	Mean	0.005	0.004	0.010	0.010	0.005	0.007
		s.d.	0.002	0.002	0.005	0.004	0.004	0.005
Fore short setae	#	Mean	0.006	0.011	0.009	0.009	0.006	0.012
		s.d.	0.002	0.004	0.002	0.003	0.002	0.004
Mid short setae	#	Mean	0.004	0.005	0.002	0.004	0.003	0.010
		s.d.	0.002	0.003	0.001	0.001	0.003	0.003
Hind short setae	#	Mean	0.007	0.009	0.008	0.008	0.006	0.012
		s.d.	0.004	0.005	0.003	0.003	0.005	0.005