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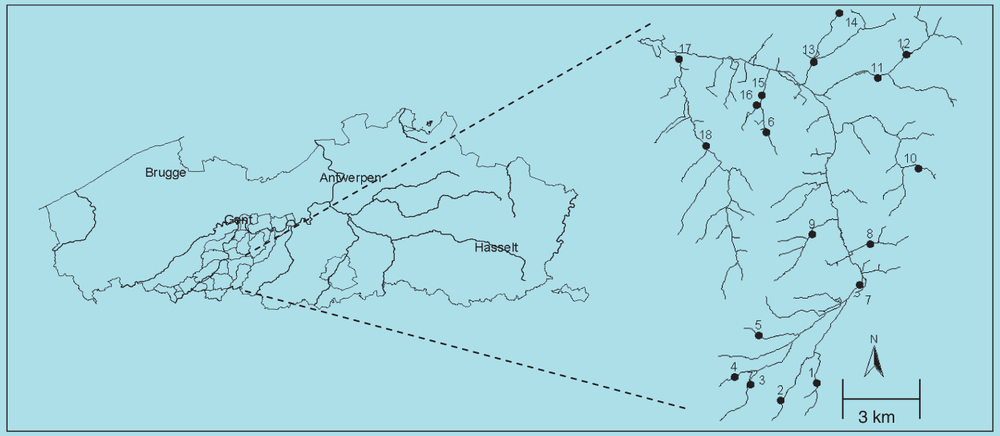

BELGIAN JOURNAL OF ZOOLOGY

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ERRATUM

Biogeographical observations on four scolytids (Coleoptera, Scolytidae) and one lymexylonid (Coleoptera, Lymexylonidae) in Wallonia (Southern Belgium)

Jean-Marc Henin[1], **Olivier Huart and Jacques Rondeux**

[1] **Unité de Gestion et Economie forestières, Faculté universitaire des Sciences agronomiques, Passage des Déportés 2, B-5030 Gembloux, Belgium**

Corresponding author : Jean-Marc Henin, e-mail : henin.jm@fsagx.ac.be

In the introduction, end of the third paragraph :

Although not easily quantifiable, the financial losses for private and public forest owners undoubtedly exceeded 50 million m^3 .**should read**; 50 million EUR.

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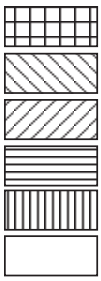
The symbols for the natural region of Wallonia do not appear. They are reproduced here :



Sandy loam region
Loess region
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Henin, J.-M., O. Huart, P. Lejeune, J. Rondeux (2003). Qualitative survey of five beech damaging Coleoptera (Scolytidae & Lymexylonidae) in Wallonia (Southern Belgium). In: McManus & Liebhold (ed.) : *Ecology, survey and management of forest insects*. Proceedings of the IUFRO WP 7.03.06, 7.03.07, 7.03.10 Meeting, 2-5 September 2002, Krakow, Poland. Gen.Tech. Rep. NE-311, Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 178 p.

Belg. J. Zool., 134 (1) : 5-8 January 2004

Winter microhabitat distribution of coots (*Fulica atra* L.1758) on gravel-pit wetlands in the Garonne river floodplain, Southwest France

Frédéric Santoul and Sylvain Mastrorillo

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ABSTRACT. Human population growth, urbanisation and conversion of land to agriculture have led to loss of natural wetlands throughout the World, making artificial wetlands such as gravel pits, dam lakes or rice fields important for waterbirds. In south-west France, the increasing abundance of gravel pits has allowed several bird species to colonize the region. These "new" wetlands have become substitutes for the natural habitats of waterbirds. Coots (*Fulica atra* L. 1758) colonised the Midi-Pyrénées area when gravel pits were created in the 1970's. Coot populations were censused weekly from October 1996 to February 1997 and from October 1997 to February 1998 on three gravel pits near Toulouse, SW France. Each winter, the number of coots was recorded on each gravel pit, and the microhabitat used by coots identified according to environmental variables (water depth, bank slope, bank vegetation, vegetation between watermarks, macrophytes, human disturbance, zones of open water or near the bank). Open water, which constitutes a secure habitat for this species during the wintering period, abundance of macrophytes (Characea) and presence of lawn on the bank appear as the most important factors influencing coot distribution. This information will be useful for site acquisition for nature conservation and management purposes.

KEY WORDS : Coots, *Fulica atra*, gravel pits, Garonne basin, habitat requirement, winter.

INTRODUCTION

Human activities destroy natural wetlands, but also create artificial wetlands such as rice fields, gravel pits, and dam lakes. These "new" wetlands have become substitutes for wildlife habitat (Bell et al., 1997; Fasola & Ruiz, 1997; Mori, 2001). The biological value of gravel pits, particularly for waterbirds, is increasingly recognised. Several studies and actions have been undertaken to protect and manage these artificial ecosystems (Keywood & Melluish, 1953; Olney, 1964; Milne, 1974; Andrews, 1991; Philips, 1992). However, few reports have been published on the ecology of waterbirds in French gravel pits (Frochot & Godreau, 1995; Santoul & Tourenq, 2002). Gravel extractions on the floodplains in France have increased over the last 25 years. Today, 45 gravel pits are active in the Garonne basin near Toulouse, southwest France, representing a water area of 2000 ha. Populations of waterbirds have increased significantly in the region over the last few years, especially during winter. Creation and development of gravel pits have attracted numerous species of birds, of which the coot (*Fulica atra* L., 1758) is the most abundant (Santoul & Tourenq, 2002). In the Garonne floodplain, the number of coots has increased from about ten birds in the 1980s to more than one thousand at the end of the 90s (Joachim et al., 1997).

We investigated habitat preferences of wintering coots censused weekly during two winters at three gravel pits located in the Garonne floodplain at Saint Caprais. We attempted to relate habitat characteristic with abundance of coots within gravel pits. Such information will be useful for site acquisition for conservation and management purposes.

MATERIAL AND METHODS

The gravel pits of Saint Caprais are located 25 km to the north-west of Toulouse in the Garonne floodplain (43° 46' N and 0° 58' E) (Santoul & Tourenq, 2002).

The total area of the three gravel pits is about 66 ha with a mean depth of 3m (maximum depth : 4m). Mixed-farming of corn and sunflower surrounds the gravel pits. These gravel pits are unmanaged. Waterfowl hunting and fishing are forbidden.

Weekly morning censuses of coots were carried out from October 1996 to February 1997 and from October 1997 to February 1998. The small surface area and open characteristic of the gravel pits studied permitted a total census of the populations (Tamisier, 1972), using a telescope (20x60) and binoculars (8x30). Coot numbers were recorded on each gravel pit and their position was noted on a map (Santoul & Tourenq, 2002).

To determine habitat used by coot, several parameters were recorded during wintering periods : bank slope : < 5% (1), 5-10% (2), > 10% (3); bank vegetation : absent (1), lawn (2), herbs (3), shrubs (4), trees (5); vegetation between watermarks : absent (1), low (2), high (3); human disturbance : absence of path (0), presence of path (1); macrophytes : absent (0), some (1), abundant (2); water depth : < 1m (1), 1-3m (2), 3-4m (3); zones of open

water or near the bank. The small amounts of vegetation present between watermarks only permitted us to establish classes of density (Bournaud et al., 1982). Macrophytes were sampled each year at the end of the spring period (May-June) at several points of each gravel pit. The species-habitat associations were tested with $\chi\Sigma$ tests (Chessel & Dolédec, 1993). The microhabitat profiles were calculated according to Mastrorillo et al. (1996) as the difference between the frequency of coots in samples representing a particular environmental variable and the frequency of that species in all samples. For significant species-habitat associations ($\chi\Sigma$ test, $p<0.05$), negative values indicated avoidance and positive values indicate preference.

RESULTS

The number of coots reached a maximum during October and November when more than 500 were present. At the end of the wintering period only a hundred birds were counted; an important decrease was noted in December.

Microhabitat profiles enabled us to highlight the ecological requirements of coots during the wintering period in relation to environmental availability. The three gravel pits studied were heterogeneous in terms of the type of vegetation covering the banks. Coots preferred areas with lawn over those with herbs, shrubs or trees. They occupied deep areas (3-4 m) with macrophytes and preferred areas of open water. However, the vegetation between watermarks and the presence of roads did not influence the distribution of coots during wintering periods. They avoided bank slope more than 10 % (Fig. 1). Six species of submerged macrophytes were present in the gravel pits : *Myriophyllum spicatum, Najas major, Nitella* sp., *Potamogeton natans, Ranunculus trichophyllus* and *Veronica anagallis.* However only the charophyte *Nitella* sp. was abundant.

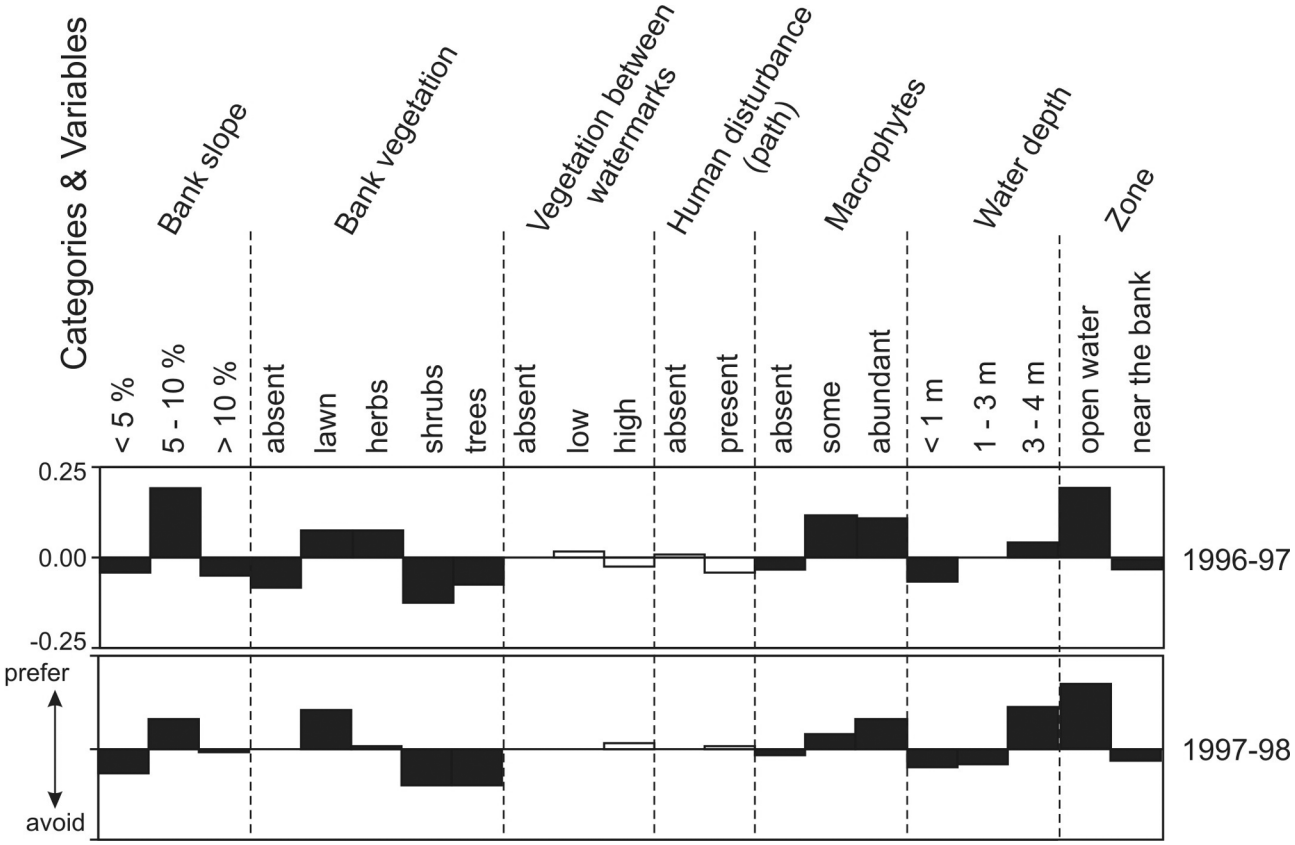

Fig. 1. – Habitat of coot during winter. Each histogram represents the difference between the frequency of coots in a group of samples having that category of environmental variable, and the frequency of that species in all samples. Significant deviations from expected between species and variables are indicated with a black histogram ($\chi\Sigma$ test, $p<0.05$).

DISCUSSION

With a population of coots reaching 600 individuals, the three gravel pits of Saint Caprais constitute an important wintering site in Midi Pyrénées. Joachim et al. (1997) have estimated the wintering populations of coots in that region to be between 2500 and 3000 birds.

Our results suggest a strong association between the availability of food and lawn zones with the presence of coots in winter. Among the factors influencing the wintering distribution of coot, the trophic factor is preponderant. Food resources are more critical in winter than in summer (Dubowy, 1988). Coots are herbivorous, feeding mainly on macrophytes such as Characeae, etc (Borowiec, 1975; Draulans & Vanherck, 1987; Irwin & O'Halloran, 1997). Bredin & Skinner (1983) emphasise the importance of aquatic plants near the surface and over large areas to attract coots. Coots remain in these foraging

zones until the complete degeneration of macrophytes (Sauer, 1982). When resources are exhausted, they move towards the lawn zones. The population decrease in December - January is due to the phenology of the species (departure of winter migrants), but is certainly accentuated by the strong depletion of trophic resources. The rare lawn areas constitute the last feeding zones used.

As did Borowiec & Jakubczyk (1975), we also noted an occupation of the central zone of the water reservoir/pond by coots. These zones, easily accessible (depth : 3-4 m) for coots (Cramp & Simmons, 1977), are rich in aquatic plants. They also constitute a safe habitat (from terrestrial predators) where birds can rest and carry out their comfort activities (e.g. preening) (Borowiec & Jakubczyk, 1975). By providing opportunities for coots to congregate far from the bank, gravel pits with large open water areas and a deep-water zone in their centre provide suitable foraging areas while reducing the risks of predation (Bell et al., 1997). Coots are not very inclined to escape by flight (Cramp & Simmons, 1977) and this also explains why, whenever possible, they avoid banks covered with shrubs and trees where predators can perch and hide. The results of the study confirm those in other areas (Borowiec, 1975; Bredin & Skinner, 1983).

Population size of coots wintering in Midi Pyrénées remains small on an international scale (about 2500 coots at the mid-winter waterbird census). However, gravel pits are the major wintering habitat in this region, and those of Saint Caprais have a high carrying capacity (Santoul, 2000). The geographical location of the gravel pits near the Pyrenees mountains also makes the Midi-Pyrénées region important as a stop-over for coots (Hoyer, 1994). The new extension of gravel pits in the Garonne River basin is of economic and environmental importance. Restoration is generally carried out to transform gravel pits to recreational areas or fishing lakes, and only rarely into habitats primarily designed for waterbird conservation.

Once considered as a simple transition between two breeding periods, the wintering period has been proven to be an essential component of the biological cycle of waterbirds (Jorde et al. 1983; Zorn et al., 1995; Tamisier & Dehorter, 1999). Preservation of wintering habitat quality for migratory species is thus necessary. Because of the increasing number of artificial wetlands (reservoirs, rice plantations, gravel pits) compared to the decreasing number of natural wetlands in Europe and worldwide, artificial wetlands have to be taken into account in conservation and management schemes for waterbird communities at both regional and international levels.

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Operculum of peppered loach, *Lepidocephalichthys guntea* (Hamilton, 1822) (Cobitidae, Cypriniformes) : a scanning electron microscopic and histochemical investigation

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ABSTRACT. *Lepidocephalichthys guntea* frequently darts and burrows in mud or sand, and spends most of the time buried in the soft bottom of water bodies. The epidermis covering its outer surface (OE) and the epithelium lining the inner surface of the operculum (EISO) differ noticeably in their surface architecture seen under SEM, and in their glycoprotein secretions analysed histochemically in whole mounts. This indicates their important physiological activity as mediators for extrinsic factors to which these are exposed. Micro-ridges arranged compactly may impart firm consistency to the free surfaces of the epithelial cells in the OE as an adaptation to mechanical stress during burrowing. In the EISO, which is not prone to abrasion during burrowing, in contrast, widely spaced micro-ridges suggest less rigid surfaces. Mucous cells are of two types, A and B, in the OE, and only one, type C, in the EISO. Most mucous cells in the OE are type A, and elaborate mainly sulphated glycoprotein, which is associated with an increase in the viscosity of mucus providing protection against possible mechanical damage during burrowing and against pathogens. In contrast, glycoproteins with oxidisable vicinal diols and with carboxyl groups elaborated by type C mucous cells in the EISO are considered to lower viscosity of the mucus. Mucus with lower viscosity is washed away more easily by water currents, preventing accumulation on the surface that may obstruct or disturb the smooth flow of respiratory current across the branchial chamber. Presence of prominent taste buds in the OE is considered as an adaptation to locate food with increased efficiency. Presence of a large number of taste buds in the EISO, is regarded as an adaptation to detect other chemicals that could enter the buccal cavity during respiration. Possible functional significance of glycoprotein secretions in the taste buds are discussed.

KEY WORDS : *L. guntea,* opercular surfaces, SEM, whole mount, glycoprotein histochemistry.

INTRODUCTION

The operculum in teleosts is an important structure covering the branchial chamber, primarily providing protection for the gills and preventing water entering the gill cavity during inspiration. The epithelium lining the inner surface of the operculum (EISO) in fishes is only in contact with the water entering the gill cavity, whereas the epidermis covering the outer surface of the operculum (OE) contacts all kinds of media, including the substrates into which fish burrow. This difference could impart modifications in the organisational patterns of the surfaces of the OE and the EISO.

A review of the literature reveals that comparative studies on aspects of the organisational patterns of the OE and the EISO have not drawn the attention of many researchers. GARG et al. (1995), using scanning electron microscopy, reported significant differences in the surface organisation of the OE and the EISO of an air breathing catfish *Clarias batrachus* Linnaeus, 1758.

The objective of this study was to compare the surface architecture of the OE and the EISO of *Lepidocephalichthys guntea* Hamilton, 1822 (Common names : Peppered loach, Guntea loach or Torential loach), using scanning electron microscopy, and to visualise glycoproteins, using histochemical methods, in whole mount preparations in light microscopy. Such a comparison may enable determination of whether or not the dichotomy in these tissues reflects differences in their functional organisation, and may promote better understanding of their roles in relation to the habit and habitat of the fish.

Loaches, in general, inhabit flowing or even stagnant waters, preferably parts that are not too deep and have a soft bottom. The fish has a habit of suddenly burrowing into the mud or sand on the bottom and in this way protects itself from predators. Food consists chiefly of worms and insect larvae, obtained by very sagacious hunting (GÜNTHER, 1989). *L. guntea* spends most of the time buried in the sandy bottom, but often comes to the surface with swift movements to gulp atmospheric air (MISHRA & AHMAD, 1986).

MATERIAL AND METHODS

Live specimens of *L. guntea* (mean length 70 ± 5 mm, n = 10) were collected from the river Ganges at Varanasi, India and were kept in a laboratory aquarium, with a layer

of sand at the bottom, at 25 ± 2 ºC for up to 24 - 48h. The fish were cold anaesthetised, following MITTAL & WHITEAR (1978), to excise the opercular pieces for this study.

Scanning electron microscopy

Opercular pieces were treated and prepared for scanning electron microscopy following PINKY et al. (2002). Critical point dried opercular pieces, attached to stubs with the OE or EISO facing upwards, were coated with gold and examined under a Scanning Electron Microscope (Leo, 435 VP, England).

Whole mount study

Opercular pieces were rinsed in physiological saline, fixed in Carnoy's fluid or alcoholic Bouin's fluid, washed and stored in 70% ethyl alcohol. Intact sheets of OE and EISO were dissected from the opercular pieces, using fine forceps and needles, under a Nikon stereoscopic microscope (model SMZ - 1B) following MITTAL & GARG (1988). The sheets were hydrated in a descending ethanol series and stained with the histochemical methods along with their controls for glycoproteins summarised in Table 1. The stained sheets were dehydrated in graded ethanol in ascending concentrations, cleared in xylene, and mounted in Harleco synthetic resin (HSR) or in Kirkpatrick and Lendrum's distrene dibutylphthalate xylene (DPX). Observations were made on a Leitz microscope (model Laborlux S). Photomicrographs were taken with a Leitz camera system for automatic microphotography (model Vario Orthomat 2).

Densities of mucous cells and taste buds in the OE and the EISO were calculated using a stage micrometer (1 division = 0.01mm) and an eyepiece graticule with a square grid (5 x 5 = 25 squares) (Carl Zeiss, Jena). Ten randomly selected sites of each sample were analysed. Data thus obtained for each, the OE or the EISO, from five fishes were pooled separately and results were expressed as mean value ± SD throughout.

TABLE 1

Summary of the histochemical methods for visualisation of glycoproteins in different types of mucous cells and the taste buds in the OE and the EISO of *L. guntea* (Carnoy's fluid fixed tissues).

Histochemical methods	Reactions	Interpretation of reactions	References	Mucous cells Types			Taste buds
				A	B	C	
1. WO/ S	M	Free aldehydes		_	_	_	_
2. PAS	M	GPs with oxidisable vicinal diols and/or glycogen	McMANUS, 1948	1-2M	3-4M	3-4M	2M
3. Acetylation/PAS	0(M)	Same as '2'	LILLIE & FULLMER, 1976	_	_	_	_
4. α-amylase/PAS	M	GPs with oxidisable vicinal diols	SPICER et al., 1967	1-2M	3-4M	3-4M	2M
	0(M)	Glycogen					
5. AB2.5	T	GPs with carboxyl groups and/or with O- sulfate esters	MOWRY, 1956	3-4T	#	3-4T	2T
6. Active methylation AB2.5	0	GPs with carboxyl groups and/or with O- sulfate esters	SPICER et al., 1967	-	-	-	-
7. Active methylation/ saponification AB2.5	T	GPs with carboxyl groups	SPICER et al., 1967	1-2T	#	2-3T	1T
	0(T)	GPs with O-sulfate esters					
8. AB1.0	T	GPs with O-sulfate esters	LEV & SPICER, 1964	2-3T	#	1-2T	1T
9. Active methylation AB1.0	0	GPs with O-sulfate esters	SPICER et al., 1967	-	-	-	-
10. AB2.5/PAS	M	GPs with oxidisable vicinal diols and/or glycogen	MOWRY, 1963	3-4TP	2-3M	3-4P	2P
	T	GPs with carboxyl groups and/or with O-sulfate esters					
	P	GPs with oxidisable vicinal diols and/or glycogen, GPs with carboxyl groups and/or with O-sulfate esters					
11. AB1.0/PAS	M	GPs with oxidisable vicinal diols and/or glycogen	SPICER et al., 1967	2-3TP	2-3M	3-4MP	2MP
	T	GPs with O-sulfate esters					
	P	GPs with oxidisable vicinal diols and/or glycogen, GPs with O-sulfate esters					

Methods : WO/S, without oxidation Schiff's; PAS, periodic acid/Schiff; AB2.5, alcian blue at pH2.5; AB1.0, alcian blue at pH1.0.
Reactions : #, could not be distinguished; M, magenta; MP, magenta with purple tinge; P, purple; T, turquoise; TP, turquoise with purple tinge; -, no reaction; 1 to 4, feeble to very strong reactions.

RESULTS

Scanning electron microscopy

The surfaces of the opercular epidermis (OE) and the epithelium lining the inner surface of the operculum (EISO) in *L. guntea* were a mosaic of irregularly polygonal epithelial cells of varied dimensions (Fig. 1 a, b). The free surfaces of the epithelial cells were differentiated into micro-ridges forming characteristic patterns. In the OE, the microridges were compactly arranged, sinuous with jagged surface, short and branched with abrupt ends

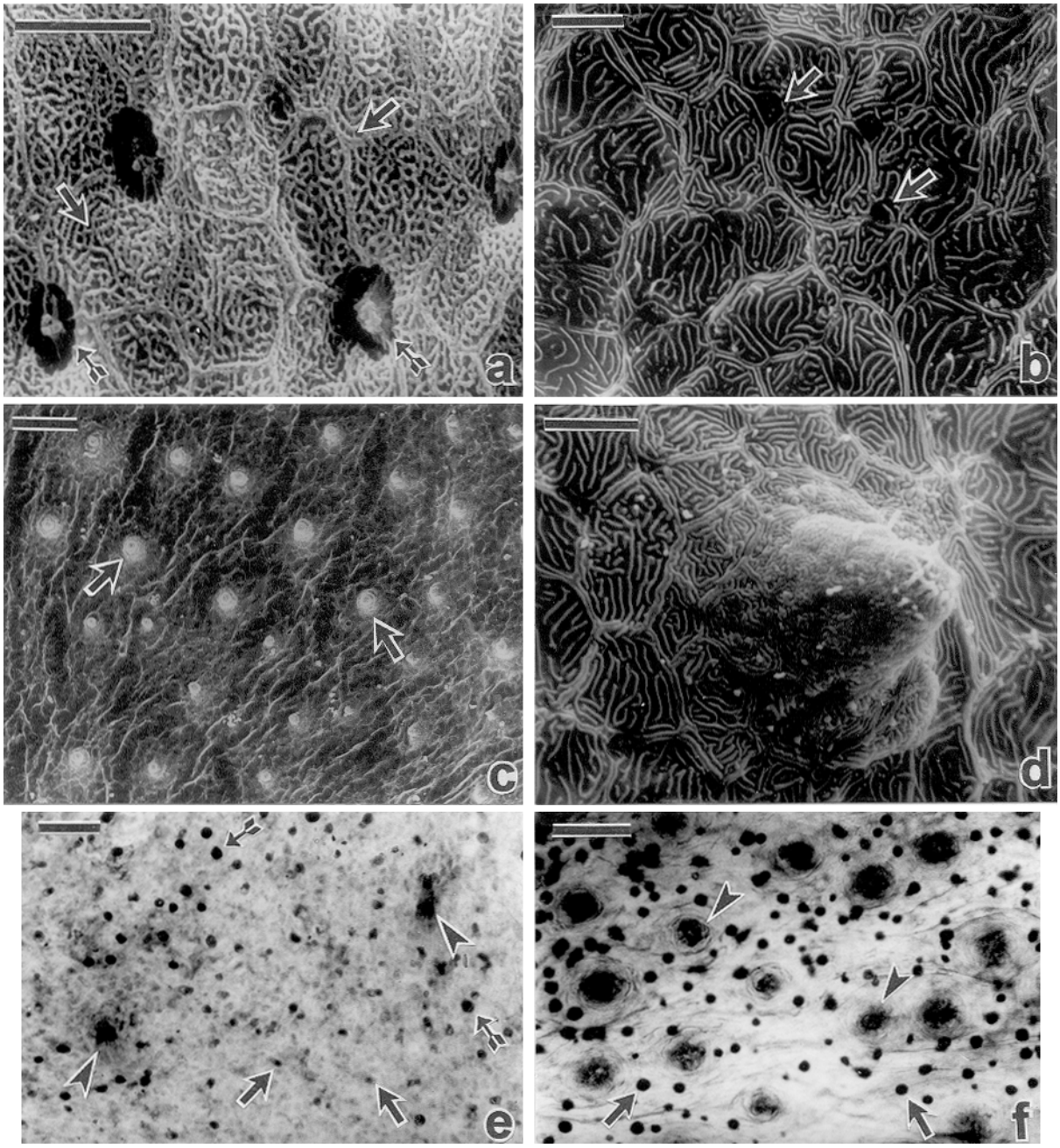

Fig. 1 (a-d). Scanning electron photomicrographs showing surface architecture of (a) OE and (b-d) EISO of *L. guntea*. **(a)** OE showing mosaic of epithelial cells with prominent boundaries. Microridges, often with fine microbridges (arrows) on the surface of the epithelial cells are compact and irregularly interwoven to form complex patterns. Note the presence of mucous cell apertures (winged arrows) laden with blobs of oozed mucus. (Scale bar = 5 μm). **(b)** EISO. Compare with OE (a). The microridges are without microbridges and are widely spaced to form simplified patterns on the surface of the epithelial cells. The mucous cell apertures are smaller (arrows). (Scale bar = 5μm). **(c)** The surface of the EISO is irregularly folded with high density of taste buds (arrows). (Scale bar = 40μm). **(d)** EISO showing conical epidermal elevation with a taste bud. Note microvilli, representing taste hairs, projected at the summit. (Scale bar = 5μm).

Fig. 1 (e-f). Photomicrographs of whole mount preparations of (e) OE and (f) EISO of *L. guntea*. **(e)** Showing a large number of type A mucous cells (arrows) (turquoise with purple tinge in original). Type B mucous cells are relatively few (winged arrows) (magenta in original). A few taste buds (arrowheads) (purple in original) are also discernible. (AB 2.5/PAS). (Scale bar=40μm). **(f)** Showing type C mucous cells (arrows) (deep purple in original) and a large number of taste buds (arrowheads) (purple in original). (AB2.5/PAS). (Scale bar = 40μm).

or irregularly interwoven to form intricate mesh-like patterns (Fig. 1a). The microridges were often interconnected with fine transverse connections –microbridges (Fig. 1a). Boundaries between adjacent epithelial cells were demarcated by double rows of intimately associated microridges that often gave a braided appearance (Fig. 1a).

The surface of the EISO, in contrast, was irregularly folded (Fig. 1c). The microridges on the surfaces of the epithelial cells appeared smooth, elongated, extensive or sometimes fragmented, and often oriented regularly almost parallel to each other with wide spaces between them to form simplified patterns (Fig. 1b). Microbridges were absent. Furthermore, the double row of microridges at boundaries between adjacent epithelial cells appeared smooth and extensive (Fig. 1b).

Interspersed between the epithelial cells, mucous cell apertures and prominent taste buds were distinguished on the surfaces of both the OE and the EISO. The mucous cell apertures, generally, were rounded and occurred at the border of three or four epithelial cells (Fig. 1a, b). These apertures in the OE, as compared to those in the EISO, appeared wide, often laden with blobs of oozed mucus (Fig. 1a). Each taste bud was situated on a characteristic epidermal elevation (Fig. 1c), which in general, appeared conical and jutted out at the surface (Fig. 1d). At the summit of each elevation, closely packed microvilli representing taste hairs projected through a rounded taste pore (Fig. 1d).

Whole mount study

In the whole mount preparations of OE and EISO, the mucous cells, in general, appeared rounded (Fig. 1e, f), each opening to the surface through a narrow secretory pore. Histochemical methods employed to demonstrate glycoproteins differentiated three categories of mucous cells : for convenience designated as type A, B and C mucous cells (Table 1). In the OE, type A and type B mucous cells were distinguished (Fig. 1e), most belonging to type A, while type B mucous cells were sporadic. In the EISO, in contrast, only type C mucous cells were visualised (Fig. 1f). The density of mucous cells in the EISO was high, compared to that in the OE (Table 2).

TABLE 2

Density of mucous cells and the taste buds in the OE and the EISO of *L. guntea*

Region	Density (number/mm^2)			
	Mucous cells			Taste buds
	Type A	Type B	Type C	
OE	1392.00 ± 155.83	108.80 ± 95.64	–	35.20 ± 32.67
EISO	–	–	2443.64 ± 333.56	252.80 ± 73.34

Values = mean ± SD; N = 5 fish; –, absent

Analysis of reactions with the combination of histochemical methods and their controls (Table 1) indicate that type A mucous cells contain high amounts of glycoproteins with O-sulphate esters, together with relatively small amounts of glycoproteins with carboxyl groups and glycoproteins with oxidisable vicinal diols. Type B mucous cells contain high amounts of glycoproteins with oxidisable vicinal diols. Type C mucous cells contain high amounts of glycoproteins with oxidisable vicinal diols, and glycoproteins with carboxyl groups, together with low amounts of glycoproteins with O-sulphate esters.

Taste buds were conspicuous in the whole-mounts of OE and EISO. The epithelial cells around each taste bud were characteristically arranged in concentric whorls (Fig. 1f). The cluster of sensory cells and the tuft of taste hairs of each taste bud were stained positively for glycoproteins (Fig. 1e, f). Analysis of these reactions (Table 1) shows that the taste buds contain moderate amounts of glycoproteins with oxidisable vicinal diols together with glycoproteins with carboxyl groups, and low moieties of glycoproteins with O-sulphate esters. Density of taste buds was much higher in the EISO than in the OE (Fig. 1 e, f) (Table 2).

DISCUSSION

Noticeable differences exhibited in the patterns of microridges on the epithelial cells, composition of glycoproteins elaborated by mucous cells and distribution of taste buds on the surfaces of the OE and the EISO of *L. guntea* may be considered as modifications relating to possible difference in the functional requirements at the two locations.

Microridges have been reported to vary considerably in configuration and disposition, constituting varied patterns at different locations in different fish species, and have been implicated to play variable roles. These include to retain mucous secretions to the cell surface, to increase the surface area for excretion and absorption through the skin, to facilitate the spread of mucus away from goblet cells, to aid in producing laminar flow, to provide reserve surface area for stretching, and so on (see review of Whitear, 1990). In the OE of *L. guntea,* compactly arranged microridges forming intricate mesh-like patterns, may in addition impart firm consistency or rigidity to the free surfaces of the epithelial cells. This could be considered as an adaptation to withstand mechanical stress and protect the surface of the fish, which has the characteristic habit of frequently darting and burrowing in sand or mud. Furthermore, these microridges may gain a firm base and support from a dense network of fine filaments - the terminal web, which, as shown by Schliwa (1975), Mittal et al. (1980) and Whitear (1986), is differentiated characteristically at the apical regions of the surface epithelial cells. The microbridges interconnecting the microridges may further reinforce renitency of the surfaces of epithelial cells in the OE of *L. guntea*. Microbridges, variously named as cross connections (Whitear, 1990) or interconnections (Reutter et al., 1974) in fish epidermis, and as microbridges (Karlsson, 1983) or crossbridges (Avella & Ehrenfeld, 1997) in gill epithelia, have also been reported previously. In the EISO, in contrast, widely spaced microridges forming simplified patterns and the absence of microbridges between them

suggest less rigid surfaces of the epithelial cells. This is interesting since the EISO, unlike the OE is not prone to abrasion during burrowing.

The characteristically folded surface of the EISO in *L. guntea* may further be considered to impart stretchability to the epithelium, required for expansion of the branchial chamber during respiratory movements of the operculum. Furthermore, it may account for increased surface area as well for respiration in the fish.

Acid glycoproteins have been shown to coincide with increased viscosity of mucus in the alimentary tract of fish *Arrhamphus sclerolepis krefftii* Steindachner, 1866 (Tibbetts, 1997), in airway epithelia of mammals (Jones et al., 1973; Iravani & Melville, 1974) and in corals (Miekle et al., 1988). The elaboration of mainly sulphated glycoproteins by type A mucous cells, the most common type in the OE in *L. guntea*, could thus be related to increased viscosity of the mucus and lubrication of the surface of the fish. This could play a vital role in providing protection to the body against mechanical damage to which these fishes are highly vulnerable during burrowing in mud or sand. Mittal et al. (1994 a, b) also correlated the release of large amounts of epidermal glycoproteins with O-sulphate esters to provide heavy lubrication to protect the body against mechanical damage in *Monopterus cuchia* Hamilton, 1822 and *Mastacembelus pancalus* Hamilton, 1822 during their peculiar wriggling movements on moist grass and during burrowing in mud. Elaboration of sulphated glycoproteins by superficial layer epithelial cells and most mucous cells in the epidermis of semi-amphibious *Blenny sanguinolentus* Pallas, 1811 (Zaccone, 1983) and *Blennius pholis* Linnaeus, 1758 (Whitear & Mittal, 1984) both with the habit of creeping about with the aid of paired fins, further supports this view.

Furthermore, sulphation of complex carbohydrates has also been shown to result in increased resistance to their enzymatic breakdown by bacterial glycosidases (Mian et al., 1979; Tsai et al., 1992), to play a role in defence against pathogens (Solanki & Benjamin, 1982) and to prevent the proliferation of pathogenic microorganisms (Tsukise & Yamada, 1981; Suprasert et al., 1986, 1987). Thus high proportions of sulphated glycoproteins in the mucous cell secretions on the surface of the OE in *L. guntea* may also confer high resistance against pathogens and protect the fish.

Sibbing & Uribe (1985) reported that sialomucins are less viscous than sulphomucins produced by mucous cells in the pharynx of *Cyprinus carpio* Linnaeus, 1758. Further, Tibbetts (1997) has shown that neutral glycoproteins are less viscous than the acid glycoproteins produced by mucous cells in the alimentary tract of *A. sclerolepis krefftii.* Elaboration of glycoproteins with oxidisable vicinal diols (neutral glycoproteins) and glycoproteins with carboxyl groups (sialomucins) in high concentrations by the type C mucous cells in the EISO of *L. guntea,* would thus lower the viscosity of the mucus in this region. Mucus with lower viscosity is considered to be fairly easily washed away with the respiratory water current. This prevents the accumulation of mucus on the surface of the EISO that could otherwise obstruct or disturb the smooth flow of respiratory current across the branchial chamber. Generally, visibility is poor at the muddy bottoms inhabited by *L. guntea*, owing to depth and increased turbidity, caused by the disturbance of the bottom mud due to the characteristic habit of the fish. The presence of prominent taste buds in the OE might be considered to increase the probability of accurately detecting and locating prey concealed by darkness or turbidity, and may also permit the accurate location of small food particles, which would be missed otherwise. Presence of a large number of conspicuous taste buds on the interior face of the operculum i.e. the EISO as well, could play a role in detecting other chemicals that could enter the buccal and gill cavity during respiration and that could potentially be noxious to gills. Garg & Mittal (1990), however, reported the absence of taste buds in the EISO of C. batrachus. Barrington (1957) reported that taste buds are often present even in fish oesophagus and postulated that it is an indication of the probable importance of this region in the selection and rejection of food.

Characteristic concentric whorls of epidermal cells encircling the taste buds in *L.* guntea are interesting. Such arrangements of epithelial cells have also been reported in *C. batrachus* (Garg et al., 1995) and in cod (Harvey & Batty, 1998). Harvey & Batty (1998) stated that the characteristic ring of epithelial cells may make it possible to locate and count taste buds even when their apex was damaged or missing.

Presence of glycoproteins indicates a secretory function of the taste buds of *L. guntea*. Grover-Johnson & Farbman (1976) and Reutter (1971), using transmission electron microscopy, reported two types of gustatory cells - light and dark - in the taste buds of *Ictalurus punctatus* Rafinesque, 1818 and *Ameiurus nebulosus* LeSuer, 1819. Grover-Johnson & Farbman, (1976) postulated that the dark cells were secretory in function. Witt & Reutter (1990) have shown, using lectin histochemistry for the detection of carbohydrates, that the dark sensory cells in the taste buds in European catfish *Silurus glanis* Linnaeus, 1758 are secretory. Glycoproteins with sialic acids have also been demonstrated in the taste buds in rabbit tongue (Witt & Miller, 1992). The secretions in the taste buds have been postulated to play different roles e.g. to maintain and regulate the chemical microenvironment (Grover-Johnson & Farbman, 1976), to protect mucus or other glycoproteins in the taste bud cells and inside the taste pore from premature enzymatic degradation and to have a hormone-like paraneuronal function (Witt & Miller, 1992), and to play a role in recognition phenomena on the plasmalemmata of the taste bud sensory cells and recognition processes directed to bacteria or viruses (Witt & Reutter 1990). Fujita (1994) and Zaccone et al. (1999) also suggested a paraneuronal role of the gustatory cells in taste buds.

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Macrofaunal community structure and zonation of an Ecuadorian sandy beach (bay of Valdivia)

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ABSTRACT. The sandy beach macrofauna of the Bay of Valdivia (Ecuador) was sampled in August-September 1999 along six replicate transects between the high and low water line. The sediment consisted of well-sorted, fine to medium sand. Taking into account the dimensionless fall velocity (Ω) and the relative tidal range, the beach was characterized as an exposed, low tide terrace - rip beach. The distribution of the macrofauna was mainly determined by the elevation on the beach. Thirty-one taxa were found throughout the study, varying between 10 and 22 taxa per transect. Molluscs were the most dominant taxon (overall average : 285 ind/m^2, max. : 2135 ind/m^2), followed by crustaceans. The gastropod *Olivella semistriata* (overall average : 243 ind/m^2, max. : 2131 ind/m^2) was the most abundant species. The crustaceans were the most diverse taxon (10 spp.); *Haustorius* sp., *Excirolana braziliensis* and *Emerita rathbunae* were the most abundant species. Densities of polychaetes were low in comparison with the previous two taxa mentioned (overall average : 13 ind/m^2). The total average density over the entire bay was 370 ind/m^2. Three zones of macrofaunal distribution along the tidal gradient could be distinguished : an upper beach, a middle beach and a lower beach assemblage. The upper beach assemblage consisted mainly of *Excirolana braziliensis* and ghost crabs. The middle beach assemblage had highest macrofaunal densities and was dominated by *Olivella semistriata* and *Haustorius* sp. The most diverse assemblage was found on the low beach, with representatives of different taxa, but with lower densities. These results are in conformity with other studies along the Pacific coast of South America and fit into the zonation scheme of Dahl (1952). Given that many other studies from South America were done at temperate latitudes, we conclude that, although differences in species composition were found, the general pattern of macrobenthos zonation on sandy beaches is similar in tropical regions.

KEY WORDS : Macrobenthos, sandy beach, Ecuador.

INTRODUCTION

Macrofaunal zonation on sandy beaches is a distinctive and well-described phenomenon of intertidal zones (McLachlan & Jaramillo, 1995). The existence of species zonation on exposed sandy beaches is thought to be mainly caused by species-specific responses to swash climate and sedimentology, with a less critical role of biological interactions (McLachlan, 1983a; McLachlan et al., 1993; McLachlan & Jaramillo, 1995).

Different generalizing zonation schemes for sandy beach macrofauna have been proposed (Davenport, 1903; Mortensen, 1921; Schulz, 1937) with the schemes of Dahl (1952) and Salvat (1964 and 1967) being the most commonly used. Dahl (1952) suggested a distinction between three zones, defined in terms of a typical crustacean fauna inhabiting each zone, while Salvat proposed a four zone system based on physical conditions.

In all of the studies describing intertidal zonation within different South American Atlantic and Pacific regions, three zones have been recognized (McLachlan & Jaramillo, 1995 and references herein), supporting Dahl's scheme. Several studies (Clarke & Peña, 1988; Defeo et al., 1992; Dexter, 1974, McLachlan & Jaramillo, 1995) showed that crustaceans are the most diverse taxon on South American sandy beaches. The upper parts of tropical and subtropical beaches are characterized by ocypodid crabs, while hippid crabs, bivalves and amphipods dominate the lower beach. Cirolanid isopods *(Excirolana* spp.*)* are abundant on the midshore, together with opheliid and spionid polychaetes; the bivalves *Mesodesma* spp. and *Donax* spp. are other characteristic organisms. To our knowledge, no information is available about sandy beach macrofauna in Ecuador. The studies of sandy beach macrofauna nearest to Ecuador were conducted to the south in Peru (8° S) by Bocanegra et al. (1985) and to the north in Colombia (1° 48' N) by Riascos & Rallón (2001), locations with different environmental conditions. Because the Ecuadorian coastal waters are divided by two opposite currents, the warm El Niño-current coming from the north, and the cold Humboldt-current coming from the south, and because of the tropical location of Ecuador, a different macrobenthic community structure might be expected.

In this study the intertidal zonation and assemblage structure of the macrofauna of the Bay of Valdivia (Ecuador; 1-2˚ S) were investigated. This bay was chosen because of the importance of the beach for harvesting shrimp larvae, which are used in one of Ecuador's largest economies.

This paper presents the distribution patterns of the macrofauna in order to set up a larger project on the influence of ENSO on macrobenthic communities of sandy beaches in Ecuador.

MATERIAL AND METHODS

Study site

The study area is located in the Bay of Valdivia, Ecuador (1°54'00" - 1°58'20" S and 80°46'00" - 80°45'30" W), approximately 50 km west of Guayaquil (Fig. 1). This 10 km long bay consists of 8 km of exposed sandy beaches with rock formations on either side.

The beaches have a semi-diurnal, mesotidal regime (Davies, 1964) with a tidal range of 2.5-3 m (average : 2.6 m). The modal breaker height (H_b) is 0.49 m. The modal wave period (T) is 14 s (unpublished pers. comm. S. Guartatanga).

The Ecuadorian coastal climate is characterised by two seasons : a dry-cool season (May-December) and a wet-warm season (January-April) and is influenced by currents in the Pacific Ocean. From July until October the area is subjected to the relatively cold (<22 °C) Humboldt-current, heading North, while during the months January until April the warm (>25 °C) El Niño-current, heading South, dominates.

Additional to the normal seasonality, with a periodicity of three to seven years the climatologic and oceanographic phenomenon ENSO dramatically alters the conditions along the Ecuadorian coast, with higher temperatures and precipitation levels during El Niño and lower temperatures during the subsequent La Niña.

Sampling and laboratory work

Sampling took place between 31 August and 5 September 1999. To cover habitat variability over the bay, six transects, distributed over the whole bay and perpendicular to the waterline, were sampled (Fig. 1 : A-F). Each transect was sampled at six stations : five stations were situated in the intertidal zone, while a sixth one was located on the dry beach. Sampling of the intertidal zone always started at high tide, following the receding water down the beach. To distribute the stations evenly across the intertidal gradient, the transect was sampled in the swash zone every 90 minutes (Fig. 2). At each station three replicate samples were collected by excavating a metal frame (sampling surface area : 0.1026 m^2) to a depth of 15 cm. The samples were sieved alive over a 1 mm mesh-sized sieve. The organisms retained were stored in 8 % formaldehyde-seawater solution.

At each station, one sediment sample was collected for grain size analysis using a core with diameter 3.6 cm. In addition, the relative elevations of the different stations were measured using an altimeter. Distances between all sample sites were measured.

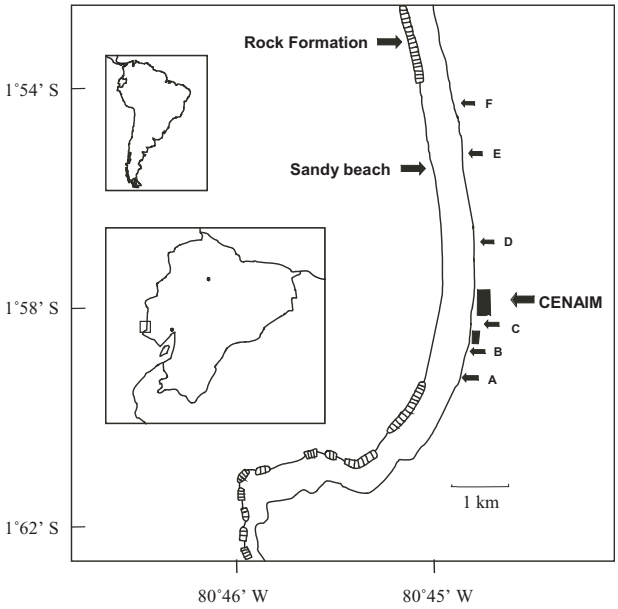

Fig. 1. – Geographical situation of the six sample transects (A-F) from the Bay of Valdivia, Ecuador (modified from Boothroyd et al., 1994).

Laboratory treatment

Macrobenthic organisms were counted and identified to species level, where possible.

The sediment grain size distribution between 2 and 850 µm was determined with a laser COULTER LS and classified according to the Wentworth scale (Buchanan, 1984). The median grain size of the sand was largely biased by the mixture with shell fragments present in all samples, while the modal grain size remained unaffected. Hence, the modal grain size was preferred over median grain size as a good representation of the sediment's main characteristic.

Mathematical analyses

Combining the relative elevation of the lowest station of each transect with the data from the tide table for La Libertad (INOCAR) allowed estimation of their absolute elevation (relative to the mean low water level at spring tide, MLWS), from which the absolute elevation of all higher stations was calculated and beach profiles were obtained. By means of these beach profiles, the mean slope between low and high water of every transect was calculated. In this study, beach width is defined as the distance between the low water line and the lowest edge of the terrestrial vegetation.

The morphodynamic state of each transect was assessed by calculating the dimensionless fall velocity ($\Omega = H_b / w_s T$) (Dean, 1973) and the relative tide range ($RTR = MSR/H_b$) (Masselink & Short, 1993). Sediment fall velocity (w_s) was obtained from sediment particle size after Gibbs et al. (1971). Mean spring tidal range (MSR) was obtained from the tide table (INOCAR).

For each sample the species richness (N_0) (Hill, 1973) and diversity (Shannon-Wiener diversity index, H') were determined (Shannon & Weaver, 1949). Community analysis was done by means of Cluster-analysis (Clif-

FORD & STEPHENSON, 1975), Canonical Correspondence Analysis (CCA) (TER BRAAK, 1988) and Indicator Species Analysis (DUFRÊNE & LEGENDRE, 1997). Correlations between environmental variables were analysed by means of the non-parametric Spearman rank correlation coefficient (CONOVER, 1971).

To visualize zonation patterns of density and the number of species (N_0), polynomial functions were fit to the data according to the distance-weighted least squares smoothing procedure, using STATISTICA 5.1 (STATSOFT, 1996).

RESULTS

Environment

The beach width ranged from 70 to 172 m, while the width of the intertidal zone ranged from 42 to 109 m. The beach slope varied between 1:25 and 1:54. Ω was found between 1.172 and 1.541; RTR had a value of 5.306.

Although all transects were situated on the same beach, some variation in beach profile was observed (Fig. 2).

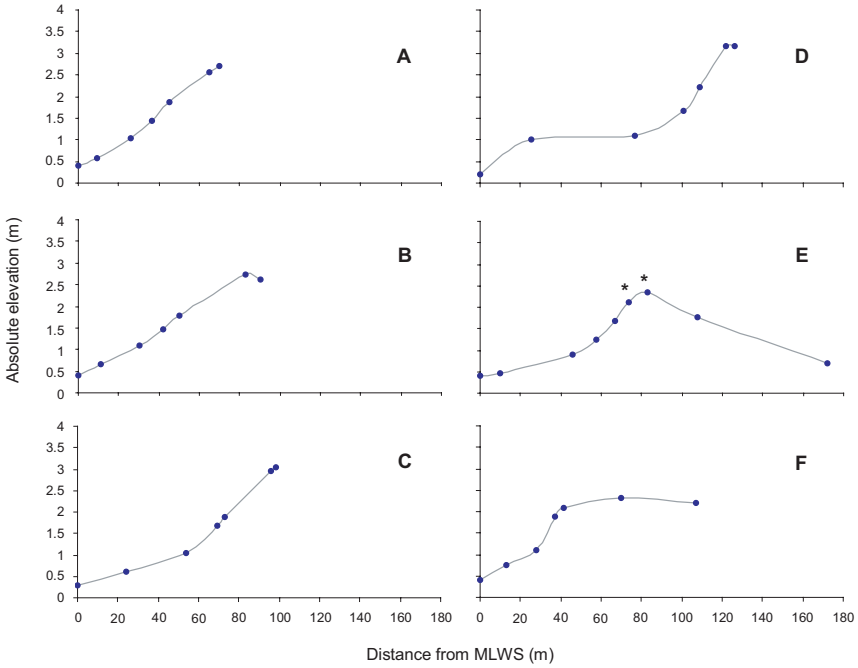

Fig. 2. – Beach profiles at the six transects (*: additional measuring points; the most right point is the vegetation line).

Furthermore, an alternation of ridges and runnels was present at transect F, while all other transects showed a quite featureless beach profile. Transects C and D showed a break in their slope. The upper part of transect C was steeper than the lower part (1:14 versus 1:39). The middle part of transect D had a gentle slope compared to the lower and upper part.

Fine to medium sands (125-500 µm) dominated the sediment. The stations' modal grain size ranged between 171 µm and 262 µm, except for the lowest stations of the two northern-most transects (modal grain size: 325 µm). The fraction of silt and clay (<63 µm) was low (0-3.2 %) and was negatively correlated with the elevation ($r=-0.707$; $p<0.0001$) as was the fraction of coarse sand (>500 µm) ($r=-0.428$; $p<0.0001$).

Macrofauna

A total of 31 macrofaunal taxa (ten taxa of crustaceans, ten of molluscs, eight of polychaetes and five others) were collected (Table 1 : species list), ranging from ten to 22 taxa per transect and varying between nil and ten taxa per station. H' diversity index ranged from 0 to 2.87. Overall average macrobenthic density was 370 ind/m^2. Molluscs dominated the fauna (average : 285 ind/m^2, max. : 2135 ind/m^2), followed by crustaceans (average : 66 ind/m^2, max. : 413 ind/m^2). The gastropod *Olivella semistriata* was the most dominant species of the whole beach (average : 243 ind/m^2, max. : 2131 ind/m^2). Other abundant species were the gastropods *Mazatlania hesperia, Mazatlania* sp., the polychaetes *Nephtys* sp., *Hemipodus* sp. and *Lumbrineris* sp., the haustoriid amphipod *Haustorius* sp., the cirolanid isopod *Excirolana braziliensis,* the anomuran crabs *Emerita rathbunae* and *Lepidopa deamae* and spat of bivalves.

Along the whole beach, highest species richness (N_0) was situated at the lower beach (Fig. 3) : species richness generally decreased towards the upper beach. Highest densities (up to 2400 ind/m^2) were found between 1.6 and 2.2 m above MLWS. The stations on the dry zone of the beach (>2.4 m) had the lowest densities (maximum : 100 ind/m^2). Below 1.6 m, densities remained more or less equal (generally : 80-600 ind/m^2) : no obvious density increase towards MLWS was observed.

Multivariate techniques, CCA, Cluster analysis and species indicator analysis consistently distinguished between three station groups (Fig. 4). Station elevation

TABLE 1

Species list (* not sampled and not counted in analyses)

		Family	Species
Annelida	Polychaeta	Maldanidae	Maldanidae sp.
		Lumbrineridae	*Lumbrineris* sp. (Blainville, 1828)
		Magelonidae	*Magelona* cf. *mirabilis* (Johnston, 1865)
		Pisionidae	*Pisione* sp. (Grube, 1857)
		Glyceridae	*Hemipodus* sp. (Quatrefages, 1865)
		Nephtyidae	*Nephtys* sp. (Cuvier, 1817)
		Spionidae	*Scolelepis* sp. 1 (Blainville, 1828)
			Scolelepis sp. 2 (Blainville, 1828)
Crustacea	Decapoda	Albuneidae	*Lepidopa daemae* (Benedict, 1903)
		Hippidae	*Emerita rathbunae* (Schmidt, 1935)
		Paguridae	*Pagurus* sp. (Fabricius, 1775)
		Ocypodidae *	*Ocypode occidentalis* (Stimpson, 1860)
		Portunidae	*Arenaeus mexicanus* (Gerstaecker, 1856)
	Amphipoda	Haustoriidae	*Haustorius* sp. (Müller, 1775)
			Bathyporeia sp. (Lindström, 1855)
	Isopoda	Cirolanidae	*Excirolana braziliensis* (Richardson, 1912)
		Sphaeromatidae	*Paracerceis* sp. (Hansen, 1905)
	Mysidacea	Mysidae	*Bowmaniella* sp. (Bacescu, 1968)
			Metamysidopsis sp. (Tattersall, 1951)
Echinodermata	Echinoidea	Mellitidae	*Mellita longifissa* (Michelin, 1858)
	Stelleroidae		Ophiurae sp.
Mollusca	Bivalvia	Donacidae	*Donax mancorensis* (Olssen, 1961)
			Donax sp. (Linnaeus, 1758)
		Tellinidae	*Strigilla chroma* (Salisbury, 1934)
	Gastropoda	Collumbellidae	*Mazatlania hesperia* (Pilsbry & Lowe, 1932)
			Mazatlania sp. (Dall, 1900)
		Olividae	*Olivella semistriata* (Gray, 1839)
		Terebridae	*Hastula luctuosa* (Hinds, 1844)
		Vitrinellidae	*Anticlimax willetti* (Hertlein & Strong, 1951)
Hexapoda	Insecta		Insecta sp.
Nemertea			Nemertea sp.

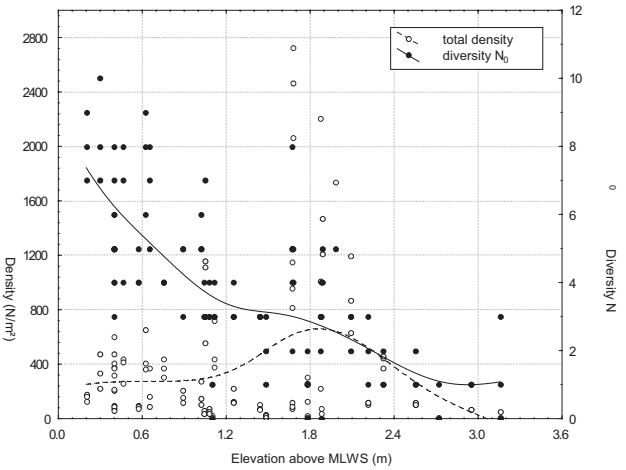

Fig. 3. – Total density and N_0 diversity set against elevation.

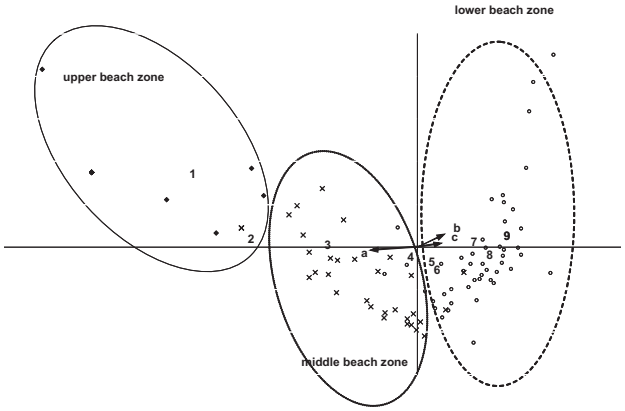

Fig. 4. – CCA-output with the three distinguished zones; group division is based on the outcome of all three applied multivariate techniques (eigenvalue axis 1: 0.500; axis 2: 0.183); a: elevation, b: % silt and clay (<63 µm), c: % very fine sand (63 – 125 µm); 1: Insecta sp., 2: *Excirolana braziliensis*, 3: *Haustorius* sp., 4: *Olivella semistriata*, 5: *Nephtys* sp., 6: *Emerita rathbunae*, 7: *Nemertea* sp., 8: *Mazatlania* sp., 9: *M. hesperia*; ◦: lower beach (47 stations), x: middle beach (33 stations), ♦: upper beach (19 stations).

was the most determining variable as far as upper and middle beach zones were concerned, % mud and % very fine sand (<125 µm) distinguished the lower beach fauna. The most important indicator species were *Excirolana braziliensis*, *Olivella semistriata*, *Haustorius* sp., Bivalvia spat and Nemertea sp.

The first group of 47 replicates was found between 0.3 and 1.3 m above MLWS, the lower beach zone (Fig. 4 and table 2). The fine sand fraction (49 %) dominated the sediment. Relatively high percentages of mud (average : 1 %) and coarse sand (average : 9 %) were present. In total, 27 taxa were found, evenly divided over the molluscs, polychaetes and crustaceans (Fig. 5). Macrofaunal

TABLE 2

Characteristics of the three distinguished zones

		Lower beach zone	Middle beach zone	Upper beach zone
Elevation (m above MLWS)		0.3-1.3	1.0-2.1	1.8-3.2
Sediment	% silt and clay (<63µm)	1.3	0.4	0.1
	% very fine sand (63-125µm)	8.1	3.9	3.1
	% fine sand (125-250µm)	48.5	50.5	57.0
	% medium sand (250-500µm)	31.3	37.9	37.0
	% coarse sand (500-800µm)	9.0	6.3	2.0
Average N_0		5.38	3.47	1.42
Number of species	All taxa	27	15	7
	Mollusca	8	3	2
	Polychaeta	7	4	1
	Crustacea	9	7	3
Average density (ind/m^2)	All taxa	268.0	662.0	154.0
	Mollusca	221.0	577.0	2.6
	Polychaeta	18.8	11.2	0.5
	Crustacea	14.5	111.0	148.0
Percentage of all taxa within zone (%)	Mollusca	82.5	82.5	1.7
	Polychaeta	7.0	1.6	0.3
	Crustacea	5.4	15.9	96.0
Dominant taxa (>2%)		*Olivella semistriata* Bivalvia spat *Mazatlania hesperia* *Mazatlania* sp. *Emerita rathbunae* *Nephtys* sp. Nemertea sp.	*Olivella semistriata* *Haustorius sp.*	*Excirolana braziliensis* Insecta sp.

density (average : 268 ind/m^2) was dominated by molluscs (83 %), mainly *Olivella semistriata* (48 %). Other abundant macrofauna comprised spat of bivalves (28 %), *Mazatlania* sp. (4 %), *Emerita rathbunae* (3 %), nemerteans (3%), *Nephtys* sp. (2 %), and *Mazatlania hesperia* (2 %).

The second group (33 replicates) was situated between 1.0 and 2.1 m above MLWS, the middle beach zone. Sediment was mainly composed of fine sand (50.5 %), with 0.4 % of mud and 6.0 % of coarse sand on average. Fifteen taxa, of which seven were crustaceans, were present. This zone was characterized by a high density (average : 662 ind/m^2). Molluscs, especially the gastropod *Olivella semistriata* (82 %), dominated the macrofauna (Fig. 5). The amphipod *Haustorius* sp. was the second most abundant species (13 %).

The third group (19 replicates) was found between 1.8 and 3.2 m above MLWS (upper beach zone), including the upper intertidal and supralittoral zone (high tide mark at 2.4 m above MLWS). Sediment was dominated by fine sand (average : 57.0 %) with low mud and coarse sand contents (average : 0.1 and 2.0 %, respectively). Seven taxa were found, with an average macrofaunal density of 154 ind/m^2. This zone was dominated by crustaceans (96 %), mainly *Excirolana braziliensis* (Fig. 5). Next to crustaceans, several insect species were present. *Excirolana braziliensis* was found both on the dry beach and at the highest intertidal station. The insects were only present on the dry beach, together with high numbers of ocypodid crabs.

The mole crabs *Emerita rathbunae* and *Lepidopa deamae* were found across the entire intertidal gradient.

DISCUSSION

This study was initiated as a pilot study for macrofauna research on Ecuadorian sandy beaches. Because only one beach was sampled, generalization of the results to all Ecuadorian beaches cannot be made. Further, since the beach was only sampled in one short period, no inference about seasonal trends can be made. It has to be emphasized that the macrobenthic community structure and zonation pattern, which are obtained by data collected in a short period of time, do not necessarily represent the distribution during the rest of the year (Haynes & Quinn, 1995; Brazeiro & Defeo, 1996). In this study, the macrofaunal zonation during the dry, cool season is documented.

Sampling took place during a very strong La Niña phase of the ENSO cycle, and the preceding year was one of the strongest El Niño years ever recorded (Chavez et al., in press). There is some evidence that ENSO, and more specifically a strong El Niño, has a substantial influence on macrobenthic communities of sandy beaches (Tarazona et al., 1988; Tarazona & Paredes, 1992). It is thus very likely that the situation encountered in this study was altered by the abnormal climatologic conditions of the two preceding years. Regardless of these shortcomings, if interpreted with caution, the present study provides a first overview on the community structure and zonation of Ecuadorian sandy beach macrofauna.

Environment

According to the morphodynamic classification scheme of Masselink & Short (1993), all investigated transects can be classified as low tide terrace-rip beaches

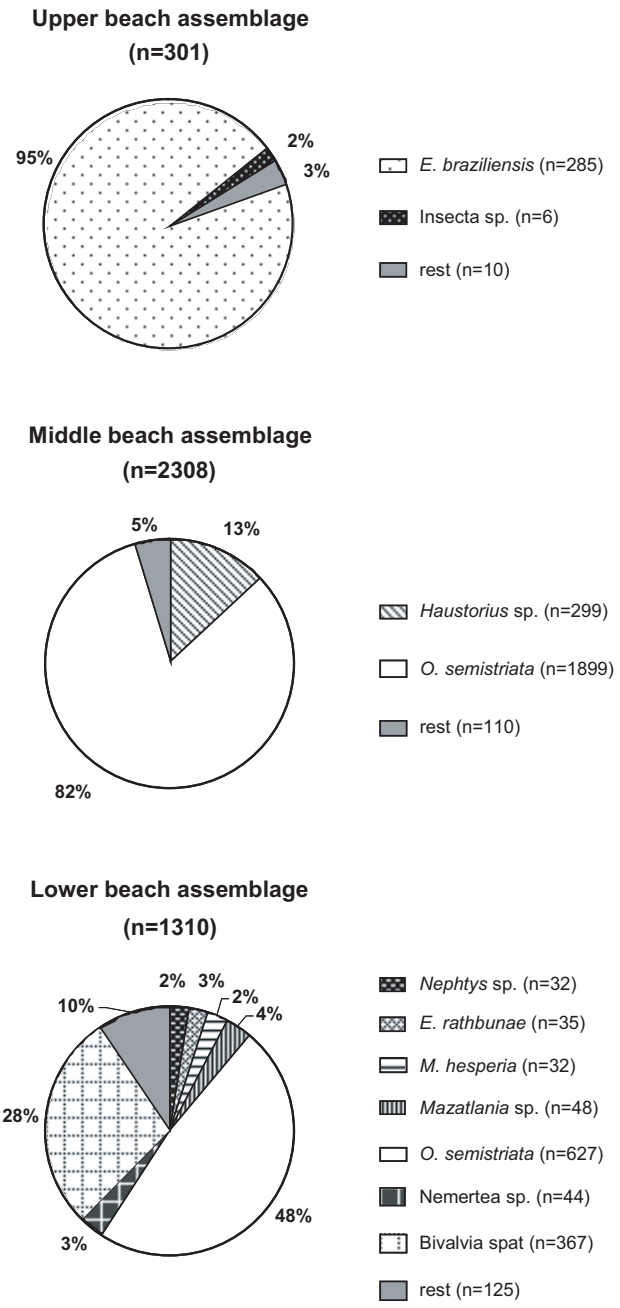

Fig. 5. – Taxon distribution for the three distinguished assemblages.

(Ω : 0-2; RTR : 3-7). Although differences between the different transects exist, the six transects were regarded as replicates of the same beach, rather than transects along six different beaches. Therefore, the zonation patterns might be less clear, but can be considered as representative for the whole bay (Degraer et al., 1999). The differences in beach profile for the six sampled transects of the same beach (Fig. 2) show that it could be dangerous to only sample one transect when investigating the macrofauna of a sandy beach.

Macrofauna : General

Molluscs, crustaceans and polychaetes have been reported to be the three most abundant macrofaunal taxa on sandy beaches worldwide (Souriea, 1957; Pichon, 1967; Dexter, 1969; McLachlan, 1983b). Crustaceans tend to dominate exposed beaches, polychaetes sheltered beaches, while molluscs reach maximum densities in intermediate situations (Dexter, 1983). The investigated beach had a high richness of crustaceans (ten taxa) but was, in terms of density, dominated by molluscs. Especially the gastropod *Olivella semistriata* proved to be very abundant in this study (66 % of the overall macrofauna). So far, this genus has only been found in low numbers on sandy beaches in Peru (Suarez Calvanapón, 1981), Columbia (Riascos & Rallón, 2001) and the Pacific coast of Mexico (Cupul-Magana & Téllez-Duarte, 1997). The high numbers found might be an overestimation of the actual density of this species, since this gastropod is thought to appear in high density patches (Riascos & Rallón, 2001). Still, preliminary research on other sandy beaches in Ecuador supports the statement that *O. semistriata* is a very dominant species (Vanagt, unpublished). It thus seems that this species is more abundant towards the Equator.

The bivalve *Donax* sp. only appeared in low numbers in Valdivia Bay, but has been reported to be very abundant at other sandy beaches from the same geographical area (Dexter, 1974; Perez Nieto, 1980; Riascos, 2002). There might be a negative correlation between *Donax* and *Olivella semistriata*-populations. Another possibility is that *Donax* was negatively influenced or *O. semistriata* positively influenced by the strong El Niño one year before sampling or by the strong La Niña during the sampling campaign. This hypothesis is supported by the fact that another common bivalve of South American Pacific beaches, *Mesodesma* spp., was absent on the Ecuadorian beach. Tarazona & Paredes (1992) reported that in Peru *Mesodesma donacium* might disappear almost entirely after a strong El Niño.

This study shows, in agreement with different other studies (e.g. Clarke & Peña, 1988; Defeo et al., 1992; Dexter, 1974, McLachlan & Jaramillo, 1995), that crustaceans are the most diverse taxon on South American beaches along the Pacific coast. Cirolanid isopods, especially *Excirolana braziliensis*, were abundant in Valdivia Bay. This species is widely spread along the coasts of Central and South America, but shows a high spatial variability in density, attributed to variable beach temperatures (Zuñiga et al., 1985). Next to *E. braziliensis*, *Haustorius* sp. (average : 27 ind/m^2) and *Emerita rathbunae* (average : 6 ind/m^2) were abundant crustaceans as well. These latter two crustaceans groups were also found on many other South American beaches (e.g. Peru : Suarez Calvanapón, 1981; Chile : Jaramillo et al., 1993 and Uruguay : Giménez & Yannicelli, 1997). Ghost crabs (*Ocypode occidentalis*) appeared in high numbers above the drift line on the investigated beaches, as was the case in Valdivia Bay. They were not sampled because of the applied sampling technique.

Polychaete species of the genera *Hemipodus*, *Lumbrineris* and *Nephtys*, which were the most abundant polychaete taxa in this study, have been reported from Peru (Suarez Calvanapón, 1981), Chile (Clarke & Peña, 1988) and Columbia (Dexter, 1974). Spionid polychaetes were only found in very small numbers. Opheliid

polychaetes (e.g. *Euzonus furciferus*), which were found on several other South American beaches (Clarke & Peña, 1988; Gianuca, 1983; Escofet et al., 1979) were not registered in the present study.

In general, taxon composition in Valdivia Bay was similar to other South American sandy beaches. Some differences, mainly in molluscs, were found, possibly due to the location near the Equator with its tropical conditions.

Macrofauna : Zonation

In the Bay of Valdivia, three beach zones were distinguished : upper, middle and lower zone. The presence of three zones has already been demonstrated for several sandy beaches around the world (McLachlan & Jaramillo, 1995 and references herein).

Generally, the upper beach zone of South American sandy beaches is dominated by a low number of species. Cirolanid isopods (e.g. *Excirolana braziliensis*) are often encountered (Dexter, 1974; Bocanegra et al., 1985), together with large numbers of air-breathing ghost crabs (*Ocypode occidentalis*). This is typical for the transition zone between the marine and the terrestrial environment (Jaramillo, 1987).

Compared with the upper beach zone, a higher diversity of very abundant macrofaunal species is found at the middle beach. Again, crustaceans tend to be characteristic for this zone along many South American beaches (McLachlan & Jaramillo, 1995). In the present study, however, the most abundant species within the middle beach zone was the gastropod *Olivella semistriata*. Although polychaetes are rarely abundantly present in the middle beach zone of South American beaches, some individual species might be (e.g. opheliids, spionids and nephtyids) (McLachlan & Jaramillo, 1995). In Valdivia Bay, few polychaetes were found in the middle beach zone.

The lower beach zone of all exposed South American beaches is characterized by a large number of abundant species (McLachlan & Jaramillo, 1995), as was the case in the intermediate beach in our study. The lower beach zone is often regarded as an intertidal extension of the subtidal habitat. The higher diversity of the lower beach zone could be a reflection of the high subtidal diversity (Degraer et al., 1999). Also, the short period of exposure to the air allows more species to inhabit the lower beach zone.

It has to be emphasized that no sharp boundaries between the different zones were found, partly because of the morphodynamic differences between the replicate transects. Moreover, zonation on sandy beaches has to be seen as an artificial division of a continuum, with an overlap between adjoining zones (Degraer et al., 1999).

In general, the zonation pattern of the macrobenthic assemblages on the investigated tropical beach was similar to other beaches at different latitudes in South America and the rest of world.

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Microscopic studies of the paraphysis of the turtle *Trachemys (scripta) dorbigni* (Duméril & Bibron, 1835)

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ABSTRACT. A microscopic investigation was conducted on the paraphysis of the turtle *Trachemys dorbigni*. The paraphysis is a highly vascular neuroepithelial structure lined by a simple cuboidal epithelium. The prominent ultrastructural features observed were many dense bodies, mitochondria and lipid droplets. The cells of the paraphysis exhibit extensive microvillar borders, large intercellular spaces, many mitochondria, dense bodies and lipid droplets. Few PAS (periodic acid of Schiff) positive granules were found in the cytoplasm of the epithelial cells. Large intercellular spaces were seen when samples were fixed by perfusion. Scanning electron microscopy revealed that surface epithelial cells have microvilli and cilia distributed in tufts in the center of the cell. Macrophagic cells were frequently seen on the surface of the epithelial cells. The connective tissue of the paraphysis presented many sinusoid vessels, mast cells, fibroblasts, and collagen fibers. Intra-arterial administration of Evans blue showed the absence of the blood-brain barrier. This is probably related to the presence of a fenestrated endothelium, which characterizes this structure as a circumventricular organ (CVO). The presence of vesicles in the cytoplasm of epithelial cells, fenestrations, and macropinocytosis vesicles in the vascular endothelium suggest absorption and secretion functions.

KEY WORDS : paraphysis, turtle, morphology, electron microscopy.

INTRODUCTION

The paraphysis is a branchial and tubular sacciform structure of the posterior telencephalic roof. It is considered a circumventricular organ similar to the choroid plexus (Oksche, 1973). With few exceptions, this specialized neuroepithelial structure is common to all vertebrates, including humans, in an embryonic stage (Shuangshoti & Netsky, 1966; Ebbesson & Schroeder, 1975; Kondziolka & Bilbao, 1989).

In reptiles as well as in amphibians, the paraphysis is well developed and occupies a great subdural area located between the posterior borders of the cerebral hemispheres (Kelly, 1964; Ebbesson & Schroeder, 1975). Studying green sea turtles, Owens & Ralph (1978) demonstrated that the paraphysis was a continuation of the choroid plexus of the third ventricle, slightly to the enlarged distal end of the pineal gland. A fluid-filled cavity was formed inferior to the large pineal-paraphyseal complex.

Studies on the ultrastructural features of the paraphysis in different species (*Lampreta* sp. : Tsuneki, 1986; *Chondricthyes* : Shuangshoti & Netsky, 1966; *Bufo bufo* L. 1758 larvae : Farnesi et al., 1994; *Necturus* sp. : Kelly, 1964, Ebbesson & Schroeder, 1975; *Hyla versicolor* Le Conte, 1825 : Kemnitz et al., 1990 : *Natrix maura* Laurenti, 1768 : Fernández-Llebrez et al., 1982) showed that it consists of a single or pseudostratified epithelium surrounded by highly vascular connective tissue.

The most important functions related to this structure are the production of the cerebral spinal fluid (CSF) in cooperation with the choroid plexus, and the exchange of substances between the blood and the CSF (Kelly, 1964; Owens & Ralph, 1978; Fernández-Llebrez et al., 1982; Kemnitz et al., 1990; Hinton et al., 1990; Farnesi et al., 1994). Paraphysectomy in *Rana* species also showed that a substance discharged by the paraphysis, by the choroid plexus or by both structures seems to be involved in calcium regulation. It resulted in hypocalcemia, motor neuron degeneration, abnormal increased weight, and a tendency to form cysts in the parathyroid gland (Ueno et al., 1984; Nelson et al., 1985).

The aim of this work was to investigate the morphological features of the paraphysis of the turtle *Trachemys (scripta) dorbigni* (Duméril & Bibron, 1835) using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and light microscopy.

MATERIAL AND METHODS

Twenty-four adult turtles *Trachemys dorbigni* (twenty-one females and three males) weighing between 950g and 2,500g were captured over the year and used in this study with the permission of IBAMA (Registration numbers 047/97; 026/98; 004/99). The animals were fed with ground meat ad libitum.

Light Microscopy (LM)

Six animals (one male and five females) were anesthetized with 35% chloral hydrate and perfused with a saline solution followed by 4% paraformaldehyde in sodium phosphate buffer (pH. 7.4). Sections were stained with haematoxylin/eosin (HE). Evans Blue at 0.01% was added to the saline solution (adapted from Eberhardt, 1971) in two animals (females) to demonstrate the vascular permeability of the paraphysis.

Gomori's Acid Phosphatase (six females)

Cryostat sections were incubated in a solution of 0.1M trismaleate buffer (pH 5) and 1.25 % sodium β-glycerophosphate. Lead nitrate (0.2%) was added to this solution under constant stirring, followed by heating at 37º C for 5 minutes and subsequent filtration. Sections were incubated for 30 minutes at 37º C, rinsed in distilled water (three times), developed in a solution of 5% ammonium sulfide for 5 min, rinsed in distilled water and mounted in Kaiser's medium. As a control, a few sections were incubated in a medium with 1% sodium fluoride (NaF) (Pearse, 1968).

PAS (one male and four females)

After fixation in Bouin's solution, the paraphysis was dehydrated, embedded in paraffin and cut into 8 µm-thick sections, which were then deparaffinized, hydrated and subjected to 0.5% periodic acid for 15 min. After distilled water rinsing, sections were immersed in Schiff's reagent for 30 min, rinsed again in distilled water, submitted to three sulfurous baths, rinsed again in distilled water, and subsequently stained with Mayer's hematoxylin, dehydrated, and mounted with Entellan®. For control of the specificity of reaction we used sections incubated in 0.5% α-amylase at 37 °C for 3 hours (Pearse, 1968).

Electron Microscopy

The paraphyses of three females and one male were fixed by immersion and perfusion with 3% glutaraldehyde, 1.5% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). For post-fixation 2% OsO_4 was used in the same buffer. Ultrathin sections were collected on copper grids, contrasted with uranyl acetate and lead citrate (Reynolds, 1963), and examined in a JEOL 1200 EX II electron microscope at 80 KV.

The paraphyses of four females were dehydrated in acetone and dried by the critical point technique using carbon dioxide (Balzer CPD 030), sputter-coated with carbon and gold (Balzer SCD 050), and examined under a scanning electron microscope (JEOL 5800 and Cambridge Stereoscan).

RESULTS

The paraphysis of the turtle *Trachemys dorbigni* is a well-developed, often tubular organ. It is located in the interhemispheric fissure (Fig. 1). At the superior and posterior faces (Fig. 2) it is bordered by the proximal and distal portions of the pineal gland, laterally by the cerebral hemispheres, and by the third ventricle at the inferior side. However, below the pineal-paraphysis complex there is a CSF-filled cavity connected to the third ventricle by an orifice (Fig. 3).

Histochemical study showed a few PAS-positive granules throughout the cytoplasm (Fig. 4). The basal membranes of the endothelium and epithelium of the paraphysis became stained after treatment with Schiff's reagent. Pre-treatment with α-amylase reduced the number of PAS-positive granules in the epithelium, indicating the presence of glycogen. The presence of lysosomes was demonstrated through Gomori's acid phosphatase technique (Fig. 5).

The paraphysis is lined with a simple, cuboidal epithelium, the cells of which have a round, central nucleus (5.3 to 7.5 µm in diameter) occupying a large part of the cytoplasm. The (usually singular) nucleolus is very prominent and spherical (0.08-0.12µm in diameter) (Fig. 7). The cytoplasm of paraphyseal cells contained several mitochondria with transversal crests and dense bodies (0.8-1 µm in diameter) (Fig. 6).

In the tubules of the paraphysis (Fig. 8), epithelial cells presented an apex with numerous microvilli (3.5 µm long and 0.2 µm in diameter). Cilia (6.5-9 µm long and 0.18-0.23 µm in diameter) were usually detected at the central surface of the cell (Fig. 9). The numbers of cilia varied from four to 11 per cell. Cells with a morphology resembling macrophage cells were detected on the ventricular surface of these cells.

Mast cells were found in the connective tissue located under the epithelium (Fig. 6). Collagen fibers and fibroblasts processes were also detected (Fig. 10).

The paraphysis presents a large number of blood vessels in the connective tissue. By injecting Evans Blue intra-arterially, we observed that the paraphysis allowed the passage of this dye to its interior, showing that this structure has no blood-brain barrier (Fig. 1). The vascular endothelium presents many fenestrations from 50 to 75 nm in diameter, covered by a thin diaphragm (Fig. 11). Cytoplasmic projections of the endothelium were observed in both apical and basal portions. On the luminal face, these projections formed macropinocytosis vesicles with diaphragms. The presence of these vesicles was clearly seen in the cytoplasm (Fig. 12). The basal layer of these capillaries could be traced along large extensions, even if in a few points it was discontinuous.

DISCUSSION

Analysis of the serial sections of the paraphysis (sagittal, coronal, and horizontal) showed that this structure of the turtle *Trachemys dorbigni* is formed by a tubular cavity which is enlarged in the proximity of the pineal gland and is covered by epithelial cells and meninges. This cavity decreases its diameters through the communication with the third ventricle. A similar cavity was reported by Owens & Ralph (1978) in marine turtles. Due to the close relationship between this cavity, the pineal gland and the paraphysis, Owens & Ralph called it pineal-paraphyseal cavity. In the snake *Natrix maura*, this cavity was also observed and was named central ventricular cavity (Fernández-Llebrez et al., 1982).

According to several authors (KAPPERS, 1956; SHUANGSHOTI & NETSKY, 1966; EBBESSON & SCHROEDER, 1975; KEMNITZ et al., 1990; FARNESI et al., 1994), the lining epithelium is continuous between the paraphysis and the choroid plexus. In our study we confirmed this finding and also observed a communication between the lumen of the paraphysis and the third ventricle. This communication, which occurs through a small orifice, had also been described in other animals, such as the salamander *Amblystoma mexicanum* Shaw, 1789 (KAPPERS 1950), the frog *Hyla versicolor* (KEMNITZ et al., 1990) and larvae of the toad *Bufo bufo* (FARNESI et al. 1994). These findings demonstrate that the paraphysis is not an isolated organ but is connected to the CSF of the other ventricular cavities.

In the cytoplasm we observed an accumulation of mitochondria, dense bodies (probably secondary lysosomes), and lipid droplets, particularly in the apical pole of epithelial cells. These lipid droplets were also found in the basal portion of the cell in the salamander *Necturus* sp. (KELLY, 1964; SHUANGSHOTI & NETSKY, 1966; EBBESSON & SCHROEDER, 1975).

Few histochemical assays have been done on the paraphysis. The most common studies are those investigating the presence of glycogen, especially those describing the paraphysis in amphibians and reptiles. Histochemical and biochemical studies demonstrated that the quantity of glycogen in the cells of the paraphysis and in other cells of the CNS is related to environmental conditions and to the different seasons of the year. From spring through winter there is a gradual increase in the total amount of glycogen in the cells of the CNS as well as in the paraphysis (FERNÁNDEZ-LLEBREZ et al., 1982; PARTATA & MARQUES, 1994). With TEM we observed few glycogen granules in the paraphysis of *Trachemys dorbigni.* There were no variations in the amount of glycogen in animals prepared in spring as compared to autumn. Our results are in disagreement with the findings of KAPPERS (1956), who described the epithelium of the paraphysis of salamanders as being rich in glycogen. The presence of glycogen in the paraphysis was also described in fishes, amphibians, and other reptiles (WOLFF, 1962; SHUANGSHOTI & NETSKY, 1966; MCNULTY, 1976), but in *Natrix maura* it was not detected (FERNÁNDEZ-LLEBREZ et al., 1982).

Through Gomori's acid phosphatase method we observed the presence of positive granules in the paraphyseal epithelium, mainly in areas close to the cell apex. They appear to be lysosomes and dense bodies. The elements that reacted with acid phosphatase are likely to be the same ones that had reacted with PAS, and this is in agreement with KOENING & BARROW (1962), who demonstrated that Schiff's reagent also reacts with lysosomes. EBBESSON & SCHROEDER (1975), in a study involving the paraphysis of *Necturus* sp., reported the presence of granules similar to lysosomes in the apical regions of cells of the paraphyseal epithelium.

The examination of the surface of the paraphyseal epithelium of *Trachemys dorbigni* by SEM showed that this surface is covered by numerous short microvilli and cilia distributed in tufts. Besides these structures, we also found macrophagic cells similar to the epiplexus cells reported by LING et al. (1998) in rats. These authors proposed that a typical cell of the epiplexus has from three to five cytoplasmic processes emanating from the cell body. In our study the cells observed on the ventricular surface of the paraphysis usually had three processes.

In the literature there are no SEM data of the paraphysis. From our observations we highlight the presence of dilations at the end of both microvilli and cilia. We suspect that such dilations are artifacts of the preparations, since they were not detected in the TEM examination.

Elements of the conjunctive tissue that are notable in the formation of the tubules of the paraphysis can vary between different species of amphibians and reptiles. The most prominent structures found in the turtle *Trachemys dorbigni* are mast cells, collagen fibers, and fibroblast processes. Mast cells were numerous in the dorsal region of the paraphysis lying closer to the pineal gland. No macrophages were found, nor were any nerve fibers.

The paraphysis is a highly vascular structure presenting many fenestrated sinusoid capillaries, indicating possible absence of the blood-brain barrier. This absence was demonstrated through its permeability to Evans Blue. The sinusoidal vascularization was described in the amphibians *Ambystoma* sp. (ROOFE, 1936; KAPPERS, 1950, 1956; EBBESSON & SCHROEDER, 1975), *Necturus* sp. (KELLY, 1964), *Rana catesbiana* Schreber, 1782 and *R. pipiens* (HINTON *et al*., 1990), the turtle *Chrysemys picta marginata* Agassis, 1857 (WARREN, 1911), and the snake *Natrix maura* (FERNÁNDEZ-LLEBREZ et al., 1982). The presence of fenestrations in the endothelium, which confirm the blood-brain barrier absence, was described in *Necturus* sp. (KELLY, 1964), *R. catesbiana* and *R. pipiens* (HINTON et al., 1990), larvae of *Bufo bufo* (FARNESI et al., 1994), and in *Natrix maura* (FERNÁNDEZ-LLEBREZ et al., 1982).

Circumventricular organs (CVOs) of the brain are characterised as highly vascular structures, with no blood-brain barrier, and in contact with one of the cerebral ventricles (WEINDL et al., 1972; LOW, 1982, PETERS et al., 1991). Based on these criteria, we consider the paraphysis of the turtle *Trachemys dorbigni* to be a CVO. A number of authors (FERNÁNDEZ-LLEBREZ et al., 1982; TSUNEKI, 1986; KEMNITZ et al., 1990) also described the paraphysis as a CVO, due to its pronounced vascularization and its contact with the cerebral ventricular system.

The connection with the ventricle and some morphological features suggest possible functions of the paraphyse. The presence of vesicles in the cytoplasm of the epithelial cells, the fenestrations and the macropinocytosis vesicles in the vascular endothelium are indications of substance exchange activities, may be also secretion. In this way, the paraphysis may be able to release substances into the cerebro-spinal fluid of the ventricular system. At present there are few data in the literature about the functions of the paraphysis. When its physiological role has been determined by further studies, it may become possible to explain the atrophy of this structure in humans (KONDZIOLKA & BILBAO, 1989).

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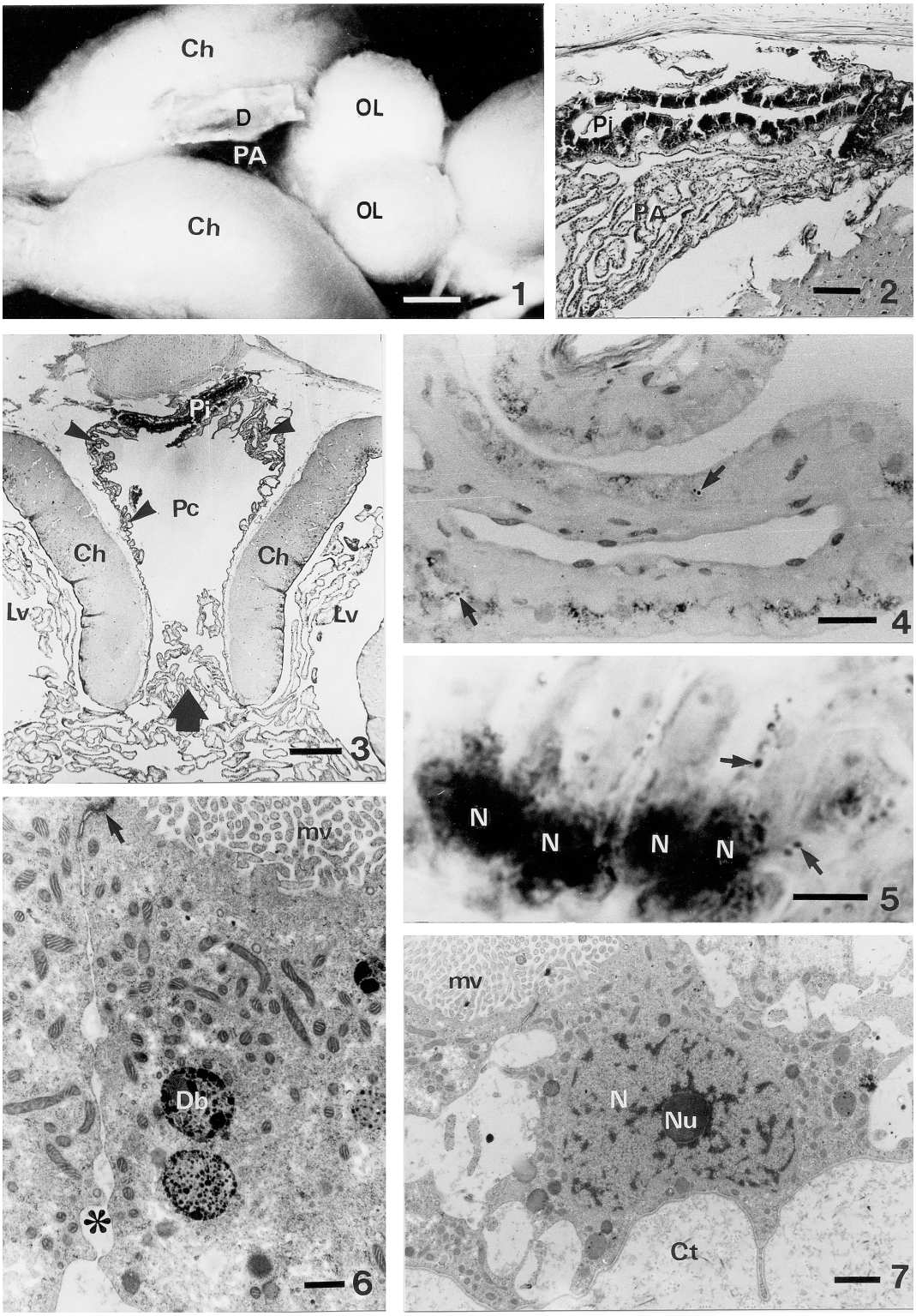
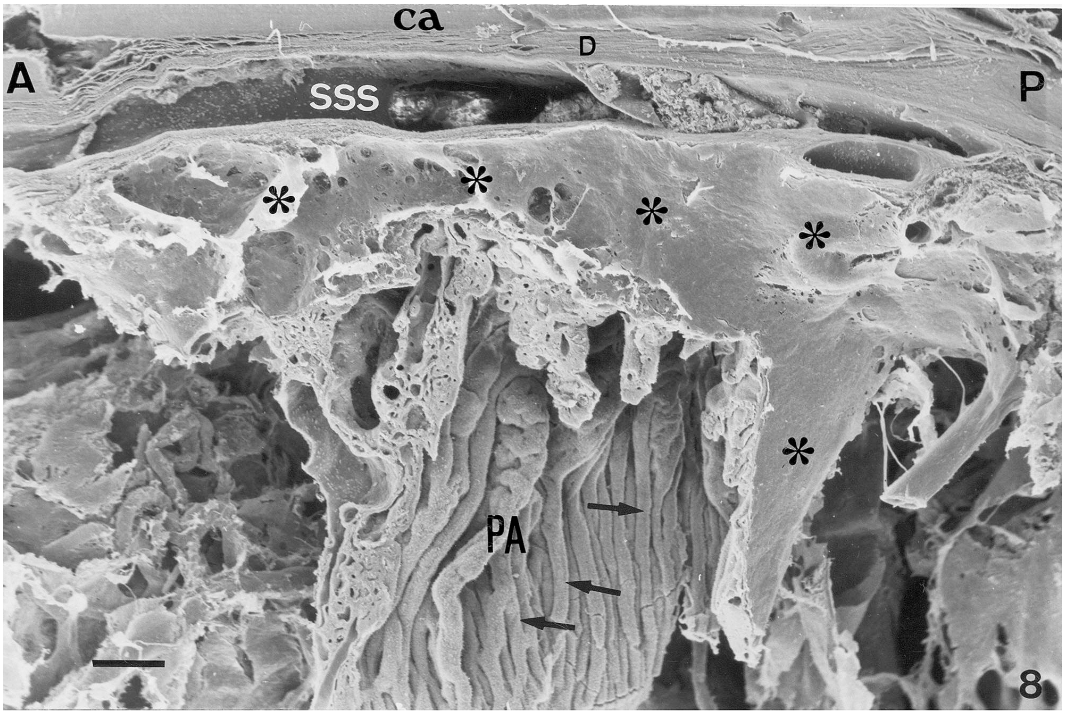
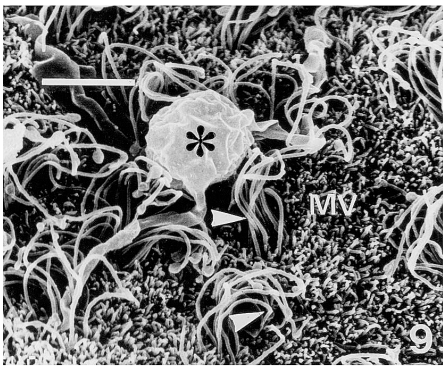
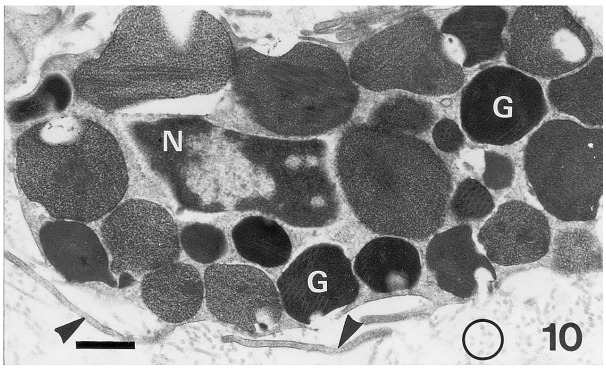
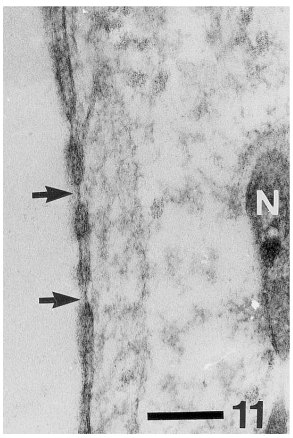


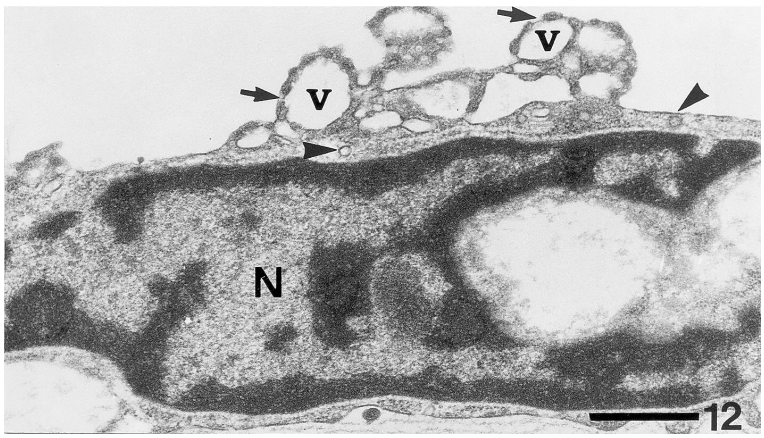
Fig. 1-7. – **1.** Micrograph of the *Trachemys dorbigni*'s brain (dorsal view) intra-arterially injected with Evans Blue, showing that the paraphysis (PA) presents no blood-brain barrier : cerebral hemisphere (Ch), optical lobe (OL), dura mater (D); bar : 0.16 mm. – **2.** Photomicrograph of a sagittal section showing the location of paraphysis and the structures to which it is anatomically related. Pineal (Pi) and paraphys¬is (PA). LM section, material included in paraffin and stained with HE; bar : 50 µm. – **3.** Coronal section of brain showing the relation between the paraphysis, lateral ventricles, and the third ventricle. Orifice (arrow) that links the paraphyseal cavity (Pc) with the third ventricle, cerebral hemisphere (Ch), pineal (Pi), paraphysis (arrowheads) and lateral ventricle (Lv). LM section, stained with HE; bar : 250 µm. – **4.** Paraphysis sections processed for PAS technique; PAS-positive granules (arrows) are very scantily found in the paraphyseal epithelium. LM; bar : 30µm. – **5.** Photomicrograph showing lysosome staining through the technique of Gomori's acid phosphatase; Lysosomes (arrows), nucleus (N). LM; bar : 10 µm. – **6.** Electron micrograph of a section of the paraphyseal epithelium showing cytoplasmic structures : dense bodies (Db) and intercellular spaces (*). Desmosome (arrow), microvilli (mv). TEM; bar : 500nm. – **7.** Electron micrograph showing the nucleus (N) and the nucleolus (Nu) of an epithelial cell of paraphysis. In the apical portion of the cell are microvilli (mv). Underlying the epithelium is the layer of connective tissue (Ct). TEM; bar : 1µm.











Figs 8-12. – **8.** Para-sagittal section of paraphysis (SEM) : anterior (A) and posterior (P) part of paraphysis (PA); in the top see the location of the pineal gland (*). Dura-mater (D), superior sagittal sinus (SSS), cartilage (ca), tubules of paraphysis (arrows). SEM; bar : 200 µm – **9.** Surface of paraphysis observed under SEM : macrophagic cell (*), cilia (arrowheads), microvilli (MV). SEM; bar : 5 µm. – **10.** Mast cells in the cytoplasm of paraphyseal cells. Granules (G), nucleus (N), fibroblasts processes (arrowheads), cluster of collagen fibrils (circle). TEM; bar : 500 nm. – **11.** Vascular endothelium. Note diaphragms (arrows) in the fenestrations along the endothelium of epithelial cells of paraphysis. Nucleus (N). TEM; bar : 200 nm. – **12.** Vascular endothelium showing the nucleus (N). On the luminal surface note the presence of macropinocytosis vesicles (v) and diaphragms (arrows). Also, note the presence of macropinocytosis vesicles close to the nucleus (arrowhead). TEM; bar : 500 nm.

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Potential of bio-indication of chironomid communities for assessment of running water quality in Flanders (Belgium)

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ABSTRACT. The distribution of chironomid species in small lowland rivers and brooks in the crenal zone of the Zwalm river basin (Flanders, Belgium) was studied. We examined whether a more refined identification of Chironomidae would result in an added value to the biological assessment of the Zwalm river basin, currently based on the Belgian Biotic Index. At the 18 sampling sites, a total of 31 different taxa of chironomids were identified. Identification was at species, aggregate, group, subgenus or genus level. The diversity of chironomid communities and their indicator role for assessment of particular river types and water quality were examined by means of multivariate analysis. Direct ordination of the identified chironomid taxa resulted into three groups of indicators related to the assessment of the water quality of a brook : (1) indicators of a good water quality, (2) indicators of waters enriched with nutrients and organic matter compounds and (3) taxa that were indifferent to water quality. The more refined identification levels provided useful information on the ecology of these organisms and their role as indicator organisms of specific water quality states. Because of the more labour-intensive procedure, however, chironomid identification at these levels will be more difficult to apply within a rapid bio-assessment protocol of running waters.

KEY WORDS : Chironomidae, macroinvertebrates, bio-indicators, brooks, rivers, biomonitoring.

INTRODUCTION

Numerous human activities have an impact on the quality of surface waters and consequently on the organisms living in these habitats. This aquatic fauna, therefore, can serve as convenient biological indicators of the various environmental stresses on these ecosystems (De Pauw & Hawkes, 1993). Unlike physical-chemical measurements, biological assemblages reflect long-term exposure to varying water quality conditions, and benthic macroinvertebrates in particular are well suited for use within rapid bioassessments (Rosenberg & Resh, 1993). Among freshwater macroinvertebrates, chironomid larvae are considered as promising indicators of water quality because of their ubiquity, high abundance and high diversity in aquatic ecosystems (Saether, 1979; Lindegaard, 1997). Moreover, the effects of pollution on chironomid communities have been extensively covered in literature and chironomid taxa have been shown to differ in their tolerance of specific pollution sources (Fitter & Manuel, 1986). Nutrient overload, for example, may serve as an extra food source but at the same time it will result in an oxygen depletion stress in slow running waters because of algal blooms (Lenat, 1983). As a consequence of their large differences in tolerance of eutrophication and organic enrichment, chironomids, in common with oligochaetes, will have indicative representatives in polluted and unpolluted waters (King & Bal, 1964; Ladle & Bird, 1980; Lenat, 1983; Rodriguez & Armas, 1983). Furthermore, the potential of chironomids as biological indicators of heavy-metal contamination has been assessed by several studies based on detected morphological mouthpart deformities (Meregalli et al., 2000; Martinez et al., 2002) and metal adaptation studies (Groenendijk et al., 2002). Finally, besides these water quality aspects, preference of chironomids for certain habitat characteristics (Buskens & Moller Pillot, 1992) can also provide information on the quality of particular hydro-morphological characteristics of a watercourse.

Ecological studies revealing preferences of organisms for certain environmental conditions are often carried out at the species level because of the detailed information contained at this level. Also for chironomid communities in rivers, various studies stress the response at the species level to natural- or human-caused disturbances in rivers (Bazzanti & Bambacigno, 1987; Verdonschot et al., 1992; Reinhold-Dudok van Heel & den Besten, 1999; Orendt, 2000).

Some biotic indices do indeed integrate the information of chironomid communities at the species level (Saether, 1979; Barzerque et al., 1989; Eulin et al., 1993; Evrard, 1996). In Flanders, Belgium, the response of chironomid species to environmental perturbation is, however, not well documented. The biological quality of waters is mainly assessed by means of the Belgian Biotic Index (BBI) (De Pauw & Vanhooren, 1983). This biotic index is based on the taxonomic diversity and the presence/absence of particular indicator taxa. For calculation

of the BBI, macroinvertebrates are identified up to family, genus or group level. The family Chironomidae is split into two groups based on the presence or absence of thummi (the *thummi-plumosus* and the *non thummi-plumosus* group) in correspondence with their clearly different responses to the stress of oxygen depletion.

In the present study we examined whether a more refined identification of chironomids would result in an added value to the biological assessment of running waters in Flanders, Belgium. For this reason, the diversity of chironomid communities and their indicator role in assessment of particular river types were examined by means of multivariate analysis. The sampling sites were clustered based on their chironomid populations, and the principal environmental variables structuring these different chironomid communities were identified. Potential bio-indicators were identified, and the added value of the more refined identification in river assessment and ecological research discussed.

MATERIAL AND METHODS

Study area

The study was performed in the Zwalm river basin, which is part of the Upper-Scheldt basin (Flanders, Belgium), and consists mainly of numerous small brooks (Fig. 1). The Zwalm river basin has a total surface of 11650 ha. The Zwalm river itself has a length of 22 km. The southern part of the Zwalm river basin consists of small brooks situated in the crenal zone, where groundwater flows in the brooks at the source. These brooks are expected to be unpolluted. Because of the specific geomorphology in this area, they have a unique fauna (Goethals & De Pauw, 2001). The northern part is influenced by punctual and diffuse domestic pollution as well as diffuse pollution originating from agricultural activities. Habitat degradation of the watercourses in this region is mainly caused by erosion effects. Sampling sites in the Zwalm river basin are shown in Fig. 1.

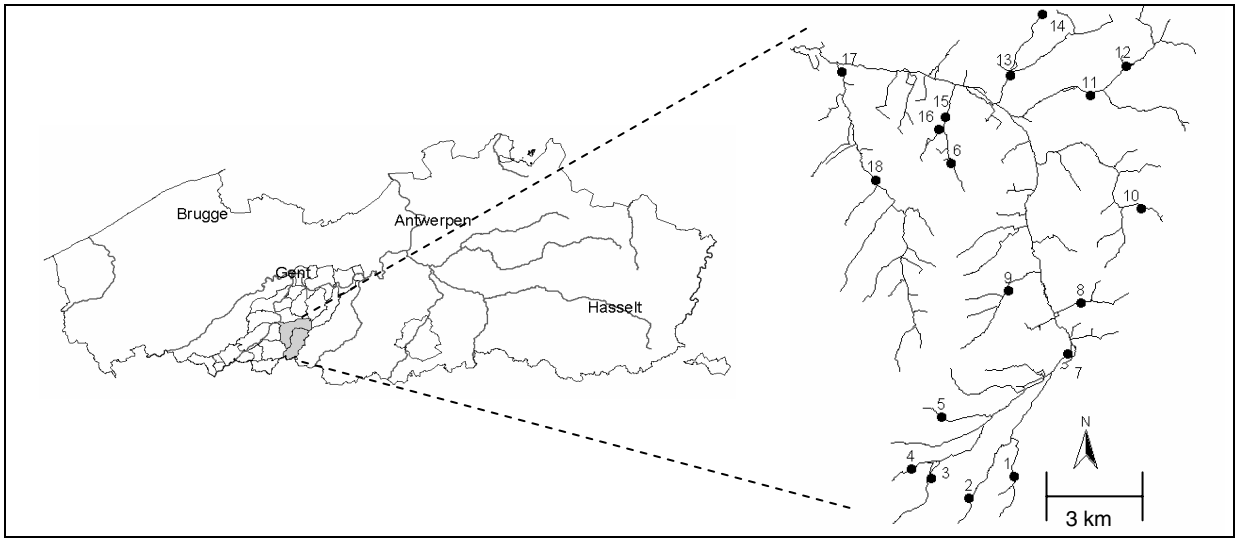

Fig. 1. – Location of (left) and sampling sites in (right) the Zwalm river basin in Flanders, Belgium.

Environmental variables

The measured physical-chemical variables and habitat characteristics are given in Table 1.

TABLE 1

Environmental variables, habitat characteristics and measuring units

Variables	Measuring units
temperature	°C
pH	- log [H+]
conductivity	µS/cm
suspended solids	mg/l
dissolved oxygen	mg O_2/l
chemical oxygen demand	mg O_2/l
total nitrogen	mg N/l
nitrate	mg NO_3^--N/l
ammonium	mg NH_4^+-N/l
total phosphorus	mg P/l
phosphate	mg PO_4-P/l
meandering	6 categories (1 (well developed) to 6 (absent))
hollow banks	6 categories (1 (well developed) to 6 (absent))
pools / riffles	6 categories (1 (well developed) to 6 (absent))

Biological water quality assessment

The biological water quality of the sampling sites was assessed using the Belgian Biotic Index (BBI) in which the macroinvertebrates were identified up to the genus, family or group level (De Pauw & Vanhooren, 1983; IBN, 1984). The BBI is based on taxa diversity as well as the sensitivity of particular groups in relation to pollution (De Pauw & Vannevel, 1993).

The BBI index values are divided into five water quality classes, ranging from very good (BBI value 9-10), good (BBI value 7-8), moderate (BBI value 5-6) to bad (BBI value 3-4) and very bad (BBI value 0-2) quality. These classes can be visually represented by a colour code (respectively blue, green, yellow, orange, red).

Chironomid taxa

Chironomids were identified up to species, aggregate, group, subgenus or genus level, depending on the external morphology of the instar stage of the chironomid. Sampling of the chironomids was done together with the other macroinvertebrate taxa by means of a handnet (mesh size

= 350µm) (De Pauw & Vanhooren, 1983) and samples were sieved the day after sampling. The following identification keys were used : Paine (1956), Klein (1967), Mason (1968), Moller Pillot (1978), Klink (1981), Cranston (1982), Klink (1983), Wiederholm (1983) and Moller Pillot (1984). Availability of suitable identification keys for the larval stages (instars) and the presence of larvae not reaching the 4th larval stage, were often the limiting factors. Chironomids that could not be identified up to species level were identified up to the lowest practical level attainable (i.e. aggregate, group, subgenus or genus level).

Data analysis

Data were processed based on the methodology described in Fig. 2.

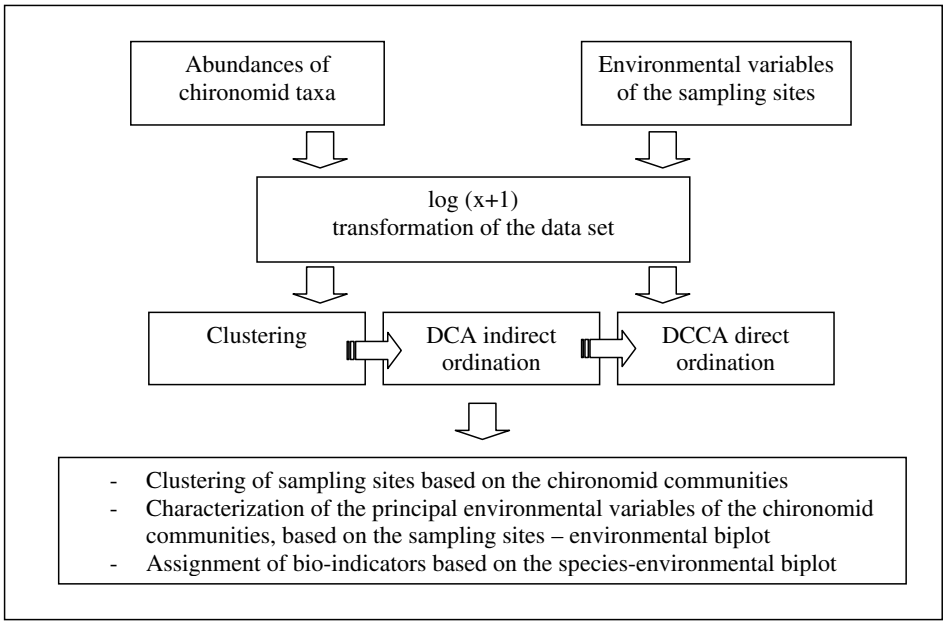

Fig. 2. – Treatment and methodology of multivariate analyses of the biological and environmental data.

Biological and environmental data were log(x+1) transformed, except for the categorical variables (meandering, pool/riffle, and hollow bed development) and the pH. The transformed data were processed by means of several multivariate analyses, more specifically a clustering by making use of FLEXCLUS (van Tongeren, 1986) as well as an indirect (Detrended Correspondence Analysis (DCA)) and a direct ordination (Detrended Canonical Correspondence Analysis (DCCA)) based on CANOCO (ter Braak, 1986; ter Braak & Smilauer, 1998).

Clustering with the programme FLEXCLUS (Van Tongeren, 1986) is based on the Sørensen similarity ratio (Sørensen, 1984) where the most similar samples of the biological data are placed next to each other based on the biological data. The Sørensen similarity ratio (*SR*) used for the clustering is as follows :

$$SRi_j = \sum_k y_{ki} y_{kj} / \left(\sum_k y_{ki^2} + \sum_k y_{kj^2} - \sum_k y_{ki} y_{kj} \right)$$

where y_{ki} = the abundance of the k^{th} species at site i and y_{kj} = the abundance of the k^{th} species at site j. The initial, non-hierarchical clustering is a means for handling noise and redundancy by combining several samples into groups following the algorithm of Sørensen (1984). Samples are fused into clusters when their similarity is higher than the given threshold value. Refinement of the initial clustering by reallocation leads to a final clustering, which is a combination of fusion and division of clusters based on the distance of a sample to the cluster centroid. The ordering of clusters is obtained by reciprocal averaging (Hill, 1973; Van Tongeren, 1986).

DCA was used for indirect ordination analysis and detrending was done by segments. Because species abundance or probability of occurrence is often a unimodal function of the environmental variables, for direct analysis we opted for the unimodal DCCA ordination method (ter Braak & Verdonschot, 1995) when the gradient length of the first DCA axis > 2. DCCA was used for direct unimodal analysis, and detrending was done by polynomials. In this analysis, habitat characteristics such as meandering-, pool/riffle- and hollow beds-development, were included as categorical variables.

RESULTS

Chironomid biodiversity and biological assessment

In total, 31 different chironomid taxa were found in the 18 sampling sites (Table 2).

Among these 31 taxa, *Macropelopia,* cf. *Conchapelopia* and *Prodiamesa olivacea* (Meigen, 1830) appeared in all samples. Group *Conchapelopia* consists of four different genera, each with a different habitat preference. Some taxa such as *Brillia modesta* (Meigen, 1830)*, Polypedilum* cf. *breviantennatum* (Tsjernovskij, 1949)*, Polypedilum* gr. *laetum, Polypedilum pedestre* agg. and *Zavrelimyia* were only found in the crenal zone of the brooks.

TABLE 2

Taxonomic level, taxon name and abbreviations of the monitored chironomid taxa in the Zwalm river basin

Taxonomic level	Taxon name	Abbreviations
Species	*Apsectrotanypus trifascipennis* (Zetterstedt, 1838)	Aspe trif
	Brillia longifurca Kieffer,1921	Bril long
	Brillia modesta (Meigen, 1830)	Bril mode
	Odontomesa fulva (Kieffer, 1919)	Odon fulv
	Parametriocnemius stylatus (Kieffer, 1924)	Para styl
	Prodiamesa olivacea (Meigen, 1818)	Prod oliv
	Psectrotanypus varius (Fabricius, 1787)	Psec vari
	Rheocricotopus fuscipes (Kieffer, 1909)	Rheo fusc
Aggregate (Agg.)	Eukiefferiella discoloripes agg	Euki disc
	Paraphaenocladius impensus agg.	Para impe
	Polypedilum pedestre agg.	Poly pede
	Paracladopelma camptolabis agg.	Para camp
Grouplevel (Gr.)	*Chaetocladius* gr. *piger,*	Chae piger
	Chaetocladius gr. *vitellinus,*	Chae vite
	Cryptochironomus gr. *defectus,*	Cryp defe
	Dicrotendipes gr. *notatus,*	Dicr nota
	Dicrotendipes gr. *nervosus*	Dicr nerv
	Polypedilum gr. *laetum*	Poly laet
	Polypedilum gr. *scalaenum*	Poly brev
Subgenus	*Procladius (Holotynapus)*	Proc holo
Genus	cf. *Conchapelopia,*	Cf. Concha
	Chironomus,	Chironom
	Limnophyes,	Limnophy
	Macropelopia,	Macropel
	Micropsectra,	Microspe
	Paratanytarsus,	Paratany
	Phaenopsectra,	Phaenops
	Rheotanytarsus,	Rheotany
	Stempellinella,	Stempeli
	Tanytarsus,	Tanytars

The biological water quality of the Zwalm river basin based on the BBI is illustrated in Fig. 3A. The results show that most of the sampling sites (13 of 18) were moderately polluted. Even for the brooks in the crenal zone, no classification as "very good" was reached. Two sampling sites, located in the small brooks of the crenal zone, reached the basic water quality standard for Flanders (BBI value ≥7). Bad conditions were present at two sites, located near and in the Zwalm river. One sampling site was depleted of macroinvertebrates and was classified as having a very bad quality.

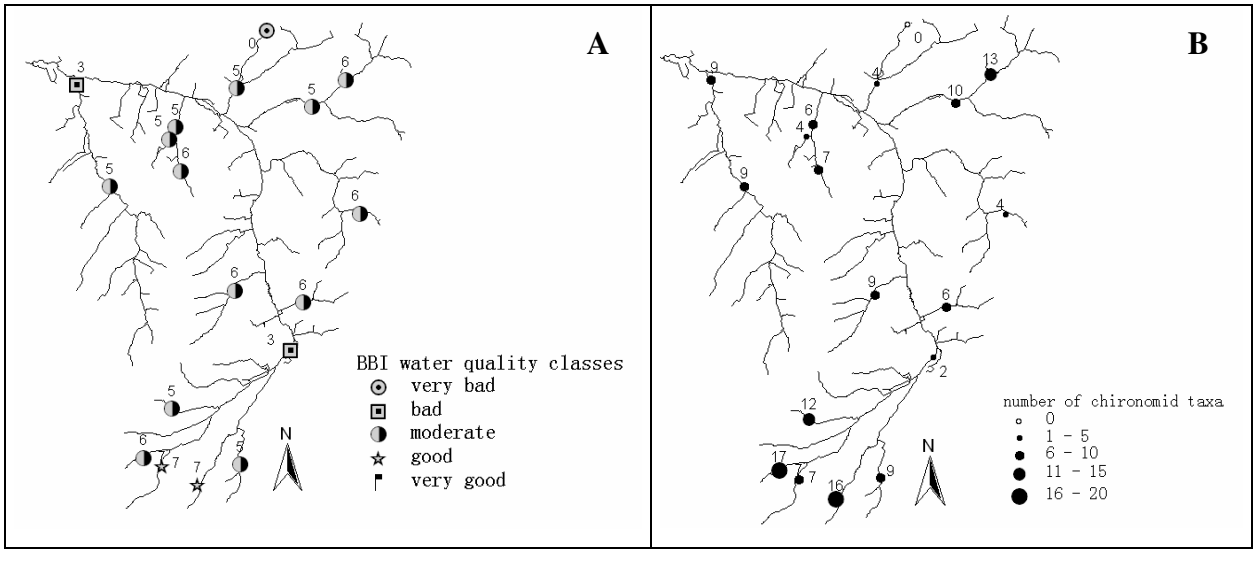

Fig. 3. – BBI water quality values (A) and number of chironomid taxa sampled (B) in 18 sites of the Zwalm river basin.

Fig. 3B also shows the number of chironomid taxa found in each site. A high chironomid biodiversity was found at the sites located near the source. This taxa richness, however, was not reflected by the BBI. For example, one of the sampling sites, classified as "good", contained only seven chironomid taxa, while another sample also classified as "good" had 16 taxa. Samples collected in the Zwalm river itself contained only chironomid taxa, present in low numbers, and no other macroinvertebrate taxa.

Multivariate analyses of the Zwalm river basin data

The output of the DCA analysis revealed a cumulative variance explained by the first two axes of respectively 22.3% and 31.6%. The gradient length of the first DCA axis was 2.331 and therefore a unimodal direct analysis (DCCA) was chosen.

The cumulative variance of the first two axes in the direct DCCA analysis, which explained the role of the environmental variables in the structure of the chironomid communities, was 24.4% and 35.6%, respectively. The eigenvalues of the first two DCCA axes (0.355 and 0.163, respectively) were very close to those obtained from the DCA analysis (0.360 and 0.150, respectively). Therefore, it can be said that the measured environmental variables are important parameters determining the structure of the chironomid communities. The DCCA biplot of sampling sites (assigned by cluster membership) and environmental variables is shown in Fig. 4.

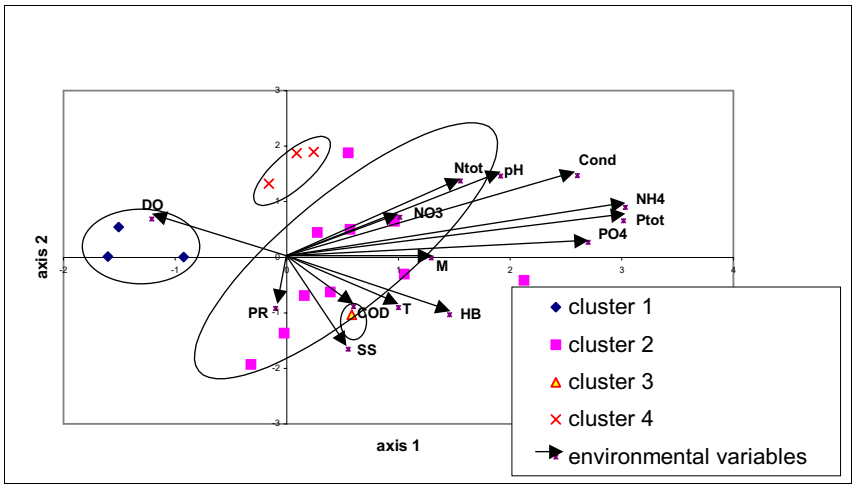

Fig. 4. – DCCA biplot of sampling sites, characterized by their cluster membership based on chironomid communities and environmental variables measured at 18 sites in the Zwalm river basin. (T = temperature, SS = suspended solids, DO = dissolved oxygen, COD = chemical oxygen demand, N tot = total nitrogen, Cond = electrical conductivity, NH4 = ammonium, P tot = total phosphorus, PO4 = phosphate, M = meandering development, HB = hollow beds development, PR = pool/riffle development).

On the basis of the chironomid abundance data, the sampling sites could be subdivided into four clusters by means of the Sørensen similarity ratio clustering, and these results were confirmed by the ordination method revealing the same clustering structure (Fig. 4). The main explanatory variables proved to be ammonium and phosphorus concentrations, electrical conductivity, dissolved oxygen concentration, and pH of the water. The cluster membership of the different sites in the Zwalm river basin is given in Fig. 5.

TABLE 3

Characterization of the different clusters of sampling sites in the Zwalm river basin, based on the chironomid communities

Cluster n°	Number of sampling sites	Number of chironomid taxa	Location in the Zwalm river basin	Environmental characterization of the cluster
Cluster 1	3	+++	near the source	*Fast running non-polluted streams* high dissolved oxygen concentrations low nutrient and organic concentrations
Cluster 2	10	++	small brooks near the mouth of the Zwalm	*Streams subjected to diffuse agricultural pollution* high nutrient level and organic contents high suspended solids concentrations high water temperature values high pH values
Cluster 3	1	---	the Zwalm river	
Cluster 4	3	--	head brooks in agricultural area	low ammonium concentrations high nitrate concentrations

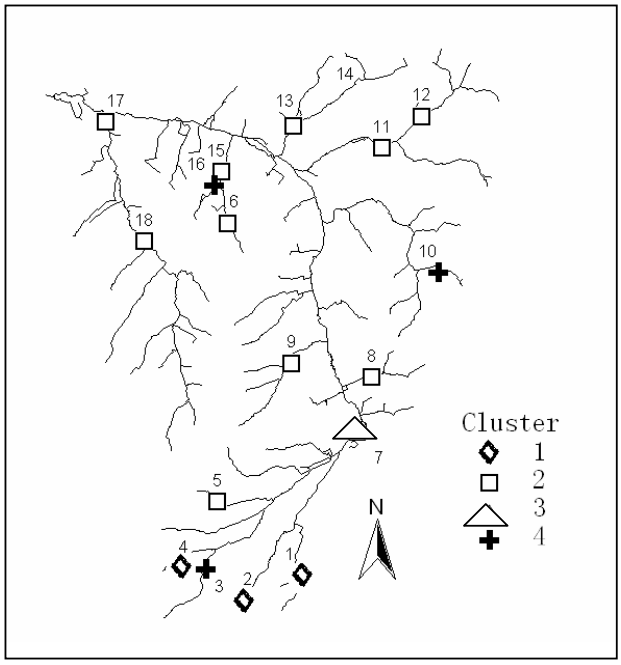

Fig. 5. – Cluster membership of the different sampling sites in the Zwalm river basin based on the Sørensen similarity ratio clustering of the chironomid data, based on FLEXCLUS.

An overview of the most important characteristics of each cluster based on the clustering and direct ordination analysis (Fig. 4) and their location in the Zwalm river basin (Fig. 5) is given in Table 3.

Cluster 1 contained *Brillia longifurca* (Kiefer, 1921), *Procladius holotanypus* (Robback, 1982) and *Tanytarsus*. *Brillia longifurca* was highly frequent and strongly positively correlated with dissolved oxygen concentration. In cluster 2, *Chironomus* was the most frequent taxon, positively correlated with organic load in the water. Cluster 3 contained only one site located in the river. At this site no macroinvertebrate taxa were found except for two chironomid taxa, i.e. *Chironomus* and *Prodiamesa olivacea*. Cluster 4 was not characterized by any specific chironomid taxon.

In Fig. 6, the DCCA biplot of chironomid taxa and environmental variables is shown. Environmental variables and sampling sites are plotted together in a two-dimensional space, oriented by the first two axes, which explain the most important part of the variation in the dataset.

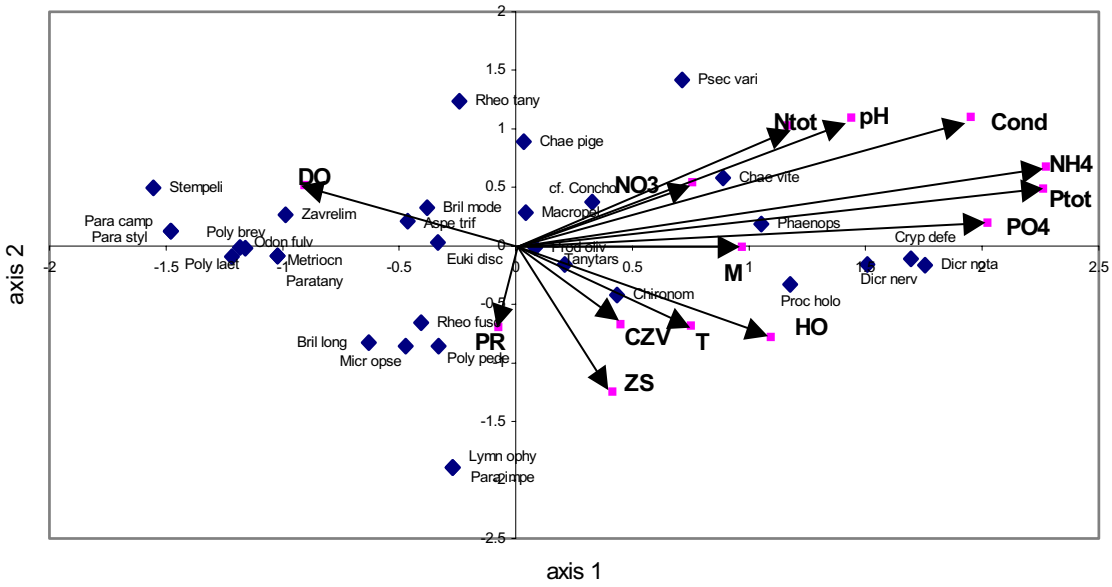

Fig. 6. – DCCA biplot of chironomid taxa and environmental variables monitored in the Zwalm river basin (T = temperature, SS = suspended solids, DO = dissolved oxygen, COD = chemical oxygen demand, N tot = total nitrogen, Cond = electrical conductivity, NH4 = ammonium, P tot = total phosphorus, PO4 = phosphate, M = meandering development, HB = hollow beds development, PR = pool/riffle development). The legend of the abbreviations of the chironomid taxa is given in Table 2.

DISCUSSION

Although distribution patterns of the family Chironomidae are well documented in Belgium (Goddeeris & Behen, 1991; Evrard, 1995a, 1995b), thorough knowledge about the major environmental factors that control their distribution is still lacking. In the present study, several taxa such as *Parametriocnemus stylatus, Limnophyes* (Eaton, 1875), *Brillia modesta* and *Polypedilum* cf. *brevi-antennatum* were only found in the brooks in the crenal zone. In the study of Verdonschot (2000b), these taxa were assigned as source brook indicator taxa. Taxa characteristic of headwaters (cf. Verdonschot, 2000c; personal communication) are very scarce in the present study. This is probably due to the combination of erosion, diffuse pollution and habitat degradation in the Zwalm river basin, drastically reducing chironomid diversity. The typical succession of chironomid taxa from source to

mouth can be recognized, but the higher the impact of water deterioration the less transparent was the succession in the Zwalm data (cf. Rossaro, 1991).

No correlation could be found between the number of chironomid taxa and the BBI. This can be explained by (1) the absence of pollution-sensitive macroinvertebrate taxa. As do most of the biotic indices, the BBI assesses water quality on the basis of the taxonomic diversity of the benthic macroinvertebrate community and the presence or absence of indicator taxa (De Pauw & Vanhooren, 1983). Sensitive indicator taxa such as Plecoptera and Heptageniidae weigh the most heavily. These two taxa, however, are seldom found in the Zwalm river basin (e.g. between 2000 and 2002, Plecoptera were only found in two sampling sites in 2002, Heptageniidae were found only once in four sampling sites in 2000 and 2002; both taxa were found only twice and in very low abundance). The absence of these two taxa, which could partly be explained by the heterotrophy in the crenal zone, thus lowers the BBI values of the brooks located in the zone, where, however, the highest chrironomid diversity was found; (2) differences in response to environmental changes between chironomid taxa abundance and the whole macroinvertebrate community expressed as the BBI. Similar results have been reported by Bazaanti & Bambacigno (1987) who did not find a correlation between the number of chironomid taxa and any biological index applied on the Italian streams.

Multivariate analysis, such as ordination and clustering, is often used to analyse environmental preferences of macroinvertebrate communities (Verdonschot et al., 1992; Ter braak & Verdonschot, 1995; Cao et al., 1997; Verdonschot, 2000a). In this way, river management can be focused on the mitigation of certain pollution sources that cause e.g. acidification, eutrophication, habitat degradation. The agglomerative clustering method FLEXCLUS allowed grouping of sampling sites, revealing the same pattern of groups as in the ordination analysis with CANOCO. In our study, some sites of the data matrix had a similar chironomid community but differed in abundance for a large part of the matrix (e.g. cluster 2 and cluster 4), and as such, clusters overlapped each other. This equalising effect is probably caused by pollution, which excludes specific chironomid taxa and promotes the dominance of tolerant ubiquitous chironomid taxa. This was also referred to in the research of Verdonschot et al. (1992).

The ecological preferences of the taxa in our study within the different clusters have been compared with the ecological study of chironomids in the Netherlands (Moller Pillot & Buskens, 1990). *Brillia longifurca*, although not very typical for brooks in the crenal zone, is, however, strongly correlated with dissolved oxygen concentration, a correlation that also could be deduced from our results. Although *Procladius holotanypus* (Robback, 1982) is highly frequent in different stream types, and is very tolerant for differing physical-chemical conditions, it was only found in three places, in one source brook. *Tanytarsus* species can be found in different water types under different conditions, and was found in our research at the brooks of the crenal zone and the mouth of the Zwalm river, probably representing different *Tanytarsus* species.

In general, natural chironomid communities in the brooks of the crenal zone could be separated from the other communities by means of clustering (Fig. 5). This agrees with the expected unpolluted conditions of the crenal zone (southern part) in the Zwalm river basin.

The present study shows that the main explanatory variables for interpreting the results of the direct ordination were ammonium and phosphorus concentrations, electrical conductivity and pH of the water (Fig. 4 and Fig. 6). Nutrient concentrations (e.g. ammonium and phosphorus) were also reported as the main explanatory variables in several other studies e.g. Moller Pillot & Buskens (1990). Based on the DCCA species biplot of the Zwalm data (Fig. 6) and the ecological information provided by Bazzanti & Bambacigno (1987) and Moller Pillot & Buskens (1990), indicator taxa for eutrophication, which can be of use for rivers in Flanders, can be suggested. Indicators of oligotrophic conditions are *Paracladopelma camptolabis* agg. and *Parametriocnemus stylatus*. Indicators of waters enriched with nutrients and organic compounds are *Chironomus, Psectrotnaypus varius* and *Dicrotendipes* gr. *nervosus* and taxa appearing in eutrophic as well as oligotrophic conditions are, for example, *Prodiamesa olivacea.* Beside these taxa, other chironomid indicator taxa were proposed by Bazzanti & Bambacigno (1987) and Moller Pillot & Buskens (1990) and should receive attention in future research.

Besides the nutrients, the role of pH as an influencing variable for chironomid communities in running waters has been stressed (Orendt, 1998, 2000). In our study, no specific pH indicator taxa were detected (Fig. 6). According to Verdonschot et al. (1992) habitat variables are more important than physical-chemical variables influencing the distribution of chironomids, which was also noted by Rae (1985) and Buskens & Moller Pillot (1992). A more detailed analysis of the role of chironomids as indicators of habitat quality will, therefore, be necessary in the future when habitat restoration becomes of greater importance in river management. More refined analysis of habitat preferences of chironomid species, e.g. for hydraulic and sediment characteristics, could yield important additional information (Verdonschot et al., 1992).

It has been reported that a grab sampler is an appropriate instrument for collecting chironomid organisms from the bottom sediment (Int Panis et al., 1995). From our results it appeared that the chironomid taxa, which are buried in the sediment, could also representatively be sampled with a 350µm handnet (e.g. *Chaetocladius* gr. *piger*). However, a biased view of the biological communities could be obtained because certain smaller chironomid taxa may seem absent when sampled with a handnet of 350µm (Storey & Pinder, 1985). Regarding the sampling season, it has been demonstrated that during winter a lesser number of species can be found because of the formation of resting stages of some chironomid taxa (mackey, 1977; Goddeeris, 1987; Rossaro, 1991). Thus, sampling in summer, as was done in the present study, should be appropriate to ascertain chironomid biodiversity (Moller Pillot, 1978).

In the present study, chironomid taxa were identified at the lowest level possible to analyse the ecological information gathered for the running waters in the Zwalm river basin. The more refined identification level of the chironomids could provide useful information for application within water quality assessment, but also for obtaining information about aspects such as the ecology of indicator taxa in specific water types and a better ecological characterization of the brooklets. The chironomid community structure can also serve as an indication of the habitat and the water quality of the river, based on the absence/presence of some indicator species (Verdonschot, 2000b, 2000c). It could even be possible to construct specific chironomid indices, reflecting the habitat quality (Verdonschot et al., 1992). One has to keep in mind, however, that an ecological evaluation based on chironomids should include type specific reference conditions.

The advantage of using species-level data to obtain more meaningful information for the assessment of the biological condition of a site has been addressed by several authors (Bowman & Bailey, 1997; Guerold, 2000). The present study shows that identification of chironomids at a more detailed level than family level provides additional information on the water quality of the Zwalm river basin when compared to the BBI. However, for application of this refined identification in routine monitoring, time and effort (expertise/personnel) required for such work should be taken into consideration (personal communication). Identification of chironomids at a more detailed level can, therefore, probably not be part of a rapid bioassessment procedure for rivers in Flanders.

CONCLUSION

In this study, chironomid taxa could be assigned as indicators for natural running waters in Flanders (e.g. brooks in the crenal zone) and nutrient conditions. These results were obtained by multivariate analyses based on data collected within the Zwalm river basin. These techniques were very useful to detect relationships between chironomid taxa and environmental variables in a river basin. In the future, physical characteristics of the watercourses should also be included in such studies because of the importance of the habitat conditions for chironomid communities. A more refined identification of the chironomids provided useful information for various applications, including the study of the ecology of these indicator organisms in specific water types and a better ecological characterization of the brooklets where they were collected. Identification of the chironomid communities is, however, very laborious and as such, due to its relatively high energy-input, not directly suitable for rapid bio-assessments.

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Diet composition in relation to morphology in some African anguilliform clariid catfishes

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ABSTRACT. Genera of the catfish family Clariidae that have an extreme anguilliform body shape (e.g. *Dolichallabes*, *Gymnallabes* and *Channallabes*) are also characterized by an extreme hypertrophy of the jaw closing muscles. Whereas it has been demonstrated that the hypertrophy of these muscles results in increased bite performance, the adaptive significance of these enlarged jaw muscles remains unclear. Given that hypertrophied forms bite harder it was suggested that the hypertrophy of the jaw adductors might be correlated with changes in diet or an altered feeding pattern. To test this hypothesis, stomach contents were analysed from specimens of a few more anguilliform species, as well as in a limited set of specimens from some of the less anguilliform clariid species. The results show that specimens with a more anguilliform body-shape have a different feeding pattern with a special preference for coleopterans. However, the absence of bite-marks on the prey retrieved from the stomachs indicated that jaw morphology and bite performance are not likely to be related to the dietary composition in the species with hypertrophied jaw adductors.

KEY WORDS : anguilliformity, Clariidae, diet, ecomorphology, Siluriformes.

INTRODUCTION

Members of the Clariidae, one of the 35 presently recognised families of catfishes (DE PINNA, 1998), mainly occur on the African continent, but can also be found in Minor Asia, the Indian subcontinent and southeast Asia (TEUGELS, 1996). Clariids are primarily freshwater fishes, but they also tolerate brackish water. Together with their ability to utilize atmospheric air through a suprabranchial organ and to "walk" on land, this tolerance partially explains their large area of dispersion (ROBERTS, 1975; TEUGELS, 1996). Clariids, and many other families of Siluriformes, are also characterized by a more benthic and/or nocturnal life-style, combined with morphological adaptations for such a life-style (ALEXANDER, 1965). These alterations are mainly : (1) small eyes, in combination with a highly specialised Weberian apparatus, and the presence of oral barbels, and (2) the dorso-ventral flattening of the head (as an adaptation towards a more benthic life-style) (FINK & FINK, 1981; ADRIAENS & VERRAES, 1997).

Within the Clariidae, both genera with a more fusiform and genera with a distinct anguilliform body shape exist, as well as intermediate forms. The more anguilliform genera show several transformations within the cranial morphology associated with hypertrophy of the adductor mandibulae complex (CABUY et al., 1999; DEVAERE et al., 2001). Although it has been shown that the hypertrophy of the jaw adductors results in an increased bite performance (HERREL et al., 2002), the question remains whether this is an actual adaptation or simply the result of the miniaturization of the skull (DEVAERE et al., 2001).

As the hypertrophy of the jaw adductors results in an increase in bite force, an often suggested and plausible explanation would be that this is an adaptation to a dietary specialisation involving the crushing of hard prey. This explanation can be tested by comparing the diet of hypertrophied forms with that of non-hypertrophied ones. Whereas the diet of some more fusiform genera that show no hypertrophy of the jaw adductors (e.g. *Clarias* sp., SCOPOLI, 1777) has been well investigated (SPATARU et al., 1987; FAGBENRO et al., 1991), little or no information is available concerning the diet of the genera with more hypertrophied jaw adductors.

Thus, the aims of this study were : (1) to determine, based on stomach-content analysis, the overall composition of the diet of different clariid species, (2) to investigate whether the species with hypertrophied jaw adductors show changes in diet compared to species with less hypertrophied jaw adductors and (3) to examine whether the hypertrophy of the jaw adductors can be linked to shifts in diet.

MATERIAL AND METHODS

Sampling

For this study, a total of 306 specimens from six species of clariids were examined, focussing largely on the species with the most extreme hypertrophy of the jaw

adductors : *Channallabes apus* (Günther, 1873) (173 specimens), *Gymnallabes alvarezi* Roman, 1970 (85 specimens) and *Gymnallabes typus* Günther, 1867 (25 specimens). For comparative purposes, the diets of some of the intermediate species of clariids were also examined : *Platyallabes tihoni* (Poll, 1944) (13 specimens), *Clariallabes melas* (Boulenger, 1887) (five specimens) and *Clariallabes longicauda* (Boulenger, 1902) (five specimens). However, certain geographically distinct populations of *C. apus* and *G. alvarezi* might be in need of transfer from one species to the other. For the purpose of the present paper, however, this issue is of minor importance as the main objective was to determine whether the species with strongly hypertrophied jaw adductors such as *C. apus*, *G. alvarezi*, *G. typus* and *Dolichallabes microphthalmus*, have specialized diets that might be related to the observed hypertrophy of the jaw adductors. Some of the specimens used were obtained from the Koninklijk Museum voor Midden-Afrika/Musée Royal de l'Afrique Centrale (MRAC), Tervuren, Belgium and some from an expedition to Gabon in September 2000. Due to the scarcity of material, no conclusions as to seasonality or locality of the diet could be made. From all specimens the content of the stomach was removed, sorted and identified up to the level of order.

To assess whether animals actually selected certain prey, several pitfall traps were placed at two distinct sites in Gabon where the fish (half of the *C. apus* specimens and almost all *G. typus* specimens) were collected. These sites were chosen in such a way that they reflected the overall composition of the habitat of the anguilliform clariid species. Pitfalls were placed, for 48 hours, in the swamp area where fish were collected, and randomly arranged at each site. Four aluminium guides were placed perpendicularly around each pitfall to increase capture success. The contents of the traps were sorted and identified as mentioned above.

As it was expected that animals with hypertrophied jaw adductors would preferentially feed on harder prey, the hardness of the prey collected in the pitfalls was determined. By comparing prey in the stomach of the fishes with the hardness data of the available prey we were able to determine whether fish actually selected for harder prey. Prey hardness was measured using a Kistler force transducer (type 9203, Kistler, Zwitserland), connected to a Kistler charge amplifier (type 5058A, Kistler, Zwitserland) and a PC equipped with an electronic measuring board (PC-Scope T512, IMTEC, Germany), as described in Herrel et al. (1999). A screw with narrow pointed tip was attached to the transducer and slowly pushed onto the prey until failure of the exoskeleton of the hardest part of the body (usually the head and prothorax). The maximal force recorded just before failure of the prey was considered to be the hardness of that prey. Differences in prey hardness between dietary categories were compared by means of pairwise Mann-Whitney U tests.

As the formaldehyde solution used to preserve arthropods in the pitfalls might affect hardness, we experimentally tested the effects of preservation on the hardness of two prey types : mealworms and crickets. Thirty house crickets (*Acheta domestica* L., 1758) and twenty large mealworms (*Tenebrio molitor* L., 1758) were selected for testing. Each prey group was randomly subdivided in half, and euthanised. One half of the prey groups was subsequently preserved in a 10% aqueous formaldehyde solution for five days. The other half of each group was measured (length and width) using Mitutoyo (type CD 20DC) electronic callipers, and weighed to the nearest 0,001 gram using a Denver Instruments (M220) electronic balance. Next, prey hardness was measured for each individual prey item using a Kistler (type 9203) force transducer attached to a portable Kistler charge amplifier (type 5995) as described in Herrel et al. (2001). Preserved prey were taken out of the formaldehyde, rinsed, blotted dry and measured, weighed and crushed as described above. Two-way analysis of variance was used to estimate the effects of preservation and prey type on prey hardness (SPSS v. 10.05).

Data analysis

To describe and compare diets among species, several indices per food item were calculated :

– Relative importance index (Pinkas et al., 1971) :

$$IRI = (\%N + \%V) \,.\, \%Oc$$

where %N and %Oc are, respectively, numeric abundance and the frequency of occurrence, and %V is the volumetric percentage of the prey type. In this research the %V was replaced by %DW as suggested by Koné (2000). In addition, %IRI was calculated, being the proportion of IRI of each prey type in relation to the total IRI value.

– Electivity index (Kohler & Ney, 1982) :

$$E_i = (\%N - \%N_{pit}) / (\%N + \%N_{pit})$$

where %N and $\%N_{pit}$ are as mentioned above. This index compares the abundancies of the prey items as found in the stomachs with those in the pitfalls, thus providing an indication of the degree to which the diet is selective.

In addition, the diets from the two species with the largest sample size, *C. apus* and *G. alvarezi*, were compared using a randomisation test designed by Mantel (1967) and used by Patterson (1986) and Sevenster & Bouton (1998) for the comparison of diets. This procedure uses the mean percentage overlap as a test statistic. The percentage overlap between two individuals *a* and *b* is calculated as :

$$O_{ab} = \sum_i \min (p_{ai}, p_{bi})$$

where p_{ai} is the proportion of prey type *i* used by specimen *a* and min (p_{ai}, p_{bi}) is the smallest of p_{ai} and p_{bi} . The statistic for interspecific overlap is the mean of the overlaps in all possible heterospecific pairs of individuals, and the statistic for intraspecific similarity is the mean of the overlaps in all possible pairs of individuals of the same species (Sevenster & Bouton, 1998).

RESULTS

The dietary results are summarized below and are sorted per species as they were defined by Poll (1942).

Species diet

Table 1 shows the results of the stomach content analysis. For *C. apus* the table shows that Coleoptera is the species' main prey type (%IRI = 89,31). In comparison with

the number of Coleoptera found in the pitfalls the species shows actual selection for the given prey item (E_i = 0,69), which also seemed to be the case for Isoptera (E_i = 0,75). The latter value is, however, of little significance and will be dealt with further in the discussion. Hymenoptera (E_i = -0,66) and also Hemiptera, Diptera and Araneae are underrepresented. Furthermore, *C. apus* has a high vacuity index and a low Shannon-Wiener index or small diet breadth.

TABLE 1

Diet composition in *C. apus* and *G. alvarezi*

Prey category	N	%IRI	%Pit	E_i	N	%IRI	%Pit	E_i
Mollusca								
Gastropoda	3	0.36	-	-	-	-	-	-
Malacostraca								
Isopoda	2	0.7	-	-	-	-	-	-
Arachnida								
Araneae	5	1.90	7.80	-0.27	-	-	-	-
Insecta								
Ephemeroptera	3	0.21	-	-	-	-	-	-
Odonata	6	2.01	-	-	1	0.96	1.00	0.41
Dictyoptera	1	0.34	0.49	0.30	-	-	-	-
Isoptera	15	0.51	1.95	0.75	-	-	-	-
Hemiptera	5	1.24	9.76	-0.37	1	0.09	4.81	-0.35
Diptera	8	1.84	9.76	-0.15	5	1.86	3.85	0.50
Hymenoptera	7	1.23	31.22	-0.66	1	0.12	37.50	-0.88
Coleoptera	54	89.31	8.78	0.69	34	96.97	31.73	0.43
Actinopterygii								
Cypriniformes	2	0.65	-	-	-	-	-	-
Aves								
Sp.	-	-	-	-	1	-	-	-
							85	
No. of stomachs examined			173					
Vacuity index		68.79				75.29		
Shannon-Wiener index			0.57				0.16	

Also for *G. alvarezi* Coleoptera form the majority of the diet (%IRI = 96,97) and a positive selection towards this prey type is present (E_i=0,43). Other results have little significance, since the absolute numbers of prey eaten were low. Remarkable, however, is the small bird found in one of the *G. alvarezi* stomachs. The results also show a high vacuity index and low Shannon-Wiener index.

Table 2 combines the results of the other species examined. The numbers of prey found in these analyses were, however, so low that no significant conclusions could be made. Nevertheless the data are presented for comparison and can be considered as indicative. The high percentage of dipteran larvae and Ephemeroptera is remarkable for *G. typus* and *P. tihoni*. The high abundance of fish prey in the analysis of *C. melas* is another interesting point of possible divergence compared to the two diets previously mentioned. The results for *C. longicauda*, however, only show Coleoptera within the diet.

TABLE 2

Diet composition in *G. typus*, *P. tihoni*, *C. melas* and *C. longicauda*

Species	Prey Category	N	%N
G. typus	Insecta		
	Diptera	10	90.91
	Coleoptera	1	9.09
Vacuity index = 88,0 %			
P. tihoni	Insecta		
	Ephemeroptera	5	71.43
	Diptera	2	28.57
Vacuity index = 76,9 %			
C. melas	Insecta		
	Odonata	2	40.00
	Diptera	1	20.00
	Actinopterygii		
	Gonorhynchiformes	2	40.00
Vacuity index = 40,0 %			
C. longicauda	Insecta		
	Coleoptera	3	100.00
Vacuity index = 60,0 %			

Prey hardness

Fig. 1 shows the results obtained on the relative hardness of different prey types that occur within the habitat of the investigated species. These results were analysed by means of pairwise Mann-Whitney U tests, from which the respective p-levels are given in Table 3. This table shows that Hymenoptera are significantly harder than all other investigated prey types, followed by Coleoptera and Hemiptera, which in turn are significantly harder than all remaining prey, except for Dictyoptera. Gryllidae, Araneae and Decapoda are in turn significantly softer than all other prey items, except for Dictyoptera. Supplementary tests also showed that effects of preservation on prey hardness were present but had no influence on the relative proportions of prey hardness.

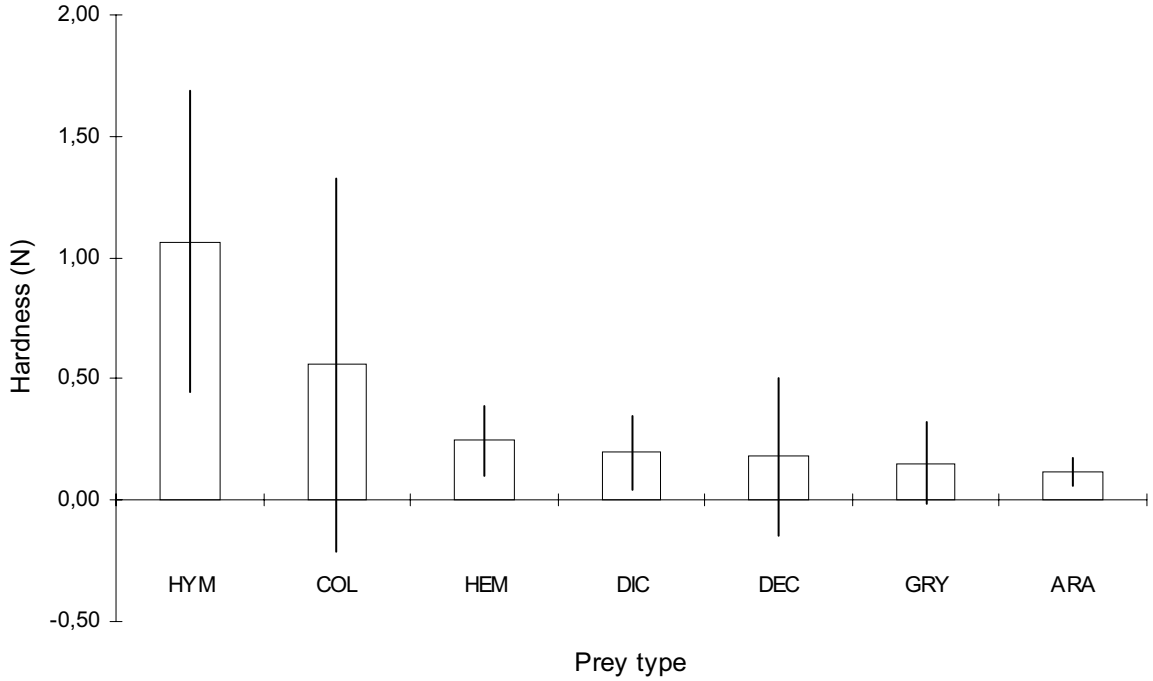

Fig. 1. – Comparison of hardness of prey from pitfalls (HYM : Hymenoptera; COL : Coleoptera; HEM : Hemiptera; DIC : Dictyoptera; DEC : Decapoda; GRY : Gryllidae; ARA : Araneae) The column height displays the average, the whiskers indicate standard deviation.

TABLE 3

Pairwise comparison of U-values (bottom) and p-levels (top) in Mann-Whitney U test concerning relative hardness of possible prey types. (DEC : Decapoda; ARA : Araneae; GRY : Gryllidae; DIC : Dictyoptera; HEM : Hemiptera; HYM : Hymenoptera; COL : Coleoptera) *p < 0.05; ** p < 0.005.

	DEC	ARA	GRY	DIC	HEM	HYM	COL
DEC		0,136	0,096	0,148	*	**	**
ARA			0,835	0,440	*	**	*
GRY				0,250	*	**	*
DIC					0,444	*	0,353
HEM						**	0,372
HYM							**
COL							

Dietary overlap

The Schoener index mainly indicated dietary overlap between *C. apus* and *G. alvarezi*, a hypothesis that was further tested in a randomisation test, of which the results are represented in Table 4. The table shows that the diets of *C. apus* and *G. alvarezi* do not differ significantly (p = 1.00). The results also show a high intraspecific overlap in *C. apus* (p = 0.001) and a low intraspecific overlap in *G. alvarezi* (p = 1.000).

TABLE 4

Observed (Obs.) and expected (Exp.) overlaps, and estimated significance, based on 1000 simulations.

Overlap	Obs.	Exp.	Significance	
Between groups	36.8	31.5	p = 1.000	(100.0% of simulations < observed)
Within *C. apus*	22.6	31.3	p = 0.001	(0.1% of simulations > observed)
Within *G. alvarezi*	60.6	31.7	p = 1.000	(100.0% of simulations > observed)

DISCUSSION

This study indicates that both *C. apus* and *G. alvarezi* mainly feed on Coleoptera and that their diets in general do not differ significantly. This is indicated by the Schoener index as well as in the randomisation test. The latter, however, reveals a much higher intraspecific overlap in *C. apus* than in *G. alvarezi*, which could imply that *G. alvarezi* is a more specialised feeder, whereas *C. apus* has a more generalised feeding habit (Sevenster & Bouton, 1998). The fact that the abundance of Coleoptera was lower in the pitfalls than in the stomachs of both species nevertheless indicates that both species tend to have a preference for Coleoptera (E_i = 0.69 and 0.43). The fact that both diets are characterized by a rather low Shannon-Wiener index demonstrates that the diet is highly specialised (Labropoulou et al., 1997), which is confirmed in the randomisation test for *G. alvarezi* but not for *C. apus*. Feeding intensity and frequency are negatively related to the number of empty stomachs, thus the high vacuity index that was found in *C. apus* as well as in *G. alvarezi* indicates that in both species intensity and frequency of feeding are very low (Bowman & Bowman, 1980). In addition, feeding intensity and frequency are directly correlated with meal size and digestion time (Fange & Grove, 1979). All these observations suggest that both species mainly feed on large or relatively indigestible food (*e.g.* hard prey), which tallies with the observation that mainly Coleoptera are eaten. On the other hand, Table 3 showed that not only Coleoptera but also

Hymenoptera and Hemiptera were significantly harder than most other preys. The latter two prey types, however, were underrepresented in the stomachs, and thus a negative preference towards both prey types seems to be present. This is also confirmed by the E_i of both prey types in *C. apus* as well as in *G. alvarezi* (Table 1). As to Hymenoptera, it may be mentioned that they were mainly ants; ants produce venom, which makes them less attractive (Taylor et al., 2002). The same may be suggested for Hemiptera, since eight of the ten families of Hemiptera that are considered aquatic have specialised glands that emit pungent, protective secretions against potential predators (Scrimshaw & Kerfoot, 1987). Within the Coleoptera, however, no species are known to be venomous (Blum, 1981).

The results in other clariids, discussed below, have to be considered with care since data on these were few, although they provide some interesting indications.

In *G. typus*, for instance, the results show a different feeding pattern. The vacuity index is very high (Table 2), so the species may also feed on large or hard prey items. The data on the stomach contents, however, do not confirm this, since mainly small Diptera were eaten (Table 2). For *P. tihoni* the vacuity index is again very high (Table 2). Again little or no evidence of a diet of large and/or relatively indigestible prey is found. In both *Clariallabes*-species, again relatively high vacuity indices were found. Also in *C. longicauda* large and relatively indigestible prey were found, comparable to *C. apus* and *G. typus*, which is also indicated by a high Schoener index between the latter two species and *C. longicauda* (Table 2).

The data of *C. apus* and *G. alvarezi* seem to confirm the assumption that the hypertrophy of the main jaw muscles in these anguilliform species is an actual adaptation towards dietary changes, which is to a high extent confirmed by the data on relative prey hardness. However, when examining the prey items that were found within the stomachs, it was noticed that most of them were intact and had no bitemarks. This indicates that both species swallow their prey whole without prior oral or pharyngeal processing, apart from buccal transport. The swallowing movements are mainly accomplished by hyoid depression and through the action of protractor hyoidei, hyohyoideus inferior and sternohyoideus (Schaefer & Lauder, 1986; Lauder, 1980). In conclusion it can be stated that the current analysis of the diet does not support the hypothesis that hypertrophism of the musculus adductor mandibulae complex is an adaptation to feeding on larger or harder prey. Yet, these hypertrophied muscles may enable the fish to occasionally eat larger or harder preys, as in the example of the small bird that was eaten by one of the *G. alvarezi* specimens. The fact remains, however, that the diet of both *C. apus* as *G. alvarezi* is very selective towards coleopteran prey, since intensive feeding on Coleoptera is not widespread among teleosts. Even when regarding the diet of an other eel-shaped freshwater teleost such as *Chendol keelini* Kottelat & Lim, 1994 (Chaudhuriidae), the number of coleopteran prey only represented an average of 2.3 % of the diet (Kerle et al., 2000). Also in data on diets of other Clariidae as *Heterobranchus bidorsalis* Geoffroy St. Hilaire, 1808 and *Clarias gariepinus* (Burchell, 1822) low numbers of coleopteran prey are found. In *Clarias gariepinus* a figure of 6.8 % (Fagbenro et al., 1991) is mentioned and in *Heterobranchus bidorsalis* an exact number of coleopteran prey was not given but the total number of insect prey only added up to an average of 19.2 % of the diet (Spataru et al., 1987).

As far as the diet results of *G. typus* are concerned, a different diet was found, mainly consisting of mosquito-larvae. However, the high vacuity index found in the specimens examined precludes justifiable interpretation of these results.

In conclusion, this study shows that the diet of both *C. apus* and *G. alvarezi* is a very selective one. Both species mainly feed on Coleoptera, which are generally harder than other prey items. Although these results could indicate that the main jaw muscles are extremely enlarged as an adaptation towards an altered and specialized feeding pattern, the absence of bite marks on hard prey items suggests that both species presumably feed by swallowing rather then biting. This suggestion is further confirmed by the results of Van Wassenbergh (2003), who stated that features in favour of an increased biting force (such as enlarged jaw muscles) have consequences on suction feeding mechanisms. As a consequence, this still leaves the question on the functional significance of these altered jaw muscles open, which could confirm the hypothesis by Devaere et al. (2001) that they could well be just the result of the miniaturization of the skull.

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A new parrot from the Miocene of Germany, with comments on the variation of hypotarsus morphology in some Psittaciformes

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ABSTRACT. A new taxon of parrot (Psittaciformes) is described from the Middle Miocene of Southern Germany. *Bavaripsitta ballmanni* gen. et sp. n. is known from an almost complete tarsometatarsus which resembles the corresponding bone of some small Psittaculini (*Polytelis*, *Alisterus*) and Platycercini (e.g., *Psephotus* spp.) in general shape and morphology. Although the new taxon cannot be assigned to any of the modern groups of parrots, together with other fossil specimens it shows that there was a considerable diversity of parrots in the Lower and Middle Miocene of the Old World. Our study further provides the first detailed survey on the variation of hypotarsus morphology within extant Psittaciformes. A derived hypotarsal structure is described that supports monophyly of a clade including the genera *Psephotus*, *Eunymphicus*, *Cyanoramphus*, *Northiella*, *Prosopeia*, *Barnardius*, *Platycercus*, and *Melopsittacus*, to the exclusion of *Neophema* and *Neopsephotus* (all Platycercini). Also well characterized by a derived morphology of the hypotarsus are Loriinae and Cyclopsittacini. A shared derived hypotarsal morphology may further support sister group relationship between *Agapornis* and *Loriculus* (Psittaculini).

KEY WORDS : Aves, Psittaciformes, *Bavaripsitta ballmanni* gen. et sp. n., Miocene, Nördlinger Ries, hypotarsus, phylogeny.

INTRODUCTION

Parrots (Psittaciformes) are a morphologically quite uniform group of birds, which today has its greatest diversity in the Australasian and Neotropic region. Crown group parrots (i.e. the taxon comprising the last common ancestor of all extant Psittaciformes as well as all its extant and extinct descendants) are currently divided into the Australasian Cacatuidae and the Psittacidae (del HOYO et al., 1997). Within the Psittacidae, COLLAR (1997) distinguished two subgroups, the Australasian Loriinae (Lories) and the Psittacinae. The latter include the New Zealandian Nestorini (Kea and Kaka) and Strigopini (Kakapo), the New Guinean Psittrichadini (Pesquet's parrot), the Australasian Platycercini (platycercine Parrots), Cyclopsittacini (Fig parrots), and Micropsittini (pygmy-parrots), the Psittacini (Afrotropical parrots), the Psittaculini (psittaculine parrots), which occur in large parts of the Old World, and the Neotropic Arini (New World parrots).

Although a good fossil record exists for Eocene stem group representatives of the Psittaciformes (i.e. more basal taxa outside the crown group, see MAYR & DANIELS, 1998, MAYR, 2002), fossil crown group parrots still are very rare. Most Tertiary remains are from Miocene deposits of Europe. The first taxon described is *Archaeopsittacus verreauxi* from the Lower Miocene of France (MILNE-EDWARDS, 1867-1871), which is known from a complete tarsometatarsus and a few other referred bones, and which was tentatively referred to the Psittaculini by MLÍKOVSKÝ (1998). Also known from a tarsometatarsus is *Xenopsitta fejfari*, which was reported by MLÍKOVSKÝ (1998) from the Lower Miocene of the Czech Republic and which was considered by this author to be a member of the Psittacini. CHENEVAL (2000) described isolated bones, including an incomplete tarsometatarsus, of a parrot from the Middle Miocene of France which he referred to as *Pararallus dispar* (Milne-Edwards, 1869-71). According to MLÍKOVSKÝ (1998) this name is not available for the parrot remains that he (MLÍKOVSKÝ, 2002) instead listed as *Psittacus lartetianus* Milne-Edwards, 1872. However, the distal humerus selected by CRACRAFT (1973) as lectotype of *Pararallus dispar* is part of the syntypical series that includes the proximal tarsometatarsus selected by MLÍKOVSKÝ (2002) as lectoype of *Psittacus lartetianus* (contra MLÍKOVSKÝ 1998); a lectotype can only be changed by decision of the International Commission of Zoological Nomenclature (we thank C. Mourer-Chauviré for drawing our attention to this). HEIZMANN & HESSE (1995) further mentioned the presence of parrots in the Middle Miocene deposits of Steinheim in Germany.

Only a few Tertiary psittaciform taxa have been described from non-European localities. WETMORE (1926) assigned a humerus from the Miocene of Nebraska to a new species of the recently extinct taxon *Conuropsis*, and TONNI & NORIEGA (1996) described a well-preserved skull from the Pliocene of Argentina as a new species of the extant taxon *Nandayus*. From fossil deposits in Australia, BOLES (1993) described a rostrum of a Miocene

cockatoo and, also from Australia, BOLES (1998) identified Pliocene remains of the budgerigar, *Melopsittacus undulatus*.

Here we describe a tarsometatarsus of a fossil parrot from the Middle Miocene (about 15-13.5 million years ago, see STEININGER, 1999) freshwater deposits of the Nördlinger Ries in Germany. The occurrence of parrots at this site was already noted by BALLMANN (1979, 1983) and HEIZMANN & HESSE (1995), but the specimens have remained undescribed until now. We further comment on the variation of hypotarsus morphology in some extant Psittaciformes.

MATERIAL AND METHODS

Skeletons of the following representatives of the Psittaciformes in the collection of Forschungsinstitut Senckenberg, the Staatssammlung für Anthropologie und Paläoanatomie München, and the collection of Jürgen Bosch (Seewald-Besenfeld, Germany) were examined : Cacatuidae : *Cacatua* (*galerita*, *leadbeateri*, *moluccensis*, *sulphurea*), *Callocephalon fimbriatum*, *Eolophus roseicapillus*, *Nymphicus hollandicus*, *Probosciger aterrimus*. Psittacidae : Loriinae : *Chalcopsitta cardinalis*, *Charmosyna* (*papou*, *rubronotata*, *placentis*), *Eos* (*cyanogenia*, *histrio*, *reticulata*), *Lorius domicellus*, *Neopsittacus pullicauda*, *Oreopsittacus arfaki*, *Trichoglossus haematodus*, *Vini australis*; Psittrichadini : *Psittrichas fulgidus*; Nestorini : *Nestor notabilis*; Strigopini : *Strigops habroptilus*; Cyclopsittacini : *Cyclopsitta diophthalma*, *Psittaculirostris* (*desmarestii*, *edwardsii*); Platycercini : *Barnardius barnardi*, *Cyanoramphus novaezelandiae*, *Eunymphicus cornutus*, *Melopsittacus undulatus*, *Neophema* (*chrysogaster*, *elegans*, *pulchella*, *splendida*), *Neopsephotus bourkii*, *Northiella haematogaster*, *Platycercus* (*elegans*, *eximius*, *icterotis*), *Prosopeia tabuensis*, *Psephotus* (*chrysopterygius*, *haematonotus*); Psittaculini : *Agapornis* (*fischeri*, *lilianae*, *nigrigenis*, *personata*, *roseicollis*), *Alisterus* (*amboinensis*, *chloropterus*, *scapularis*), *Eclectus roratus*, *Loriculus stigmatus*, *Polytelis* (*alexandrae*, *anthopeplus*, *swainsonii*), *Prioniturus platurus*, *Psittacula* (*alexandri*, *cyanocephala*, *eupatria*, *himalayana*), *Psittinus cynanurus*, *Tanygnathus lucionensis*; Psittacini : *Coracopsis vasa*, *Poicephalus* (*cryptoxanthus*, *rufiventris*, *senegalus*), *Psittacus erithacus*; Arini : *Amazona* (*aestiva*, *amazonica*, *arausiaca*, *autumnalis*, *brasiliensis*, *festiva*, *imperialis*, *ochrocephala*, *pretrei*, *rhodocorytha*, *versicolor*, *vinacea*, *vittata*, *xanthops*), *Anodorhynchus hyacinthinus*, *Ara* (*ararauna*, *chloroptera*, *macao*, *rubrogenys*), *Aratinga* (*acuticaudata*, *leucophthalmus*, *pertinax*, *solstitialis*, *wagleri*, *weddellii*), *Bolborhynchus lineola*, *Brotogeris* (*chrysopterus*, *cyanoptera*, *pyrrhopterus*, *versicolorus*), *Cyanoliseus patagonus*, *Diopsittaca nobilis*, *Enicognathus (ferrugineus*, *leptorhynchus*), *Forpus* (*coelestis*, *conspicillatus*), *Geoffroyus geoffroyi*, *Guarouba guarouba*, *Myiopsitta monachus*, *Nandayus nenday*, *Pionites melanocephalus*, *Pionus sordidus*, *Pyrrhura* (*cruentata*, *leucotis*, *perlata*, *picta*).

Nomenclature and classification of the extant taxa follow ROWLEY (1997) and COLLAR (1997). Anatomical terminology follows BAUMEL & WITMER (1993) and VANDEN BERGE & ZWEERS (1993); measurements are in millimeters.

SYSTEMATIC PALEONTOLOGY

Psittaciformes Wagler, 1830
Bavaripsitta gen. n.
(Figs. 1A-D, 2A)

Type species : *Bavaripsitta ballmanni* gen. et sp. n.

Etymology : The name is derived from *Bavaria* (Lat.) : bavaria, and *psitta*, a diminutive of *Psittacus*.

Differential diagnosis : The tarsometatarsus of *Bavaripsitta* gen. n. differs from the corresponding bone of :

– all Nestorini and Strigopini in being much smaller and in the presence of a well-developed medial foramen vasculare proximale.

– Psittrichadini in being much smaller and less stout; in *Psittrichas* the canals of the deep flexor tendons are further fused to form a single canal.

– all Cacatuidae, Arini, and Psittacini investigated in this study in being less stout and in the presence of a well developed medial foramen vasculare proximale. In many Arini the canals of the deep flexor tendons (i.e., those of musculus flexor digitorum longus and m. flex. hallucis longus) are further fused to form a single canal.

– all Loriinae and Cyclopsittacini, all Platycercini included in this study except *Neophema* and *Neopsephotus*, some Psittaculini (*Prioniturus*, *Agapornis*, *Loriculus*), as well as *Bolborhynchus* (Arini) in the plesiomorphic morphology of the hypotarsus (see discussion and Figs. 1 and 3). In the derived (see discussion) condition present in the aforementioned taxa there is a large sulcus plantar to the canals of the deep flexor tendons, which confines the tendons of musculus flexor perforans digiti III and of the musculi flexores perforati digitorum III et IV (Figs. 3A-I); the tendon of musculus flexor perforatus digiti II is enclosed in a bony canal. The hypotarsus of the Loriinae and of *Agapornis* and *Loriculus* is further modified in that the canals of the deep flexor tendons are fused and the sulcus plantar thereof is closed to form a large canal (Figs. 3B, C, F).

– *Neophema* and *Neopsephotus* (Platycercini) in that the medial rim on the plantar surface of the trochlea metatarsi II is more pointed and medially protruding. In both extant taxa the medial foramen vasculare proximale is further strongly reduced and in *Neopsephotus* the trochlea metatarsi III protrudes much farther distally relative to the other trochleae.

– Micropsittini (of which only skins were available for comparison) in its much larger size. According to MLÍKOVSKÝ (1998 : 338), the hypotarsus of *Micropsitta* exhibits the derived condition found in the Loriinae (see above) and thus differs from that of *Bavaripsitta* gen. n.

– all Psittaculini investigated in this study in the absence of a sulcus for the tendon of musculus flexor perforatus digiti II; it is also much smaller than all studied Psittaculini except *Agapornis* spp. and *Loriculus* spp. The trochlea metatarsi II of *Bavaripsitta* gen. n. is further not greatly enlarged and not strongly medially protruding as in *Psittacula*, *Psittinus*, *Tanygnathus*, *Geoffroyus*, and *Eclectus* (see Fig. 2F).

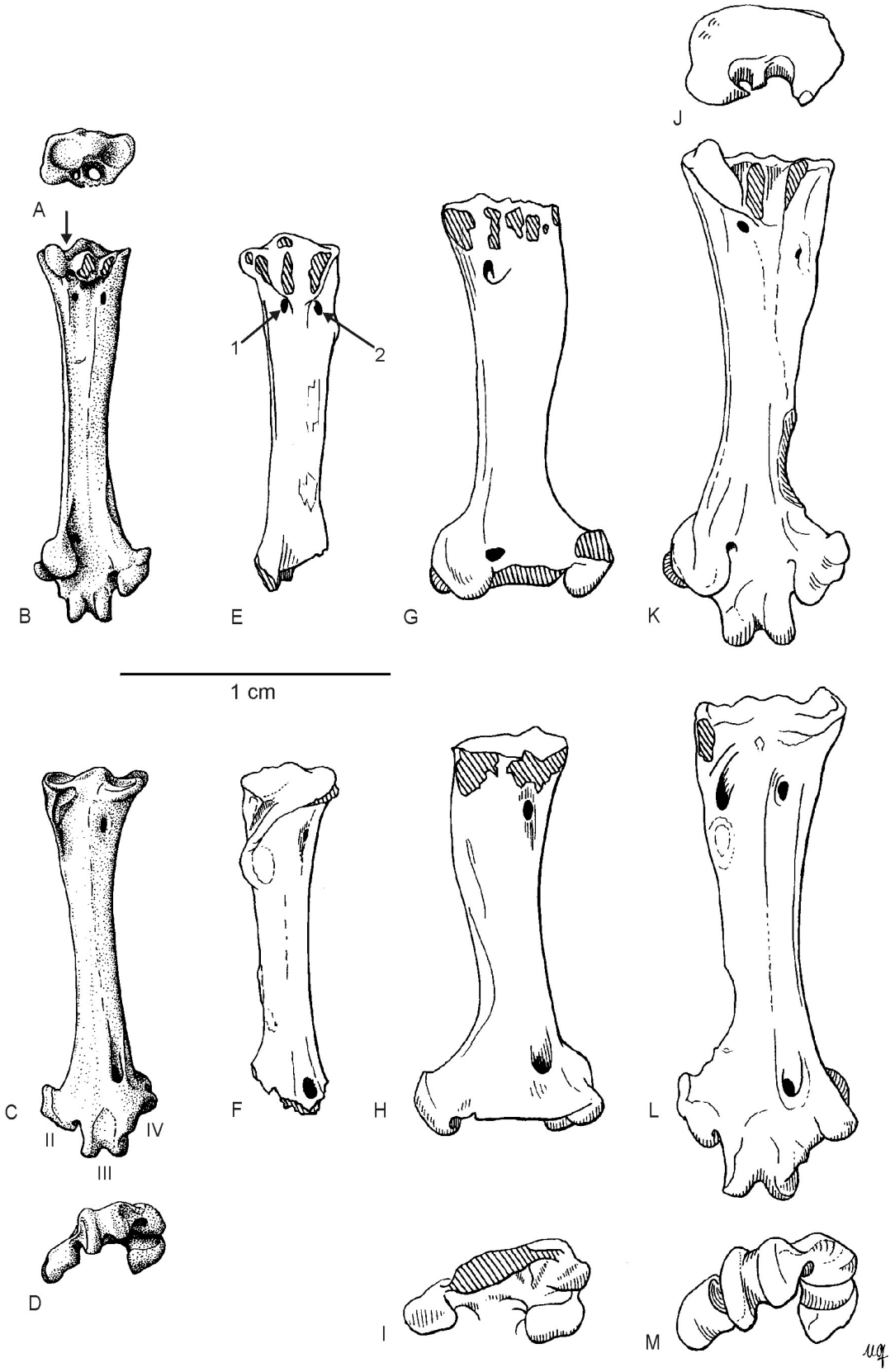

Fig. 1. – Tarsometatarsus of fossil parrots in comparison. (A-D) *Bavaripsitta ballmanni* gen. et sp. n. (holotype), (E, F) *Pararallus dispar* (Milne-Edwards, 1869) (reversed to appear to be from the left side), (G-I) *Xenopsitta fejfari* Mlíkovský, 1998 (reversed to appear to be from the left side, after Mlíkovský, 1998), (J-M) *Archaeopsittacus verreauxi* (Milne-Edwards, 1871). A, J, proximal end in proximal view, B, E, G, K, plantar view, C, F, H, L, dorsal view, D, I, M, distal end in distal view. The arrow in B indicates the canal for musculus flexor hallucis longus, which in *Bavaripsitta* gen. n. is not closed plantarly over its entire length, the arrows in E point to the lateral (1) and medial (2) foramen vasculare proximale. The trochleae metatarsorum II-IV are indicated by Roman numerals.

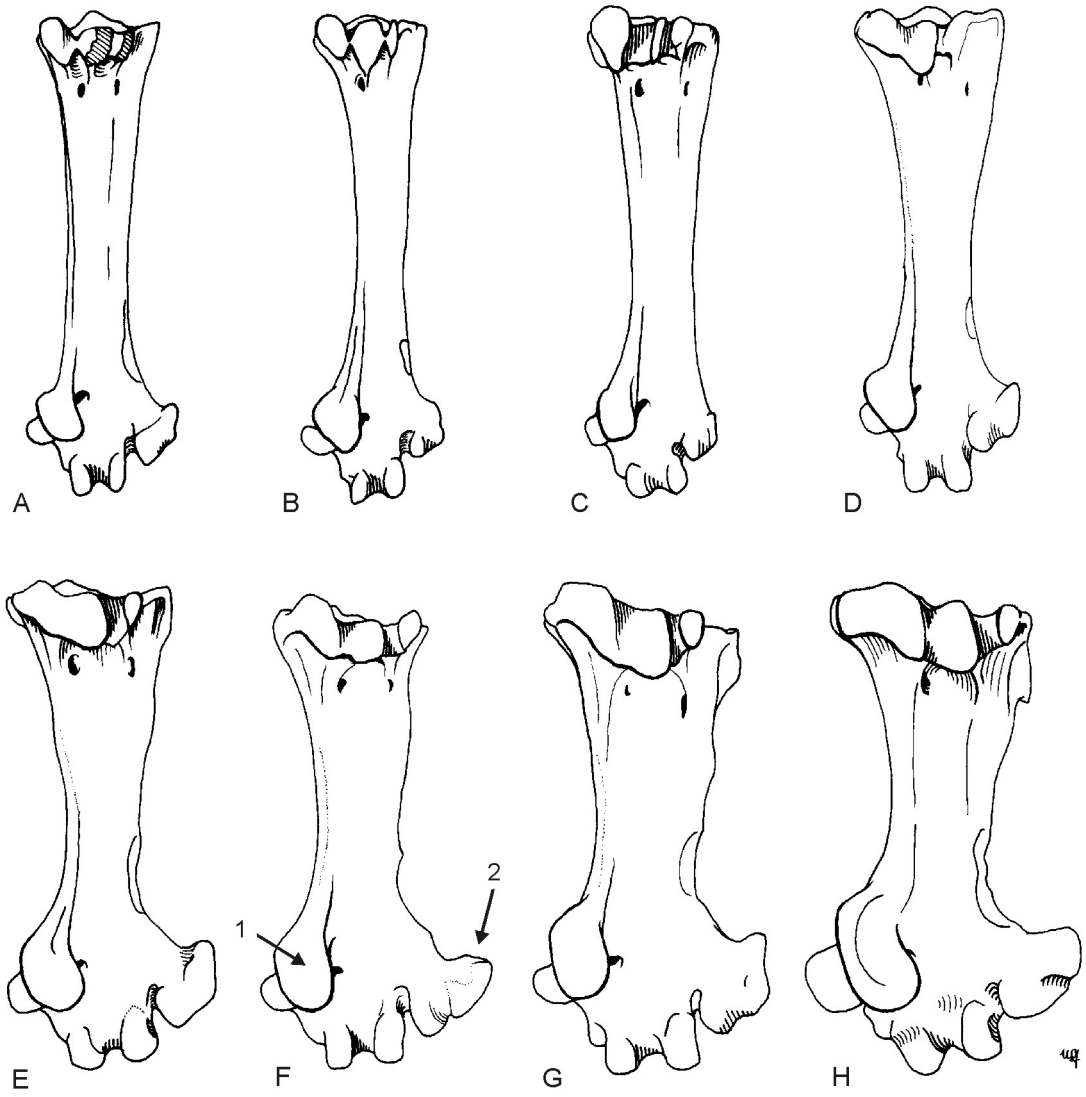

Fig. 2. – Left tarsometatarsus of some psittaciform birds in comparison (plantar view). (A) *Bavaripsitta ballmanni* gen. et sp. n. (holotype), (B) *Neophema elegans* (Platycercini), (C) *Psephotus haematonotus* (Platycercini), (D) *Alisterus scapularis* (Psittaculini), (E) *Coracopsis vasa* (Psittacini), (F) *Tanygnathus lucionensis* (Psittaculini), (G) *Amazona xanthops* (Arini), (H) *Cacatua moluccensis* (Cacatuidae). The arrows indicate the large trochlea accessoria (1) which is a synapomorphy of crown-group Psittaciformes, and the trochlea metatarsi II (2) which is exceptionally large in *Psittacula*, *Psittinus*, *Tanygnathus*, *Geoffroyus*, and *Eclectus* (Psittaculini). Not to scale.

– the Lower Miocene *Archaeopsittacus* Milne-Edwards, 1871 and *Xenopsitta* Mlíkovský, 1998 in being much smaller, less stout, trochlea metatarsi II with medial rim on plantar surface more pointed and medially protruding; it further differs from *Xenopsitta* in the presence of a well-developed medial foramen vasculare proximale.
– the Middle Miocene taxon *Pararallus dispar* (Cheneval, 2000, contra Mlíkovský, 1998, 2002) in the proximo-distally shorter hypotarsus, the less medially protruding tuberositas musculi tibialis cranialis, and the fact that the canal for musculus flexor hallucis longus is not completely closed over its length.

Remarks : Our taxon sampling includes 57 of the 84 extant psittaciform genera recognized by Rowley (1997) and Collar (1997). Fourteen of the missing genera are Neotropic, and it is unlikely for biogeographic reasons that *Bavaripsitta* n. gen. is congeneric with one of these taxa. Of the following 13 Old World taxa no skeletons were available for comparisons : *Calyptorhynchus* (Cacatuidae), *Pseudeos*, *Psitteuteles*, *Phigys*, *Glossopsitta* (Loriinae), *Micropsitta* (Micropsittini), *Bolbopsittacus* (Cylopsittacini), *Purpureicephalus*, *Lathamus*, *Pezoporus*, *Geopsittacus* (Platycercini), *Psittacella*, *Aprosmictus* (Psittaculini). All of these taxa occur either in Australia, New Guinea or on Southeast Asian islands and it is also not very likely that one is most closely related to a Miocene parrot from Germany.

Bavaripsitta ballmanni gen. et sp. n.

Holotype : Almost complete left tarsometatarsus, housed in the Bayerische Staatssammlung für Paläontologie und Geologie, München, collection number BSP 1970 XVIII 899 (Fig. 1A-D).

Type locality and horizon : Steinberg in the Nördlinger Ries, Germany (see BALLMANN, 1979 and HEIZMANN & FAHLBUSCH, 1983 for information on the locality), Middle Miocene (stratigraphic unit MN6, see HEIZMANN & HESSE, 1995). The specimen is from a single block of travertine, which contained several thousand vertebrate remains and which probably represented a filling of a Karstic cavity in the Tertiary limnic sinter of Nördlinger Ries.

Etymology : The species has been named after Peter Ballmann in recognition of his work on the fossil birds from Nördlinger Ries. The specimens described here were picked out of the fossil material from Steinberg and first identified as parrots by him.

Diagnosis : Same as for genus.

Tentatively referred specimen : Distal end of right humerus from the same block of travertine as the holotype, housed in Bayerische Staatssammlung für Paläontologie und Geologie, München, collection number BSP 1970 XVIII 900.

Measurements (in mm) : Tarsometatarsus (holotype) : maximum length, 13.6, proximal width, 3.5, distal width, 4.2. Humerus (referred specimen BSP 1970 XVIII 900) : distal width 4.7.

Description and comparison : The holotypical tarsometatarsus is about the size of the corresponding bone of the extant *Melopsittacus undulatus*. In general shape and proportions it resembles the tarsometatarsus of extant *Polytelis* spp. (Psittaculini) and, apart from the plesiomorphic morphology of the hypotarsus (see below), many Platycercini (e.g. *Neophema*, *Psephotus* spp.). Many other extant Psittaciformes have a stouter tarsometatarsus with wider proximal and distal ends (Fig. 2, the relative squatness of the tarsometatarsus of parrots does not appear to be significantly related to allometric changes due to different body size). Compared to other fossil parrots, the tarsometatarsus of *Bavaripsitta ballmanni* gen. et sp. n. is most similar to that of *Pararallus dispar* (CHENEVAL, 2000), which differs, however, in the morphology of the hypotarsus (see differential diagnosis and Fig. 1B, E).

The impressiones retinaculi extensorii on the dorsal surface of the proximal end are distinct and border a marked sulcus as in most extant Psittaciformes. The tuberositas musculi tibialis cranialis is situated at the medial margin of the shaft. There are two foramina vascularia proximalia, whereas in many of the extant taxa investigated (e.g., *Psittacus*, *Poicephalus*, most Cacatuidae, Arini, and Platycercini), the medial foramen vasculare proximale is greatly reduced or completely absent.

The hypotarsus of *B. ballmanni* is proximo-distally short and encloses two canals for the tendons of musculus flexor digitorum longus and m. flexor hallucis longus. Unlike most extant Psittaciformes (Fig. 3), there is no well-developed sulcus/canal for the tendon of musculus flexor perforatus digiti II. The canal for musculus flexor hallucis longus is not closed plantarly over its entire length, as in extant *Neophema*, but unlike virtually all other modern parrots we examined. The hypotarsus of *B. ballmanni* lacks a deep sulcus or canal for the tendons of musculus flexor perforans digiti III and of the musculi flexores perforati digitorum III et IV, which is found in all extant Loriinae and Cyclopsittacini, all Platycercini included in this study except *Neophema* and *Neopsephotus*, some Psittaculini (*Prioniturus*, *Agapornis*, *Loriculus*), and *Bolborhynchus* (Arini) (see discussion).

At the distal end of the bone, the trochlea metatarsi II does not bear a well-developed sulcus, in contrast to, e.g., *Melopsittacus* or *Alisterus*. This trochlea is further not greatly enlarged and not strongly medially protruding as in the probably closely related (e.g. SMITH, 1975; HOMBERGER, 1980; CHRISTIDIS et al., 1991) extant taxa *Psittacula*, *Psittinus*, *Tanygnathus*, *Geoffroyus*, and *Eclectus* (Psittaculini, see Fig. 2F). As in all crown group Psittaciformes (see MAYR, 2002), the trochlea metatarsi III is slightly asymmetric with the lateral rim being smaller than the medial one. The rims are narrow and widely separated, delimiting a marked sulcus. The trochlea metatarsi IV bears a large trochlea accessoria, which exhibits the unmistakable highly derived morphology typical of crown group Psittaciformes (Fig. 2, see also MAYR, 2002). The trochlea accessoria does not reach farther distally than the trochlea metatarsi IV. The fossa metatarsi I is essentially situated on the plantar surface of the bone whereas it is located on the medial margin of the shaft in many extant Psittaciformes.

The tentatively referred humerus is slightly larger than the corresponding bone of the extant *Melopsittacus undulatus* and closely resembles the distal humerus of extant Psittaciformes, which is very similar in the members of this taxon.

DISCUSSION

Archaeopsittacus, *Xenopsitta*, and the new taxon *Bavaripsitta* show distinct differences in the morphology of the tarsometatarsus (Fig. 2) that indicate there already was a considerable diversity of parrots in the Miocene of the Old World. Unfortunately, assignment of these fossils to any of the modern taxa is hindered by the absence of diagnostic derived features in the known bones (mainly tarsometatarsi).

Almost certainly, a hypotarsal morphology as exemplified by *Archaeopsittacus* and *Bavaripsitta* (the hypotarsus of *Xenopsitta* is broken) is plesiomorphic within crown group Psittaciformes, i.e. only the deep flexor tendons (those of musculus flexor digitorum longus and m. flex. hallucis longus) are enclosed in bony canals and are well separated. With slight modifications, this type of hypotarsus (Fig. 3K) occurs in numerous unrelated psittaciform taxa (Fig. 3J-L) and can be derived from the condition in fossil stem group Psittaciformes in which the hypotarsus bears two sulci for the deep flexor tendons (MAYR & DANIELS, 1998 : text-fig. 5, MAYR, 2002 : fig. 6).

Surprisingly, although there are many comparative studies on parrot morphology (e.g., VERHEYEN, 1956; BRERETON, 1963; HOLYOAK, 1973; SMITH, 1975; HOMBERGER, 1980; GÜNTERT, 1981), the striking variation in the structure of the hypotarsus received virtually no attention. MLÍKOVSKÝ (1998 : 338) appears to be the first to have briefly mentioned hypotarsal variation in some Psittaciformes, but his description is not very detailed and contains some inaccuracies (for example, he erroneously

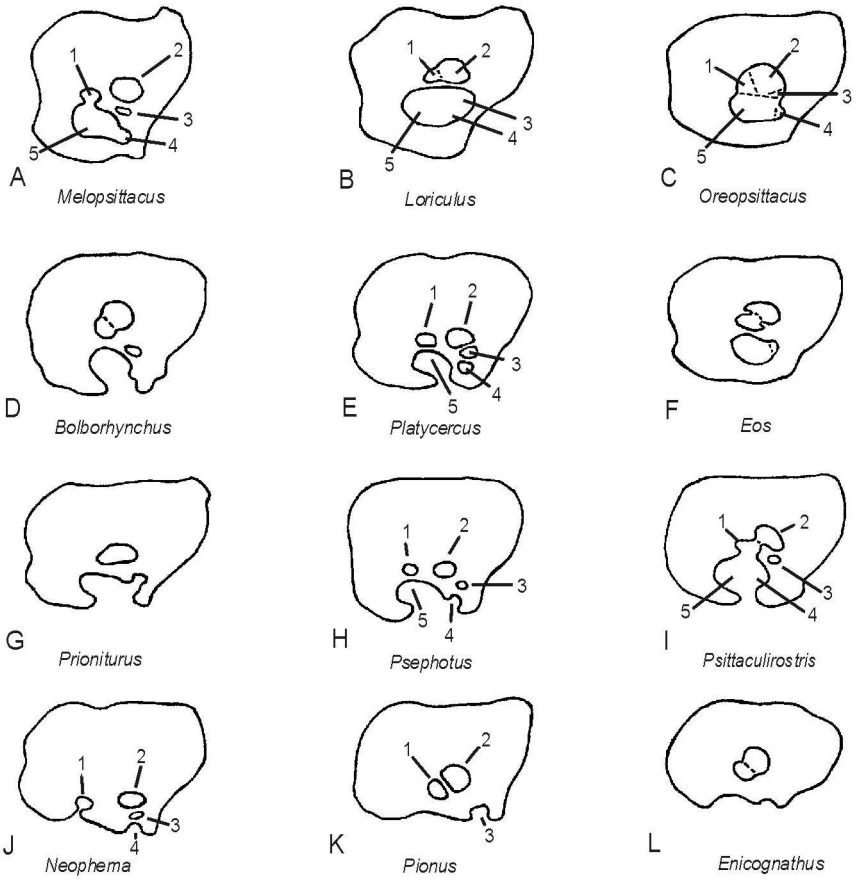

Fig. 3. – Different types of hypotarsi of extant parrots in comparison (left tarsometatarsus). (A) *Melopsittacus undulatus* (Platycercini), (B) *Loriculus stigmatus* (Psittaculini), (C) *Oreopsittacus arfaki* (Loriinae), (D) *Bolborhynchus lineola* (Arini), (E) *Platycercus elegans* (Platycercini), (F) *Eos reticulata* (Loriinae), (G) *Prioniturus platurus* (Psittaculini), (H) *Psephotus haematonotus* (Platycercini), (I) *Psittaculirostris desmarestii* (Cyclopsittacini), (J) *Neophema elegans* (Platycercini), (K) *Pionus sordidus* (Arini), (L) *Enicognathus leptorhynchus* (Arini). Not to scale; the dashed lines indicate non-ossified membranes. The numerals indicate the canals and grooves for the tendons of : 1 - musculus flexor hallucis longus, 2 - m. flex. digitorum longus, 3 - m. flex. perforatus digiti II, 4 - m. flex. perforans et perforatus digiti II, 5 - m. flex. perforans digiti III and musculi flexores perforati digitorum III et IV.

considered the hypotarsus of *Nymphicus*, *Psittrichas*, and New World parrots to be similar to that of the Loriinae).

Well characterized by a derived hypotarsal morphology are the Loriinae (Figs. 3C, F) and Cylopsittacini (Fig. 3I). In these taxa, the canals for the deep flexor tendons (m. flexor hallucis and m. flexor digitorum) are fused, and the superficial tendons are situated in a canal (Loriinae) or in a nearly closed, deep sulcus (Cyclopsittacini).

A hypotarsal morphology identical to that of the Loriinae is also found in *Agapornis* and *Loriculus* (Fig. 3 B) and may support a close relationship between these two taxa that are currently (e.g., Collar, 1997) classified into the Psittaculini (pro, e.g., Brereton, 1963; contra, e.g., Homberger, 1980).

Within the Platycercini, a derived hypotarsal morphology in which the tendons of musculus flexor perforans digiti III and musculi flexores perforati digitorum III et IV are situated in a deep sulcus (Fig. 3 E, H) is found in the genera *Psephotus*, *Eunymphicus*, *Cyanoramphus*, *Northiella*, *Prosopeia*, *Barnardius*, *Platycercus* and supports monophyly of these taxa (pro, e.g., Renzoni & Watters, 1972; Holyoak, 1973; Homberger, 1980, 1991; contra, e.g., Brereton, 1963 [who included *Prosopeia* in the Psittacini]; Smith, 1975 [who included *Prosopeia* in the Psittaculini]; Christidis et al., 1991; Miyaki et al., 1998). A slightly modified, similar hypotarsus is also found in *Melopsittacus* (Fig. 3A) and supports inclusion of this taxon into the Platycercini (pro, e.g., Renzoni & Watters, 1972; Holyoak, 1973; Homberger, 1980; Collar, 1997; contra, e.g., Christidis et al., 1991; Miyaki et al., 1998). *Neophema* (Fig. 3J) and *Neopsephotus*, however, which are generally also classified in the Platycercini (e.g., Smith, 1975; Güntert, 1980; Homberger, 1980; Collar, 1997) exhibit the presumably plesiomorphic (see above) morphology of the hypotarsus.

Although *B. ballmanni* is clearly distinguished from all other known psittaciform taxa (see differential diagnosis), because of the absence of diagnostic derived characters it cannot be reliably assigned to any of the extant psittaciform taxa. Hypotarsal morphology clearly prevents classification of the fossil taxon into the Loriinae, Cyclopsittacini, and Platycercini except *Neophema* and

Neopsephotus. However, being plesiomorphic it does not give positive clues on the phylogenetic affinities of this taxon. A closer relationship between *Bavaripsitta* and the Neotropic Arini or Australian Cacatuidae is not supported by the morphology of the tarsometatarsus and also not very likely for biogeographic reasons. As detailed in the differential diagnosis, *Bavaripsitta* is further distinguished from any of the extant African (*Psittacus*, *Poicephalus*, *Agapornis*, *Psittacula*) or continental Eurasian (*Psittacula*, *Loriculus*) psittaciform taxa. Apart from being smaller and slightly more slender, in its overall shape and morphology, the tarsometatarsus of *B. ballmanni* most closely resembles that of *Polytelis* and *Alisterus* (Psittaculini - as noted above, in the other studied taxa of the Psittaculini the trochlea metatarsi II is greatly enlarged). However, these similarities (relatively slender tarsometatarsus, morphology of the hypotarsus and the distal end of the bone) may well be plesiomorphic within crown group Psittaciformes, as the tarsometatarsus of *Bavaripsitta* for example also exhibits similar proportions to that of Eocene stem group representatives of the Psittaciformes (see MAYR & DANIELS, 1998; MAYR, 2002).

Assignment of *Archaeopsittacus* to the Psittaculini (MLÍKOVSKÝ, 1998) was also based on plesiomorphic characters, i.e. the presence of the medial foramen vasculare proximale and the similar morphology of the hypotarsus. Although the tarsometatarsus of *Archaeopsittacus* does resemble that of some extant Psittaculini (compare Figs. 1K and 2D), the phylogenetic position of this taxon needs to be substantiated with derived characters as it is conceivable that certain Psittaculini (*Polytelis* and *Alisterus*) retained a primitive tarsometatarsal morphology. Apart from a smaller trochlea metatarsi II, the tarsometatarsus of *Archaeopsittacus* for example also resembles that of the Madagascan *Coracopsis* (Fig. 2E).

Assignment of *Xenopsitta* to the Psittacini (MLÍKOVSKÝ, 1998) which include the African genera *Coracopsis*, *Poicephalus*, and *Psittacus* was based on the "general shape" of the bone and on the absence of the medial foramen vasculare proximale (which is also reduced in *Psittacus* and *Poicephalus* but present in *Coracopsis*). As far as comparable, the incomplete tarsometatarsus of *Xenopsitta* indeed resembles the corresponding bone of *Psittacus* and a close relationship to some of the African parrots would also not be unlikely for biogeographic reasons. However, at least judging from the illustration in MLÍKOVSKÝ (1998), the tarsometatarsus of *Xenopsitta* also appears to have a similar shape to that of, e.g. *Tanygnathus*, *Amazona*, or *Cacatua* (compare Figs. 1 and 2) and the medial foramen vasculare proximale is reduced in many unrelated taxa of modern Psittacidae (see above).

Knowing the exact systematic position of the European psittaciform taxa unquestionably would be of great interest concerning the biogeography and early evolution of parrots. However, due to our incomplete understanding of the relationship between the extant taxa and the limited fossil material available, we do not consider it possible to reliably assign either *Bavaripsitta*, *Xenopsitta*, or *Archaeopsittacus* to any taxon of modern parrots. Just because of its great implications such an assignment needs to be based on well-defined derived characters, such as the modifications of the hypotarsus described in this study, rather than on overall morphology and general shape of few bones.

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Intra and inter-specific mating options for gynogenetic reproduction of *Carassius gibelio* (Bloch, 1783) in Lake Pamvotis (NW Greece)

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ABSTRACT. Gibel carp *Carassius gibelio* exhibits gynogenetic reproduction by utilising the sperm of other species. Over a period of twelve months, the spawning behaviour of gibel carp in Lake Pamvotis (NW Greece) was monitored. Females almost exclusively (97.7%) composed the population, indicating gynogenesis using the sperm of other species. Reproduction began in March and lasted through April. Contrary to the situation reported in other ecosystems, common carp was not present in the spawning grounds during the spawning season of gibel carp; the most abundant other species present at that time was *Rutilus ylikiensis*. To evaluate the sperm donating results of *R. ylikiensis* as compared with other species of the lake, eggs of gibel carp were fertilised with sperm of *R. ylikiensis* (group 1), *Carassius auratus* (group 2), and *Cyprinus carpio* (group 3). Also, gibel carp eggs attached to natural substrates were collected from the spawning grounds (group 4). Hatching success ranged from 9-29% in group 4, compared to 95-98% in groups 1, 2 and 3. Over a period of 60 days after hatching, mortality ranged from 35% (group 1) to 56% (group 3), and specific growth rate from 2.4% (group 3) to 2.9% (group 1). We conclude that gibel carp can successfully utilise sperm of ylikiensis roach, which was the best available sperm donor option for the gynogenetically reproducing gibel carp of the lake.

KEY WORDS : gynogenesis, reproduction, fish.

INTRODUCTION

Carrasius gibelio (Bloch, 1783) can survive and thrive under adverse environmental conditions where other species rarely succeed (Muus & Dahlstrom, 1999; Holcik, 1980). As a consequence, it is widely distributed and flourishing from Europe, including Greece (Economidis, 1991; Kottelat, 1997), to the Japanese Islands.

In Greek lakes, gibel carp exhibits rapid growth during the first years of its life and reaches maturity in the second year of its life (Leonardos et al., 2001). Recently, a rapid increase in the commercial catch of gibel carp in Pamvotis lake NW Greece has been observed (Paschos et al., 2002). Gibel carp became abundant in Lake Pamvotis in the early 1980's. Since then, it gradually increased in numbers and is currently the dominating species in fisheries landings, while landings of species of commercial interest such as common carp, eel and the indigenous south European minnow *Phoxinellus epiroticus* have declined (Perdikaris et al., 2003).

According to Economidis et al. (2000), the indigenous species of the Lake are : *Anguilla anguilla (*Linnaeus, 1758*), Leuciscus cephalus* (Linnaeus, 1758), *Tinca tinca* (Linnaeus, 1758), *Pseudophoxinus stymphalicus* (Valenciennes, 1844), *Phoxinellus epiroticus* (Steindachner, 1895), *Economidichthys pygmaeus* (Holly, 1929), *Cobitis hellenica* (Economidis, 1991), *Rutilus ylikiensis* (Stephanidis, 1939) and *Barbus albanicus (*Steindachner, 1870). The introduced species are : *Carassius gibelio* (Bloch, 1783), *Carassius auratus* (Bloch, 1783), *Cyprinus carpio* (Linnaeus, 1758), *Silurus aristotelis* (Agassiz, 1856), *Silurus glanis* (Linnaeus, 1758), *Gambusia affinis (Baird and Girard, 1854), Hypophthalmichthys molitrix (Val. 1844), Ctenopharyngodon idella* (Cuvier and Valenciennes 1844), *Aristichthys nobilis* (Richardson 1845),

It is not clear which factors have contributed to this thriving of the gibel carp population in the lake, but in similar ecosystems environmental degradation and decreased density of predating species have been identified as important causes (Holcik, 1980).

Interestingly, gibel carp is one of the few fish species with stocks composed almost exclusively of females, reproducing gynogenetically using the sperm of other species (Riehl & Baensch, 1991; Zhou et al., 2000). Gynogenetic reproduction has some potential benefits : it allows the biomass of a population to be composed mainly of females, and available ecological resources can be used solely for egg production.

Shallow water and low vegetation characterise the spawning grounds of cyprinid fishes. In similar lake ecosystems, gibel carp and other species utilise the same spawning grounds. This is because of the availability of substrates and oxygen for the eggs, and increased microinvertebrate densities, which contribute to the survival of

early life stages and enhance recruitment. In a recent pilot study, it became evident that the gibel carp population in Lake Pamvotis is composed mainly of females, and that *Rutilus ylikiensis* was abundant in the spawning grounds of gibel carp, (Paschos et al*.,* 2001).

The purpose of the present work was to determine the relative densities of potential sperm-donating fish species in the spawning habitat of gibel carp in Lake Pamvotis. Subsequently, this information was used for experimental evaluation of gynogenetic reproduction and for assessing the degree of potential "sexual parasitism" of gibel carp in Lake Pamvotis.

MATERIAL AND METHODS

This study was carried out in Lake Pamvotis NW Greece (Fig. 1), which is a natural, eutrophic, holomictic lake. It is relatively shallow (mean depth about 4 meters and maximum depth not exceeding 8 meters) with a total area of 355 km^2, and is thermally stratified from April to August (Kagalou et al*,* 2001).

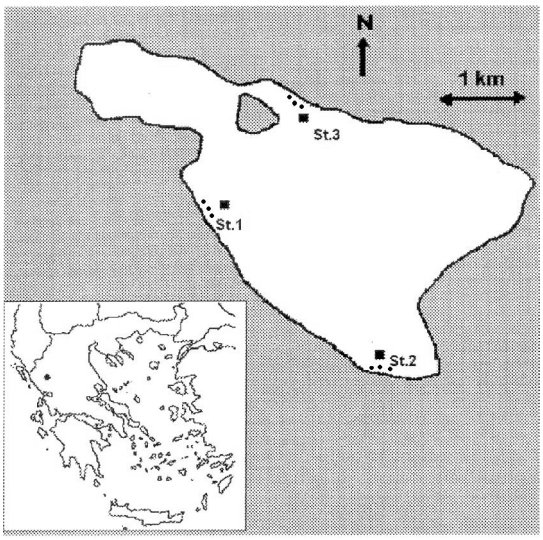

Fig. 1. – Map of Pamvotis Lake. Sampling sites (St.1 St.2, St.3) of gill nets (squares) and locality of spawning grounds (circles).

Typical spawning grounds of gibel carp and other members of the Cyprinidae family are characterised by shallow depth and the presence of vegetation (Varley 1967; Lagler 1972; Muus & Dahlstrom 1999). To monitor the spawning migration of gibel carp and identify the species that can act as sperm donors for female gibel carp, sampling was, therefore, carried out from January 1999 to January 2001, using gillnets (100m long, mesh size 40-50 mm) positioned at 100 meters away from the shallows and parallel to the shoreline of three separate spawning locations (Fig. 1). Stationary fishing traps (fyke-nets, mesh size 30mm) were also placed in typical shallow spawning grounds of the species with depth from 0.5 m to 1m. Temperature was monitored throughout the period of study using max-min thermometers.

Determination of the spawning period was based on monitoring of the catches in the shallows and examination of the gonadosomatic index (GSI = GW*100/NW, where GW is the gonad weight, NW is the eviscerated weight), staging of gonadal development according to Kesteven's scale (Bagenal, 1978), and finally by the degree of pressure required to obtain eggs from captured females. Condition factor (K) was calculated according to the following equation $K = (BW/ L^3) \times 100$, where BW is the eviscerated weight, L is total length.

In addition to the gill and fyke nets, electrofishing was carried out in the shallows when, for the first time, gibel carp was found in the shallows exhibiting typical spawning behaviour of Cyprinidae (Michaels, 1988). All individuals of other species present in the shallows during the spawning of gibel carp were thus captured with electrofishing or fyke nets.

When spawning of gibel carp became evident, ten pieces of natural substrate (branches, leaves, vegetation, rough dimensions trimmed to 0.5 X 0.4m) with eggs were collected randomly from the three different sampling sites of gibel carp spawning grounds. These were taken to the lab and placed in aquariums (0.9 X 0.6 X 0.5m) supplied with Lake Water. From each substrate and group, 100 larvae were collected and placed in four trays (1.0 X 0.25 X 0.25m).

In addition, eggs from three sexually mature female gibel carp were fertilised with sperm from three male goldfish *Carassius auratus*, three ylikiensis roach, *Rutilus ylikiensis*, and three carp *Cyprinus carpio*. From each cross, a total number of 2000 eggs were placed in incubators (zugars having total volume 7lt). The rate of embryonic development, hatching percentage and growth rate were monitored for a period of 60 days for each group.

All fish were kept according to standardised procedures (Horvath et al., 1984). Initially, food was solely zooplankton, collected daily from the lake. From the 20th day, dry trout food was provided (Dabrowski et al,. 1986; Kaushik 1986). Feeding was *ad libitum* in all groups. Mortality, length and growth rates were monitored every 20 days, for a period of two months after hatching. Specific growth rate (SGR) was calculated according to the following equation $SGR = LnBW_2\text{-}LnBW_1$ days^{-1}, where BW1 is the initial body weight and BW2 is body weight after 20 days of rearing.

RESULTS

Catch Data

In total 598 gibel carp individuals were captured over the period of the study : 392 in gillnets and 206 in fyke-nets. The largest portion of the annual gibel carp catch with gillnets, was in March (19.9%). With the exception of November, December and January, when fishing was fruitless, about 10% of the total annual catch was taken in each other month. In fyke nets, gibel carp catches occurred only during March and April, with 42% and 58% of the total catch each month, respectively (Fig. 2). Females comprised 97.7% of all gibel carp captured in the spawning grounds. Over the period of the study, temperature ranged between 7°C and 27°C. Peak capture of gibel carp, with gill and fyke nets, coincided with temperatures above 12°C (Fig. 2).

Mean total length of captured females was 28.06 cm (min 13.2, max 35.5 cm), mean body weight was 464.9 g (min 88.5, max 894 g) and condition factor was 0.93 (±0.17). The gonadosomatic index of the female fish was 12.6 and the fish were in the last stage of sexual maturation. Initially the larger fish spawned, and smaller individuals followed.

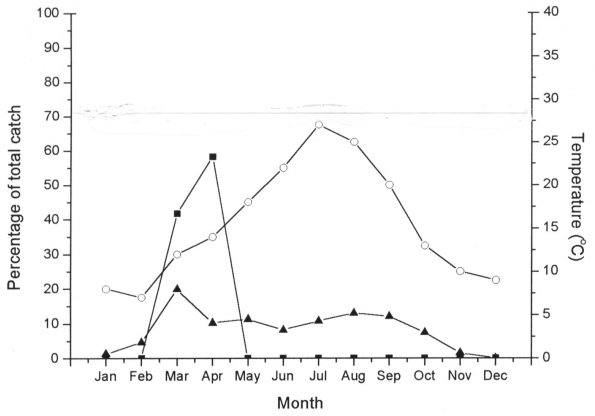

Fig. 2. – Catch data of gibel carp from Lake Pamvotis, over a period of 12 months. Number of fish caught in the spawning grounds (squares) and at 100 meters away from the shoreline (triangles), and water temperature in the shallow region of the lake (circles).

Catch data of other species in the spawning grounds

During March and April, when gibel carp spawned in the shallows, 206 fish were captured in the three sampling sites. In all sampling sites, gibel carp and ylikiensis roach were always present. All individuals of other species captured in the shallows were found to be sexually mature and the majority readily released their gametes. Gibel carp (89%), followed by ylikiensis roach (10.5 %) exhibited the highest relative abundance. The captured ylikiensis roach comprised sexually mature males (26%) and females (37%), and the rest were not sexually mature. During electrofishing operations in the shallows, *R. ylikiensis* males and females were caught in the act of mating and spawning, while simultaneously gibel carp females were observed to release their eggs.

Hatching percentage of eggs from natural substrate, under hatchery conditions

The number of eggs ranged from 118-1520 per substrate collected from the spawning grounds. Hatching occurred in all groups after three days at 14 °C, and the number of larvae ranged from 58-380. Hatching coefficient varied between samples from 7.2-29% (x^2=203, DF=9, P<0.01).

Artificial fertilisation of gibel carp eggs with sperm of other species

Viable larvae were obtained from gibel eggs fertilised with sperm from the ylikiensis roach (CrXR), goldfish (CrXG) and common carp (CrXC). Hatching occurred after three days at 14^0C in all groups. Hatching coefficient ranged from 95-98% (Table 1) and there was no significant difference between the crosses in terms of hatching coefficient (Chi-square test, x^2=0.024, DF=2, P>0.05).

Rearing of fish

Fish from the three groups of crosses (CrXR; CrXG; CrXC) and from the natural substrates exhibited similar mortality (Table 2, x^2=5.38, DF=3, P>0.05), but the cross between gibel carp and ylikiensis roach exhibited the lowest mortality of all. The specific growth rate was similar in all groups (Table 2, x^2=0.058, DF=3, P>0.05).

TABLE 1

Hatching performance of the crosses

Group	Hatching (days)	Hatching (%)
C.gibelio X R. ylikiensis	3	98
C.gibelio X C. carpio	3	97
C.gibelio X C. auratus	3	95
C.gibelio Embryos from natural substrates	3	18

TABLE 2

Specific growth rate and cumulative mortality (M) of the reared fish at 20, 40 and 60 days after hatching

Days After 1st feeding	20		40		60	
GROUP	SGR%	M%	SGR%	M%	SGR%	M%
C.gibelio X R. ylikiensis	2.4%	19%	3.4%	25%	2.9%	35%
C.gibelio X C. carpio	2.4%	30%	2.4%	44%	2.4%	56%
C.gibelio X C. auratus	2.2%	21%	3.2%	31%	2.7%	41%
C. gibelio from the lake	1.4%	18%	3.6%	33%	2.5%	47%

DISCUSSION

Spawning activity of gibel carp in Lake Pamvotis peaked during March and April. Peak capture of gibel carp with gill nets, positioned parallel to the shoreline of the spawning sampling sites, occurred during March. In spawning grounds, gibel carp were captured only during March and April. (Fig. 2). During March and April, sexually mature gibel carp exhibiting typical spawning behaviour of Cyprinidae were captured with electro-fishing, as well as with the fyke nets,

The field data indicate that spawning of gibel carp and ylikiensis roach coincided. Female gibel carp caught with gillnets during March were probably migrating towards the shallow spawning grounds. Both gibel carp and ylikienesis roach captures in the shallows (with fyke nets and electrofishing) peaked during March and April. In fact ylikiensis roach was the most abundant other species present in the spawning grounds of gibel carp. In these typical shallow spawning grounds for cyprinidae species, gibel carp were caught in the act of releasing their eggs during the spawning of ylikiensis roach.

Timing of spawning is a significant element of the reproductive strategy of all fish species. According to the field data (catch in the shallows), the spawning period of gibel carp and ylikiensis roach overlap. This period is also a period of spawning for Epirus minnow, an endemic fish species that is on the verge of extinction in the Lake (Perdikaris et al., 2003). It can be assumed that these three mentioned fish species of the lake have the same timing strategy for spawning.

Optimal conditions for hatching and growth of larvae early in the season are very important for survival and recruitment. For example, temperature and food abundance can significantly influence survival during early life stages of development (Heggberget, 1988; Hutchings, 1991). Initiation of spawning activity of gibel carp in the lake coincided with a critical increase of temperature from 7 °C in February to 13 °C in March; 13 °C appears to be on the low edge of the thermal limit of goldfish embryonic development (Wiegand et al., 1989). Successful reproductive investment and recruitment of young fish are largely influenced by environmental conditions in the nursery grounds. The timing of spawning may enhance gibel carp larval viability and growth through the increased temperature and primary and secondary productivity that is evident at the beginning of spring in the lake (Kagalou et al., 2001). Gibel carp exhibits rapid growth and development under favourable thermal conditions. For example gibel carp in Lake Lysimachia, a neighbouring Lake in West Greece, reach 41.2% of the maximum length exhibited by the population, within the first year of life (Leonardos et al., 2001).

There was some evidence of temperature related spawning migration of gibel carp in the lake. Gibel carp distribution in the shallows peaked during March and April, associated with temperature ranging from 12-14 °C. At this stage individuals were sexually mature and exhibited spawning behaviour. According to the catch data from the deeper parts of the lake, when temperature was below 12 °C or above 16 °C, gibel carp moved towards the deeper parts of the lake and returned to the shallows when temperature was between 12 and 16 °C (Fig. 2). This is in agreement with information collected from local fishermen about seasonal distribution of the species in the lake.

Contrary to the situation seen in other ecosystems (MUUS & Dahlstrom 1999), we found no evidence in Lake Pamvotis that common carp was present on the spawning ground of gibel carp. Common carp was found to be present in the shallows during May, with water temperature between 16 and 18 °C, but by this time gibel carp had released their eggs and were absent from the spawning grounds.

The laboratory results indicate lack of paternal effects on the gynogenetically reproduced gibel carp fry. Irrespective of sperm origin (goldfish, common carp or ylikiensis roach), post-hatch growth and survival were similar. In the same manner, growth and survival did not vary between fish originating from eggs collected in situ or eggs artificially fertilised in the laboratory (Table 2). This contrasts with some paternal effects observed in gibel carp crosses (Zou et al., 2001).

Under the controlled conditions and procedures of artificial egg fertilisation and incubation (mixing of eggs and sperm, eggs treated to became non sticky, optimum incubation conditions) the fertilisation ability of sperm and embryonic viability may be increased compared to natural reproduction and incubation conditions (Horvath et al., 1984). This may partially explain the fact that hatching rate did not vary significantly ($P>0.05$) between the artificially produced crosses, whereas between different egg substrates collected from the spawning grounds, hatching rate ranged from 7.2-29% and varied significantly ($P<0.01$).

The small percentage (2.3%) of male gibel carp captured during spawning indicates that, in addition to gynogenetic reproduction, bisexual reproduction is to some extent an option for the Pamvotis gibel carp. Nevertheless, the population was almost exclusively composed of females. Gynogenetic reproduction appears to be the main mode of reproduction for gibel carp in the lake. This is in agreement with reports about other aquatic ecosystems (Migdalski & Fichter 1976; Riehl & Baensch, 1991; Zhou et al., 2000).

Ylikiensis roach can contribute significantly to the gynogenetic reproduction of gibel carp in the lake. Based on the laboratory data, it can be concluded that gibel carp can potentially utilise the sperm of ylikiensis roach, which according to the field data, was the most obvious sperm donor. In fact, the results of the artificial fertilization indicate that ylikiensis roach was equally as good as the sperm of common carp or goldfish. Furthermore, based on the relative abundance data collected in the field, it can be concluded that ylikiensis roach was at least statistically the most obvious potential sperm donor for gynogenetic reproduction of gibel carp in lake Pamvotis. To our knowledge this is the first record of gynogenetic reproduction between these two fish species, and contrasts with the situation reported from other lakes, where common carp (*C. carpio*) is the principal sperm donor.

The prospects for restricting the population of gibel carp in the lake are limited. Larvae exhibit high viability and the species is almost exclusively composed of

females, which grow rapidly and spawn massively. During the last five years fisheries landings of gibel carp tripled while fisheries landings of other species were significantly reduced (Perdikaris et al., 2003).

Considering the energetic cost of sperm production in small fish such as the ylikiensis roach, it could be argued that any sexual parasitism of gibel carp on ylikiensis roach may have limited but negative consequences for the donating species. The male fish of roach species arrive first in the spawning ground and await the arrival of the female, when mating play is initiated and spawning takes place (Muus & Dahlstrom, 1999). Field observations indicate that gibel carp waits for the mating of ylikiensis roach to occur for releasing its eggs on the spawning grounds. It is, therefore, possible that some sperm of ylikiensis roach is lost because of the sexual parasitism of gibel carp, but further field work would be necessary to verify this hypothesis.

Gynogentic reproduction may have contributed to the increased gibel carp population in the lake. Nevertheless, population size is a complex product of birth, recruitment, natural and fishing mortality, carrying capacity, ability to compete for food, ecophysiological fitness or adaptation, and environmental conditions. At least two factors contribute to the thriving of the gibel carp population in the lake. One factor is the tolerance to environmental degradation (for example, reports emerged recently in local newspapers, about pollution of the lake that occurred on several occasions as a result of an overflow of the sewage pipelines of Ioannina city). Another factor is reduction in abundance of predating species (Perdikaris et al., 2003) or reduced competition for food from other species (Holcik & Zitman, 1978).

The thriving of gibel carp populations is further supported by its ability to survive in adverse environmental conditions (Holcik & Zitman 1978; Holcik, 1980). For example gibel carp fry originating from gynogenetic reproduction with sperm of *R. ylikiensis,* exhibited specific growth rates of 3.14% and 0.91% at NH_3 concentration 0.51 mg/lt and 8 mg/lt respectively (Nathanailides et al., 2003).

In conclusion, gibel carp females have little opportunity for mating with gibel carp males. Ylikiensis roach is the best available sperm donor option for the gynogenetically reproducing gibel carp of the lake.

As to the loss of biodiversity by the expansion of the gibel carp population, as is the case with other aquatic ecosystems (Holcik & Zitman, 1978; Holcik, 1980), a combination of biological and environmental factors appears to be responsible for the thriving of gibel carp in Lake Pamvotis. It would be reasonable to take action to improve ecological conditions and counteract environmental degradation, particularly in the spawning grounds.

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Movement is necessary for landmark-based navigation

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ABSTRACT. The experiments reported here were designed to find out whether mice are capable of homing by visual extra-arena landmarks, when deprived of visual access to such landmarks. Mice were placed in a circular arena, where they could view the experimental room only from a peripheral nest and the centre of the arena, while they could not access the visual cues when moving in-between. This resulted in poor homing in mice. The conclusion drawn is that mice need to view visual cues while moving for their landmark-based navigation.

KEY WORDS : Navigation; Path Integration; Homing.

INTRODUCTION

Previous work showed that mice are incapable of homing from the centre of a circular arena to their nest after viewing a distal landmark array only from a peripheral nest location (ALYAN, 1994). However, rodents are capable of homing successfully after having freely explored the arena (ALYAN & JANDER 1994), and after being passively carried from the nest to the centre of the arena (ETIENNE, 1980; MORRIS, 1981). This supported earlier claims that moving while viewing distal landmarks is necessary for successful landmark-based navigation (STAHL et al., 1987; SUTHERLAND et al,. 1987; CHEW et al., 1989; MATTHEWS & BEST, 1997).

Furthermore, ALYAN & JANDER (1997b) found that mice could home by learned landmarks, after having shuttled between the nest and the centre of a circular arena, without any exploratory phase of that arena prior to or during the experiment. This demonstrated that shuttling between only two locations, in full view of the distal landmarks, allows mice to learn distal landmarks and to home by reliance on such cues.

These findings led to one further question that is explored in this study : Can mice learn distal landmark constellations and home by them after viewing them from only two points, but not while shuttling along the path towards either point? A positive finding would indicate that mice, while shuttling, connect the disjoint landmark vistas from the two points via path integration mechanism to form a landmark cognitive representation allowing them to home accurately at a later time, using only landmark constellations.

METHODS

Subjects

Eight lactating female house mice (*Mus musculus* Linnaeus, 1758) with their pups were used. Prior to experimentation they were housed in transparent cages 25 x 50 cm. The floor was covered with wood shavings, and food and water were provided ad libitum all the time. The photoperiod was 12 h light : 12 h dark (dark 7:00 pm to 7:00 am) and all experiments were conducted between 10:00 am and 2:00 pm. The purpose of using mothers with litters was to increase their motivation to go home. Therefore, two strong motivations were exploited in this study : the mothers' motivation to take their pups back to the nest and the mothers' motivation to seek a refuge.

Apparatus

A circular arena, 1.5 m in diameter, was used in all experiments. It rested on ball bearings to allow free rotation in any direction. Four handles were attached to the arena to enable smooth manual rotation. The floor of the arena was painted with a polyurethane-sand mixture for easy cleaning and to provide purchase for the mice during movement. A hole, 3 cm in diameter, was drilled at the periphery of the arena, and led to a tube, which in turn led to a nest box located beneath the arena's floor. The edge of the arena was marked at 5° intervals to record the mice' arrival points at the edge of the arena. In addition, the arena was surrounded up to a height of 40 cm by a Plexiglas sheet that had a one-way screen made of dark-tinted Perspex glued to it. Above the centre of the arena we positioned a 40 W incandescent bulb that reflected all its light downward.

The experimental room was normally lit on one side by natural light through two large windows and from the ceiling by standard fluorescent tubes. Pieces of furniture and wall posters offered additional distal landmarks for the homing mice.

Definitions and data collection

Directions were recorded when the mouse, starting from the centre of the arena, reached the periphery of the arena. Angles were measured clockwise from 0°-360°, with 0° being the nest direction before rotation.

To test for directional tendencies, circular statistics were used (BATSCHELET, 1981; ZAR, 1974). The following parameters were used in evaluating homing performance of mice. A sample analysis yielded a mean vector, *m,* where *m* is defined by its polar co-ordinates : and *r,* where is the sample mean direction and *r* is the length of *m*. The mean vector length, *r,* serves as a measure of concentra-

tion as well as dispersion. The larger *r* is, the less dispersed the directions are around the mean angle.

Procedure

The objectives of this experiment were, first, to find out whether viewing the experimental environment from two locations, with no movement between them, would be sufficient for successful landmark-based navigation from one of these locations to the other and, second, to test whether movement between the two locations, without viewing the external environment while moving, can result in successful landmark-based navigation between the two locations.

Experiment Ia

Each of the eight female mice used in this experiment, with her pups, was taken from her cage and placed in the nest box. A clear Plexiglas cylinder (10 cm in diameter and 15 cm high) surrounded the nest entrance on the surface of the arena. The cylinder had one hole that led to a gray, opaque PVC tube (55 cm long). The other end of the PVC tube led to a hole in an identical Plexiglas cylinder placed in the centre of the arena. Both ends of the PVC tube were blocked with pieces of clear Plexiglas so that the mice could not move between the nest and the centre of the arena. Another identical PVC tube, placed on the opposite side of the central Plexiglas cylinder, led to a third identical Plexiglas cylinder placed at 180˚ from the one at the nest location. This was to ensure that the mice relied on extra-arena cues and not on certain guiding intra-arena cues, including odor trails, when later tested for spatial navigation. The arena itself was wiped with dilute alcohol solution before testing each mouse, to eliminate odor cues.

Each mouse spent 12 h in the nest box, during which she could go to the surface of the arena enclosed by the Plexiglas cylinder and view the room, which was fully illuminated, from only that location. Thus, mice were capable of viewing distal landmarks from that location. Thereafter, the mouse was placed in a dark box and rotated slowly for 1-2 min, while the experimenter walked around the room. This was done to prevent the mice from associating the nest location with any other point in space by the mechanism of path integration. The mouse was then placed inside the Plexiglas cylinder situated in the centre of the arena. She was left there for 6 h, after which she was taken on another disorientation tour, and ended up in the nest box where she stayed for another 6 h. The mouse was then taken from the nest on a third disorientation tour, during which the surface of the arena was cleaned and had no objects on it and the pups were taken from the nest box. This would eliminate orientation by intra-arena cues, including odor cues. The dark box with the mouse in it was placed in the centre of the arena and the arena, along with the boxed-in mouse, was rotated 6-7 full rotations CW or CCW. The purpose here was to prevent the mice from orienting by relying on path integration mechanism. Therefore, if the mouse navigates towards any point, it would be by means of distal visual cues. One or two pups were dropped inside the box and the box was removed leaving the mother and her pups in the centre of the bare arena. The point at which the mouse arrived at the edge of the arena was recorded for later analysis. Each mouse was tested only once.

Experiment Ib

A similar setup to that of experiment Ia was used here. The difference was that the doors at the end of the tube connecting the Plexiglas cylinders at the nest and the centre were removed. Each of the eight mice now could access the two Plexiglas cylinders through the tube within the 14-18 h they were left there. Again, the room lights were kept on during that time, so that the mice could access distal visual landmarks from their locations. To ensure that the mice would visit the centre, they were induced to retrieve pups from the centre back to the nest 30 times. In addition, a small water cup (3W x 2H cm) was left in the central area as the only source of water. After that, each mouse was placed in the dark box, and taken on a disorientation tour. The surface of the arena was cleared of all objects and the pups were taken from the nest. The mother was then placed in the centre with 1-2 of her pups, and the arena was rotated 6-7 full rotations CW or CCW. The box was then removed and the point at which the mouse arrived at the edge of the arena was recorded for later analysis.

RESULTS

Experiment Ia

The arrival points of the eight females at the periphery of the arena are shown in Fig. 1. The mice took direct paths to the edge of the arena and made no looping or circling. The sample mean angle is 89.28°, while *r* is 0.06. Therefore, the hypothesis that orientation was random cannot be rejected (V Test; $P > 0.25$).

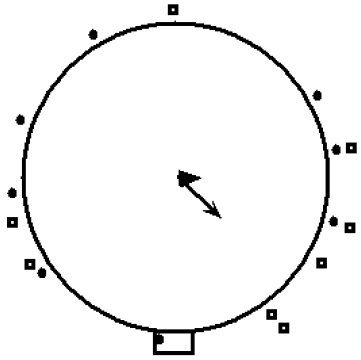

Fig. 1. – The arrival points for the eight mice used in experiment Ia are indicated by the solid arrow and circles. = 89.28°, *r* = 0.06. Arrival points for the mice that were allowed to shuttle in a dark tube (experiment Ib) are indicated by the open arrow and squares. = 47.5°, *r* = 0.37 (the rectangle indicates the nest location at 0°).

Experiment Ib

Mice arrival points at the periphery of the arena are shown in Fig. 1. Again, the mice took direct paths to the edge of the arena upon release. The sample mean angle, , is 47.5°, while *r* is 0.37. Therefore, the hypothesis that orientation was random cannot be rejected (V Test; $0.05 < P < 0.1$).

DISCUSSION

We can summarize the above findings as follows. First, allowing mice to view the environment from only two different locations results in poor landmark-based orientation. Second, this occurs regardless of whether the mice were, or were not, allowed to shuttle between the two locations, but denied access to visual landmarks while shuttling.

It has previously been shown that viewing distal landmarks from only one location cannot support later navigation to that location from another (ALYAN, 1994). What has been shown here is that viewing the distal landmarks from two locations does not allow successful landmark-based navigation, even though the mice moved between the two locations. In addition, moving between two points per se is not sufficient for successful navigation. This is in contrast to earlier findings that mice navigated successfully when they had access to visual cues while moving (ALYAN & JANDER, 1997a). Thus, the present results support earlier claims that it is necessary to view distal cues while shuttling between locations in order to navigate by such cues, (STAHL et al,. 1987; SUTHERLAND et al., 1987; POUCET & BENHAMOU, 1997). Separate vistas of distal cues from different locations, without actual movement between them, do not allow successful navigation by distal landmarks as has been claimed in various theoretical models (WILKIE & PALFREY, 1987; GALLISTEL, 1990; O'KEEFE, 1990; 1991). The present results confirm that rodents require to be fed conjointly with movement and visual information to be able to perform efficient place navigation. Altogether, these reports lend further support to the hypothesis that animals build cognitive representations of their home ranges through motor vector deduction, i.e. based on path integration, and not perceptual vector deduction (ALYAN, 1994; POUCET & BENHAMOU, 1997).

In addition, ZANFORLIN & POLI (1970), and ALYAN & MCNAUGHTON (1999) have shown that normal rats, and rats with hippocampal lesions, were capable of proper homing after shuttling between two points, by making a novel shortcut under a sandy substrate, i.e. no visual cues were accessible since rats were moving underground. The rats could view distal landmarks from the end points only, but not while shuttling. The difference between these two studies and the present results is that path integration was not specifically impaired before homing (ZANFORLIN & POLI, 1970; and ALYAN & MCNAUGHTON, 1999). Thus, both studies indicate that rats form a cognitive representation of their path, which enables them to calculate a novel shortcut by means of path integration when the original route is blocked. This also supports claims that animals could build cognitive representations through path integration.

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SHORT NOTE

Feeding habits of the temperate octocoral *Tripalea clavaria* (Studer, 1878) (Octocorallia, Gorgonaria, Anthothelidae), from sublittoral outcrops off Mar del Plata, Argentina

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Recent studies and reviews have shown the importance of passive suspension feeders in marine food webs, and noted the role that cnidarians (hydroid colonies and soft corals) play in shallow water ecosystems (1, 2).

Hydroids are assumed to be carnivorous, capturing zooplankton prey by means of nematocysts of the tentacles, although protozoans, diatoms and organic detritus can be important for some hydroid species. In contrast, gorgonians ingest and assimilate particulate and dissolved organic matter (3, 4), while other species are phytoplankton feeders (5). Capture of zooplanktonic prey has been documented in few studies (3, 6), and some researchers have postulated that the ability of these organisms to capture vagile prey is low, based on low densities of nematocysts on the tentacles (3, 4, 7).

Gorgonians are quantitatively important in certain benthic communities, and provide a good example of the role of suspension feeders in littoral food chains and of the relationship between plankton and benthos in temperate seas (8).

This study analyzed feeding habits, throughout the year, of *Tripalea clavaria*, an unbranched azooxanthellate octocoral widely distributed in the SW Atlantic Ocean (9, 10, 11). It is abundant, and a dominant component in rocky outcrops off Mar del Plata city (12). We compared the results with data on other anthozoans.

Collections and underwater observations were carried out during SCUBA dives at Banco del Medio (38º 10´S - 57º 28´W), a quartzitic rocky outcrop 18-20 m depth. Monthly samples were obtained from November 2000 to October 2001, except in July owing to persistent bad weather conditions. The gastral contents of 1072 polyps belonging to 117 colonies were examined in the laboratory under a microscope. Food items were identified to the lowest taxonomic level possible. Trophic parameters (vacuity index, frequency index of prey and percentage of prey) were calculated as in Acuña & Zamponi (13).

Table 1 lists all prey items found, ranging from small diatoms 60-80 µm in diameter to crustacean and echinoderm larvae up to 72.5 µm. The number of prey items polyp^{-1} was mostly one or two. Table 2 shows the percentage of empty gastric cavities (vacuity index), indicating that most colonies fed more actively in spring and summer in coincidence with high prey abundance. Frequency indices of prey (Table 3) show that larvae of the mytilid *Mytilus edulis platensis* d'Orbigny, 1846 were the main food item, while invertebrate eggs were a minor component except in spring when their importance as food was similar to mytilid larvae. Other food items were occasional prey only.

T. clavaria is one of a few zooplanktivore gorgonians that prey on a large variety of organisms (Table 1), mainly

TABLE 1

Number (N) total of the different prey – item and their relative abundance (%), found in the diet of *T. clavaria* in each season.* Presence, can not be identified individuals.

Item	Spring		Summer		Fall		Winter	
	N	%	N	%	N	%	N	%
Mytilus edulis platensis larvae	24	45.3	78	93.4	28	70.0	22	68.7
nauplii	1	1.9	-	-	1	2.5	1	3.1
crustacean larvae (unidentified)	-	-	-	-	1	2.5	1	3.1
Gammaridea	-	-	-	-	-	-	3	9.4
Copepoda	-	-	1	1.2	2	5.0	-	-
Ostracoda	2	3.8	-	-	-	-	-	-
crustaceans (unidentified)	1	1.9	-	-	-	-	-	-
invertebrate eggs	23	43.4	3	3.6	6	15.0	2	6.2
Nematoda	1	1.9	1	1.2	-	-	-	-
echinoderm larvae	-	-	-	-	1	2.5	-	-
Tardigrada	1	1.9	-	-	1	2.5	1	3.1
filamentous algae	*	-	*	-	-	-	-	-
diatoms	-	-	-	-	-	-	2	6.2
Total	53	100.0	83	100.0	40	100.0	32	100.0

TABLE 2

Percentage of empty gastric cavities (vacuity index).

Season	examined colonies	examined polyp	polyps with content	Vacuity index (V)
spring	24	199	42	78.89
summer	30	269	65	75.84
fall	32	305	34	85.57
winter	21	199	27	86.43

TABLE 3

Frequency index (f) and percentage of prey (Cn) of *Tripalea clavaria*.
f = n/N. n : number of gastral cavities containing a certain prey, N : the total number of gastral cavities examined. Cn= n'.100/Np. n' : total number of individuals of a certain prey, Np : the total number of prey items. * Presence, can not be identified in individuals.

Item	n	N	f	n'	Np	Cn	Result
Mytilus edulis platensis larvae	124	1072	0.12	159	222	71.62	Preferential
nauplii	4	1072	0.005	6	222	2.70	Occasional
crustacean larvae (unidentified)	2	1072	0.002	2	222	0.90	Occasional
Gammaridea	3	1072	0.003	3	222	1.35	Occasional
Copepoda	3	1072	0.003	3	222	1.35	Occasional
Ostracoda	2	1072	0.002	2	222	0.90	Occasional
crustaceans (unidentified)	2	1072	0.002	2	222	0.90	Occasional
invertebrate eggs	31	1072	0.03	35	222	15.76	Minor
Nematoda	2	1072	0.002	2	222	0.90	Occasional
echinoderm larvae	1	1072	0.001	1	222	0.45	Occasional
Tardigrada	3	1072	0.003	3	222	1.35	Occasional
filamentous algae	2	1072	0.002	*	*	*	*
diatoms	2	1072	0.002	3	222	1.35	Occasional

on larvae of *M. edulis platensis* with a 37.5-434.3 µm size range. Similar diets were observed for the gorgonian *Paramuricea clavata* (Risso, 1826), which regularly feeds on zooplanktonic prey of small size (100-200 µm) and low motility, such as nauplii and eggs (COMA et al*.,* 1994). Some species of alcyonaceans with polyps very similar in size to those of the majority of gorgonians (e.g. *Alcyonium siderium* Verrill, 1922), capture mostly small (256-345 µm) prey items of low motility, such as foraminiferans and invertebrate larvae (14).

Despite low density of tentacular nematocysts (3, 4, 7), *T. clavaria* is able to ingest many vagile organisms, implying a different mechanism for food capture, related to the transport of potential food sources by the water current ("aerosol filtration theory") (2). These results furnish evidence that gorgonians, like other benthic zooplanktivores, may play a role in the flow of energy between the plankton and the benthos (8). Further studies should be conducted to determine the extent and scale of the feeding strategies of gorgonians and other benthic zooplanktivores.

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