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Short review

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ADAPTATIONS OF PUPAE OF CHIRONOMIDAE (INSECTA: DIPTERA) TO OXYGEN-POOR HABITATS

ABSTRACT: Morphological, behavioural and physiological adaptations of chironomid pupae to oxygen-poor habitats, such as the profundal zone of lakes, are discussed. Lentic and oxy-regulator species possess developed respiratory organs (thoracic horns) and an extensive anal fringe to perform respiratory movements, whereas in rheophilic and oxy-conformer species these structures are reduced or absent. This led to the hypothesis that the number of thoracic horn branches, the number of anal macrosetae and the body size could constitute a measure of a species' oxygen requirements. These characters were analyzed in 12 species groups collected in 39 Italian lakes. Numbers were correlated to the optimum value of oxygen level, total phosphorus concentration and transparency calculated for each taxon. Taxa with plumose thoracic horns, extensive anal fringe and large body size (*Chironomus*) inhabited habitats poorer in oxygen than those inhabited by taxa with less extensive respiratory surface and fringe and small body size (*Paratendipes*, *Polypedilum*, *Microtendipes*). *Stictochironomus* was less tolerant than expected. Further studies are needed into the physiological adaptations of chironomid pupae.

KEY WORDS: lakes, morphology, autoecology, oxygen depletion, biomonitoring, thoracic horn

1. INTRODUCTION

Chironomids are widespread dipteran insects characterized by high species richness (Ashe *et al.* 1987). The immature stages are able to colonize almost every type of freshwater habitat, from streams and rivers to lakes, pools and bogs, exhibiting a great physiological tolerance to abiotic factors (temperature, pH, organic matter, oxygen – Pinder 1995). For these reasons, chironomids have been incorporated into a variety of biomonitoring programmes (Rosenberg 1992).

Conversely, the pupal stage has been somewhat neglected in earlier studies. Compared to the larva, the pupa is short-lived, rarely exceeding 72 hours (Langton 1989, Cranston 1995, Langton 1995). Nevertheless, pupae possess a variety of morphological characters which permit identification as species, even where the larvae are inseparable (Coffman 1979, Langton 1989).

The pupal structure must be highly functional, adapting rapidly to the larval evolutionary progress, in the absence of traditional selection forces. Some pupae are free-living (Tanypodinae), while most live in tubes or covering films produced by the final instar larva. In the latter case, they inhabit the same

microhabitat as the larva, and cope with the same environmental conditions (Langton 1989, 1995).

The collection of pupal exuviae for ecological studies, first proposed by Thienemann (1910), is an effective method to collect species assemblages rapidly at meso-habitat level. It was therefore proposed by Ruse (2002) as a suitable method for bio-monitoring lakes.

The profundal zone of lentic habitats, in particular, is critical in determining lake status (Wiederholm 1980, Lindegaard 1995, Ruse 2002). Since pupae do not feed, oxygen level (hypoxic/anoxic periods) is the main factor determining their survival until emergence.

Survival of species in oxygen-poor habitats involves morphological, physiological or behavioural adaptations, occurring simultaneously to determine a species' microhabitat (Int Panis *et al.* 1995).

The aim of this work is to give a short review of chironomid pupal adaptations to the profundal zone of lakes with preliminary observations from Italian lakes.

1.1 Morphological adaptations

Pupae absorb oxygen through the cuticle, but many species possess a pair of specialized respiratory organs (thoracic horns), which are an extension of the surface area and can

be directly connected to the adult tracheal system (Coffman 1979, Langton 1995). The thoracic horns in free-living species have an apical respiratory surface (plastron plate), which makes direct contact with the aerial environment at the water surface (Podonominae, some Tanypodinae). In the absence of a plastron plate, oxygen is absorbed from the water through the whole surface of the horns, which act as gills.

Tubicolous species colonizing lentic habitats are characterized by multifilamentous and plumose horns (Chironominae), whereas in rheophilic and oxy-conformer species these structures are reduced or even absent (Orthoclaadiinae) (Langton 1995) (Table 1). Thus, the thoracic horn surface has been suggested as a measure of a species' oxygen requirements (Thienemann 1954, Coffman 1979, Langton 1989).

The pupal posterior segment is modified in an anal lobe which, in free-living species, plays a role in swimming. In tubicolous taxa, however, it facilitates undulatory movements that propel respiratory current through the tube (Cranston 1995). Lotic species possess only a few strong macrosetae on anal lobes, which are used for hanging and to avoid drift. In tubicolous species, the fringe is more fully developed, making respiratory movements more effective (Table 1). The extent of setae, therefore, may be an indicator of oxygen concentra-

Table 1. Scheme of the morphological, behavioural and physiological adaptations of lacustrine chironomid pupae, according to Wiederholm (1986) and Langton (1995).

Sub-family	Thoracic horn	Anal setae	Behaviour	Haemoglobin
Tanypodinae	Present with horn sac and usually plastron	A few anal macrosetae or adhesive sheaths	Free-living	Present in some taxa
Podonominae	Present with horn sac and usually plastron	A few macrosetae	Free-living	Absent
Diamesinae	Absent or present without horn sac or plastron, simple	A few macrosetae	Tubicolous	Absent
Prodiamesinae	Present with indirect connection to the adult spiracle and without horn sac or plastron, simple	Fringe absent or present, with 3–5 distinctive macrosetae	Tubicolous	Absent
Orthoclaadiinae	Absent or present without connection to the adult spiracle, without horn sac or plastron, simple	Fringe absent or present, with usually 0–3 distinctive macrosetae	Tubicolous	Absent
Chironominae	Present without horn sac or plastron, plumose or multifilamentous	Fringe present without distinctive macrosetae	Tubicolous	Present

tion in the habitat where the species develops (Langton 1995).

The greatest development of thoracic horn and anal fringe occurs in *Chironomus* species, typically found in habitats with the lowest oxygen levels. Apparent exceptions, such as riverine pupae with an anal fringe (e.g. *Rheocricotopus*), are tubiculous inhabitants of pools and slow current zones. Lacustrine pupae without a fringe (e.g. *Cricotopus*) are colonizers of littoral zones with intense water movement. In *Thienemanniella* and *Corynoneura*, the extensive anal fringe appears to compensate for the lost thoracic horn (Langton 1995).

1.2. Behavioural adaptations

Species living in standing waters perform undulatory movements of the abdomen to drive water through the silken tube; their large body size contributes to the effectiveness of these undulations (Langton 1989, Int Panis *et al.* 1996).

Species living in well-oxygenated habitats show increased activity at a higher oxygen concentration than taxa living in hypoxic conditions (Heinis and Crommentuijn 1989, Langton 1995). In extreme oxygen depletion, *Chironomus* pupae may vacate their tubes completely and undulate the body freely in the water (Langton 1995). During anoxic periods, larvae of less tolerant species (e.g. *Tanytarsus* sp.) may migrate toward the littoral zone before moulting (Heinis *et al.* 1989).

1.3. Physiological adaptations

Profundal species, both at larval and pupal stages, possess high oxygen-affinity haemoglobin dissolved in the haemolymph (Table 1). Body size is correlated with the organism's glycogen store, which can be exploited in anaerobic conditions (Hamburger *et al.* 2000).

The relationship between vertical micro-distribution and adaptations to oxygen stress, studied by Int Panis *et al.* (1996) in littoral chironomids, revealed that larger species and individuals with higher haemoglobin concentration (e.g. *Chironomus plumosus* gr.) generally occur deeper in the sediment than others (e.g. *Tanytarsus* sp.).

Such taxa (e.g. *Chironomus*, *Dicrotendipes*, *Procladius*) have a high oxy-regulatory capacity due to their ability to maintain high oxygen uptake with decreasing concentrations of environmental oxygen. They are able to survive for several months without oxygen by switching to anaerobic metabolism, combined with dormancy (Lindegaard 1995, Brodersen *et al.* 2004).

On the other hand, taxa without oxygen storage devices (e.g. *Stictochironomus*, *Micropsectra*, *Hydrobaenus*) must switch over to anaerobic pathways, despite the availability of low residual environmental oxygen concentration (Mangum and Van Winkle 1973, Heinis and Crommentuijn 1989).

2. OBSERVATIONS FROM ITALIAN LAKES

Benthic macroinvertebrates were collected from 39 Italian lakes by different authors, using Ponar and Petersen grabs (database L.I.M.N.O. at <http://www.ise.cnr.it/limno/>; our database at <http://users.unimi.it/~roma1999/rossaro.html>). Physical and chemical variables were measured at the sampling depth: water temperature, transparency, percentage of oxygen saturation, conductivity, pH, alkalinity, phosphorus (TP and P-PO₄) and nitrogen (N-NO₃). Chironomid larvae were identified at genus or species group level. These data were used to calculate the optimum value of oxygen saturation, TP and transparency for each species group, as a mean value of environmental variables weighted for taxa abundance (Table 2) (B. Rossaro – unpublished, Marziali *et al.* in press).

Pupal exuviae were collected in 17 prealpine lakes in 2004 using a Brundin net along the shore. Exuviae were mounted on slides and identified at species level. The 20 most abundant species were analyzed. All belonged to the tribe Chironomini. Numbers of horn branches and anal macrosetae were counted and body size was measured (Table 3). These characters were correlated with the physical and chemical optima calculated for each species group (12 in all).

In all, 262 chironomid species were identified as pupal exuviae. The percentage of hypolimnetic oxygen saturation was the main

Table 2. Optimum value of percent hypolimnetic oxygen saturation, TP and transparency for each species group, calculated as mean of environmental variables weighted for taxa abundance.

Taxa	Oxygen saturation (%)	Total phosphorus ($\mu\text{g L}^{-1}$)	Transparency (m)
<i>Chironomus anthracinus</i> gr.	84.0	15.4	4.1
<i>Chironomus plumosus</i> gr.	77.7	47.9	2.3
<i>Cladopelma</i>	95.5	18.4	4.3
<i>Cryptochironomus</i>	98.0	15.5	4.7
<i>Demicryptochironomus</i>	93.2	11.6	3.4
<i>Dicrotendipes</i>	92.7	16.1	4.5
<i>Endochironomus</i>	100.1	24.0	6.2
<i>Microtendipes</i>	93.6	13.5	5.9
<i>Paracladopelma</i>	81.9	11.3	4.4
<i>Paratendipes</i>	103.6	22.0	6.3
<i>Polypedilum</i>	103.2	18.2	5.9
<i>Stictochironomus</i>	109.5	25.1	7.0

Table 3. Mean value of number of thoracic horn branches, number of anal macrosetae and abdomen length for the 12 taxa considered.

Taxa	Thoracic horn branches	Abdomen length (μm)	Anal macrosetae
<i>Chironomus anthracinus</i> gr.	147	8044.6	113
<i>Chironomus plumosus</i> gr.	146	9970.8	160
<i>Cladopelma</i>	60	3529.7	53
<i>Cryptochironomus</i>	122	5824.3	70
<i>Demicryptochironomus</i>	108	7037.9	121
<i>Dicrotendipes</i>	65	5605.6	51
<i>Endochironomus</i>	38	6297.0	128
<i>Microtendipes</i>	6	5807.7	51
<i>Paracladopelma</i>	61	4645.0	59
<i>Paratendipes</i>	8	3842.6	40
<i>Polypedilum</i>	9	4623.7	35
<i>Stictochironomus</i>	117	5858.0	48

factor determining species assemblage in lakes of similar size (volume and depth). It was inversely correlated with TP.

Morphological characters proved to be very different between species. The number of horn branches varied from 6 in *Microtendipes pedellus* (De Geer 1776) to 225 in *Chironomus riparius* Meigen 1804 (Fig. 1). The anal fringe varied from 35 macrosetae in *Polypedilum nubeculosum* (Meigen 1804) to 185 in *Glyptotendipes pallens* (Meigen 1804). Abdomen length (body size) varied from 3.12 mm in *Cladopelma bicarinatum* (Brundin 1947) to 8.04 mm in *C. anthracinus* Zetterstedt 1860.

Mean values for morphological characters were calculated for each species group

and were correlated with weighted means for each species group (Table 4). Morphological characters were correlated positively: taxa with larger size had more branches and anal macrosetae. These relationships were considered to support strongly the hypothesis of a coevolution of these structures to cope with extreme environmental conditions, such as oxygen depletion in the profundal zone of lakes (Mangum and Van Winkle 1973, Langton 1995).

The number of horn branches was negatively correlated with transparency; the number of anal macrosetae was negatively correlated with percentage of oxygen saturation and transparency; body size was negatively correlated with percentage of oxygen saturation

Table 4. Correlation coefficient between morphological characters and weighted means of environmental variables. In bold: significant correlations: ** = $P < 0.01$; * = $P < 0.05$.

Species group morphological characters	Thoracic horn branches	Anal macrosetae	Abdomen length
Anal macrosetae	0.68*	-	-
Abdomen length	0.73**	0.86**	-
Species group optima			
Percent hypolimnetic oxygen saturation	-0.43	-0.56*	-0.57*
Total phosphorus	0.32	0.52	0.56*
Transparency	-0.59*	-0.62*	-0.58*

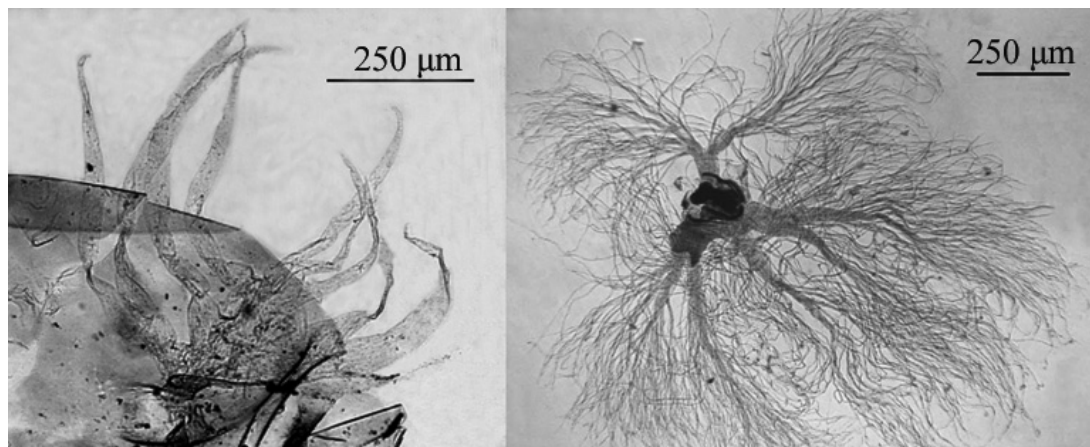


Fig. 1. Thoracic horn of *Polypedilum nubeculosum* with 10 branches (left) and *Chironomus plumosus* with 140 branches (right).

tion and transparency and positively with TP (Table 4).

In general, taxa with high numbers of horn filaments and anal setae and large body size (*Chironomus plumosus* gr., *Chironomus anthracinus* gr.) were more resistant to oxygen depletion than others (*Paratendipes*, *Polypedilum*, *Microtendipes*). Some exceptions were detected. *Stictochironomus*, for example, was less tolerant than expected, although it is known to occur in deep sediment layers. Int Panis *et al.* (1995) hypothesized that it may migrate between the sediment surface, where it stores oxygen via hemoglobin, and the deeper sediment layer, where it seeks its food supply and protection (Heinis and Crommentuijn 1989).

3. CONCLUSIONS

The most oxygen-tolerant taxa were characterized by large respiratory surface and high undulatory efficiency, due to the extensive fringe of anal macrosetae and the large body size (Langton 1995). Several exceptions require further study. In particular, the physiological devices used by species to cope with extreme conditions have scarcely been investigated. For example, haemoglobin content and enzyme activity are unknown for most species (Berra *et al.* 2004).

These results suggest a phylogenetic trend toward increasing regulation of aerobic metabolism in response to declining environmental levels, as animals acquire

oxygen storage devices (Mangum and Van Winkle 1973). With further investigation and understanding, this knowledge could constitute the basis for a biomonitoring programme for lentic waters, based on the pupal stage.

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