CONTENTS

The **BELGIAN JOURNAL OF ZOOLOGY** is covered in **Current Contents** and in Science Citation index.

ISSN : 0777-6276

1999 129 (2)

Belgian Journal of Zoology

BELGIAN JOURNAL OF ZOOLOGY

Volume 129 (2)

July, 1999

Published with the financial support of the « Vlaamse Gemeenschap – Ministerie van Onderwijs », the « Direction générale de l'Enseignement supérieur et de la Recherche scientifique », and the « Universitaire Stichting van België » / « Fondation Universitaire de Belgique ».

Publié avec l'aide financière de la « Direction générale de l'Enseignement supérieur et de la Recherche scientifique », du « Vlaamse Gemeenschap – Ministerie van Onderwijs » et de la « Fondation Universitaire de Belgique ».

Gepubliceerd met de financiële hulp van de « Vlaamse Gemeenschap – Ministerie van Onderwijs », van de « Direction générale de l'Enseignement supérieur et de la Recherche scientifique » en van de « Universitaire Stichting van België ».

NEW GUIDE FOR AUTHORS

From volume 130 (2000) on, the Belgian Journal of Zoology will change format. The page size will be 17 x 23 cm, in two columns of 8 cm. Please keep this in mind, in particular for the size of figures and tables.

1. The Belgian Journal of Zoology publishes, in English or French, original papers and notes in all fields of zoology. Reviews on topics of general interest or current importance may be considered, after invitation by or agreement with the editors. Manuscripts must contain significant new findings and must not have been published elsewhere nor be simultaneously under consideration by any other journal. Single-species descriptions will not be accepted unless they have broader relevance, such as implications for phylogeny or biogeography. Papers in French must include English translations of the title and of the abstract.
2. Authors benefit from a waiver of page charges up to eight printed pages. This waiver is subject to annual consideration by the Council of the Society. Additional pages are charged to authors at cost price. Authors will receive 50 reprints free of charge. Additional reprints can be ordered at the time of return of the galley proofs.
3. Each manuscript is reviewed by at least two referees, usually one Belgian and one non-Belgian referee. After acceptance, articles remain the full responsibility of authors.
4. Authors are requested to follow rigorously the lay-out as shown in the articles in this issue, paying particularly attention to the format of references. Papers should be written in clear, concise language and consist of an abstract (summarising the essential results and conclusions), introduction, material and methods, results and discussion, and provide a short running title and keywords (separated by commas). Sections and sub-sections should not be numbered, but authors may suggest a printing format to provide structure to the article. Author names in text citations should be in SMALL CAPITALS and in the following format: PERRIN & KOKKINN (1985) for two authors or WILSON et al. (1987) for more than two authors. Consecutive references must be separated by a semicolon (;). Figures in the text are referred to by "Fig. n" or "Figs n-m". Names of species (and names of higher taxa in taxonomic papers) should be followed, on the first occasion of use, by the name of the author who described the species or introduced the taxon name (which should be in lower case) and the year. *Species names* are in italics.
5. Short notes, not exceeding two printed pages (incl. figures), with new findings of immediate importance, comments on papers published in the journal, new records for (*e.g.*) the Belgian fauna, curiosa, etc. will be published in the next issue whenever possible. They should be written as continuous text without the various divisions of regular papers. References for Notes must be indicated in the text by number, and listed and numbered in the order of their appearance in the text. Short notes will be reviewed as normal papers.
6. All parts of the manuscript, including text, references, tables and legends for illustrations, should be typewritten, double spaced (on one side of the page only) and all pages numbered. Figures, tables and legends should remain separated from the text. Scale bars should be drawn on figures where appropriate, and size indicated in the legend. Three copies of text and three quality copies of figures must be provided, as well as the originals. Photographs should be mounted and clearly labelled. Hand made drawing should be in black ink (clearly readable after reduction). All figures should be labelled in pencil on the reverse side with the number of the figure, the name of the author(s), title of the paper, and intended degree of reduction. Photocopies of photographs or figures are not acceptable. Indicate on the manuscript the author's e-mail address for correspondence. Following acceptance of the manuscript, authors must provide one hard copy of the final version and a copy on diskette. Whenever possible, authors are asked to also include on the diskette(s) computer generated or scanned figures and photographs, with an indication of the program and system used. Indicate on the hard copy where figures and tables are to be inserted. Overseas authors can submit their manuscript by e-mail (Ernest.Schockaert@luc.ac.be).

The royal Belgian zoological Society organizes meetings by invitation of the members.
The contribution for members is 1000 BEF (25 Euro) annually. Students benifit from a contribution of 300 BEF. Members receive the journal (two issues per year, resp. in January and July and the occasional special issues) free of charge.

The price for one volume (two issues) is 2.000 BEF.

C.C.P. 000-0049113-31, Société Royale Zoologique de Belgique, Bruxelles.

P.R. 000-0049113-31, Koninklijke Belgische Vereniging voor Dierkunde, Brussel

Requests for exchange and other library matter : Prof. Dr. J. Deligne (library administrator), ULB-CP 160/11, Av. Roosevelt, 50, B-1050 Brussels (Belgium).

General information on the society and the journal can be found on http://kbvd-www.uia.ac.be/kbvd/.

Belgian Journal of Zoology

(formerly : « ANNALES DE LA SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE — ANNALEN VAN DE KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE »)

Published by the « KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE — SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE »

Volume 129 (2)

(July, 1999)

Editor :

Prof. Dr. E. Schockaert
Department SBG
Limburgs Universitair Centrum
B-3590 Diepenbeek (Belgium)

Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 327-337 - Brussels 1999

Received : 15 April 1998

A NEW GENUS AND TWO NEW SPECIES OF CHYDORIDAE (BRANCHIOPODA : ANOMOPODA), FROM KORUP NATIONAL PARK, CAMEROON

ELS CAMMAERTS AND JOHAN MERTENS

Lab. Animal Ecology, Zoogeography & Nature Conservation, Dept. Biology, University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium)
e-mail : johan.mertens@rug.ac.be

Abstract. Among the small crustaceans occurring in temporary waterbodies in the primary rain forest of Korup National Park in Cameroon, two new chydorid « cladoceran » species were found : *Hydrospilus degreefi* gen. et spec. nov. and *Bryospilus culverwelli* spec. nov.. Both species lack a compound eye. Apart from these new species, *Bryospilus repens,* which was thought to be restricted to epiphytic moss, was also found. *Hydrospilus degreefi gen. et spec. nov.* bears three setae on the inner distal lobe of P1, suggesting a relationship with *Alona*.

Key words : Cladocera, *Bryospilus*, Cameroon, Korup National Park

INTRODUCTION

In 1980 a new anomopod genus, *Bryospilus,* was described as a member of the moss fauna in wet forests (FREY, 1980). Along with the definition of the new genus, FREY (1980) described two new species, one from Venezuela and Puerto Rico *(B. repens),* another from New Zealand *(B. bifidus).* Both were obtained from epiphytic moss from elfin forests. We found three *Bryospilus* species in pristine primary rainforest of equatorial Africa (Korup National Park, Cameroon). Korup National Park is a lowland evergreen forest, typical of tropical wet coastal areas (ODNRI, 1989).

All sampling sites are located in remnants of what was once the great tropical belt of rain forest in Africa. Korup National Park lies mainly in the Ndian division of SouthWest Province. The protected area covers about 122,000 ha, located between 4°56'N-5°01'N and 8°48'E -8° 56'E. Most samples were taken from localities about 50m above sea level, belonging to the drainage basins of the Korup river, the Akpasang river, the Ndian river, and the Cross river.

During two sampling trips to the area, special attention was focused on ephemeral water bodies, such as small puddles and even empty nut shells of a *Strichnos* vine (SEGERS & MERTENS, 1997). Of 110 samples examined, six contained three different *Bryospilus* species, among them *B. repens* Frey, 1980 and an undescribed species. Material of the third species of *Bryospilus* was too scarce to enable full morphological study, but most probably represents a third undescribed *Bryospilus* species. Since the original description,

no new records were made of representatives of this genus, until we found them, together with *Alonella exigua* (Lilljeborg, 1853), *Alona eximia* (Kiser, 1948), and an unknown genus which is also described, in samples from the Korup National Park.

MATERIAL AND METHODS

A first series of samples was taken at the beginning of the rainy season (May, mean temperature 28°C), the second in the middle of the wet season (August, mean temperature 25°C). Samples collected by dragging through aquatic vegetation (plankton nets 50 µm and 100µm mesh) were preserved in 4% formalin. Specimens were examined under a Wild M5 stereomicroscope, and drawn using an Olympus CH2 compound microscope equipped with a camera lucida. For Scanning Electron Micrographs, specimens were dissected, dehydrated in an alcohol series, critical point dried, mounted on stubs, coated with gold and observed using a JEOL JSM-840 SEM.

DESCRIPTION OF NEW TAXA

Hydrospilus gen. nov.

Diagnosis: Body slightly elongated. Three broadly connected headpores. No lateral headpores. Postero-dorsal corner of valve without denticles. An ocellus present, but a compound eye lacking. Trunk limb 1 with three setae on inner distal lobe, two setae in the lateral group. Postabdomen distinctly broad. Basal spine about half length of claw, bearing eight spinules, the distalmost being the largest. Between the main striae, finer striation present ventrally; polygons present mainly posteriorly and dorsally. Antennal formula: 0-1-3(1)/0-0-3(1).

Differential diagnosis: *Hydrospilus* has three connected median headpores, and lacks lateral pores. In contrast *Bryospilus* has the median headpores not connected by any channel or line, and possess very small lateral pores. The trunk limb 1 in *Hydrospilus* bears three setae on the inner distal lobe, whereas the *Bryospilus* species have only two. The antennal formula differs between genera: 0-1-3(1)/0-0-3(1) (*Hydrospilus*) and 0-1-2(1)/0(1)-0-3(1) (*Bryospilus*).

Hydrospilus resembles *Bryospilus* in lacking a compound eye, and in having a somewhat similar gross appearance of the postabdomen. The absence of a compound eye and the presence of only two lateral setae in the lateral group of P1 in *Hydrospilus* suggest affinities with *Bryospilus*, while the three connected headpores and three setae on the inner distal lobe of P1 are reminiscent of *Alona*. Moreover, the headpores and postabdominal shape of *H. degreefi* is in particular in concordance with *A. eximia*. The presence of six rather than seven spines on the exopodite of P3 is an obvious difference between *Hydrospilus* and *Alona* (incl. *A. eximia*). So *Hydrospilus* can be related to its sister taxon *Bryospilus*, and to *Alona*.

Etymology: From (Gr.) *hydro*, water and (Gr.) *spilus*, spot. Analogous to *Bryospilus* and *Monospilus*, which also lack a compound eye and hence have only one eye spot.

Type species: *Hydrospilus degreefi* spec. nov., by monotypy.

***Hydrospilus degreefi* nov. gen., nov. spec.** (Figs 1-3)

Material examined: Ten parthenogenetic females, two juveniles.

Holotype: A parthenogenetic adult female on a slide, mounted in glycerine, and deposited at the Royal Institute of Natural Sciences, Brussels (KBIN: IG 28510a).

Paratypes: Dissected trunk limbs of a parthenogenetic female on a slide mounted in glycerine. The remaining individuals are preserved in formalin at the Dept. Biology, University Gent.

Type locality: Pamol Estate pond, Mundemba, Cameroon (5°01′N-8°56′E, leg. J. Mertens, 26/05/1995 in RUG.)

Diagnosis: Slightly elongated body shape with very broad ending rostrum. Headshield with three connected and median headpores. Postabdomen very broad (width/length = 0.5).

Etymology: the species name is after Willy De Greef, Science co-ordinator of Korup Project at the time, who invited us to Korup National Park and took part in the sampling trips.

Description

Size: Mean length 0.38mm (n=9), range 0.33-0.43mm. Mean length-width ratio of adult females is 1.43, range 1.23-1.67.

Shape: Slightly elongated (Fig. 1a; Fig. 3a), length about 1.4 times maximal height. Dorsal margin of valves anteriorly more curved than posteriorly, ventral margin anteriorly curved, posteriorly straight, posteroventral corner of valve broadly rounded and without denticles, posterodorsal corner not distinct. Ventral margin of valve adorned with feathered setae throughout, differentiated in three zones of different setae-length (Fig. 1b; Fig. 3f). At implant of each of the 19 posteriormost setae, are four to five short spinules.

Head shield terminating anteriorly in very broadly rounded rostrum (Fig. 1c; Fig. 3c). Three median headpores connected by a broad field surrounding them (Fig. 1f; Fig. 3b). Lateral headpores not found. Ocellus but no compound eye. Antennule with nine aesthetascs, one exceeds length of antennule. Antenna with two setae as long as the three segments together, one seta longer and apparently split. Antennal segments longer than wide. Antennal formula: 0-1-3(1)/0-0-3(1). Labrum with rather obtuse tip reaching far beyond rostral tip. Labral plate long, ending bluntly, and lacking setation.

Trunk limbs:

P1 (Fig. 2a, b): Three setae on inner distal lobe, one nearly half the length of the others, being subequal in length. Endite with three groups of spines and setae distally. Inner group consisting of four elements, with outermost two spines more heavily chitinized, forming a two-pronged fork-like configuration. All four elements bilateral feathered. Both innermost setae are equally weakly developed. The middle group consists of three elements, two are almost equally strongly developed, the third is much weaker. The spine in the middle of the group shows a complex setulation pattern. At the base of these elements are several strongly chitinized denticles. Only two setae in lateral group.

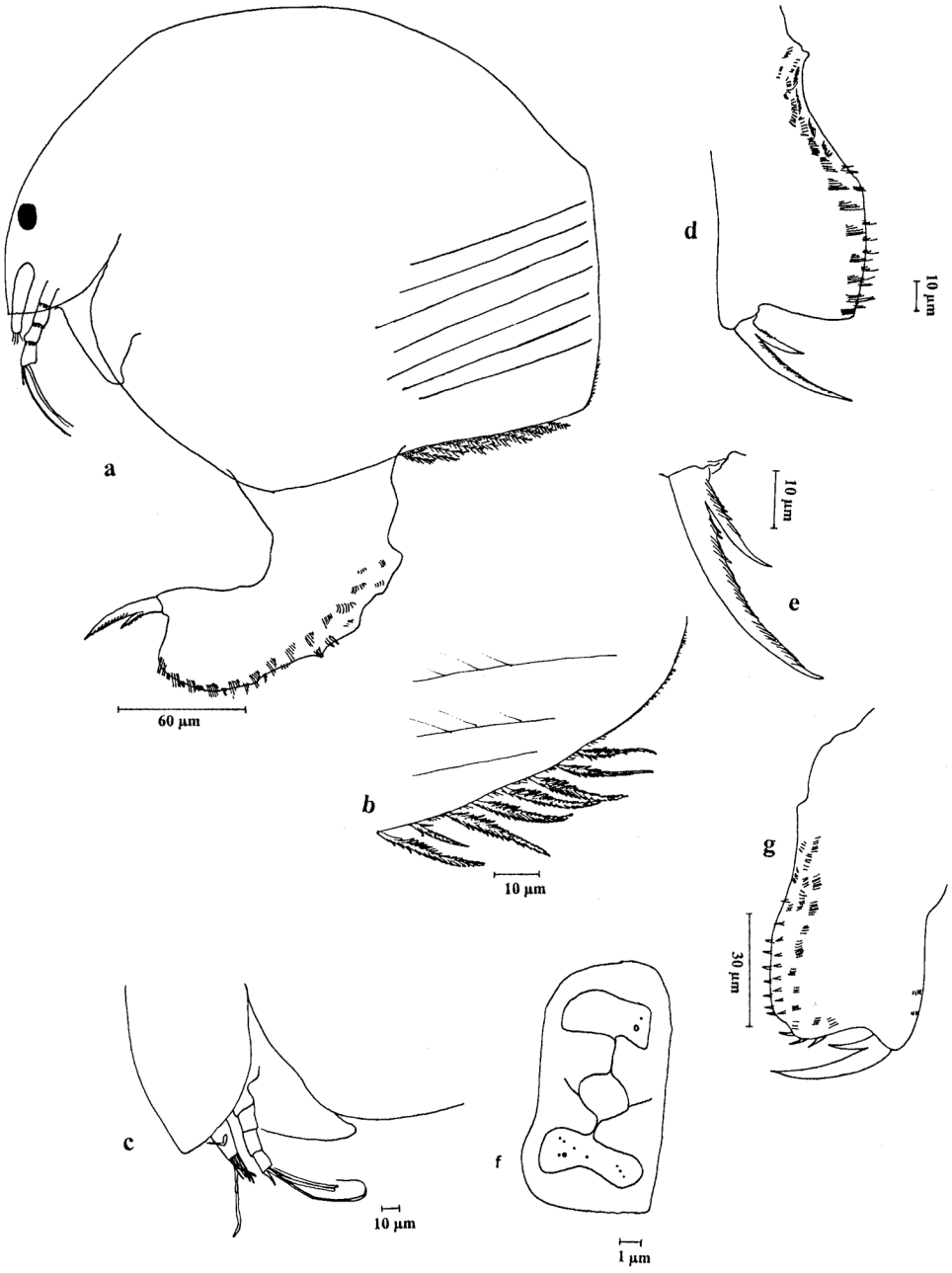


Fig. 1. – *Hydrospilus degreefi* sp. n., female. – (a) habitus, (b) posteroventral margin of valve, (c) rostrum, (d) postabdomen, (e) postabdominal claw, (f) headpores, (g) postabdomen of juvenile.

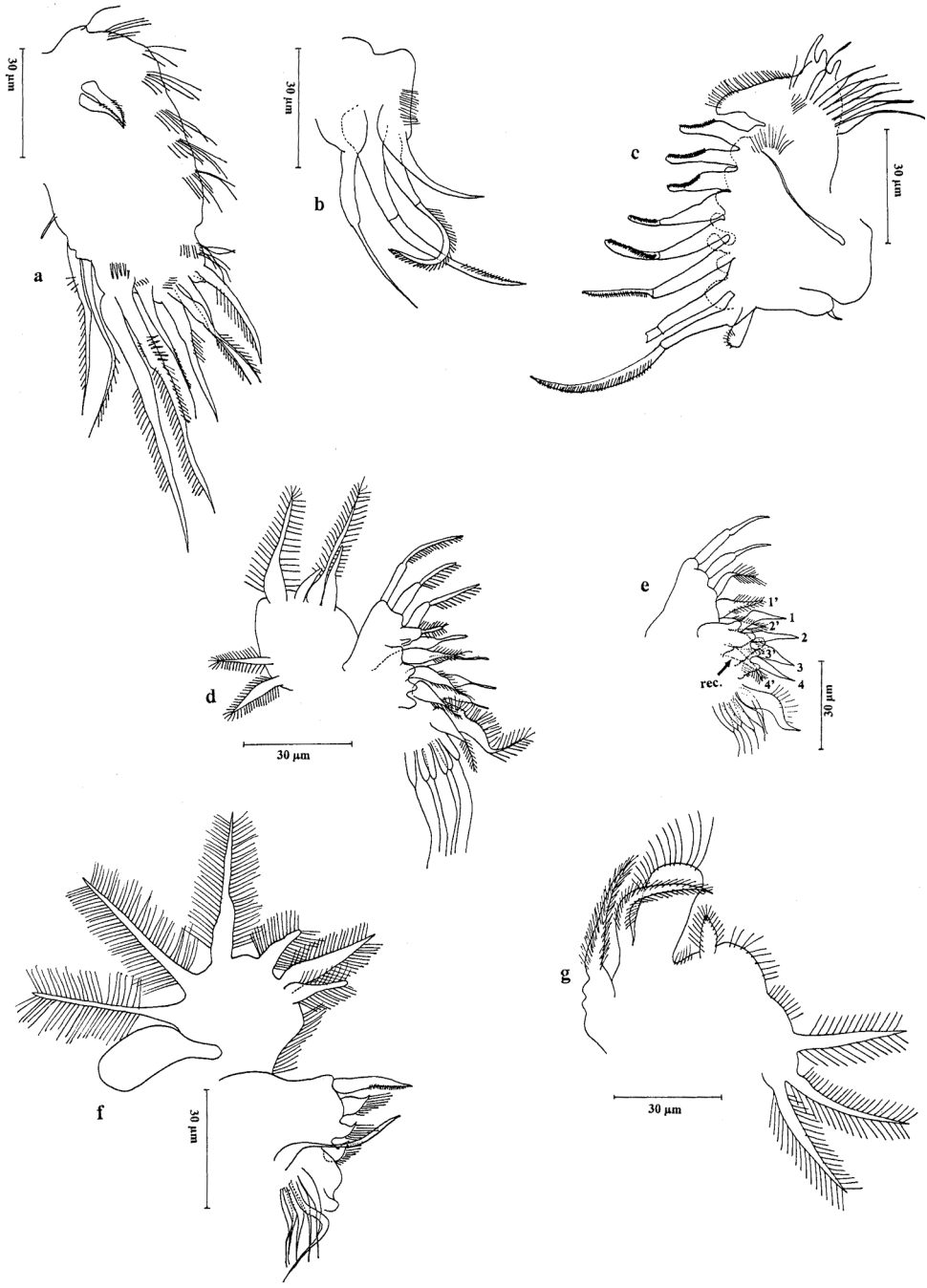


Fig. 2. – *Hydrospilus degreefi* sp. n. nov., female – trunk limbs (a) P I without outer ramus, (b) P1 outer ramus, (c) P2, (d) P 3 with exopodite, (e) P 3 showing receptor, (f) P 4, (g) P 5.

P2 (Fig. 2c) : Somewhat pointed expanded lobe between gnathobase and scraping spines. Seven gnathobasic filtering setae, eight scraping spines regressing in length. Endite surface bears a seta, the tip of which nearly reaches the base of scraping setae.

P3 (Fig. 2d, e) : Six filtering setae, three two-segmented, bilateral feathered setae (indicated with arrow on figure). Exopodite with six setae.

P4 (Fig. 2f) : Five gnathobasic filtering setae. Exopodite with six setae.

P5 (Fig. 2g) : The setae between endite and gnathobase are subequally developed.

Postabdomen (Fig. 1d ; Fig. 3d) : Dorsal margin slightly curved with two rows of grouped spinules, the anteriormost consisting of five, the distalmost consisting of four spinules, dorsal margin almost straight distally, anal margin curved. Anal groove concave, postanal zone almost straight. Margin of preanal zone distinctly S-shaped bearing teeth, as well as lateral setae. Dorsal margin of postanal zone with nine groups of anal spinules, the distal one the largest. Pre-anal margin with five groups of denticles ; the distalmost consisting of two stout denticles, the following four consisting of five each. Distal vertical side bearing two groups of setules. Flanks of postabdomen with 13 groups of lateral setae, linearly regressing in length proximally. Each group is composed of five to ten setae.

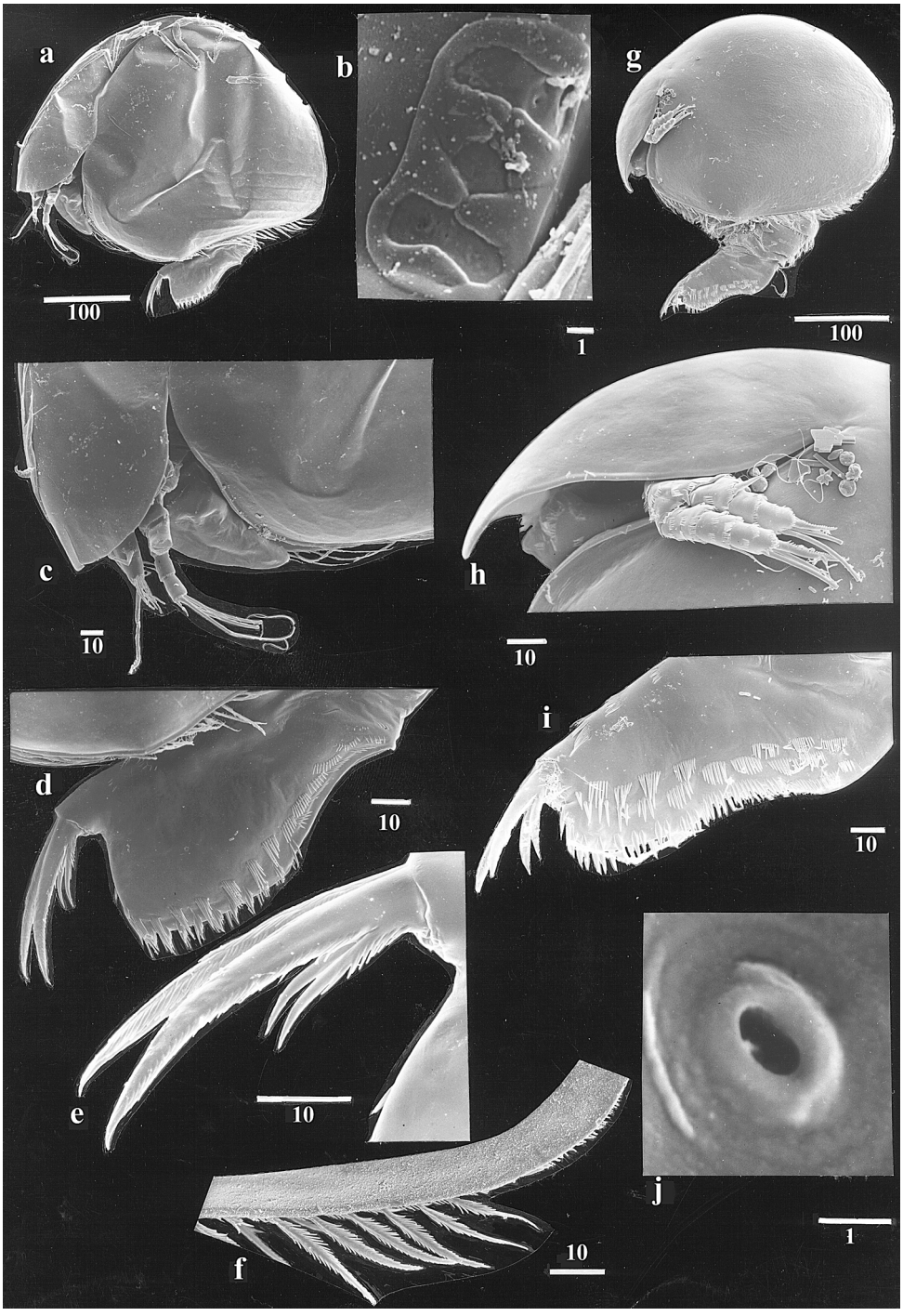
Postabdominal claw (Fig. 1e ; Fig. 3e) rather slender and evenly curved, concave surface carrying two longitudinal rows of spinules. Basal spine half length of claw, spinules bearing eight setules, the distalmost the largest.

Ecology and distribution

Hydrospilus degreefi n. sp. is only known from the type locality. The species was found together with *Alonella exigua* and *Alona eximia*, in a small shady temporary stream, with weak current, and little vegetation.

Legend to Figure 3 (see opposite page)

Fig. 3. – Scanning electron micrographs. – (a-f) *Hydrospilus degreefi* sp. n., female : (a) habitus, (b) headpores, (c) rostrum, (d) postabdomen, (e) postabdominal claw, (f) posteroventral margin, (g-j). – *Bryospilus repens*, female : (g) habitus, (h) rostrum, (i) postabdomen, (j) headpore.


a
100
b
1
g
100
c
10
h
10
d
10
i
10
e
10
f
10
j
1

***Bryospilus culverwelli* nov. spec.** (Fig. 4)

Material examined: One parthenogenetic female, one adult male

Holotype: Parthenogenetic female on a slide, mounted in glycerine, and deposited at the Royal Institute of Natural Sciences, Brussels (KBIN: IG 28511a).

Paratype: Male individual, mounted in glycerine. (KBIN: IG 28511b).

Type locality: Temporary pond, Mundemba, Cameroon (5°04´N-8°49´E, leg. J. Mertens, 16/08/1995, in RUG).

Diagnosis: Postabdomen narrowing distally. Aesthetascs of antennule not reaching apex of rostrum. Antenna with three terminal setae.

Differential diagnosis: *Bryospilus culverwelli* n. sp. is probably a close relative of *B. repens*, but the distal end of the postabdomen is much narrower. The claws have two basal spines instead of one long, slightly-curved basal spine as in the latter species. The three terminal setae of the antenna in *B. culverwelli* are longer than those of *B. repens*. The aesthetascs of the antennule does not reach to the apex of the rostrum in *B. culverwelli*.

Etymology: Named after James Culverwell (Park Adviser of Korup Project at that time), who assisted us during our stay in Cameroon.

Description

Female: Body oval (Fig. 4a). Length 0.33mm, maximal height 0.23mm; ratio 1.4. Maximal height in the middle of the body. Posteroventral and posterodorsal corners of valves not distinct. Ventral margin of valves with continuous row of feathered setae. Labrum rather short and bluntly ending. Ocellus present, but no compound eye. Two median headpores not connected. Antennule merely reaching beyond half length of rostrum. Aesthetascs not reaching apex of rostrum. Antenna with three terminal setae, and one spine. Postabdomen narrowing distally (Fig. 4b). Lateral setules abundant, two rows of stout spines along the ventral margin, followed by three rows of grouped setules. Anal groove carries small spines. Claws with two basal spines, one longer than half length of claw, the other very tiny. Concave surface of claw with spinules.

Male: More elongate than female (Fig. 4c), length 0.30mm, maximal height 0.19mm; ratio 1.6. Antennule relatively longer in female, but still not reaching apex of rostrum. Ventral margin of valves with a row of feathered setae, roughly distinguishable into three groups; anteriormost shortest, middle group longest. Postabdomen narrowing distally (Fig. 4d). Dorsal margin crenulate, carrying anal denticles (much less stout than in female), organised in only one row (*versus* two rows in female), followed by two rows of grouped lateral spinules. At distalmost extremity of postabdomen one large spine. Claws broader than in female; only one basal spine, about half length of claw.

Ecology and distribution

Bryospilus culverwelli n. sp. has not been found outside its type locality. The species was found together with *Acroperus harpae* (Baird, 1834), in clear running water.

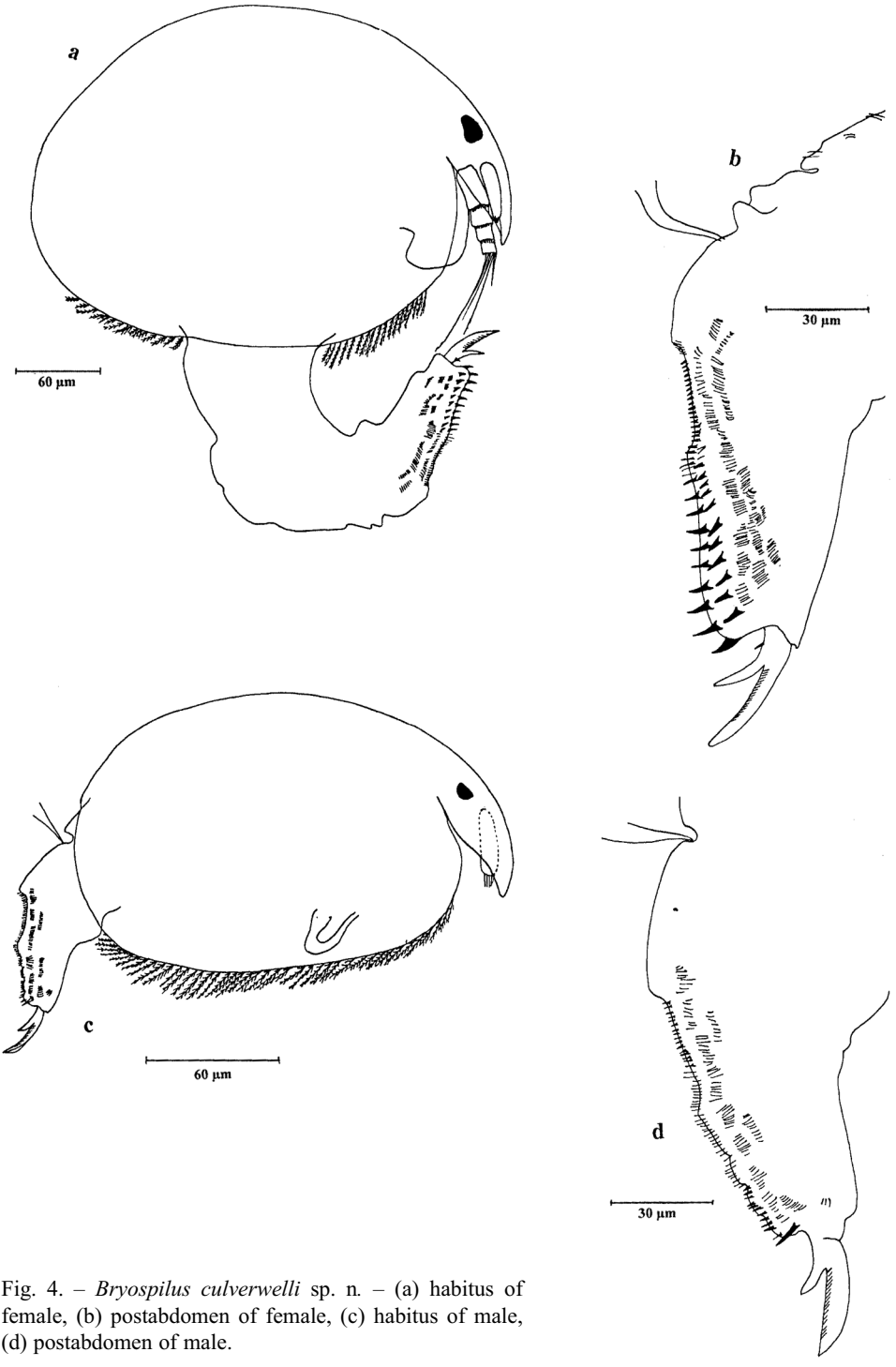


Fig. 4. – *Bryospilus culverwelli* sp. n. – (a) habitus of female, (b) postabdomen of female, (c) habitus of male, (d) postabdomen of male.

Bryospilus repens **(Frey, 1980)** (Figs 3, 5)

Material examined: Five parthenogenetic females

Observed characters are consistent with FREY's (1980) description. Two separated headpores surrounded by an annular thickening, antenna short and stubby, labrum with nodular thickening and small groups of setae, P1 with only two setae on the inner distal lobe, postabdomen of consistent structure and setation.

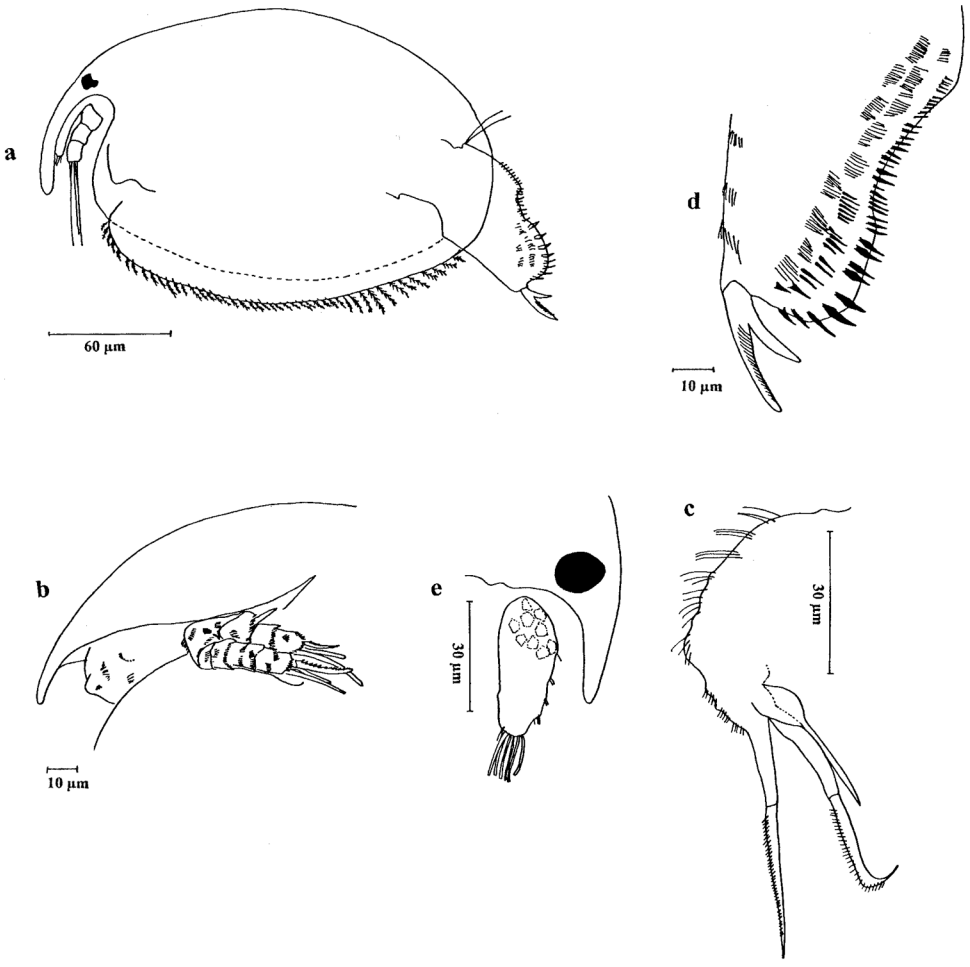


Fig. 5. – *Bryospilus repens* female. – (a) habitus, (b) rostrum, (c) outer ramus of P 1, (d) postabdomen, (e) antennule.

DISCUSSION

The known distribution of *Bryospilus* is now expanded from New Zealand and Neotropical rain forests (Venezuela, Puerto Rico) (FREY, 1980) to the Paleotropics. The habitat of the genus is no longer restricted to the epiphytic damp moss vegetation, as thought by FREY (1980). Together with the representatives of the related new genus *Hydrospilus*, at least two *Bryospilus* species inhabit the waters of the tropical forest floor in Western Africa.

ACKNOWLEDGEMENTS

We are obliged to our guide, Ferdinand Namata, without whom we would never have found our way in the rain forest of Korup. Our sincere gratitude goes to Prof. N. Smirnov who taught the first author how to identify Chydoridae. We also wish to thank Willy De Greef and James Culverwell who provided logistic support, and guidance in the forest. Our thanks also go to the Korup Project for welcoming us, and giving permission to sample the project area. Prof. E. Schockaert is acknowledged for his useful comments on the manuscript.

REFERENCES

FREY, D.G. (1980) – The non-swimming Chydorid Cladocera of Wet forests, with Description of a New Genus and two new species – *Int. Revue ges. Hydrobiol*. **65**, No.5 : 613-641.

ODNRI, (1989) – Republic of Cameroon – The Korup Project. Plan for Developing the Korup National Park and its Support Zone. : 3.11

SEGERS H. & J. MERTENS (1997) – New Rotifera from the Korup National Park, Cameroon. *J. Nat. Hist.,* **31** : 663-668.

Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 339-352 - Brussels 1999

Received : 11 May 1998

CIRCADIEL PATTERNS IN THE TIDAL PLANKTON OF A SANDY BEACH IN ZEEBRUGGE (BELGIUM)

KOEN LOCK, BREGJE BEYST AND JAN MEES
Marine Biology Section, Zoology Institute, University of Gent
Ledeganckstraat 35, 9000 Gent, Belgium
e-mail : Jan.Mees@rug.ac.be

Abstract. The intertidal fauna of a Belgian sandy beach was sampled on an hourly basis with a hand-pushed sledge to study circadiel patterns in species composition. Four assemblages could be distinguished with classification and ordination techniques. A first division separated the samples taken during daytime from the night samples. Both assemblages were subsequently divided into an ebbtide and a floodtide situation. Light intensity and tidal height were the most important variables explaining the variation in the Canonical Correspondence Analysis.

Key words : tidal plankton, circadiel patterns, Belgium.

INTRODUCTION

Since the study by RUSSELL (1925) of the diurnal vertical movements of macro-plankton, there have been several papers reporting vertical movements in intertidal waters. Early plankton collections using tow nets at the edge of sandy beaches showed that certain species of amphipods swim to the surf zone during the night (ELMHIRST, 1932 ; WATKIN, 1939 ; 1941). A distinction can be made between local endobenthic species that actively perform nocturnal vertical migrations, and tidal migrants carried in from sublittoral habitats by the tide. A very detailed study of the so-called tidal plankton was carried out on a sandy beach in Robin Hood's Bay (Great Britain) by COLMAN & SEGROVE (1955). In addition to a distinction between sand-inhabiting species and immigrants, they also found that the zonation of the sand-inhabiting species corresponded very closely to the range occupied by the same species in the sand. However, only a very small proportion of the sand-dwelling animals known to be present in the sand was caught in the water column. The tidal plankton (mainly Amphipoda, Mysidacea and Decapoda) was much more abundant near the bottom of the water column than at the surface. During rough weather, a number of species were carried between the tide marks by the turbulence of the water movements, but good swimmers like mysids avoided the surf zone in such conditions. In addition, FINCHAM (1970) reported semi-lunar and annual fluctuations in amphipod densities. Also, over rocky shores (JANSSON & KÄLLADER, 1968 ; SETRAN, 1992) and seagrass beds (LEDOYER, 1964), a variety of taxa are known to perform vertical migrations into the surf at night.

However, information about structural characteristics of the assemblages of the benthic boundary layer of intertidal areas (species composition, density, biomass, diversity, ...) is

very scarce (MEES & JONES, 1997). To date, no attempt has been made to describe the circadiel patterns of the tidal plankton as a whole. In this study the intertidal fauna was sampled hourly during 25 hours, and subsequently the samples were clustered into assemblages.

MATERIAL AND METHODS

Study area and sampling

The study was performed on a homogenous sandy beach in Zeebrugge (Belgium) where the intertidal area has recently enlarged enormously, due to the expansion of the harbour of Zeebrugge in which a long jetty causes a lot of sedimentation (Fig. 1). The study area was chosen because in Zeebrugge, the tidal plankton reached the highest density of all stations that were sampled along the Belgian coast in the summer of 1995 (unpublished data).

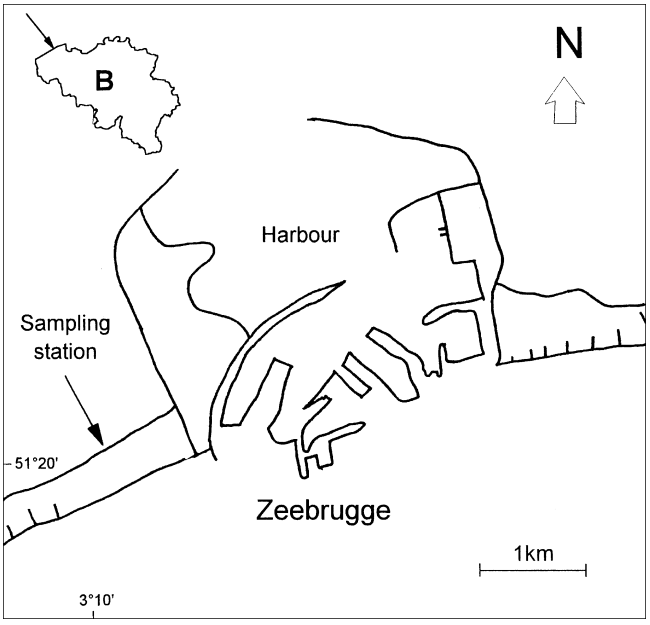


Fig. 1. – Study area, with indication of the sampling site.

On 2 and 3 November 1995, samples were collected with a 50 cm wide, hand-pushed sledge which sampled the lowest 20 cm of the water column. The net had a length of 90 cm and a mesh size of 1 x 1 mm. The total area of the mouth of the net was 0.1 m^2. The samples were immediately rinsed over a 1 mm sieve and preserved in a formalin solution, 7% final concentration. Rose Bengal was added to facilitate sorting of the organisms. On each occasion, the sledge was pushed over a distance of 50 m. Sampling depth ranged from 50 to 100 cm. In order to have an idea of the variability, each hour three successive samples were taken at the same depth. Hourly, the temperature of the sea water and the light inten-

sity were measured, and a watersample was taken for the quantification of nitrate, nitrite, ammonium, phosphate and silicon in the laboratory with the HPLC-method (MANTOURA & LLEWELLYN, 1983). The tidal height at each hour is shown in Fig. 5.

Processing of samples and data analysis

In the laboratory all animals were sorted, identified, when possible to species level, and counted. The amphipod species of the genus *Bathyporeia* and the polychaete species of the genus *Harmothoe* could not be identified to species level. Different developmental stages of decapods were treated as separate ecological species. All individuals of each species were measured (standard length : distance from base of the rostral tip to the end of the last abdominal segment for crustaceans ; from the tip of the nose to the base of the caudal fin for fishes), and their biomass was derived from length - ash-free dry weight regressions (MEES, 1994). All density and biomass data are presented as numbers of individuals (N) and mg ash-free dry weight (AFDW) per 100 m^2 ; these values correspond to a volume of 20 m^3 water filtered through the net.

Species that were caught less than 10 times during the whole sampling period were excluded from classifications and ordinations because these species are so rare they do not show a clear pattern and they do not have any influence on the analysis (DAY *et al.*, 1971). The density differences between day and night or between high and low water were tested for the dominant species with a Kruskal-Wallis ANOVA.

Diversity was calculated as Hill's diversity numbers (HILL, 1973). This set of indices incorporates the most widely used diversity measures in a continuum of indices of the orders $-\infty$ to $+\infty$. The indices differ in their tendency to include or to ignore the relatively rarer species : the impact of dominance increases and the influence of species richness decreases with an increasing order of the diversity number. Of particular interest are :

$N_0 = S$ with S = the number of species

$N_1 = e^H$ with H = Shannon-Wiener index
$H = -\sum p_i \ln(p_i)$ (p_i = the relative abundance of the i^{th} dominant species)

$N_2 = SI^{-1}$ with SI = Simpson's dominance index
$SI = \sum p_i^2$

$N_\infty = p_1^{-1}$ with p_1 = the relative abundance of the most abundant species.

To quantify the similarity between the three replicates taken per hour on the one hand, and the similarity between the samples of the subsequent hours on the other hand, a group-average sorting cluster analysis with Bray-Curtis similarities (BRAY & CURTIS, 1957) was performed on the fourth root transformed density and biomass data.

The samples were classified into clusters according to species composition, using the classification program TWINSPAN (Two-Way INdicator SPecies ANalysis) (HILL, 1979). TWINSPAN also yields indicator species characterising the various assemblages. The cut levels used in the analysis were 0, 1, 2, 5, 20 and 40 for the density data and 0, 0.1, 0.3, 0.9, 5 and 40 mg for the biomass data. These cut levels correspond with the densities and the biomass of the data pooled per hour, which are measures of the amount of animals per 75 m^2. To check the stability of the TWINSPAN results, the Canonical Correspondence Analysis

(CCA) option from the program package CANOCO (TER BRAAK, 1988) was applied on the fourth root transformed data. A Correspondence Analysis (CA) was applied on the same data in order to see if the parameters used in the CCA explained the variation in the data.

RESULTS

Exploration of the data matrix

A total of 44 ecological species was recorded (Table 1). These included species generally referred to as mesozooplankton, macrozooplankton, macrobenthos or hyperbenthos. The dominant taxa were Mysidacea (6 species, 38 % of the total number of individuals caught) and Cnidaria (1 species, 25 %). Isopoda (2 species, 9.6 %), Decapoda (7 species, 8.9 %), Cumacea (1 species, 7.8 %), Copepoda (4 species, 4.3 %) and Amphipoda (12 species, 3 %) were also common. Fishes (4 species, 1.5 %), Chaetognatha (1 species, 1.4 %), Polychaeta (5 species, 0.5 %) and Ctenophora (1 species, 0.4 %) were rare.

The temporal variation in the densities of the most abundant species can be related to the waterlevel (Fig. 5) and the light regime. Night fell at 19 h and lasted until 9 h the next morning. Dusk and dawn only lasted for one hour before and after the night respectively.

Planktonic species like *Mitrocomella polydiademata* (Cnidaria) (Fig. 2a) and *Pleurobrachia pileus* (Ctenophora) showed no clear pattern of occurrence. Despite the fact that calanoid copepods cannot be sampled efficiently with a 1 mm mesh net, *Calanus helgolandicus* (Fig. 2b), *Centropages typicus* and *Temora longicornis* were caught regularly. They all reached highest densities during the night, but only for *C. helgolandicus* was this difference significant (Kruskal-Wallis ANOVA, $p < 0.05$).

The cumacean *Cumopsis goodsiri* (Fig. 2c) was most abundant during ebbtide ($p < 0.001$); the species was, however, most abundant in the midlitoral. The mysid *Mesopodopsis slabberi* (Fig. 2d) was mainly found during the day ($p < 0.001$). It was by far the most abundant species (densities upto 1333 individuals per 100 m^2). Another mysid species, *Neomysis integer* (Fig. 2e) was mainly found during the night ($p < 0.01$) and during high water ($p < 0.05$). The isopod *Eurydice pulchra* (Fig. 2f) was predominantly found during the night ($p < 0.001$) and at floodtide ($p < 0.001$). The amphipods were the most diverse group during the sampling period : 12 species were recorded. Most of them were, however, very rare. Only *Gammarus crinicornis* once reached a density of more than 10 animals per 100 m^2. This species was most abundant during the night ($p < 0.05$). Adult *Crangon crangon* (Fig. 2g), as well as its postlarva, were most abundant during the night (both $p < 0.01$). This shrimp was the most important species in terms of biomass, with a maximum of 2067 mg AFDW per 100 m^2. *Pomatoschistus microps* (Fig. 2h) was the only fish that was caught regularly. It was most common during the night ($p < 0.05$).

TABLE 1

List of species with indication of the used abbreviations and the classification

Name and stage	Abbreviation	Classification
Cnidaria		
Mitrocomella polydiademata (Romanes, 1876)	Mitr poly	Macrozooplankton
Ctenophora		
Pleurobrachia pileus (Müller, 1776)	Pleu pile	Macrozooplankton
Polychaeta		
Harmothoe species	Harm Spec	Hyperbenthos
Lanice conchilega (Pallas, 1766)	Lani conc	Macrobenthos
Lanice conchilega (Pallas, 1766) (aulophorelarva)	Lani Aulo	Macrozooplankton
Scolelepis squamata (Müller, 1789)	Scol squa	Macrobenthos
Spio filicornis (Müller, 1766)	Spio fili	Macrobenthos
Crustacea		
Copepoda		
Calanus helgolandicus (Claus, 1863)	Cala helg	Mesozooplankton
Centropages typicus (Kröyer, 1849)	Centr typi	Mesozooplankton
Labidocera wollastoni (Lubbock, 1857)	Labi woll	Mesozooplankton
Temora longicornis (Müller, 1792)	Temo long	Mesozooplankton
Cumacea		
Cumopsis goodsiri (van Beneden, 1851)	Cumo good	Hyperbenthos
Mysidacea		
Gastrosaccus spinifer (Goës, 1864)	Gastr spin	Hyperbenthos
Mesopodopsis slabberi (van Beneden, 1861)	Meso slab	Hyperbenthos
Neomysis integer (Leach, 1814)	Neom inte	Hyperbenthos
Praunus flexuosus (Müller, 1776)	Prau flex	Hyperbenthos
Schistomysis kervillei (Sars, 1885)	Schi kerv	Hyperbenthos
Schistomysis spiritus (Norman, 1860)	Schi spir	Hyperbenthos
Isopoda		
Eurydice pulchra (Leach, 1815)	Eury pulc	Hyperbenthos
Idotea pelagica (Leach, 1815)	Idot pela	Hyperbenthos
Amphipoda		
Bathyporeia species	Bath Spec	Hyperbenthos
Corophium acherusicum (Costa, 1851)	Coro ache	Hyperbenthos
Corophium insidiosum (Crawford, 1937)	Coro insi	Hyperbenthos
Gammarus crinicornis (Stock, 1966)	Gamm crin	Hyperbenthos
Haustorius arenarius (Slabber, 1769)	Haus aren	Hyperbenthos
Jassa marmorata (Holmes, 1903)	Jass marm	Hyperbenthos
Microprotopus maculatus (Norman, 1867)	Micr macu	Hyperbenthos
Pariambus typicus (Kröyer, 1845)	Pari typi	Hyperbenthos
Perioculodes longimanus (Bate & Westwood, 1868)	Peri long	Hyperbenthos
Pontocrates altamarinus (Bate & Westwood, 1862)	Pont alta	Hyperbenthos
Pontocrates arenarius (Bate, 1858)	Pont aren	Hyperbenthos
Stenothoe marina (Bate, 1856)	Sten mari	Hyperbenthos
Decapoda		
Carcinus maenas (L., 1758) (zoea)	Carc Zoea	Mesozooplankton
Carcinus maenas (L., 1758) (megalopa)	Carc Mega	Mesozooplankton
Crangon crangon (L., 1758)	Cran cran	Hyperbenthos
Crangon crangon (L., 1758) (postlarva)	Cran Post	Hyperbenthos
Diogenes pugilator (Roux, 1828) (megalopa)	Diog Mega	Mesozooplankton
Portumnus latipes (Pennant, 1777)	Port lati	Hyperbenthos
Portumnus latipes (Pennant, 1777) (megalopa)	Port Mega	Mesozooplankton
Chaetognatha		
Sagitta setosa (Müller, 1847)	Sagi seto	Mesozooplankton
Pisces		
Ammodytes tobianus (L., 1758)	Ammo tobi	Hyperbenthos
Chelon labrosus (Risso, 1962)	Chel labr	Hyperbenthos
Pleuronectes platessa (L., 1758)	Pleu plat	Hyperbenthos
Pomatoschistus microps (Kröyer, 1838)	Poma micr	Hyperbenthos

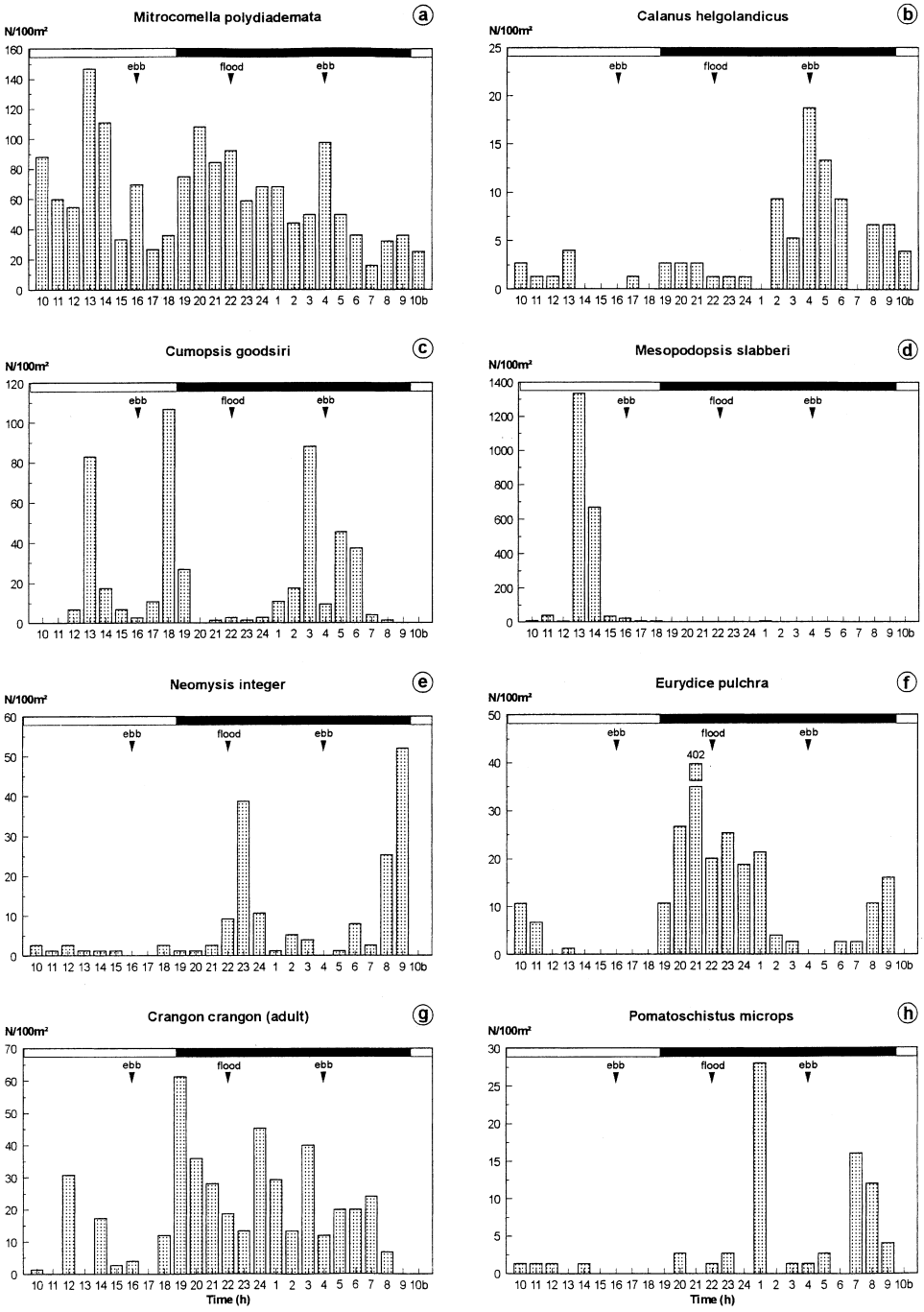


Fig. 2. – The abundance of some intertidal animals during the course of the cycle.

Analysis of the temporal patterns

The cluster analyses with the density data, as well as with the biomass data, separated the day samples from the night samples. The samples that were taken at a similar tidal height were often clustered together. However, the similarity between the three successive samples taken each hour was not much greater than the similarity between samples taken at approximately the same tidal height. Therefore the three replicates per hour were pooled for TWINSPAN and for the ordinations.

The result of the TWINSPAN of the density data is presented in Fig. 3. In the first division, the samples taken during daytime were separated from the samples taken at night. Indicator species for the night samples were *Calanus helgolandicus* (cut level 2), *Crangon crangon* (cut level 3), *Pomatoschistus microps* (cut level 2), *Eurydice pulchra* (cut level 2) and *Gammarus crinicornis* (cut level 2). The indicator species for the day samples was *Mesopodopsis slabberi* (cutlevel 2). Both the clusters of day and night were subsequently divided into groups of flood- and ebbtide samples. At night, the indicator species for the flood cluster was *Eurydice pulchra* (cut level 3), while *Cumopsis goodsiri* (cut level 3) was the indicator species for the ebb cluster. Also during the day, the indicator species for the ebb cluster was *C. goodsiri* (cut level 1). The analysis with the biomass data yielded the same four clusters (not figured).

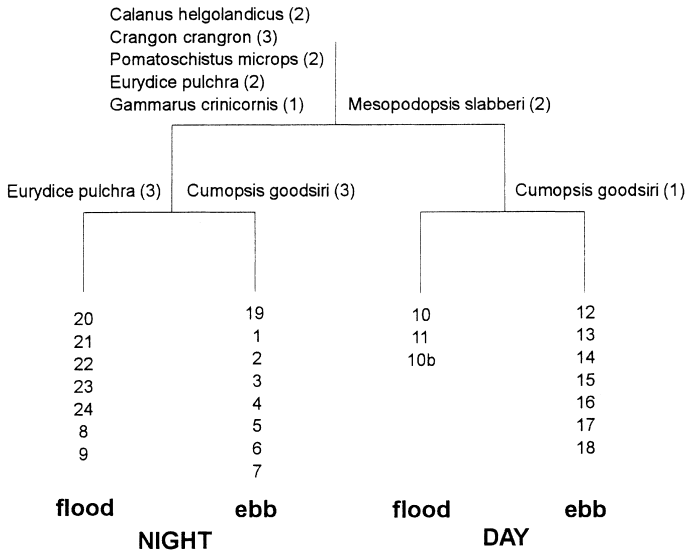


Fig. 3. – TWINSPAN with the density data. Indicator species with pseudo-species cut level are given for each division.

The direct gradient analysis of the fourth root transformed density data was in general agreement with the classification : the four clusters identified by TWINSPAN could also be identified in the ordination plane formed by the first (eigenvalue 0.12) and the second (eigenvalue 0.06) axes (Fig. 4, right). The eigenvalues for the third axis were much lower (0.03), and yielded no additional information. Along the first and most important axis, the

day and night samples were spatially segregated. Along the second axis, the samples taken at low and at high water were segregated. The tidal height and the light intensity were the largest vectors, which explained most of the variation in the ordination plane. The other parameters explained only a little of the variation between the different samples.

For the indirect gradient analysis of the density data, the first axis (eigenvalue 0.15) was plotted against the third axis (eigenvalue 0.07) (Fig. 4). When the first axis was plotted against the second axis (eigenvalue 0.08), the samples from low and high water were not clearly separated from each other. Just as for the direct gradient analysis, the

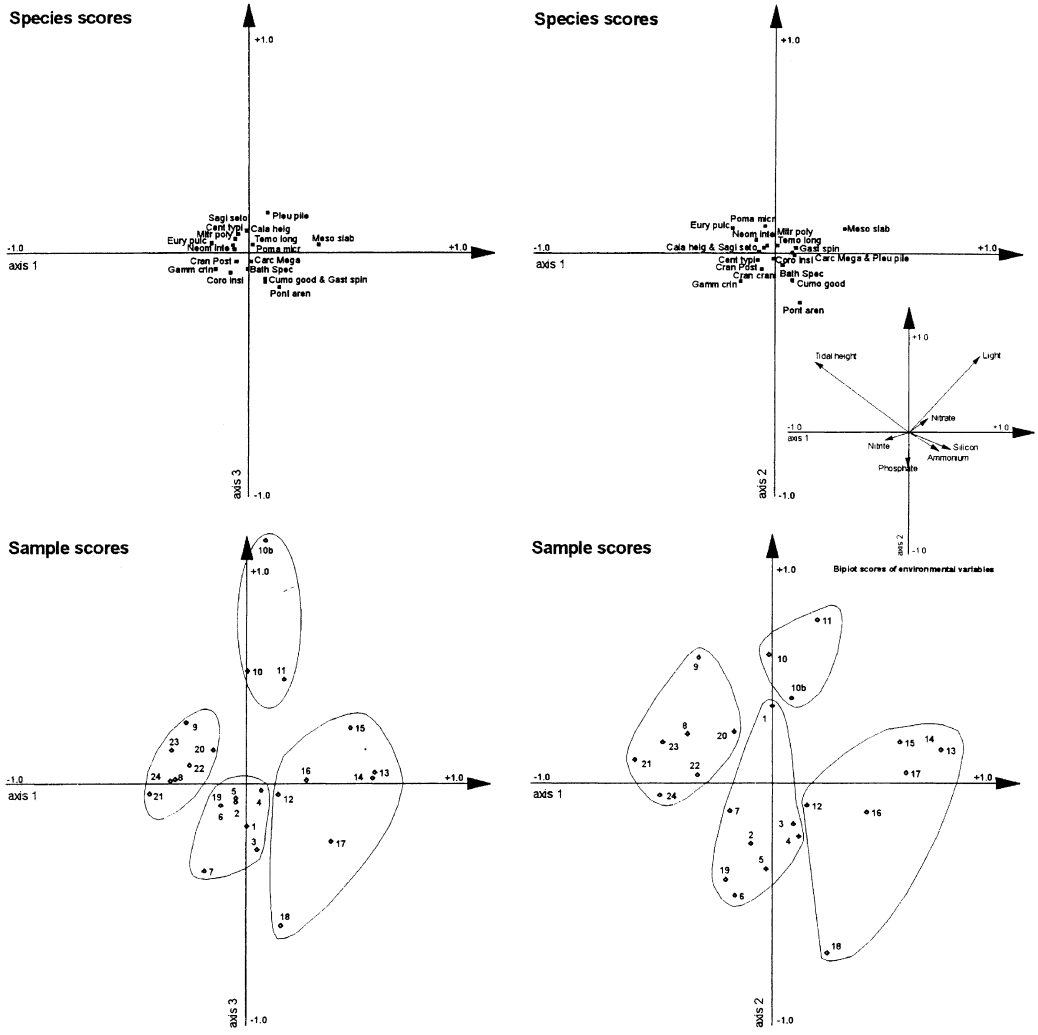


Fig. 4. – Correspondence Analysis (left) and Canonical Correspondence Analysis (right) of the fourth root transformed density data.

TWINSPAN clusters were clearly separated in the ordination plane formed by the first and the third axis (Fig. 4). The similarity between the sample scores from the direct and the indirect gradient analysis indicates that the most important environmental parameters were measured. The light intensity and the tidal height were indeed shown to be the most important parameters in the explanation of the circadiel patterns of the intertidal fauna.

The direct and the indirect gradient analysis of the biomass data gave the same pattern after removing the outliers *Pomatoschistus microps* and *Gammarus crinicornis* (not presented).

As can be seen in Fig. 5, the identified assemblages were separated very well into a day versus a night situation. The same held true for the differentiation between the floodtide and the ebbtide situation: the edge between those assemblages was found to be at a tidal height of approximately 3 m above ELWS.

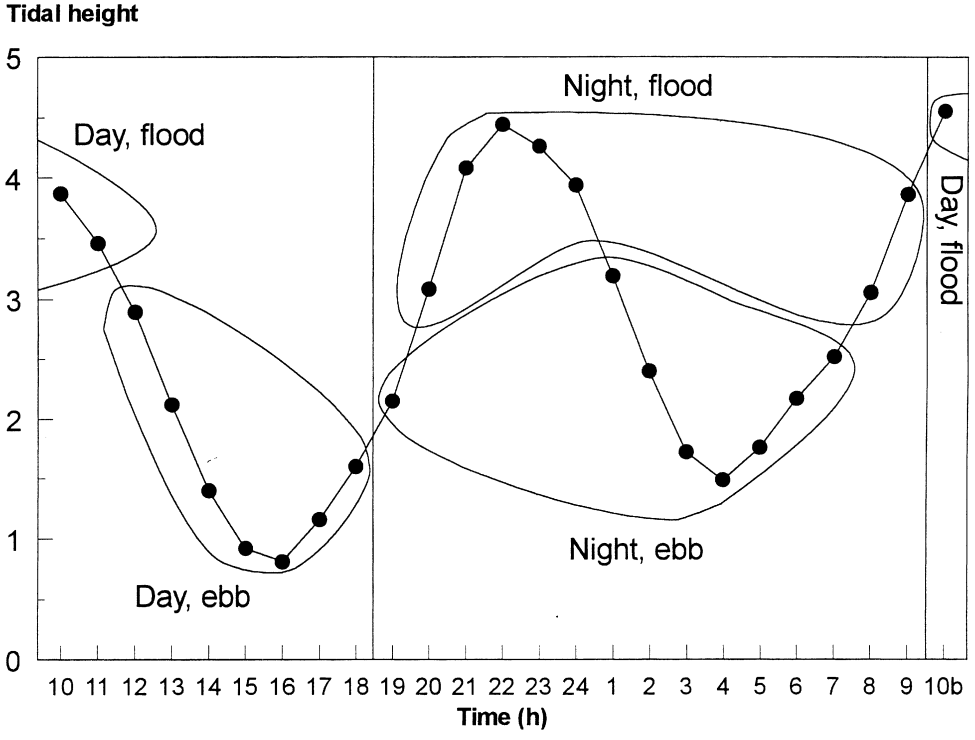


Fig. 5. – The waterlevel per hour with indication of the distinguished assemblages.

Characterisation of the assemblages

The average abundance and biomass of the different assemblages, as identified by TWINSPAN and confirmed by the ordination, are shown in Fig. 6. In the pie charts, the faunistic composition of each cluster is shown.

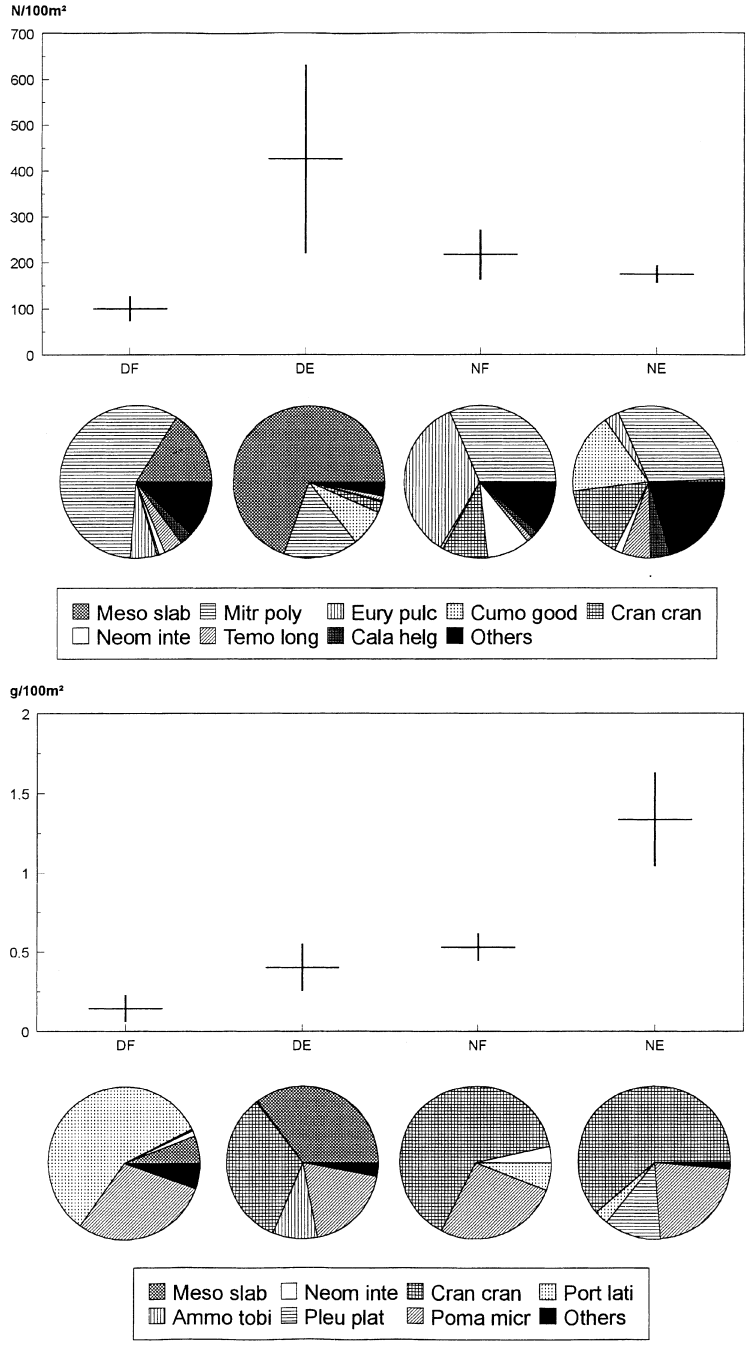


Fig. 6. – Average density and biomass of the four assemblages, as identified by the multivariate analyses with indication of the standard error. The species composition of each assemblage is shown in the pie charts below each graph.

During the day flood tide, the fauna was dominated by *Mitrocomella polydiademata*, *Mesopodopsis slabberi* and, to a lesser extent, *Eurydice pulchra*. Biomass on the other hand, was mainly made up of *Portumnus latipes*, *Pomatoschistus microps* and *Mesopodopsis slabberi*. The mean density, as well as the biomass, was very low in comparison with the other assemblages.

The ebbtide situation of the day was characterised by very high densities of *Mesopodopsis slabberi*, but also of *Mitrocomella polydiademata* and *Cumopsis goodsiri*. *Mesopodopsis slabberi*, *Crangon crangon*, *Pomatoschistus microps* and *Ammodytes tobianus* were most important in terms of biomass.

At night and flood tide, *Eurydice pulchra*, *Mitrocomella polydiademata*, *Cumopsis goodsiri* and *Neomysis integer* were the most important taxa, while the biomass was made up almost exclusively of *Crangon crangon* and *Pomatoschistus microps*.

The ebbtide situation of the night was most diverse, and *Mitrocomella polydiademata*, *Crangon crangon*, *Cumopsis goodsiri*, *Temora longicornis* and *Calanus helgolandicus* all contributed considerably to the density. The biomass of this assemblage was much higher than that of the other assemblages. This was mainly because of *Crangon crangon*, *Pomatoschistus microps* and *Pleuronectes platessa*.

The average Hill's diversity numbers per assemblage are presented in Table 2. For all diversity indices, the night samples had higher values than the day samples. Within those night samples, the diversity was higher during ebbtide.

TABLE 2

Mean Hill's diversity numbers of the four assemblages, as identified by the multivariate analysis, with indication of the standard error

	DF	DE	NF	NE
N_0	11,67 (2,72)	11,29 (0,80)	14,71 (0,72)	17,38 (1,33)
N_1	3,93 (0,49)	3,68 (0,46)	5,68 (0,61)	7,91 (0,49)
N_2	2,49 (0,29)	2,50 (0,28)	3,86 (0,48)	5,30 (0,40)
N_∞	1,73 (0,18)	1,76 (0,16)	2,38 (0,25)	3,12 (0,27)

DISCUSSION

This is a first attempt to quantify the tidal plankton of a sandy beach in the Southern Bight of the North Sea. We concentrated on the macro-component of the zooplankton (animals larger than 1 mm). A community approach, though often used in studies of the subtidal hyperbenthos (*e.g.* MEES, 1994) has not yet been applied to intertidal assemblages. In this pilot study, a high number of species has been found to utilise the beach habitat at specific times of the tidal cycle or day. The fauna mainly consisted of fast swimming species like mysids. These typically live subtidally in the benthic boundary layer or hyperbenthal (MAUCHLINE, 1980; MEES & JONES, 1997; ZOUHIRI *et al.*, 1998), and their densities are

notoriously difficult to estimate. Many species are known to form aggregations (MAUCHLINE, 1980) and, especially in intertidal areas, it is difficult to distinguish between increases in population numbers resulting from immigration and those resulting from a disaggregated population aggregating or swarming in a small area. We tried to avoid this problem by taking three replicates per hour. Also, the biomass data presented in this study should be interpreted with care, since incidentially caught large epibenthic animals (mainly adult crabs and fish) sometimes contributed significantly to the total biomass. The present study covers only one situation and does not include seasonal or semi-lunar patterns. Further research is needed to determine and to understand the impact of this and other factors on the tidal plankton communities.

Despite the extreme conditions in the intertidal, a lot of species occur between the tidemarks. Decreased predation pressure and optimal feeding conditions are the most obvious factors. In this study *Eurydice pulchra* (JONES & NAYLOR, 1967) and *Cumopsis goodsiri* (JONES, 1976) are good examples of sand-dwelling species that clearly have a restricted range in the intertidal, and are only rarely found in subtidal areas (HAMERLYNCK & MEES, 1991 ; MEES & HAMERLYNCK, 1992 ; CATTRIJSSE *et al.*, 1993). Some other species are typically estuarine, like *e.g. Neomysis integer* (TATTERSALL & TATTERSALL, 1951) or *Pomatoschistus microps* (NIJSSEN & DE GROOT, 1987 ; ELLIOTT & DEWAILLY, 1995). Both species are euryhaline, with a wide range of salinity tolerance and considerable powers of adaptation to the changing salinity of the waters they frequent. MAUCHLINE (1971) has often found *N. integer* in the intertidal, whereas according to NIJSSEN & De GROOT (1987), *P. microps* is common and abundant in the shallow gullies (0.2-2 m) of the Wadden Sea.

The intertidal fauna clearly showed circadiel and tidal distribution patterns. Each situation (day-flood, day-ebb, night-flood, night-ebb) was characterised by a different faunal assemblage. Moreover, the floodtide situation during the day was characterised by both the lowest densities and biomasses in comparison with the other periods, whereas the ebbtide situation during the night was the most diverse. Circatidal activity rhythms of planktonic organisms in the intertidal area have hardly been studied as such. Most studies only cover rocky shores (*e.g.* JANSSON & KÄLLANDER, 1968 ; SAWARA, 1992), or concentrate on only one taxonomic group (*e.g.* COLMAN & SEGROVE, 1955 : amphipods ; TAKAHASHI & KAWAGUCHI, 1997 : three mysid species), and mostly they discuss only sand-burrowing species (*e.g.* COLMAN & SEGROVE ; DE RUYCK *et al.*, 1991 ; TAKAHASHI & KAWAGUCHI, 1997). However, the activity rhythms of most species were in general agreement with the available literature. *Eurydice pulchra* for example, emerges from the sand as the tide comes in and re-buries after high tide (a.o. ALHEIT & NAYLOR, 1976). *Neomysis integer* is known to avoid light in seawater (TATTERSALL & TATTERSALL, 1951). MAUCHLINE (1971) often found the species in the intertidal at high water. Also in this study, *N. integer* was mainly found during the night during high water.

ACKNOWLEDGEMENTS

We would like to thank Leen Durwael for her help during sampling, Dirk Van Gansbeke for analysing the water samples, and two anonymous referees whose comments and suggestions greatly

improved the manuscript. Research supported by the University of Gent (contract BOF 98-03, 12050398).

REFERENCES

ALHEIT, J. & E. NAYLOR (1976) – Behavioural basis of intertidal zonation in *Eurydice pulchra* Leach. *J. Exp. Mar. Biol. Ecol.*, **23** : 135-144.

BRAY, J.R. & J.T. CURTIS (1957) – An ordination of the upland forest communites of southern Wisconsin. *Ecol. Monogr.*, **27** : 325-349.

CATTRIJSSE, A., J. MEES & O. HAMERLYNCK (1993) – The hyperbenthic Amphipoda and Isopoda of the Voordelta and the Westerschelde estuary. *Cah. Biol. Mar.*, **34** : 187-200.

COLMAN, J.S. & F. SEGROVE (1955) – The tidal plankton over stoupe beck sands, Robin Hood's Bay (Yorkshire, North Riding). *J. Anim. Ecol.*, **24** : 445-462.

DAY, J.H., J.G. FIELD & M.P. MONTGOMERY (1971) – The use of numerical methods to determine the distribution of the benthic fauna across the continental shelf of North Carolina. *J. Anim. Ecol.*, **40** : 93-126.

DE RUYCK, A.M.C., T.E. DONN Jr. & A. MCLACHLAN (1992) – Distribution of three intertidal cirolanid isopods (Flabellifera : Cirolanidae) on a South African sandy beach. *Cah. Biol. Mar.*, **33** : 147-168.

ELLIOTT, M. & F. DEWAILLY (1995) – The structure and components of European estuarine fish assemblages. *Neth. J. Aquat. Ecol.*, **39** : 397-417.

ELMHIRST, R. (1932) – Quantitative studies between tide-marks. *Glasgow Nat.*, **10** : 56-62.

FINCHAM, A.A. (1970) – Amphipods in the surf plankton. *J. mar. biol. Ass. U.K.*, **50** : 177-198.

HAMERLYNCK, O. & J. MEES (1991) – Temporal and spatial structure in the hyperbenthic community of a shallow coastal area and its relation to environmental variables. *Oceanologica Acta*, **11** : 205-212.

HILL, M.O. (1973) – Diversity and eveness : a unifying notation and its consequences. *Ecology*, **54** : 427-432.

HILL, M.O. (1979) – A FORTRAN program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Ecology and systematics. Cornell University Ithaca, New York (66 pp).

JANSSON, B.O. & C. KÄLLANDER (1986) – On the diurnal activity of some littoral peracarid crustaceans in the Baltic Sea. *J. exp. mar. Biol. Ecol.*, **2** : 24-36.

JONES, N.S. (1976) – British Cumaceans (Arthropoda : Crustacea), Identification of the Species. Synopsis of the British Fauna (New Series) 7, The Linnean Society of London, Academic Press, London and New York (66 pp).

JONES, D.A. & E. NAYLOR (1967) – The distribution of *Eurydice* (Crustacea : Isopoda) in Bristish waters, including E. Affinis new to Britain. *J. Mar. Biol. Ass. UK*, **47** : 373-382.

LEDOYER, M. (1964) – Les migrations nycthémérales de la faune vagile aux sein des herbiers de *Zostera marina* de la zone intertidale en Manche et comparaison avec les migrations en Méditerranée. *Rec. Trav. St. Mar. End. Bull.*, **34** : 241-247.

MANTOURA, R.F.C & C.A. LLEWELLYN (1983) – The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. A*nalytica Chemica Acta*, **151** : 297-314.

MAUCHLINE, J. (1971) – Seasonal occurrence of mysids (Crustacea) and evidence of social behaviour. *J. mar. biol. Ass. U.K.*, **51** : 809-825.

MEES, J. (1994) – *The hyperbenthos of shallow coastal waters and estuaries : community structure and biology of the dominant species*. PhD Thesis, University of Gent (212 pp).

MEES, J. & O. HAMERLYNCK (1992). Spatial community structure of the winter hyperbenthos of the Schelde estuary, the Netherlands, and the adjacent coastal waters. *Neth. J. Sea Res.*, **29** : 357-370.

MEES, J. & M.B. JONES (1997) – The hyperbenthos. *Oceanogr. Mar. Biol. Annu. Rev.*, **35** : 221-255.

NAYLOR, E. (1972) – British marine isopods. Synopsis of the British Fauna (New Series) 3, The Linnean Society of London, Academic Press, London and New York (86 pp).

NIJSSEN, H. & S.J. DE GROOT (1987) – De vissen van Nederland. Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging, Utrecht (224 pp).

RUSSELL, F.S. (1925) – The vertical distribution of marine macroplankton. I. An observation on diurnal changes. *J. mar. biol. Ass. U.K.*, **13** : 769-809.

SAWARA, Y. (1992) – Differences in activity rhythms of juvenile gobiid fish, *Chasmichthys gulosus*, from different tidal localities. *Jap. J. Ichthyol.*, **39** : 201-209.

SETRAN, A.C. (1992) – A new plankton trap for use in the collection of rocky intertidal zooplankton. *Limnol. Oceanogr.*, **37** : 669-674.

TAKAHASHI, K. & K. KAWAGUCHI (1997) – Diel and tidal migrations of the sand-burrowing mysids, *Archaeomysis kokuboi*, *A. Japonica* and *Iiella ohshimai*, in Otsuchi Bay, northeastern Japan. *Mar. Ecol. Prog. Ser.*, **148** : 95-107.

TATTERSALL, W.M. & TATTERSALL, O.S. (1951) – *The British Mysidacea*. Ray Society, London (460 pp).

TER BRAAK, C.J.F. (1988) – CANOCO – a FORTRAN program for canonical community ordination by (partial) (detrended) (canonical) correspondence analysis, principal components analysis and redundancy analysis (version 2.1). Agricultural Mat. Group, Ministry of Agriculture and Fisheries (Neth.), Wageningen (95 pp).

WATKIN, E.E. (1939) – The pelagic phase in the life-history of the amphipod genus *Bathyporeia*. *J. mar. biol. Ass. U.K.*, **23** : 467-481.

WATKIN, E.E. (1941) – Observations on the night tidal migrant crustacea of Kames Bay. *J.mar. biol. Ass. U.K.*, **25** : 81-96.

ZOUHIRI, S., C. VALLET, P. MOUNY & J.-C. DAUVIN (1998) – Spatial distribution and biological rhythms of suprabenthic mysids from the English Channel. *J. Mar. Biol. Ass. U.K.*, **78** : 1181-1202.

Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 353-362 - Brussels 1999

Received : 16 September 1998

CHARACTERIZATION OF ACETYLCHOLINE-INDUCED LUMINESCENCE IN *AMPHIPHOLIS SQUAMATA* (ECHINODERMATA : OPHIUROIDEA)

NANCY DE BREMAEKER, FERNAND BAGUET AND JEROME MALLEFET

Laboratory of Animal Physiology, Université catholique de Louvain, Place Croix du Sud 5, B-1348 Louvain-la-Neuve, Belgium
e-mail :debremaeker@bani.ucl.ac.be

Abstract. *Amphipholis squamata* is a luminescent polychromatic ophiuroid species. The population from Normandy (France) exhibits six different coloration patterns (orange, beige, dark brown, grey, spotted and black). Each variety of ophiuroid exhibits a different pattern and intensity of luminescence as induced by acetylcholine. The luminescence of dark brown and of black specimens was investigated over six months. A significantly higher intensity of luminescence was observed in February for both varieties.

Key words : *Amphipholis squamata*, echinoderm, ophiuroid, luminescence, acetylcholine.

INTRODUCTION

For almost two centuries (VIVIANI, 1805 ; HARVEY, 1952) it has been known that echinoderms are able to produce light. There are many luminous echinoderms, but only in ophiuroids have *in situ* observations been possible and rapid flashing reported. Most studies were conducted on two species, *Ophiopsila californica* (Clark) and *O. riisei* (Lütken). The most common explanation for the role of bioluminescence in ophiuroids is defence against potential predators. The light is used either to stun by intense flashes (BASCH, 1988), to warn by aposematism (GROBER, 1988a,b), or to lure by leaving behind one or more autotomized luminescent arms (GOTTO, 1963).

Amphipholis squamata (Delle Chiaje, 1828) is a small cosmopolitan viviparous ophiuroid, which was first described as luminous in 1805 by Viviani. This species is polychromatic ; six different coloration patterns have been described (BINAUX & BOCQUET, 1971). A recent study has shown these varieties to exhibit different luminescent capabilities (DEHEYN *et al*., 1997) and seasonal variations (DEHEYN, 1998) in response to KCl stimulation. Given that the luminescence in *A. squamata* is under cholinergic nervous control (DE BREMAEKER *et al.* 1993 ; 1996), the aim of this study was to investigate the acetylcholine-induced luminescence in the different colour varieties, and the variation of this light production over six months for two of these varieties.

MATERIAL AND METHODS

Specimens of *A. squamata* were collected intertidally at Langrune-sur-Mer (Normandy, France). Two sets of experiments were conducted. First, experiments were designed to investigate light emission according to coloration pattern ; specimens were collected in June 1997. Next, experiments were performed to study the luminescence variation over a six month period ; specimens were collected from October 1996 to May 1997. A recent study (DEHEYN *et al.*, 1997) showed that brooding and non-brooding ophiuroids exhibited a difference in intensity of light production, hence only brooding adult specimens (disc diameter > 1.6 mm ; EMSON & WHITFIELD, 1989) were used for our experiments.

Specimens were maintained for one day in open-circuit marine aquaria at the marine station in Luc-sur-Mer, and then transferred to our laboratory in Louvain-la-Neuve, Belgium. Luminescence measurements were investigated no more than two days following the transfer, to ensure the luminescence performance was not affected (DUBUISSON, unpubl.). During these two days, specimens were kept in a closed-circuit marine aquarium under field conditions of temperature and salinity.

The arms are the only luminescent parts of *A. squamata* (BREHM & MORIN, 1977). After anaesthetization of the ophiuroid by immersion in artificial sea water (ASW) containing 3.5 % w/w $MgCl_2$, arms were removed and placed in small chambers containing 200 µl ASW with the following composition (in mM) : 400.4 NaCl ; 9.6 KCl ; 52.3 $MgCl_2$; 9.9 $CaCl_2$; 27.7 Na_2SO_4 ; 20 Tris HCl ; pH 8.3. The arms were stimulated by application of exogenous acetylcholine (Ach) (Sigma Chemical Co.) at a final concentration of 10^{-3}M.

Light emission was monitored with either a PM 270 D photomultiplier connected via an amplifier IL500 (International Light, USA) to a chart recorder (Servogor S, Germany), or a 1250 Bioorbit luminometer (Bioorbit, Finland) connected to a computer.

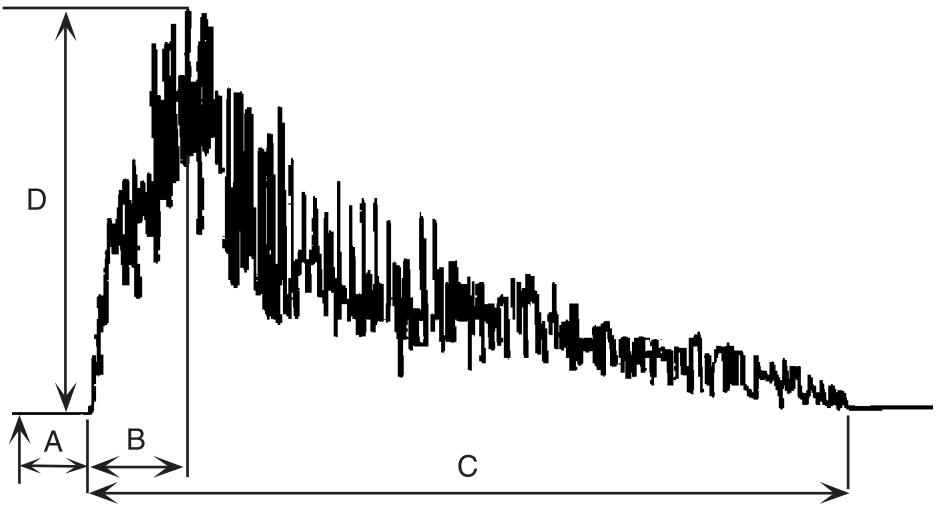


Fig. 1. – *Amphipholis squamata*: parameters of ACh-induced luminescence. – (A) TL: the latency time expressed in seconds; (B) TLmax: the time to reach the maximal light intensity in seconds; (C) Tresp: the total response time in seconds; (D) Lmax: the maximal light intensity in Mq/s/mm.

The parameters used to characterize the luminescence were (Fig. 1): (i) the latency time (TL) expressed in seconds, (ii) the time expressed in seconds to reach the maximal light intensity (TLmax), (iii) the total response time in seconds (Tresp) and (iv) the maximal light intensity (Lmax) expressed in Megaquanta/sec per millimetre of arm length (Mq/s/mm). Statistical analyses were performed using analysis of variance (anova); each mean value is expressed with its standard error of mean (mean ± s.e.) and number of preparations (n).

RESULTS

Light emission according to coloration pattern

The pattern (Fig. 2) and the parameters (Table 1) of luminescence of ophiuroids varied from one variety to another.

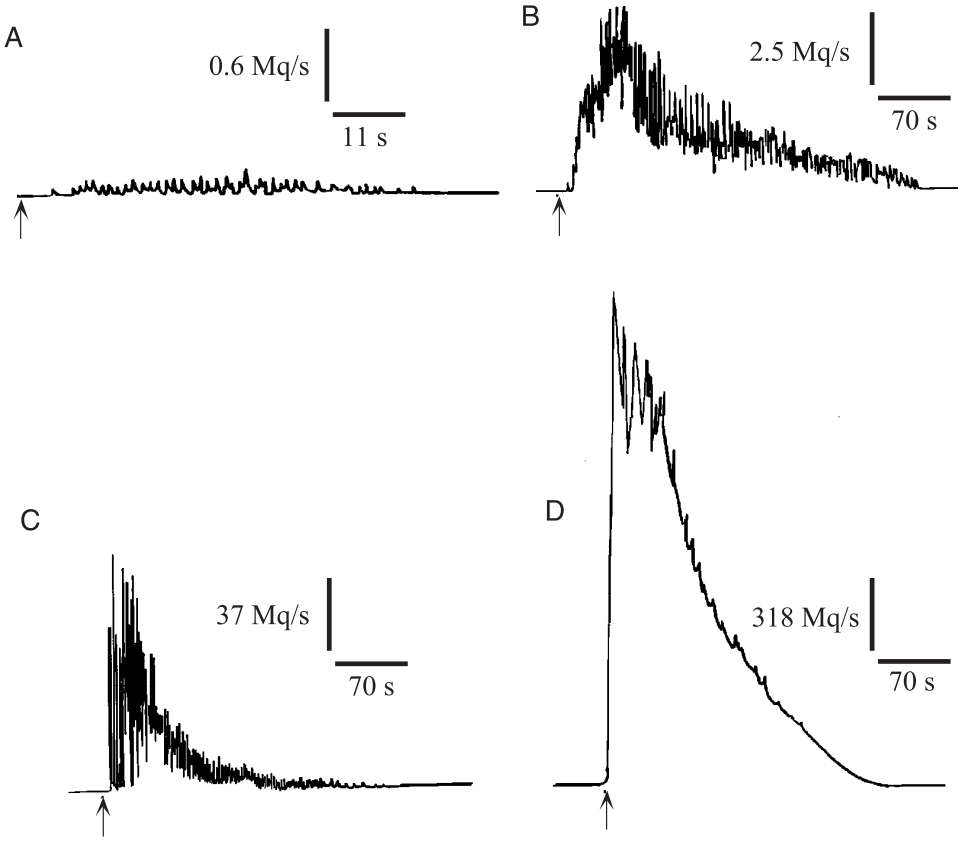


Fig. 2. – Luminescence patterns of ophiuroids from different colour varieties in response to ACh stimulation. – (A) orange specimens, (B) dark brown specimens, (C) spotted specimens, (D) black specimens. Arrow: injection of ACh.

Orange and beige specimens : only 33 % and 50 % of stimulated arms, respectively, emitted light in response to ACh. In addition, the maximal light production (Lmax), which was similar in orange and beige specimens, was significantly lower than for the other varieties ($P < 0.01$, calculated after logarithmic transformation of the n values as indicated by Zar, 1984 when heteroscedasticity occurs) (Table 1). Although the latency time (TL : 9.05 ± 1.57 s) and total response time (Tresp : 40.05 ± 3.21 s) of light emission for orange specimens was significantly different ($P < 0.01$) from the TL (4.46 ± 0.65 s) and Tresp (133.13 ± 9.29 s) of beige specimens, the luminescence pattern for both varieties was similar. The original recording, represented in Fig. 2A, showed a succession of individual flashes of very low intensity.

Dark brown and grey specimens : nearly all of the stimulated arms emitted light ($n = 28$ and 29, respectively). The parameters (Table 1) and the pattern of luminescence (Fig. 2B) were very similar for both types of specimens, except that Lmax was significantly higher ($P < 0.01$) for the grey specimens (2.53 ± 0.37 Mq/s/mm) than for the dark brown (0.72 ± 0.07Mq/s/mm). The luminescence pattern was a series of superimposed light flashes of increasing amplitude that reached a maximal value and then decreased in magnitude.

Spotted specimens : all of the stimulated arms emitted light. The latency time and maximal light production were, respectively, shorter and higher than for the other varieties ($P < 0.01$) except for the black specimens. The luminescence pattern (Fig. 2C) consisted of a series of individual flashes of increasing amplitude that reached a maximal value and then decreased in magnitude.

Black specimens : all of the stimulated arms emitted light. The luminescent response was very fast with a significantly shorter TL and Tlmax, whereas Lmax was significantly higher than for the other varieties ($P<0.01$) (Table 1). The luminescence pattern (Fig. 2D) was monophasic-like, although several peaks of decreasing intensity were observed.

TABLE 1

Values of luminescent parameters for the six colour varieties of Amphipholis squamata. *For each variety, arms from six specimens were stimulated, giving a total of 30 stimulated arms per variety (mean ± s.e.; *= P< 0.05, **= P< 0.01; n= number of responding arms).*

Colour varieties	**Orange**	**Beige**	**Dark Brown**	**Grey**	**Spotted**	**Black**
TL	9.05 **	4.46	4.11	4.17	2.4 **	0.76 **
(s)	± 1.57	± 0.65	± 0.48	± 0.27	± 0.35	± 0.05
TLmax	28.40	20.0	21.4	22.77	18.77	1.68 **
(s)	± 6.66	± 5.08	± 4.83	± 5.02	± 2.25	± 0.23
Lmax	0.03 **	0.08 *	0.72 *	2.53 **	13.14 **	238.26 **
(Mq/s/mm)	± 0.006	± 0.01	± 0.07	± 0.37	± 2.42	± 38.53
Tresp	40.05 **	133.13 *	169.96 *	168.48 *	140.17 *	148.73 *
(s)	± 3.21	± 9.29	± 10.10	± 3.31	± 5.95	± 19.26
n	10	15	28	29	30	30

Variation in luminescence performance over six months

Only dark brown and black ophiuroids were investigated. They were both chosen because of their differences in luminescent properties and their relative abundance in the population.

Dark brown specimens exhibited Lmax values which did not significantly vary between the months October, December 1996 and January, March, April, May 1997. Lmax values ranged from 0.13±0.03 Mq/s/mm in December 1996 to 0.66 ± 0.31 Mq/s/mm in January 1997. Conversely, a peak of significantly higher intensity of light production ($P < 0.01$) was observed in February 1997, reaching a value of 5.04±0.54 Mq/s/mm (Fig. 3A).

Black specimens showed Lmax values which varied from 336.4 ± 57.2 Mq/s/mm in October 1996 to 138.1 ± 37.1 Mq/s/mm in April 1997. A peak of significantly higher inten-

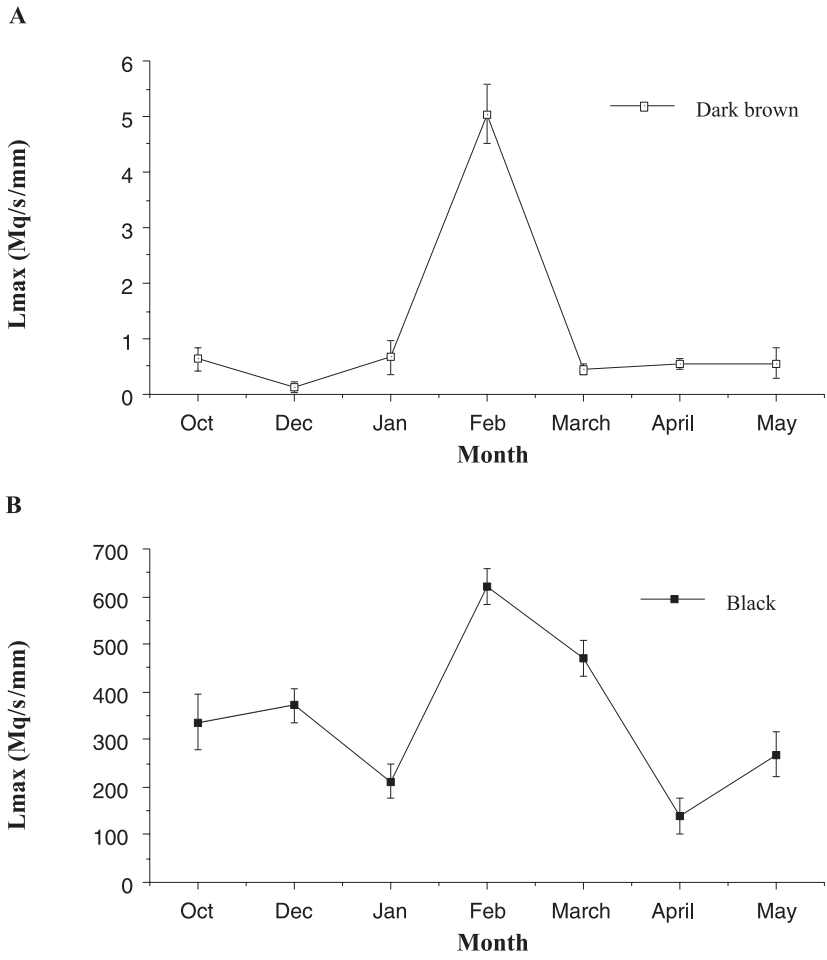


Fig. 3. – Variation of light intensity (Lmax in Mq/s/mm) from October 1996 to May 1997 of (A) dark brown specimens, (B) black specimens.

sity of light production ($P < 0.01$) was observed in February 1997, reaching a value of 621.8 ± 37.6 Mq/s/mm (Fig. 3B). The Lmax value for March 1997 was not significantly different from the Lmax value for October and December 1996.

It is noteworthy that the pattern of light emission varied through the year : with an increasing Lmax we usually observed a reduced latency time, a shorter TLmax and superimposed flashes which, for the black specimens in periods of maximal light emission, led to a monophasic curve.

DISCUSSION

Light emission according to coloration pattern

Ophiuroids of each colour variety differed in their luminescence parameters. The latency time was the shortest for black specimens and the longest for orange specimens. The following ranking was observed : black < spotted < grey = dark brown = beige < orange. TLmax was significantly shorter for black specimens whereas the other varieties exhibited similar values (black << orange = beige = dark brown = grey = spotted). The total light response time was significantly shorter for orange specimens and longer for grey and dark brown specimens (orange << beige = spotted = black < grey = dark brown). The maximal light production differed significantly from one variety to another. The orange and beige specimens exhibited the lowest levels of luminescence, followed in order of intensity by dark brown, grey, and spotted specimens, with a maximal intensity reached by black specimens (orange = beige < dark brown < grey < spotted << black).

Compared with the results obtained using KCl stimulation (DEHEYN *et al.*, 1997), ACh-induced luminescence differed in many ways from KCl-induced luminescence. Firstly, the pattern of light emission induced by ACh did vary from one variety to another whereas the pattern of KCl-induced luminescence was a monophasic curve, similar for all varieties. Secondly, the maximal amplitude of luminescence was lower for ACh-induced luminescence. And finally, like the results obtained with ACh, the maximal light production induced by KCl differed significantly from one variety to another, but the spotted specimens showed the highest intensity for KCl-induced luminescence while it was the black specimens for ACh-induced luminescence.

So far, the most common role described for bioluminescence in ophiuroids is defence against predators (BASCH, 1988 ; GROBER, 1988a,b ; GOTTO, 1963). Assuming this function to be true for *A. squamata*, the fact that colour varieties show significant differences in light intensities would mean that some individuals in the population would be more protected than others. This seems not to be the case, as individuals producing the most intense light (spotted and black varieties) have been shown not to be the most abundant in the population (DEHEYN *et al.*, 1997).

The difference of light emission between polychromatic ophiuroids may be explained either by a variable amount of luminescent tissue in the arm, or by a different quantity of substrate and/or enzyme for the light reaction in the luminescent cells. Conversely, pharmacological studies showed that there were differences in the subtype of cholinergic

receptors and in the neuromodulation of luminescence between dark brown and black specimens (De Bremaeker *et al.,* 1996 ; Mallefet *et al.*, 1994). These results support the view that there are distinctive functional differences in the neuromodulatory control of luminescence between dark brown and black specimens.

Variation in luminescence performance over 6 months

Both dark brown and black specimens of *A. squamata* displayed variation of luminescence performance over the investigated period of the year ; the light production in response to ACh stimulation was significantly higher in February. Since only brooding adult specimens were considered, the variation of luminescence performance could not be related to sexual maturity.

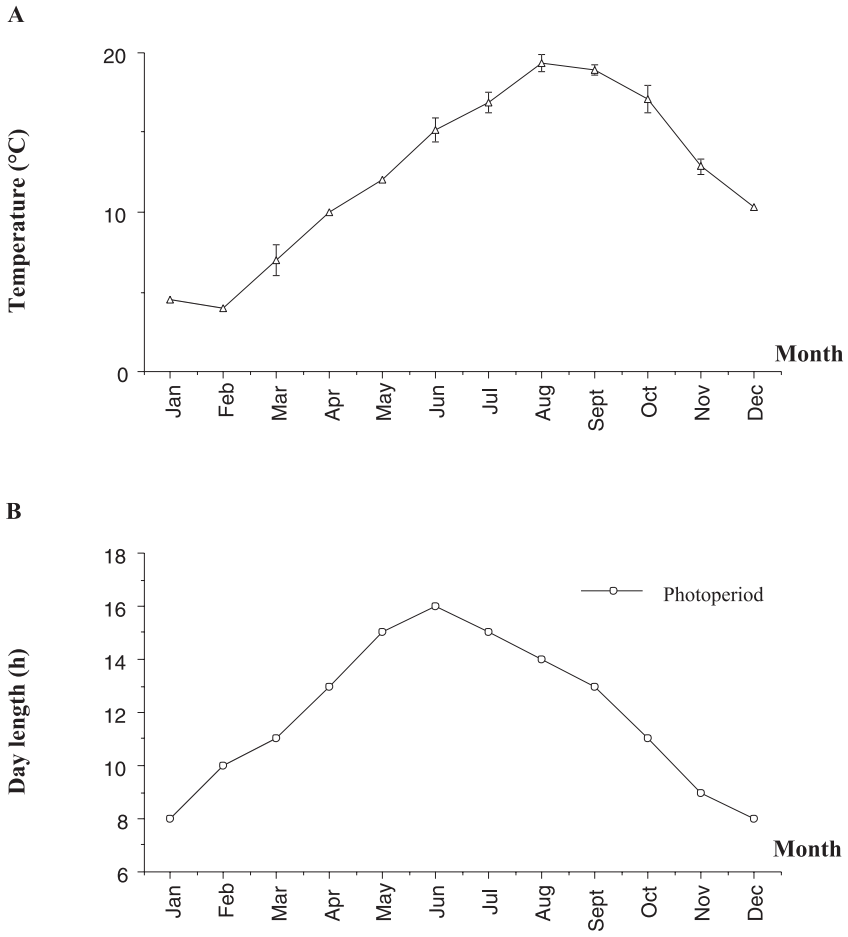


Fig. 4. – Seasonal variations from January 1997 to December 1997 of (A) water temperature, (B) photoperiod.

Other factors could affect luminescence. In this study, three abiotic field parameters were measured : surface salinity, surface temperature and photoperiod. Salinity was not significantly different from one month to another, ranging from 32.4 to 33.5 ‰ (not shown). Hence this factor was not likely to affect luminescence. Conversely, temperature and photoperiod exhibited large variations (Fig. 4A-B) ; temperature and photoperiod being low (4 °C ; 10 :14, Light : Dark) in February when a peak of luminescence was observed. These two factors may influence directly or indirectly the luminescence. This hypothesis agrees with recent field investigations and laboratory experiments on the effect of exogenous factors on the luminescence induced by KCl depolarisation, which showed that salinity had no main effect whereas the temperature and photoperiod significantly affected the KCl-induced luminescence (DEHEYN, 1998).

Seasonal photoperiod changes are known to guide the reproductive cycle of many organisms, including the echinoderms (BAY-SCHMITH & PEARSE, 1987 ; BOULAND & JANGOUX, 1988 ; XU & BARKER, 1990). Although *A. squamata* produce fertile gametes all year (Fell, 1946 ; Emson & Whitfield, 1989), the reproductive effort varies according to season, and all the gonads reach complete sexual maturity in winter. The maximal reproductive effort thus coincides with the coldest months, *i.e.* February-March for northern temperate locations (JONES & SMALDON, 1989 ; EMSON & WHITFIELD, 1989 ; EMSON *et al.* 1989 ; ALVA, 1996). As a consequence, the seasonal variation of the luminescence in *A. squamata* could be influenced by an endogenous factor guided, by the photoperiod, causing the luminescence performance to increase in winter.

Seasonal variation of light production could be due to seasonal changes in the level of neuromodulators. Seasonal changes in the levels of monoamines (serotonin, dopamine and noradrenaline) occur in invertebrates and vertebrates (SPAFFORD & PENGELLEY, 1971 ; YORK & TWAROG, 1973 ; STEFANO & AIELLO, 1975). In the invertebrate bivalve mollusc *Mytilus edulis*, these monoamines were higher during the summer and lower during the winter (STEFANO & CATAPANE, 1977). In two echinoderm species, the starfish *Asterias amurensis* and the sea urchin *Strongylocentrotus intermedius*, seasonal changes of catecholamine levels have also been reported. The levels of dopamine were higher in the winter and tryptamine higher in the summer (KHOTIMCHENKO & DERIDOVICH, 1988). Preliminary pharmacological studies have shown that catecholamines inhibited ACh-induced luminescence in *A. squamata*. Further studies will investigate the neuromodulatory processes occuring in *A. squamata* luminescence.

ACKNOWLEDGEMENTS

We thank I. Cogneau for technical assistance, D. Deheyn and E. Lamarque for field assistance and Professor J. Avoine for providing facilities at the Marine Laboratory of Luc-sur-Mer (France). The temperature and salinity field data were obtained from IFREMER (Port en Bessin, France). This work was supported by FRFC 6.231.85. Contribution of the « Centre Interuniversitaire de Biologie Marine » (CIBIM). J. Mallefet is Research Associate of the National Fund for Scientific Research (FNRS, Belgium).

REFERENCES

ALVA, V. (1996) – Reproduction, développement, incubation et dynamique de population de l'ophiure *Amphipholis squamata* (Echinodermata) en baie de Seine. Thèse de doctorat, Université Libre de Bruxelles, Belgique.

BASCH, L.V. (1988) – Bioluminescent anti-predator defense in a subtidal ophiuroid. In *Echinoderm Biology*, R.D. Burke, P.V. Mladenov, P. Lambert & R.L. Parsley (eds), Balkema, Rotterdam : 503-515.

BAY-SCHMITH, E & J.S. PEARSE (1987) – Effect of fixed daylengths on the photoperiodic regulation of gametogenesis in the sea urchin *Strongylocentrotus purpuratus*. *Int. J. Invert. Reprod. Develop*., **11** : 287-294.

BINAUX, R. & C. BOCQUET (1971) – Sur le polychromatisme de l'ophiure *Amphipholis squamata* (Delle Chiaje). *C.R. Acad. Sc. Paris*, **273** : 1618-1619.

BOULAND, C. & M. JANGOUX (1988) – Investigation of the gonadal cycle of the asteroid *Asterias rubens* under static condition. In : *Echinoderm Biology*, R.D. Burke, P.V. Mladenov, P. Lambert & R.L. Parsley (eds), Balkema, Rotterdam : 169-175.

BREHM, P. & J.G. MORIN (1977) – Localization and characterization of luminescent cells in *Ophiopsila californica* and *Amphipholis squamata* (Echinodermata : Ophiuroidea). *Biological Bulletin. Marine Biological Laboratory, Woods Hole,* **152** : 12-25.

DE BREMAEKER, N., J. MALLEFET & F. BAGUET (1993) – Involvement of a cholinergic control in *Amphipholis squamata* (Echinodermata) luminescence. *Arch int Physiol Bioch.*, **101** : 30.

DE BREMAEKER, N., J. MALLEFET & F. BAGUET (1996) – Luminescence control in the brittlestar *Amphipholis squamata* : Effect of cholinergic drugs. *Comp. Biochem. Physiol.*, **115C** : 75-82.

DEHEYN, D. (1998) – Etude de la bioluminescence chez l'ophiure *Amphipholis squamata* (Delle Chiaje, 1828) (Echinodermata) : structure des sites photogènes, modalités de l'expression et fonction de la bioluminescence. Thèse de doctorat. Université Libre de Bruxelles, Belgium.

DEHEYN, D., V. ALVA & M. JANGOUX (1996) – Fine structure of the photogenous areas in the bioluminescent ophiuroid *Amphipholis squamata* (Echinodermata : Ophiuroidea). *Zoomorphology*, **116** : 195-204.

DEHEYN, D., J. MALLEFET & M. JANGOUX (1997) – Intraspecific variations of bioluminescence in a polychromatic population of *Amphipholis squamata* (Echinodermata : Ophiuroidea). *J. mar. biol. Ass. UK*, **77** : 1213-1222.

DELLE CHIAJE, S. (1828) – *Memorie sulla storia e anotomia degli animali senza vertebre del Regno di Napoli*, **3** : 231 pp., Napoli.

EMSON, R.H. & P.J. WHITFIELD (1989) – Aspects of the life history of a tide pool population of *Amphipholis squamata* (Ophiuroidea) from South Devon. *J. Mar. Biol. Ass. UK*, **69** : 27-41.

EMSON, R.H., M.B. JONES & P.J. Whitfield (1989) – Habitat and latitude differences in reproductive pattern and life-history in the cosmopolitan brittle-star *Amphipholis squamata* (Echinodermata). In *Reproduction, genetics and distributions of marine organisms.* J.S. RYLAND & P.A. TYLER (eds), Fredensborg, Olsen & Olsen : 75-81.

FELL, H.B. (1946) – The embryology of the viviparous ophiuroid *Amphipholis squamata* Delle Chiaje. *Phil. Trans. Royal Soc. New Zealand*, **75** : 419-464.

GOTTO, R.V. (1963) – Luminescent ophiuroids and associated copepods. *Ir. Nat. J.*, **14** : 137-139.

GROBER, M.S. (1988a) – Responses of tropical reef fauna to brittlestar luminescence (Echinodermata : Ophiuroidea). *J.Exp. Mar. Biol. Ecol.*, **115** : 157-168.

GROBER, M.S. (1988b) – Brittlestar bioluminescence functions as an aposematic signal to deter crustaceans predators. *An. Behav.*, **36** : 493-501.

HARVEY, E.N. (1952) – Echinodermata. In *Bioluminescence*, Academic Press, New York : 472-479.

JONES, M.B. & G. SMALDON (1989) – Aspects of the biology of a population of the cosmopolitan brittlestar *Amphipholis squamata* (Echinodermata) from the Firth of Forth, Scotland. *J. Nat. Hist.*, **23** : 613-625.

KHOTIMCHENKO, Y.S. & I.I. DERIDOVICH (1988) – Seasonal dynamics of biogenic monoamines in the central nervous system of the sea urchin *Strongylocentrotus intermedius* and the starfish *Asterias amurensis*. *Marine Biol. Vladivostock*, **2** : 98-103.

MALLEFET, J., N. DE BREMAEKER & F. BAGUET (1994) – Neuromodulatory effects of Gaba, S1 and S2, FMRFamide and Antho-RFamide on *Amphipholis squamata* (Echinodermata) luminescence. In : *Bioluminescence and chemiluminescence, fundamentals and applied aspects* , A.K. CAMPBELL, L.J. KRICKA & P.E. STANLEY (eds), Chichester, J. Wiley & Sons : 109-112.

MALLEFET, J., P. VANHOUTTE & F. BAGUET (1992) – Study of *Amphipholis squamata* luminescence. In *Echinoderm Research* , L. SCALERA-LIACI & C. CANICATTI (eds), Balkema, Rotterdam : 125-130.

SPAFFORD, D.C. & E.T. PENGELLEY (1971) – The influence of the neurohumor serotonin on hibernation in the golden-mantled ground squirrel, *Citellus lateralis*. *Comp. Biochem. Physiol.*, **38A** : 239-250.

STEFANO, G.B. & E. AIELLO (1975) – Histofluorescent localization of serotonin and dopamine in the nervous system and gill of *Mytilus edulis* (Bivalvia). *Biol. Bull.*, **148** : 141-156.

STEFANO, G.B. & E.J. CATAPANE (1977) – Seasonal monoamine changes in the central nervous system of *Mytilus edulis* (Bivalvia). *Experientia*, **33** : 1341-1342.

VIVIANI, D. (1805) – Quattuordecim lucescentium animalculorum novis speciebus illustrata. In : *Phosphorescentia maris*, J GIOSSI (ed.), Genova, Italy : 1-17.

XU, R.A. & M.F. BARKER (1990) – Annual changes in the steroid levels in the ovaries and the pyloric caeca of *Sclerasterias mollis* (Echinodermata : Asteroidea) during the reproductive cycle. *Comp. Biochem. Physiol.*, **95A** : 127-133.

YORK, B. & B.M. TWAROG (1973) – Evidence for the release of serotonin by relaxing nerves in molluscan muscle. *Comp. Biochem. Physiol.*, **44A** : 423-430.

ZAR, J.H. (1984) – *Biostatistical analysis*. 2nd ed. Englewood Cliffs, New Jersey : Prentice-Hall.

Belg. J. Zool. - Volume 129 (1999) - issue 1 - pages 391-404 - Brussels 1999

Received : 13 November 1998

DIFFERENTIATION OF THE EPIDERMIS OF NECK, TAIL AND LIMBS IN THE EMBRYO OF THE TURTLE *EMYDURA MACQUARII* (GRAY, 1830)

LORENZO ALIBARDI

Dipartimento di Biologia, University of Bologna,
via Selmi 3, 40126 Bologna

Abstract. The development of the skin in turtles and the differentiation of the first keratinized layers are largely unknown processes. The histology and ultrastructure of the developing skin of neck, tail and limbs of the embryo of the turtle *Emydura macquarii* were studied. This study showed that three to four embryonic layers are initially formed from the basal layer. They contain scarce bundles of 8-12 nm-thick intermediate filaments of keratin, and many coarse 28-35 nm-thick filaments of unknown nature. The coarse filaments form reticulate bodies similar to those of lepidosaurian reptiles and birds, and form large aggregations within corneocytes of the embryonic epidermis. Embryonic layers also contain mucus and vesicular bodies, the latter associated with lipid droplets. Lipids and mucus are partly discharged into the amniotic fluid. Shortly before hatching, typical α-keratinocytes, containing keratin filaments and no coarse filaments, replace the embryonic epidermis. Mucus and lipids are, however, retained among α-keratinocytes after hatching. The loose dermis of early embryonic stages rapidly turns into a dense connective tissue that strengthens the delicate epidermis. Large collagen fibrils contact the basement membrane of the epidermis of the tail. Lipidic material is also stored in dermal fibroblasts.

Key words : turtle (*Emydura macquarii*), embryo, soft skin, ultrastructure.

INTRODUCTION

Chelonia are considered among the most ancient fully terrestrial vertebrates (McFARLAND *et al*. 1979). The skin of these reptiles is adapted to protect the body from desiccation and mechanical stress on land. The epidermis varies in histological and biochemical composition according to the body regions.

In the shell (carapace and plastron), a hard and variably thick layer of horny cells is composed of β- (ϕ)-keratins (SPEARMAN, 1966 ; ALEXANDER, 1970, PARAKKAL & ALEXANDER, 1972 ; BADEN *et al.*, 1974 ; WYLD & BRUSH 1979, 1983). This hard type of keratin shows a typical X-ray diffraction pattern, a fibril periodicity of 2-3 nm, and barely stains with toluidine blue, eosine or other dyes. β-keratinocytes merge completely or partially with one another to form a syncytial β-keratin layer (ALEXANDER, 1970 ; ALEXANDER & PARAKKAL, 1969 ; LANDMANN, 1979 ; 1986 ; MADERSON, 1985 ; MADERSON *et al.*, 1998).

The specialized epidermis of the shell derives from embryonic regions where peculiar dermo-epithelial interactions take place (RUCKES, 1929; EWERT, 1985; BURKE, 1989a,b, 91). This epidermis forms placodes under which dermal cells aggregate.

In the embryonic skin of the head, neck, limbs and tail, similar dermo-epidermal interactions are not present, and the adult skin of these areas is soft, pliable and capable of folding. The epidermis of these regions is covered by α-keratinocytes (ALEXANDER, 1970; SPEARMAN, 1969; PARAKKAL & ALEXANDER, 1972; MATOLTSY & HUSZAR, 1972). Contrary to β-keratin, the soft α-keratin presents its specific X-ray diffraction pattern, a fibril periodicity of 8-12 nm, similar to that of mammalian keratins (BADEN & MADERSON, 1970). Also, mature α-keratinocytes are very thin (0.2-1.0 μm), do not merge with one another but remain separate, and are stainable with toluidine blue, eosin and other dyes.

Contrary to most turtles (adapted to a freshwater environment), in some tortoises (adapted to a more terrestrial and dry environment) also the outer part of numerous limb and neck scales contains hard β-keratin, which alternates with softer α-keratin on the inner side and hinge regions (SPEARMAN, 1966, 1969; BADEN & MADERSON, 1970). These large and tough scales enhance the mechanical and defensive protection when the animal retracts the limbs into the shell.

Although the process of keratinization has been studied histologically, histochemically and ultrastructurally in the soft skin regions of adults (SPEARMAN, 1966, 1969; HENRIKSON & MATOLTSY, 1970; MATOLTSY & HUSZAR, 1972; MATOLTSY & BEDNARZ, 1975; MATOLTSY, 1987), to date there are no reports on the modality of keratinization during skin embryogenesis up to the time of emergence of the hatchling.

The present ultrastructural study, together with others on the shell morphogenesis and on the differentiation of the skin in the carapace and plastron (ALIBARDI & THOMPSON, 1999a,b), describes the modifications of the epidermis during the passage from the liquid environment of the embryo, surrounded by the amniotic fluid, to the dry and freshwater environment of the adult.

MATERIAL AND METHODS

Eggs of the Australian short-necked pond turtle *Emydura macquarii*, Gray 1830, collected during summer in the countryside of New South Wales, were used in this study.

The tables of development by YNTEMA (1968) on the freshwater turtle *Chelydra serpentina*, were used as a reference system. Collected embryos were representative of embryonic stages (ES) 15 (n=3), ES 16 (n=2), ES 18 (n=1), ES 19 (n= 3), ES 22 (n=1), ES 23 (n=3), ES 24 (n=7), ES 25 (n=3), 1-2 post-hatching (n=2), 1 week post-hatching (n=2).

Pieces (1-5 mm large) of embryonic tissues were fixed in cold (0-4°C) 2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. After rinsing in the same buffer, the tissues were post-fixed in 1% osmium tetroxide for about 2 h (some pieces also placed in 4% uranyl acetate for 1-2 h), dehydrated and embedded in Durcupan or Spurr resins.

The skin of limbs, neck and tail, was sectioned with an ultramicrotome in cross or sagittal planes in order to collect representative areas. One to 2 μm-thick semithin sections

were stained with 0.5% toluidine blue or toluidine blue-eosine (5-10 seconds each on a hot plate). Thin, 50-90 nm-thick, sections were collected on copper or nickel grids, routinely stained with uranyl acetate and lead citrate, and observed in a transmission electron microscope Philips CM 100, operating at 60-80 kV.

RESULTS

Light microscopy

At ES 15-16, most of the epidermis covering the studied regions was bilayered, and few suprabasal cells were seen. Mitotic cells were frequently observed in the epidermis and in the outer flat peridermis (Fig. 1). Between ES 17 and ES 22, 1-2 layers of suprabasal cells were produced, which did not accumulate keratin but were rich in pale vesicles resembling lipid droplets, as previously described for the epidermis of an adult turtle (MATOLTSY & HUSZAR, 1972 ; MATOLTSY & BEDNARZ, 1975).

In the neck region, the epidermis was linear or arranged in irregular or symmetrical dermo-epidermal elevations and folds (Fig. 2). At ES 22, the epidermis appeared generally composed of 3-4 layers of cells under a flat peridermis. One to two subperidermal layers of flat cells and 1 suprabasal layer were present. The dermis, in particular near the shell openings where the neck and limbs exit, was composed of fusoid or flat fibroblasts surrounded by thick bundles of collagen fibrils (Fig. 3). The orientation of such collagen bundles was often undulated like the epidermis.

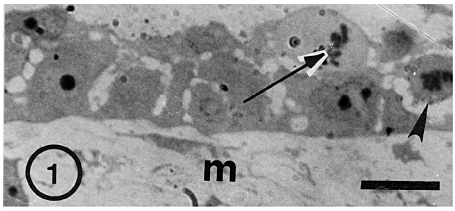
In limbs, skin foldings formed symmetric or even asymmetric scales with a longer outer side and a shorter inner side (Fig. 4). At ES 22, the whole epidermis showed the same thickness along the entire skin surface and was composed of 1-2 flattened cell layers under a superficial peridermis. Above the basal layer one suprabasal layer was present. Dermal fibroblasts and fibers followed the epidermal outline. At ES 23, there were 3-4 flattening suprabasal cell layers, both in the outer and inner sides of the scales, beneath the external peridermis, which often formed a thin, darker layer (Fig. 5). Clear droplets or vesicles were observed in the suprabasal cells.

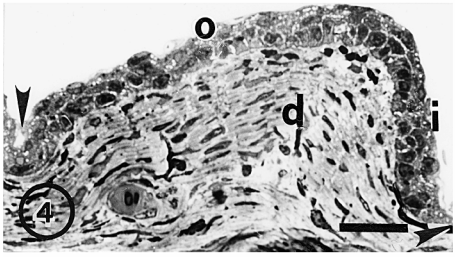
The skin of the tail showed a more or less irregular outline, or was often folded into large dermo-epidermal bumps (Figs 6, 7). At ES 22, epidermal cells in this region were cuboidal or flat, and were irregularly stratified, with a wave-like disposition. These keratinocytes showed an irregular cellular and nuclear outline.

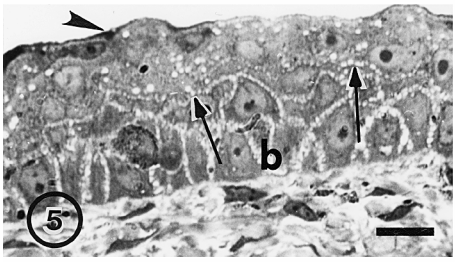
At ES 23, under the flat external peridermis, 3-4 suprabasal layers were seen. The cytoplasm of the more superficial and flattened suprabasal layers became dark in patches (Figs. 5).

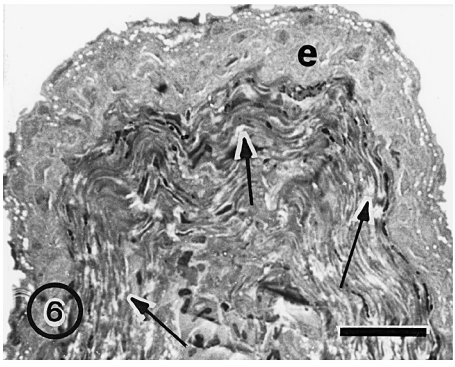
At ES 24, the epidermis contained 2-3 suprabasal cell layers beneath 2 flat subperidermal darker layers.

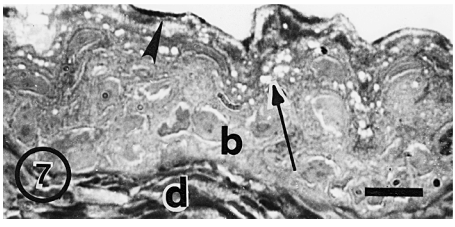
At ES 25, 5-6 layers of differentiating corneocytes were visible beneath 2-4 external dark layers (Fig. 8). The peridermis and the more external layers were very flat, anucleated, dark and largely cornified.

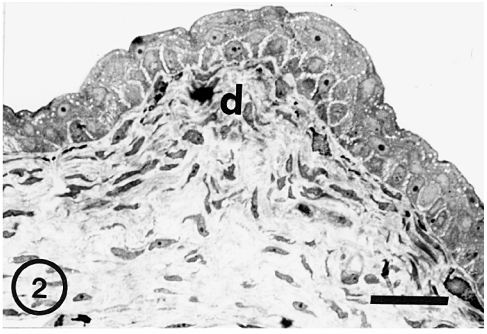

1
m

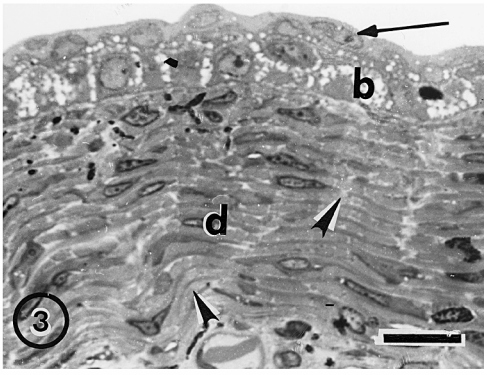

o
d
i
4

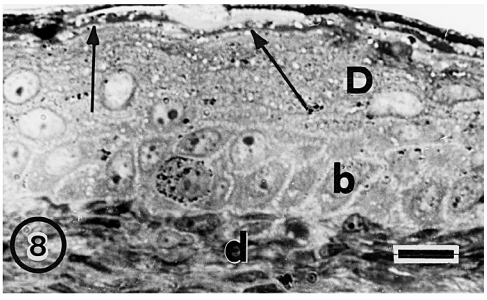

b
5

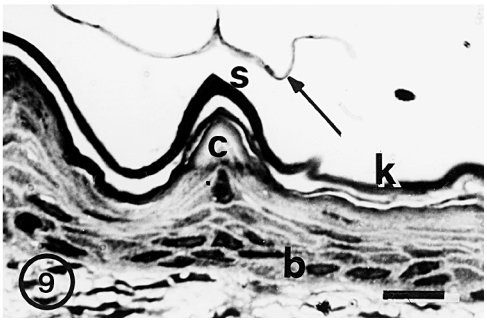

e
6


b
7
d


d
2


b
d
3


D
b
8
d


s
c
k
b
9

Finally, from 2-3 days to 1 week post-hatching, the epidermis appeared covered by a dark corneous stratum, composed of narrow layers of desquamating keratinocytes (Fig. 9). The basal layer was formed by flat to cubic cells, followed by 2-4 suprabasal layers of flat cells. Above suprabasal cells the nuclei disappeared, and a 5-10 μm-thick pale layer without keratohyaline granules preceded the corneous layer. In various regions along the epidermis corneous spurs were formed.

Electron microscopy

Between ES 15 and ES 17 the cytoplasm of peridermal and epidermal cells contained most ribosomes and few, sparse, glycogen particles. Occasional isolated 8-12 nm-thick (intermediate) keratin filaments were also present in the cytoplasm of these cells but no bundles of keratin filaments were seen. The latter appeared in suprabasal cells at ES 19, and by ES 22 they were also present in the basal cells of the epidermis. A flat peridermis remained over the stratifying epidermis up to ES 24, but peridermal cells were sometimes detached or missing on the external surface. The first 3-4 layers underneath the peridermis showed peculiar cytological characteristics and, since they disappeared after ES 24, have been termed embryonic epidermal layers (ALIBARDI & THOMPSON, 1999a).

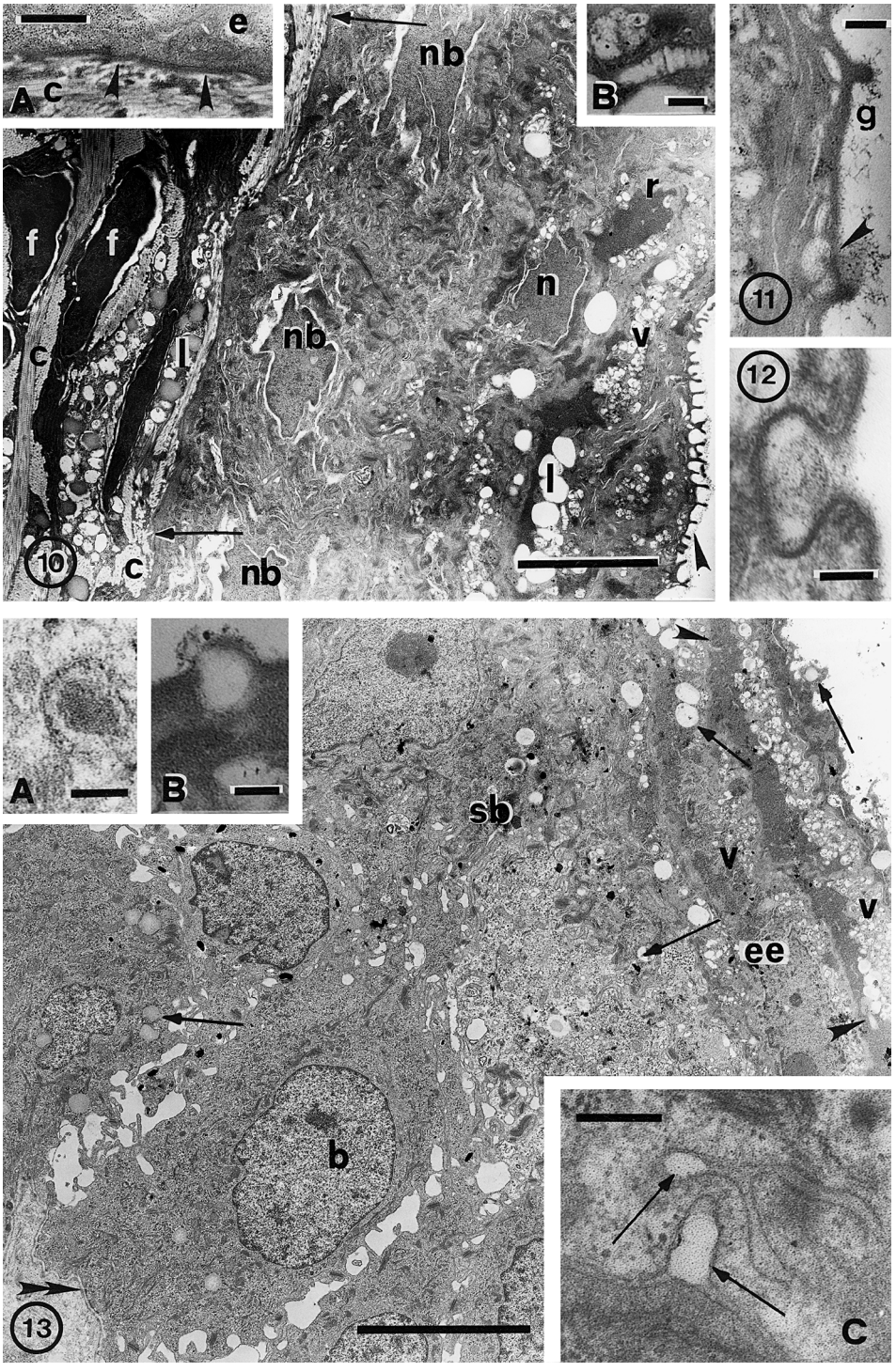
At ES 22, epidermal cells of the tail were irregular, their cell and nuclear outline was indented or irregular, forming a jig-saw puzzle-like epithelium (Figs 7, 10). As in other epidermal areas, no nuclear condensation (apoptosis) was visible in suprabasal cells up to ES 24.

The lamina densa of the basement membrane was discontinuous or lacking, and contacted by large, electron-pale collagen bundles of the dermis (Fig. 10 inset A). At ES 22, the dermis was already composed of thick criss-cross or plywood oriented collagen bundles. Lipidic material was also accumulated in some dermal cells (Fig. 10).

On the external surface of the peridermis and superficial embryonic epidermis vesicles similar to those containing mucus (PAS positive, see MATOLTSY & HUSZAR, 1972;

Legend to the figures (see page 394)

Figs 1-9. – Light microscopic observation. – 1. ES 15. Mitotic cells in the peridermis (arrow) and basal epidermis (arrowhead) of an arm. m, dermal mesenchyme. Bar=10 μm. – 2. ES 22. Epidermal bump in distal neck region, showing little epidermal stratification. The dermis (d) is composed of flat fibroblasts. Bar=20 μm. – 3. ES 22. Epidermis of proximal neck with few cells (arrow) above the basal layer (b). The dense dermis (d) shows flat fibrocytes among collagen bundles (arrowheads). Bar=20 μm. – 4. ES 22. Arm scale showing the same epidermal thickness in the outer (o) and inner (i) sides. d, oriented dermal fibroblasts. Arrowheads on hinge regions. Bar=20 μm. – 5. ES 23. Close view of leg epidermis with 3-4 suprabasal cells (arrows on pale vescicles) beneath the dark narrow peridermis (arrowhead). b, basal layer. Bar=10 μm. – 6. ES 22. Bump-like epidermal folding (e) in the tail skin with thick wavy dermis (arrows). Bar=20 μm. – 7. Close-up of tail epidermis at ES 22 showing numerous pale vesicles (arrow) localized under the dark peridermis (arrowhead). b, basal layer. Bar=10 μm. – 8. ES 25. Stratified arm epidermis of an outer scale with dark external layers (arrows). D, suprabasal differentiating cells. b, basal layer. d, thick dermis. Bar=10 μm. – 9. Digit epidermis 1 week post-hatching with a keratinized layer (k) above a pale (c) and a living flat suprabasal and basal layers (b). The embryonic epidermis (arrow) is detached. s, spurr. Bar=10 μm.


e
A
c
nb
B
g
11
r
f
f
n
v
c
l
nb
12
l
10
c
nb
A
B
sb
v
v
ee
b
13
C

MATOLTSY & BEDNARZ, 1975) were seen fused with the external membrane, suggesting they are secretory vesicles (Figs 11, 12, 13A). Microvilli, often coated with a glycocalyx, were present in the outer peridermis (Figs 10, 11, 13, 14).

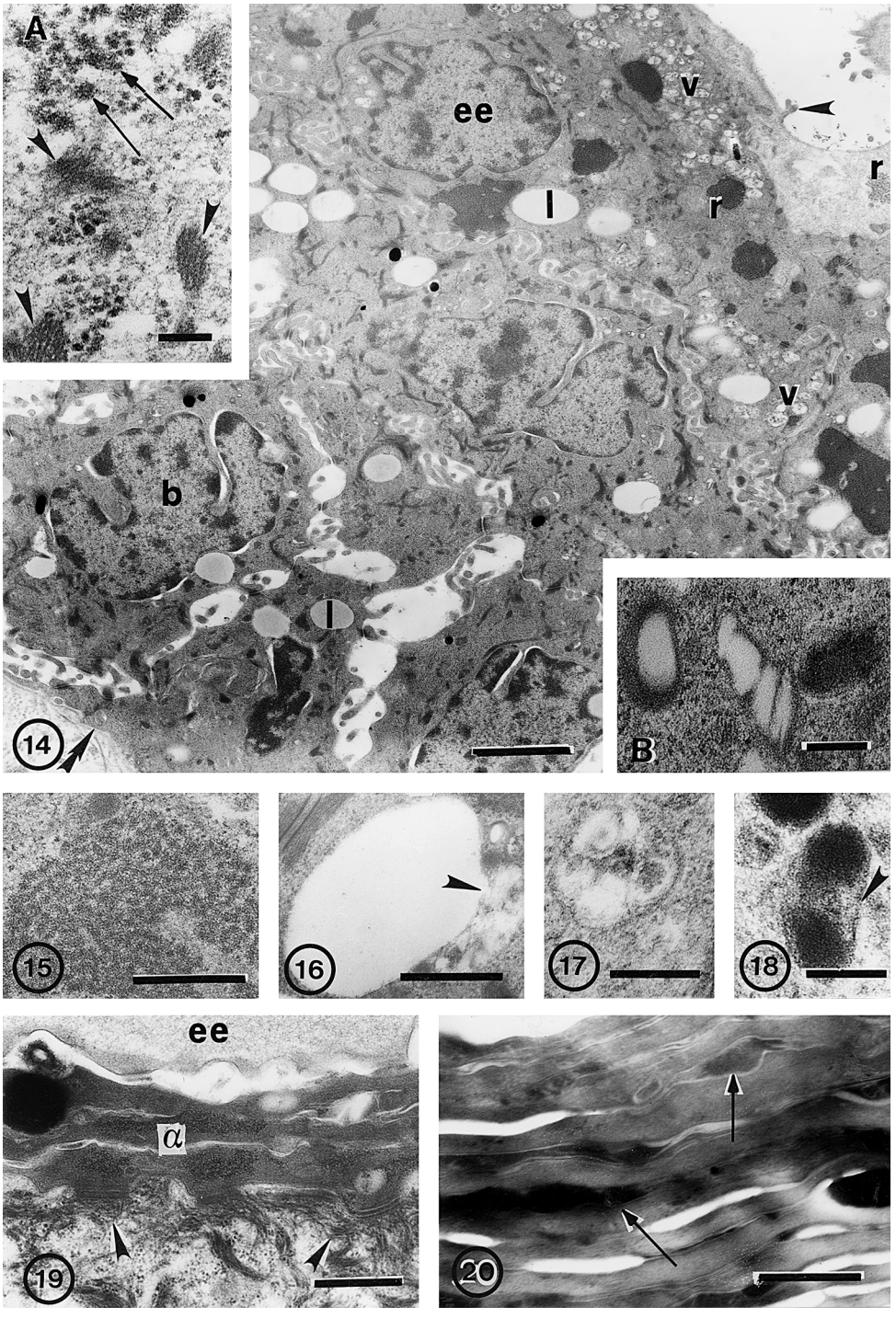
At ES 22-23, sparse bundles of intermediate filaments of keratin, and free or clumped ribosomes were visible in basal and suprabasal cells. Few ergastoplasmic cisternae, mitochondria and glycogen particles were present. In the external 3-4 epidermal layers, keratin filaments were scarce, and broad cytoplasmic areas were occupied by 28-35 nm-thick coarse filaments. The latter formed reticulate bodies that correspond to the dark patches observed in the external epidermal cells with the light microscope (Figs 5, 7, 8, 10, 13, 14, 15). Electron-pale lipid droplets (not surrounded by a membrane) and vesicular bodies (surrounded by a membrane), were sparse throughout the epidermis but were more common in the upper flat layers (Figs 10, 13, 14). Vesicular bodies were 0.2-0.4 μm-large, and contained amorphous material/membranes. These organelles were often associated with lipid droplets, with the Golgi apparatus or with the smooth endoplasmic reticulum (Figs 13 C, 16, 17). Some lipid droplets and vesicular bodies were seen on the external surface of embryonic epidermal cells, suggesting they were discharging their content onto the outer surface (Fig. 13). Other vesicular bodies containing an electron-dense material (MATOLTSY & HUSZAR, 1972 ; LANDMANN, 1986), were also present in these embryonic cells (Figs 13, 14B, 18). Some lamellation pattern was occasionally seen within the electron-dense matrix of these organelles.

At ES 24, more bundles of keratin filaments accumulated in the lower-most differentiating suprabasal cells, but no dark (apoptotic) nuclei were seen. At ES 25, beneath the first 3-4 embryonic layers, numerous bundles of keratin filaments increased in suprabasal cells and accumulated within the keratinizing cells (Fig. 19). Reticulate bodies disappeared in these forming α-keratinocytes, while vesicular bodies were reduced in number. Two layers of forming α-keratinocytes were present beneath the embryonic layers and above 2-4 layers of living suprabasal cells. These electron-dense and thin (0.1-0.5 μm-thick) keratinocytes also incorporated some melanosomes, and formed the definitive α-

---

Legend to the figures (see page 396)

Figs 10-13. – Electron microscopic observations. – Electron-micrograph of tail epidermis at ES 22. Basal (nb) and suprabasal (n) nuclei are irregularly indented. Many vesicular bodies (v) and lipid droplets (l) are present in the external layers and in dermal fibroblasts (f). r, dense reticulate bodies. c, electron-pale collagen fibrils. Arrowhead on coated microvilli. Arrows point to the basement membrane. Bar=5 μm. Inset **A** illustrates the contact (arrowheads) of pale collagen fibrils (c) with the basement membrane of epidermal cells (e). Bar=1 μm. Inset **B** on two vesicular bodies. Bar=250 nm. – 11. Embryonic tail epidermis at ES 22. Discharging mucus vesicles (arrowhead) on the external epidermal surface forming a glycocalyx (g). Bar=250 nm. – 12. Embryonic tail epidermis at ES 22. Discharging or invaginating vesicle containing a low electron-dense amorphous material. Bar=100 nm. – 13. Limb epidermis at ES 23 showing suprabasal (sb) and flat embryonic epidermis (ee) layers, rich in lipid vescicles (arrows) and vesicular bodies (v). Arrowheads on dense areas occupied by reticulate bodies. b, basal cells. Double arrowhead on the basement membrane. Bar=5 μm. Inset **A**, dense-cored mucus granule. Bar=100 nm. Inset **B**, discharging lipid-like vesicle on the epidermal surface. Bar=100 nm. Inset **C**, blebbing pale vesicles (arrows) from smooth endoplasmic reticulum. Bar=200 nm.


A
ee
v
r
l
r
v
b
l
14
B
15
16
17
18
ee
α
19
20

keratin layer. No keratohyaline granules were seen in these cells. Although some nuclei showed nuclear clumping during keratinization, the nuclear modifications during keratinization were not specifically studied in this report.

More α-keratinocytes continued to accumulate in post-hatching epidermis, which consisted of 7-15 layers (depending upon the body region) of very narrow horny cells (Figs 9, 19, 20). Extracellular dense deposits of mucus or lipidic material similar to those previously described (MATOLTSY & HUSZAR, 1972 ; MATOLTSY & BEDNARZ, 1975), were present.

DISCUSSION

Epidermis

The liquid environment of the embryo of *Emydura* is in contact with a soft, non-keratinized external peridermis, which is lost, partly in ovo and completely a few days after hatching.

Beneath the peridermis, the 3-4 layers of embryonic epidermis do not cornify by accumulation of keratin bundles, as in the adult epidermis. Instead they contain mucus, vesicular bodies and lipids, which are secreted extracellularly. This secretory epithelium is visible until ES 24, and although sparse keratin bundles were present, its main cytoskeletal components are 28-35 nm-thick coarse filaments aggregated into reticulate bodies. The latter are organelles typical of peridermal cells (MOTTET & JENSEN, 1968 ; PARAKKAL & MATOLTSY, 1968 ; DHOUAILLY & MADERSON, 1984 ; SAWYER *et al.*, 1986 ; ALIBARDI, 1997, 1998a,b ; ALIBARDI & THOMPSON, 1999a), but their molecular nature is unknown. From ES 25 onward, the condensation of reticulate bodies with the scarce keratin filaments, determines the corneification and darkening of the embryonic layers. Reticulate bodies have been described in other mucus-secreting epithelia, both embryonic (SAWYER *et al.*, 1986 ; ALIBARDI, 1998a,b) and adult (FUKUYAMA & EPSTEIN, 1973).

Legend to the figures (see page 398)

Figs 14-20. – Neck epidermis at ES 23 showing accumulation of vesicular (v) and reticulate (r) bodies in external embryonic epidermal cells (ee). The arrowhead points to a microvillus. Lipidic vescicles (l) are also present in the basal layer (b). Double arrowhead on dense basement lamella. Bar=2 mm. Inset **A** on coarse filaments (arrows) of a reticulate body. Arrowheads on keratin bundles. Bar=100 nm. Inset **B** shows vesicular bodies with dense areas. Bar=250 nm. – 15. Detail of a reticulate body of embryonic epidermal cell of the neck at ES 23. Bar=500 nm. – 16. Lipid droplet contacting a vesicular body (arrowhead) in embryonic neck epidermal cell at ES 23. Bar=500 nm. – 17. High magnification of a vesicular body in embryonic epidermal cell of neck at ES 23. Bar=200 nm. – 18. Dense body surrounded by a membrane (arrowhead) in embryonic epidermal cell of neck at ES 23. Bar=250 nm. – 19. Limb scale epidermis at ES 25 showing α-keratinized cells (α) under the embryonic epidermis (ee). Arrowheads indicate aggregation of bundles of keratin filaments in differentiating α-cell. Bar=500 nm. – 20. External narrow α-keratinocytes of an arm with dense intercellular material (arrows) at 1 week post-hatching. Bar=500 nm.

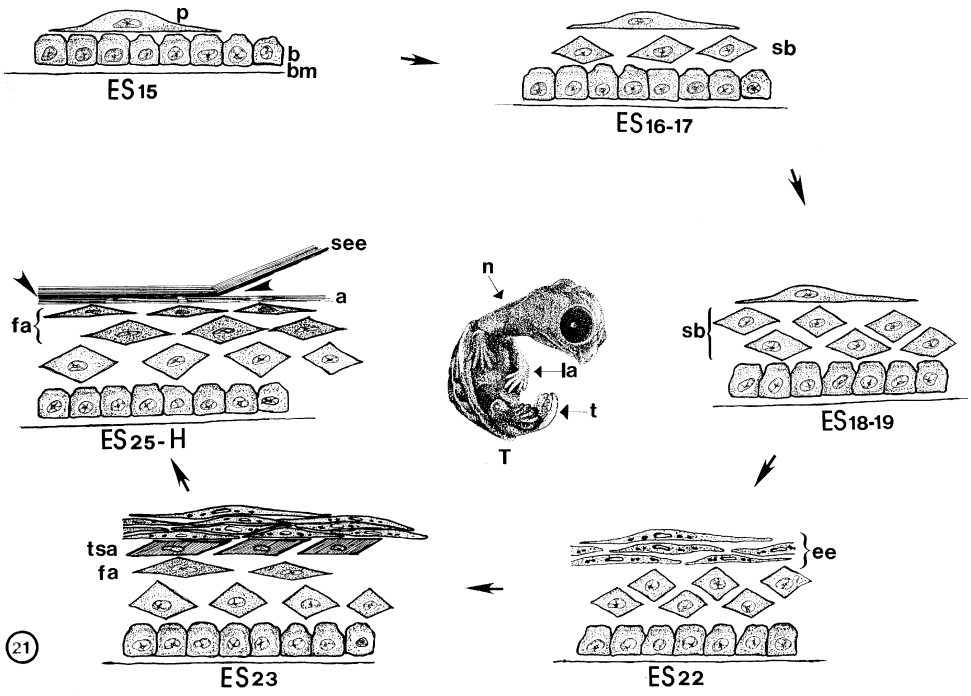


Fig. 21 – Schematic drawing of the stratification in the soft embryonic epidermis of the turtle embryo (**T**). **a**, α-keratin layer. **b**, basal layer. **bm**, basement membrane. **ee**, embryonic epidermis. **ES**, embryonic stages. **fa**, forming α-layer. **H**, hatching to about 1 week post-hatching. **la**, limb-arm. **n**, neck. **p**, periderm. **sb**, suprabasal layer. **t**, tail. **tsa**, transition α-layer between embryonic to α-keratin layer. **see**, shedding embryonic epidermis along a shedding line (**arrowheads**).

In conclusion, the embryonic epidermis shows characteristics of the mucus-secreting epidermis of amphibians (PARAKKAL & MATOLTSY, 1964; PARAKKAL & ALEXANDER, 1972; LAVKER, 1974; MATOLTSY, 1987) together with the lipid-secreting characteristics of the epidermis of aves (MATOLTSY, 1969; PARAKKAL & ALEXANDER, 1972; SAWYER & BORG, 1979; LANDMANN, 1980; SAWYER *et al.*, 1986; MENON *et al.*, 1986, 1996). Lipids, probably of polar type (MENON *et al.*, 1986), were perhaps an evolutionary addition to mucus for terrestrial life. Mucus granules with a dense core, such as those described in the adult turtle epidermis (ALEXANDER, 1970; MATOLTSY & HUSZAR, 1972), were observed less frequently in the embryonic epidermis of *Emydura*, but appeared in progressive stages of condensation (LANDMANN, 1986).

Lipid droplets in basal cells increase in number and associate with vesicular bodies. The latter resemble the lamellar bodies of adult turtle epidermis (MATOLTSY & BEDNARZ, 1975), although a regular lamellation pattern was not visible in our preparations. Since the latter organelles appeared more frequently in the more superficial embryonic layers, it seems possible that they were derived from some kind of modification of the lipid droplets present in the lower-most layers. In birds, however, lipid droplets in the intermediate to

upper epidermal layers appear to be derived from the loss of organization of lamellate bodies (MENON *et al.*, 1986 ; 1996). Vesicular bodies may also be directly produced from the smooth endoplasmic reticulum and Golgi apparatus of suprabasal cells, as in the chick epidermis (MATOLTSY, 1969 ; LAVKER, 1975), and progressively accumulate in the most superficial layers. However, the knoweldge of the precise origin of these organelles awaits more dynamic studies.

Mucus and lipids coat the epidermal surface, probably in relation to the impermeabilization of the skin to prevent inward-outward water movements in the acquatic environment.

From ES 24 onward, before the transition from the liquid to the terrestrial environment, the transformation from a mucus-lipidic-secreting epidermis to an α-keratin-producing epidermis takes place. The new epidermis is more suitable for protection against desiccation, mechanical wear, or injuries (ALEXANDER, 1970 ; HENRIKSON & MATOLTSY, 1970 ; MATOLTSY & HUSZAR, 1972). A similar epithelial transformation takes place in the dorsal lingual epithelium of freshwater turtles in contrast to more terrestrial tortoises or lizards (IWASAKI *et al*., 1996a,b), and also in the buccal epithelium of the rat (FUKUYAMA & EPSTEIN, 1973).

In the shell region, from ES 25 onward, beneath a similar embryonic epidermis a cellularized β-keratin layer is formed (ALIBARDI & THOMPSON, 1999a). The process of accumulation of β-keratin takes place with a similar modality to that described in other reptiles (ALEXANDER, 1970 ; PARAKKAL & ALEXANDER, 1972 ; LANDMANN, 1986 ; MADERSON *et al.*, 1998).

From ES 25 onward, bundles of keratin filaments increase in the newly generated α-keratinocytes, and they mix with lipids and mucus, as previously reported in the adult epidermis (MATOLTSY & HUSZAR, 1972 ; MATOLTSY, 1987). While lipids and mucus appear to decrease in quantity, reticulate bodies completely disappear in α-keratinocytes.

At 2 days post-hatching, the embryonic epidermis is reduced to a thin dark layer that is partially or totally detached from the definitive stratum corneum. At 1 week post-hatching, some 10-20 layers of α-keratinocytes are already present and the germinative epithelium becomes cuboidal or even flat (SPEARMAN, 1969 ; MATOLTSY & HUSZAR, 1972), a condition that may be related to a slowing down of the process of keratinization. In fact, in lepidosaurian reptiles the epidermal flattening that follows the loss of the old epidermis is related to the resting phase in the production of suprabasal cells (MADERSON, 1985 ; LANDMANN, 1986 ; MADERSON *et al*., 1998).

It is not known whether the soft epidermis of the neck, limbs, and tail of some Chelonia is also subject to alternating periods of high and low proliferative activity, as in the case of the epidermis of shell scutes (SPEARMAN, 1966 ; ZANGERL, 1969).

Dermis

The initial mesenchyme at ES 16 rapidly becomes a dense connective tissue, by the accumulation of thick collagen bundles among fibroblasts. At ES 22, when the epidermis is still immature, the dermis already resembles that of the adult (MATOLTSY & HUSZAR, 1972 ; LANDMANN, 1986).

The high content of collagen fibrils among oriented fibroblasts determines a reinforcement of the dermis in support of the epidermis integrity during folding of neck, limbs and tail. Dermal cells also accumulate fat.

ACKNOWLEDGEMENTS

This study was supported 60% by an Italian MURST grant, and by the University of Bologna. Many thanks to Dr. M.B. Thompson and Mr. R. Spencer (University of Sydney) for hosting my visit, and supplying me with some eggs. Mr. R. Porter (The Reptile Park, Gosford, NSW) supplied most of the eggs. Ms. L. Montanari (Disegno Anatomico, Bologna) made the drawing, and Dr. M. Zordan (University of Padova) read the English text.

REFERENCES

ALEXANDER, N.J. (1970) – Comparison of a an b keratin in reptiles. *Z. Zellforsch.*, **110** : 153-165.

ALEXANDER N.J. & P.F. PARAKKAL (1969) – Formation of a and β-type keratin in lizard epidermis during the molting cycle. *Z. Zellforsch.*, **101** : 72-87.

ALIBARDI, L. (1997) – Ultrastructural and autoradiographical analysis of setae development in the embryonic pad lamellae of the lizard *Anolis lineatopus*. *Ann Sci Naturelles (Zoologie)*, Paris, **18** : 51-61.

ALIBARDI, L. (1998a) – Differentiation of the epidermis during scale formation in embryos of lizard. *J. Anat.*, **192** : 173-186.

ALIBARDI, L. (1998b) – Glycogen distribution in relation to epidermal cell differentiation during embryonic scale morphogenesis in the lizard *Anolis lineatopus. Acta Zool.*, **79** : 91-100.

ALIBARDI, L. & M.B. THOMPSON (1999a) – Epidermal differentiation during carapace and plastron formation in the embryo of the turtle *Emydura macquarii*. *J. Anat*,. **194** (in press).

ALIBARDI, L. & M.B. THOMPSON (1999b) – Morphogenesis of shell and scutes in the turtle *Emydura macquarii*. *Austral. J. Zool*,. **47** (in press).

BADEN, H.P. & P.F.A. MADERSON (1970) – Morphological and biophysical identification of fibrous proteins in the amniote epidermis. *J. Exp. Zool.*, **174** : 225-232.

BADEN, H.P., S. SVIOKLA & I. ROTH (1974) – The structural protein of reptilian scales. *J. Exp. Zool.*, **187** : 287-294.

BURKE, A.C. (1989a) – *Epithelial-mesenchymal interactions in development of the chelonian bauplan.* In «Trends in Vertebrate Morphology». Progress in Zoology vol. **35**. (H. SPLECHTNA and H. HILGERS, eds), Gustav Fisher Verlag, Stuttgart-New York. pp 206-209.

BURKE, A.C. (1989b) – Development of the turtle carapace : implication for the evolution of a novel bauplan. *J. Morph.*, **199** : 363-378.

BURKE, A.C. (1991) – The development and evolution of the turtle body plan : Inferring intrinsic aspects of evolutionary process from experimental embryology. *Am. Zool.*, **31** : 616-627.

DHOUAILLY, D. & P.F.A. MADERSON (1984) – Ultrastructural observations on the embryonic development of the integument of *Lacerta muralis* (Lacertilia, Reptilia). *J. Morph.*, **179** : 203-228.

EWERT, M.A. (1985) *Embryology of turtles*. In « Biology of the Reptilia » (C. Gans, R. Billett, P.F.A. Maderson, eds) vol **15** A Development., John Wiley & Sons New York. chapt. 3, pp 77-244.

FUKUYAMA, K. & W.L. EPSTEIN (1973) – Heterogeneous ultrastructure of keratohyalin granules : a comparative study of adjacent skin and mucus membrane. *J. Inv. Dermat.*, **61** : 94-100.

HENRIKSON, R.C. & A.G. MATOLTSY (1970) – Turtle epidermis : electron microscopic observations on a granular component of malpighian cells. *Aust. J. biol. Sci*., **23** : 241-244.

IWASAKI, S.C., T. ASAMI & C. WANICHAON (1996a) – Fine structure of the dorsal lingual epithelium of the juvenile hawksbill turtle, *Eretmochelys imbricata bissa*. *Anat. Rec.*, **244** : 437-443.

IWASAKI, S.C., C. WANICHAON & T. ASAMI (1996) – Histological and ultrastructural study of the lingual epithelium of the juvenile pacific ridley turtle, *Lepidochelys olivacea* (Chelonia, Cheloniidae). *Ann. Anat.*, **178** : 243- 250.

LANDMANN, L. (1979) – Keratin formation and barrier mechanisms in the epidermis of *Natrix natrix* (Reptilia : Serpentes) : an ultrastructural study. *J. Morph.*, **162** : 93-126.

LANDMANN, L. (1980) – Lamellar granules in mammalian, avian, and reptilian epidermis. J. Ultrastr. Res., **72** : 245-263.

LANDMANN, L. (1986) – The skin of Reptiles : epidermis and dermis. In *Biology of the integument* vol. **2** Vertebrates. (J. BEREITER-HAHN, A.G. MATOLTSY, K. SYLVIA-RICHARDS, eds). Springer Verlag Berlin-Haidelberg-New York : 150-187.

LAVKER, R.M. (1974) – Horny cell formation in the epidermis of *Rana pipiens*. *J. Morph.*, **142** : 365-378.

LAVKER, R.M. (1975) : Lipid synthesis in chick epidermis. *J. Inv. Dermatol.*, **65** : 93-101.

MADERSON, P.F.A. (1985) – *Some developmental problems of the reptilian integument*. In Biology of the Reptilia (C. Gans, F. Billett, P.F.A. Maderson, eds) vol. **14**, Dev B, New York : John Wiley & Sons : 525-598.

MADERSON, P.F.A.,T. RABINOWITZ.,B. TANDLER & L. ALIBARDI (1998) – Ultrastructural contributions to an understanding of the cellular mechanisms in lizard skin shedding with comments on the function and evolution of a unique lepidosaurian phenomenon. *J. Morph.*, **236** : 1-24.

MATOLTSY, A.G. (1969) – Keratinization of the avian epidermis. An ultrastructural study of the newborn chick skin. *J. Ultrastr. Res*., **29** : 438-458.

MATOLTSY, A.G. (1987) – *Cell and Molecular biology of keratins. Concluding remarks and future directions.* In Topics in Developmental Biology. **22**, (A.A. MOSCONA and A. MONROY, eds) (volume editor R.H. SAWYER). Acc. Press Inc. : 255-264.

MATOLTSY, A.G. & T. HUSZAR (1972) – Keratinization of the reptilian epidermis : an ultrastructural study on the reptilian skin. J. *Ultrastr. Res.,* **38** : 87-101.

MATOLTSY, A.G. & J.A. BEDNARZ (1975) – Lamellar bodies of the turtle epidermis. *J. Ultrastr. Res.*, **53** : 128-132.

MCFARLAND, W.N., F.H. POUGH, T.J. CADE & J.B. HEISER (1979) – *Vertebrate life*. Macmillan Publishing Co. Inc New York.

MENON, G.K., B. BROWN, P.M. ELIAS (1986) – Avian epidermal differentiation : role of lipids in permeability barrier formation. *Tiss. Cell*, **18** : 71-82.

MENON, G.K., P.F.A. MADERSON, R.C. DREWES, L.F. BAPTISTA, L.F. PRICE & P.M. ELIAS (1996) – Ultrastructural organization of avian stratum corneum lipids as the basis for facultative cutaneous waterproofing. *J. Morph*., **227** : 1-13.

MOTTET, N.K. & H.M. PJENSEN (1968) – The differentiation of chick embryonic skin *Exp. Cell Res.*, **52** : 261-283.

PARAKKAL, P.F. & G.A. MATOLTSY (1964) – A study of the fine structure of the epidermis of *Rana pipiens*. *J. Cell Biol*., **20** : 85-94.

PARAKKAL, P.F. & G.A. MATOLTSY (1968) – An electron microscopic study of developing chick skin. *J. Ultrastr. Res.*, **23** : 403-416.

PARAKKAL, P.F. & N.J. ALEXANDERL (1972) – *Keratinization, a survey of vertebrate epithelia*. Accademic press, New York & London.

RUCKES, H. (1929) – Studies on chelonia osteology (II). The morphological relationships between the girdles, ribs and carapace. *Ann. N.Y. Acad. Sci.,* **31** : 81-120.

SAWYER, R.H. & T.K. BORG (1979) – Avian scale development. VI. Ultrastructure of the keratinizing cells of reticulate scales. *J. Morph*., **161** : 111-122.

SAWYER, R.H., L.W. KNAPP & M.W. O'GUIN (1986) – *Epidermis, dermis and appendages*. In Biology of the skin (J. Bereither-Hahn, A.G. Matoltsy, R. Sylvia-Richards, eds), vol. **2** Vertebrates, Springer-Verlag, Berlin : 194-237

SPEARMAN, R.I.C. (1966) – The keratinization of epidermal scales, feathers and hairs. *Biol. Rev.*, **41** : 59-96.

SPEARMAN, R.I.C. (1969) – The epidermis of the gopher tortoise *Testudo polyphemus* (Daudin). *Acta Zool.* **50** :1-9.

WYLD, J.A. & A.H. BRUSH (1979) – The molecular heterogeneity and diversity of reptilian Keratins. *J. Molec. Evol.*, **12** : 331-347.

WYLD, J.A. & A.H. BRUSH (1983) – Keratin diversity in the reptilian epidermis. *J. Exper. Zool*., **225** : 387-396.

YNTEMA, C.L. (1968) – A series of stages in embryonic development of *Chelydra serpentina*. *J. Morph.*, **125** : 219-252.

ZANGERL, R. (1969) *The turtle shell*. In «Biology of the Reptilia» (C. Gans, ed) vol. **1**, Chapter 6. Academic Press, London and New York : 311-339.

Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 405-419 - Brussels 1999

Received : 23 November 1998

DENSITY AND BREEDING BIOLOGY OF THE BARN OWL *TYTO ALBA* (AVES, TYTONIDAE) ON THE TROPICAL ISLAND OF MAYOTTE

JAN STEVENS, MICHEL LOUETTE AND MARC HERREMANS

Royal Museum for Central Africa, Department Zoology, B-3080 Tervuren (Belgium)
e-mail : louette@africamuseum.be

Abstract. *Tyto alba* is widely distributed on Mayotte, an island group of 374 km² in the Comoro archipelago. The species was studied in August 1996 (dry season) and December 1997/January 1998 (wet season). Territorial response was high in December/January, and there were fledged young in August, indicating that breeding starts in the wet season and young fledge during the dry season. The species was inventoried at the beginning of the breeding season by using playback of calls during point-transect counts. Breeding densities were high, estimated at ca. 1.03 to 2.75 territories/km², based on three different methods of calculation. Possible sources of error are discussed, but this method will most likely produce useful results in other situations where Barn Owls respond well to playback. Breeding sites were mainly cavities in trees, but cliffs and, more rarely, buildings were also used. Breeding success seemed low : 1.62 young fledged per nest.

Key words : Barn Owl, *Tyto alba*, population, density, breeding biology, Mayotte, Comoros

INTRODUCTION

The Barn Owl *Tyto alba* (Scopoli, 1769) is a cosmopolitan species. In temperate regions it is rare and declining in many places, and generally ranks highly as a nature conservation priority. It is also much used as a bioindicator for habitat quality (BUNN *et al.,* 1982 ; SHAWYER, 1987). The Barn Owl is widespread in the tropics, where it is the most important nocturnal avian predator on many tropical islands. It can be beneficial to man as an agent to control commensal rodents, or it can be undesirable as an important threat to endemic animals or seabirds (HEIM DE BALSAC, 1965). In regions such as Malaysia its proliferation was stimulated in oil palm monocultures, resulting in successful rodent control (LENTON, 1984 ; BASRI WADID *et al.,* 1986 ; DUCKETT, 1991). However, its introduction to some islands in the Indian Ocean (*e.g.* granitic Seychelles), in order to achieve the same goal, resulted in catastrophic predation on seabirds (PENNY, 1974), rather than rodent control. In the nearby Comoros (including Mayotte), where it occurs naturally, it does, however, feed to a very large extent (over 90 %) on commensal rats (LOUETTE, 1996 ; ERVYNCK *et al.,* 1998).

Accurate knowledge of Barn Owl density, and monitoring of its population are needed on Mayotte, in order to evaluate, and possibly enhance and exploit its rodent control

potential. Being a nocturnal bird, standard techniques to estimate bird densities (BIBBY *et al.,* 1992) do not apply. In this paper we discuss the application of a combination of techniques to estimate the density of Barn Owls, and we investigate some aspects of its breeding biology on Mayotte.

Mayotte, the easternmost group of islands in the volcanic Comoro archipelago, covers 374 km² (Fig. 1), attains a maximum altitude of 660 m, has fertile soils and, in 1991, had ca. 94,500 human inhabitants (BOLE & CIBARD, 1994), living mostly at the coast. Shifting cultivation and forest reserves alternate with coastal villages and mangrove tracts ; some badlands occur on eroded slopes. There is a marked gradient of rainfall in relation to altitude (RAUNET, 1992).

The subspecies *hypermetra* (BUNN *et al.,* 1992) of the Barn Owl, considered as a synonym of *affinis* (BENSON, 1960 ; LOUETTE, 1988) is known from all the Comoro islands. Observations prior to our 1996-1998 study are sparse, however. BENSON (1960) and FORBES-WATSON (1969) mentioned the species from the small annex islet of Pamandzi. LOUETTE (1988) confirmed that location, and also noted its presence at Dzaoudzi and Mamoudzou, and added that it is no doubt distributed widely on « Grande Terre ». BENSON (1960) indicated that this species is partly diurnal on Mayotte, because he observed owls at the airport of Pamandzi : one at mid-day, two others hunting over grassland an hour before sunset, and another seen on the ground devouring some prey at the same time. LOUETTE (1988) also noted Barn Owls active on Mayotte during the day. Most observations by BENSON (1960) were from forested regions, but LOUETTE (1988) observed the species also in inhabited environments. There are no breeding data available from the Comoros. Based on its nesting behaviour elsewhere, LOUETTE (1988) mentioned that the species might use cavities, cliffs and buildings.

MATERIAL AND METHODS

Questionnaire and field inventory

BIBBY *et al.* (1992) recommended two separate methods for the inventory of Barn Owls in Europe, both with the bird in its territory as the counting unit. On the one hand, they suggested the use of questionnaires on the bird, and on the other hand, the rigorous search for nests at potential nesting sites (*e.g.* useful buildings) in the survey area. However, since we lacked information on the breeding period or the breeding habitat of Barn Owls on Mayotte, we combined the questionnaire method with other inventory techniques.

In August 1996 local people were questioned. During the presentation of a stuffed specimen, we asked standard questions of a representative number of persons in 55 villages, distributed over the whole island. These questions concerned their knowledge about the presence of the Barn Owl in their neighbourhood : preferred habitat, nesting site and nesting period, population trends, and human impact on the species. We also searched for nesting sites ourselves, in restricted areas near Dembeni and M'rereni. During daytime, we checked all trees for possible cavities, owl droppings and the presence of pellets. Each nesting site, discovered from the presence of young owls or by the accumulation of pellets, was described (situation, species of tree, height and dimensions of the cavity).

Point counts

A point-transect-count technique was tested in August 1996, and repeated in December-January 1997/98. During the counts we used playback of Barn Owl vocalisations from a cassette player. In western Europe, the species is reputed to react poorly to this sound (HUSTINGS *et al.,* 1985), but SMETS (pers. comm.) observed some cases of good response in central Belgium at the beginning of the breeding season, particularly with newly established pairs and in situations of high density. In Mayotte, Barn owls did respond quite well. Playback has also been used elsewhere for the inventarisation of other owl species (*e.g.* LYNCH & SMITH, 1984 ; SOLHEIM, 1986 ; VAN NIEUWENHUYSE & NOLLET, 1990 ; KOWALSKI *et al.,* 1991). Calls were played at night during five minutes, from points at 500 m intervals along roads and tracks, between 1900 h and 2300 h. The chosen distance of 500 m between points was based on the size of territories reported in the literature. Each playback included calls of the male, the female, and young (copied from PALMER & BOSWALL's bird songs cassettes) ; playback was repeated three times for one minute, while three observers watched (using torchlight frequently) and listened for a possible response during two more minutes after playback.

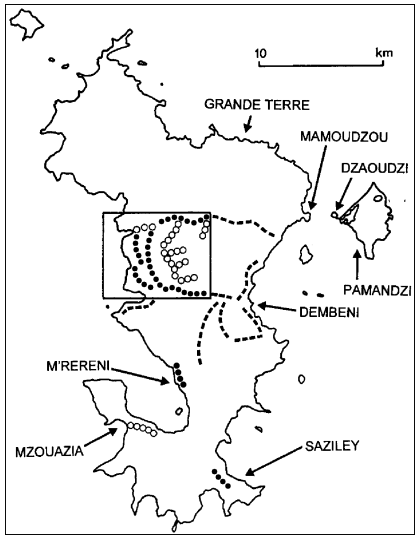


Fig. 1. – Position of the point-count transects on Mayotte. Dashed lines : counts in 1996. Open circles : counts in 1997/1998. Filled circles : counts in both years. Inset : the west-central study sector.

In 1996, counts were concentrated in the central part of the island (Fig. 1). In 1997/98, the west-central region (inset) was chosen as a restricted study area because of its comparatively dense network of roads and tracks. Outside this sector, counts were made in 1997/98 along other transects as follows : on the ridge near Saziley (south-eastern peninsula), a dry area with the vegetation dominated by Baobabs *Adansonia digitata*, near Mzouazia (south-west), a dry area dominated by *Albizzia lebbeck* trees, and near M'rereni (western coast), an area under intense cultivation.

Categories of response

Response of Barn Owls to playback of their vocalisations was classified in the following five categories :

1. *Presence of a pair*

Two adult owls were heard and/or seen during the count. The distinction between partners could sometimes be made from their behaviour (both birds present without mutual aggression but with a co-ordinated reaction in vocalisation and while flying about the playback spot), or from their vocalisations.

2. *Presence of two individuals not belonging to the same pair*

Either two males were present simultaneously, being recognised by mutual aggression, or two individuals were present which could not be assigned to one pair, according to their behaviour.

3. *Presence of a single individual*

In most cases, at each point, only a single owl responded. A distinction was made between early and late reaction and between strong and weak reaction. The intensity of the response varied from flying towards the tape and perching silently, to flying about repeatedly during the entire counting period in search of the source of the sound, while calling regularly.

4. *Presence of the same individual at different points*

In a number of cases, it was clear that the same individual responded at consecutive points. A bird could respond at one point and subsequently follow the observers to the next point (then first calling at a distance, from the direction of the previous point, followed by calls emitted progressively closer by, eventually approaching the second point).

5. *Presence of juveniles*

A number of juveniles were encountered, sometimes in groups, producing the characteristic begging call. They were also recognised by their plumage, which is more yellowish than in adults, especially on the throat and breast. Sometimes the adults were also seen nearby. A number of nests were found on subsequent visits during the day near spots where fledged juveniles had been recorded.

Calculations of density

The point counts in the west-central sector enabled us to attempt an estimate of the density of the Barn Owl population on Mayotte. Three methods were used :

1. The number of territories found, divided by the total surface studied, gave an estimate of the (minimal) density (D_t).

2. From evidence in the field (*e.g.* birds responding at two consecutive points 500 m apart and a number of field tests of audibility of calling juveniles), we concluded that birds responded from distances of up to 250 m. Dividing the number of territories by the sur-

face inventoried as such (points with a radius of 500 m), we obtained a second measure of density (D_e).

3. Finally, we used the nearest-neighbour-distances (NND). The mean NND of the (presumed) centres of territories was considered as the average diameter of the territories. If they were adjoining and covering the entire study area, the (maximal) density could be estimated.

RESULTS

Questionnaire and field inventory

For the detailed results of the questionnaires see LOUETTE *et al.* (1996). It is sufficient to mention here that the Barn Owl is known in 54 of the 55 villages of Mayotte. It is observed mostly around the perimeter of the village (15 times), in rural habitats (13 times), and infrequently in forests (five times). Most persons (21) named trees as the main nesting site, two indicated buildings, and one person indicated cliffs. Few persons (six) had (contradictory) ideas about the breeding period. Fourteen out of 15 informants indicated a declining population. In 10 out of 12 villages, people were convinced that humans, especially children, catch and kill Barn Owls, because they are widely considered to be a 'harbinger of evil'.

In the neighbourhood of Dembeni (on nearly 350 ha), 336 potential nest trees (310 Mango *Mangifera indica*, 11 West Indian Almond *Terminalia catappa*, eight Kapok *Ceiba pentandra*, six *Erythryna* sp., one Baobab) were investigated for signs of Barn Owl presence. We observed a nest in one Mango, pellets under another one and droppings under three more trees.

In M'rereni we checked 318 *Erythryna,* four Mango trees, and three West Indian Almonds, but found only one pellet.

Point counts

In 1996, owls were recorded at 21 out of 134 points (15.6%); in 1997/98 at 52 out of 135 (38.5 %). The comparison of the number of positive and negative points along the transects counted in both years shows no significant relationship ($X^2 = 0.20$, $p>0.50$). The density analysis was restricted to the data obtained in 1997/98 in the west-central study sector (Fig. 1). Here, owls were recorded at 44 out of 107 counted points (41.1%). The counting points with a pair were always considered as territories. Those with one adult were considered as territories if they were not already situated in a territory covered by neighbouring points (Fig. 2). In a number of cases, two adjoining positive recording points were included in a single territory. Very few cases left us undecided. Fig. 3 shows the position of the (presumed centres of) territories in the west-central sector as deduced from the data in Fig. 2. In an attempt to extrapolate the data from this sector to the whole island of Mayotte, the counts in Saziley, M'rereni and Mzouazia were used (LOUETTE, 1998). It is interesting that, from the ridge of Saziley in August 1996, no response of Barn Owls was obtained, but in December-January 1997/98, five counting points yielded six territories there.

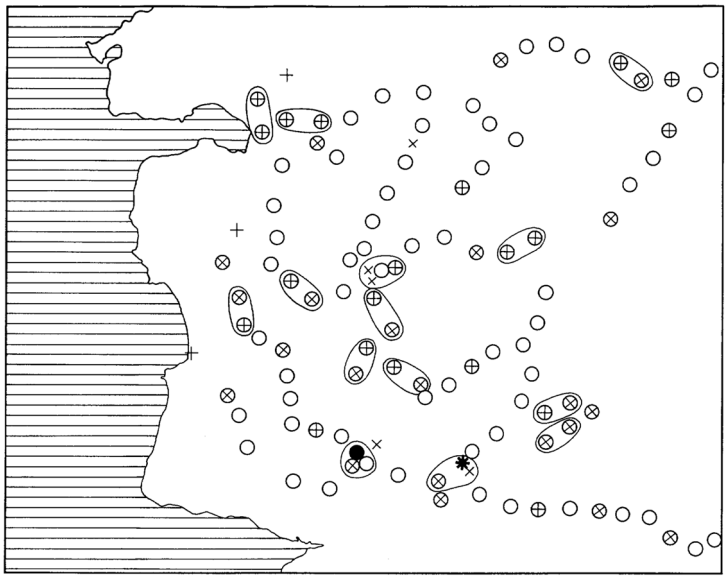


Fig. 2. – The west-central study sector on Mayotte. Results at each counting point in 1997/1998 during the transects (encircled) : open circles : no response ; + : poor response ; x : heavy response ; filled circle : response of a pair. A few observations outside the transects added (not encircled ; asterisk : response of a pair). In ellipses : adjoining points, presumed to have yielded responses from (a bird of) the same pair.

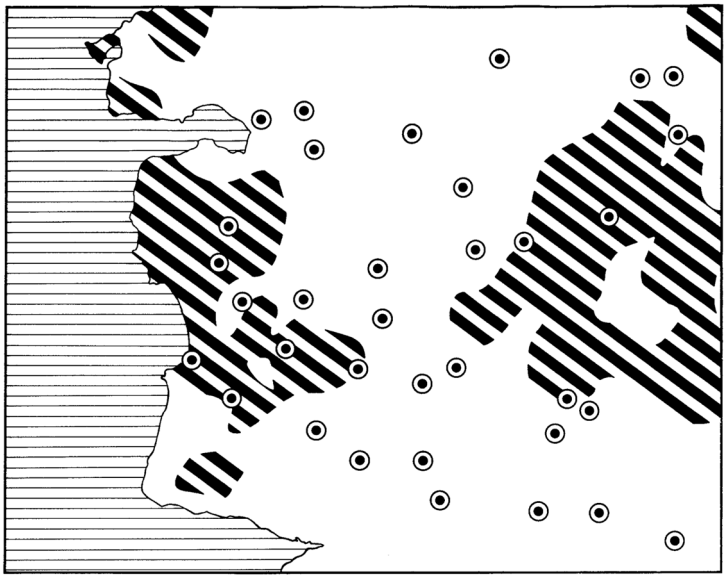


Fig. 3. – The west-central study sector on Mayotte. Forested areas shaded. Stippled circles : presumed centres of territories.

We compared the response of Barn Owls between dirt tracks (excluding one which supported intense traffic) and tar roads (LOUETTE, 1998). During both years, no significant difference in Barn Owl response could be detected between the two road types (1996 : X^2= 0.69, p>0.30 ; 1997/98 : X^2= 0.20, p>0.50).

Calculations of density

From the data of 1997/98, the different methods resulted in the following estimates :

1. D_t

The total surface in the west-central sector amounts to 33 km^2. The number of territories was 34. The corresponding (minimal) density was 1.03 territories per km^2.

2. D_e

The counting points (circles with a diameter of 500 m) represented a studied surface of 107 x 0.1962 km^2 = 20.9 km^2, yielding 34 territories. This corresponds to 1.62 territories per km^2.

3. NND

The mean NND was 647 m, which gave an average territory size of 0.363 km^2 (if hexagonal-shaped). This corresponded to a (maximal) density of 2.75 territories per km^2.

With a mean NND of 647 m and 34 territories on 33 km^2, the territories were distributed regularly (P = 0.00023 ; test for significance of departure of the observed NND from the expected NND for a randomly distributed population density via the « standardized normal deviate » – WRATTEN & FRY, 1980). Because the mean NND was considerably larger than the distance between counting points, there was no interference with the quality of the test.

Choice of nesting place

Table I lists the 20 nesting sites found on Mayotte. All nests were situated in a cavity : 16 in trees, two in buildings and two in cliffs. Of the 16 tree nests, five were in an *Erythryna*, four in a Mango, four in a West Indian Almond, two in a Baobab, and one in a Takamaka *Calophyllum inophyllum.* The cavity was, in general, high in the tree, but its dimensions varied strongly. The depth of the cavity varied from 2 cm to 2 m. In the former, the nest was simply made on an open platform in the trunk. The most aberrant nest was found in an old Baobab tree with a hollow trunk and large, hollow roots. The nest was actually situated about 1.5 m beneath ground level.

The trees containing a Barn Owl nest were situated mainly in a rural environment, near villages, frequently in valleys near water courses. Probably because of the difficulty of finding them, we located no nests in forests. The counts indicated however that the birds were present in this habitat as well, and Fig. 3 suggests that the density was no less in forests.

One nest in a building was situated on the garret, just under the roof. The entrance was about 50 cm in diameter. The two nests in cliffs were about 6 m and 20 m above the ground

TABLE I

Description of the Barn Owl nests found on Mayotte in 1996 and results of their control in 1997/1998 (plus two additional nests of 1997/1998)

Dates 1996	1997/1998	Localities	Nest support	Cavity dimension in cm	Height above ground in m	Number of adults present in 1996	Number of juveniles present in 1996	Occupied in 1997/1998 ?
27 VII	19 XII	Musicale plage	Baobab		5/6	2	1	no
29 VII	19 XII	Mramadoudou	*Erythryna*	20 x 25	4	2	2	no
29 VII	19 XII	Mtsanga Sakouli	Baobab	150	1,5	2	1	1 ad
30 VII	20 XII	Pointe Mahabou	Cliff	rock crevice	20	2		no
30 VII	21 XII	Sohoa plage	WIA (1)			2	2	no
30 VII		Barakani	Mango	25-35 x 150	3/4			
30 VII	24 XII	Ongojou	Mango	35 x 100	4		2	no
31 VII	27 XII	Bouyouni	*Erythryna*	30 x 150	7			no
31 VII		Hamjago	Garret	∅ 50	3		1	
1 VIII		Sada-Doujani	Roof		3,5			
1 VIII	28 XII	Mouanatrindri	Takamaka	30 x 80	5			(2)
1 VIII	19 XII	Kani-Be	WIA		9/10	1	1	1 ad
1 VIII	19 XII	Nyambadao	WIA				2	no
7 VIII	9 I 98	Mavingoni	*Erythryna*	30 x 100	5	2	3	no
10 VIII	24 XII	Combani	*Erythryna*	30 x 100	8		2	no
13 VIII	21 XII	Sohoa	Mango	20 x 30	5	1	1	no
15 VIII	9 I 98	Tsararano	*Erythryna*	50 x 30	6	1	2	no
19 VIII	19 XII	M'lamamani	Mango	∅ 25	8/10		1	no
	17 XII	Dzaoudzi	Cliff	rock crevice	5/6			no
	23 XII	Chembenyoumba	WIA	30 x 30	5			no

(1) WIA = West Indian Almond.
(2) Occupied by Indian Mynah *Acridotheres tristis.*

or the sea, respectively. The latter nest-site was a large ledge of some tens of m^2 in a sea cliff. The other was a typical cavity with an entrance of about 50 x 100 cm diameter, and extended several m deep.

Breeding success

The number of juvenile birds actively begging for food in the vicinity of the nest sites in August 1996 was an indication of the breeding success. In thirteen nests (including one in a building containing one nestling) we found an average of 1.62 fledglings (one with 3, six with 2 and six with 1).

DISCUSSION

Questionnaire and field inventory

The Barn Owl is indeed widespread on Mayotte. The local people in all regions of the island know the species very well. During our questionnaire, people led us to seven active nests. These data complete the scanty information previous ornithologists obtained during the past decades (BENSON, 1960 ; FORBES-WATSON, 1969 ; LOUETTE, 1988). These preliminary data allowed us to further investigate the species efficiently. However, random investigation of potential nest trees without prior consultation with local informants is definitely a time-consuming technique, yielding poor results.

Breeding period

During the counts in December-January 1997/98, many birds responded strongly to playback. We observed strong territorial aggression, sometimes between pairs. These observations indicated that the birds were in the pre-breeding or early breeding period. On the other hand, in August 1996, hardly any territorial behaviour was observed. The presence of fledglings, the observation of only one young remaining in the nest, and the presence of fresh pellets near nests indicated that the breeding season was then near its end. Furthermore, the occurrence of begging young decreased progressively during August : begging juveniles encountered on consecutive days in the first weeks were not found again during the last days of the month. This indicated that the last fledglings became independent by mid August. The breeding cycle of the Barn Owl in Europe spans about five months (seven for the whole population) (CRAMP, 1985 ; BUNN *et al*., 1992). In Madagascar, breeding is reported from April and May (GOODMAN *et al*.,1993). In southern Africa, the breeding period is extended, but is mainly in the cool, dry season (WILSON *et al*., 1988 ; MENDELSOHN, 1997).

Combining our observations of both study periods, and the knowledge of the length of time Barn Owls need to accomplish their breeding cycle, we conclude that the breeding period on Mayotte starts in December-January (the beginning of the rainy season), and the last young become independent in August (in the second half of the dry season). This means that most Barn Owls have nestlings in the period April, May and June (the start of

the dry season) and that most fledglings will leave the nests in July (the middle of the dry season). (Climatological data from BATTISTINI & VERIN, 1984). Since our observations are from two different breeding cycles, the possibility remains, however, that the breeding cycle is not strictly timed but could be tuned *e.g.* to the intensity of the rainy season.

The lower responsiveness during the counts in August in comparison with December-January was due to the season. The absence of any response during the counts in Saziley in August 1996 could have been due to an earlier or shorter breeding season in the dry southern part of the island in comparison with the breeding season in the wet parts of the island.

Choice of nesting place

In Africa, Barn Owls nest readily in rock crevices or caves, and in hollow trees, far from human habitations (ARCHER & GODMAN, 1961 ; WILLIAMS, 1963 ; SERLE *et al*., 1977) ; in certain areas in Europe nesting on sea-cliffs has been reported (CRAMP, 1985). The proportion of 80% of nests in hollow trees, as found in Mayotte, is high with respect to information from Europe (BEZZEL, 1985 ; BUNN *et al*., 1992 ; DE JONG, 1995), but probably comparable with Africa and Madagascar. The low number found in Mango trees is somewhat surprising, because this tree is the most common one over wide areas on Mayotte. However, it probably is generally a « healthy » tree, only rarely presenting cavities adequate for owl nests. Since it is mostly old trees that are hollow and represent important nesting sites, it is alarming that in some areas (*e.g.* M'rereni) they are cut and burned on a large scale for cultivation.

Breeding success

The figure of 1.62 fledglings per nest may be an underestimation of the actual breeding success. We cannot be certain that the number of juveniles observed was the number really present. Nevertheless, we probably would have heard other juveniles had they been present. They are very noisy and can be heard over large distances while begging at this stage (pers. obs.). If the number of juveniles observed did represent complete sets, breeding success on Mayotte is relatively low. In the different parts of Europe, mean breeding success varies between 2.7 and 5.2 young fledged (dependent on prey abundance : BAUDVIN, 1975 ; BRAAKSMA & BRUIJN, 1976 ; BEZZEL, 1985 ; BRUIJN, 1994 ; DE JONG, 1995).

Breeding density

Evaluation of the field methods

Accurately assessing the population density of Barn Owls in a tropical environment, during a period of a few weeks as a visiting researcher, is no easy matter, particularly because most owls breed in hollow trees in remote areas. Methods for inventory used *e.g.* in Europe (questionnaires of owners of potential buildings, and monitoring of known nesting sites) are of no use here. We decided to use point counts and playback. At variance with Barn Owl behaviour in Europe, response to playback was strong on Mayotte, which greatly improved the quality of our data. Potential sources of error are, however, numerous.

The population might be underestimated if not all territory-holders responded. During the pre-breeding period at the onset of the wet season, however, this seems unlikely in view of the strong and often prompt response of most birds.

Underestimation could also be the case if individuals belonging to different territories were considered to be one and the same. This error is unlikely to be important, because adjacent birds could often be recognised by their behaviour (aggressiveness, response of pairs). Overestimation due to double counting of the same individual is also unlikely. In a number of cases we were able to trace the same individual from one count point to the other. Such points therefore belonged to a single territory**.** If the same birds were regularly counted twice from adjacent points and later considered as belonging to different territories, we would expect our results to show a clustering of territories, which was not the case.

An overestimate due to separate registration of males and females is unlikely at this stage of the breeding cycle, when pair formation takes place. If unpaired individuals were also territorial and responded to playback, this would result in an overestimation of the breeding population. This possibility cannot be ruled out and is difficult to assess ; we assume, however, that the proportion of territorial, unpaired birds is likely to be small in a high density population.

Another factor possibly interfering with the quality of the data is the noise and disturbance from traffic during observations, possibly causing observers to be distracted and owls to be scared off, but this factor was not found to be significant.

The comparison of positive and negative points indicated that the territories found were not situated at the same sites. However, at particular points it was very clear that intense response was obtained in both years. This was especially so where villages and fields alternated. Possibly villages were good beacons between territories, and in these modified habitats the number of potential nesting trees was more limited anyhow, resulting in the same sites being re-used.

Evaluation of density calculations

According to the distance at which owls still responded and at which owls could still be recorded by the observers (either visually or acoustically), part of the 33 km^2 of the study area was, in fact, not addressed from any of the playback points, notwithstanding the dense network of roads and tracks in comparison with the rest of the island. So the first density estimate (D_t) of 1.03 territories/km^2 was an underestimation.

According to the second method (D_E), with an effectively inventoried surface of circles with 250 m radius per counting point, only 20.9 km^2 were effectively inventoried, and the 34 territories represented in that case an effective density of 1.62 territories/km^2. In suitable habitat, this would also represent the « ecological density » (*sensu* VERHEYDEN, 1991). Along with the variation according to local circumstances (*e.g.* topography and vegetation density), there remains the problem that some individuals probably could be heard from a greater distance than others. Some might have been missed. From field observations (*e.g.* birds responding at two consecutive points 500 m apart, and a number

of field tests of audibility of calling juvenile birds), the radius of 250 m seems to be the « best possible » average in the given circumstances.

For the third method, we assume that the (mean) NND between two territories equals two times the apothem of the supposedly hexagonal territory. Indeed, all territories supposedly touch one another, without gaps in between (otherwise circles would have to be used). The mean NND was 647 m, which corresponds to a density of 2.75 territories/km^2, if hexagons are used. But it is probably wrong to think that the whole surface was occupied at the same rate (= density) or that one could hope to obtain a response at each point (indeed, there were quite a number of points without response). Therefore, in reality, this density estimate is probably exaggerated. A remaining problem is that the NND could only be measured with an accuracy of ca. 500 m, because the counting points were situated at that distance from each other.

The comparison of density (D_t) with mean NND shows the owls were spread out extremely regularly in the west-central sector. No doubt this was due to territorial activity, which in turn determines the population size to a great extent. It suggests furthermore that available nesting places and the amount of food were less important determinants of population size in this sector.

Extrapolation to the whole island

The study area is situated in the wet west-central sector of Mayotte. The few transects counted in the drier parts gave the same response rate, suggesting that densities were similar. On the Saziley peninsula, the proportion of positive points was particularly high, probably because the counts from the ridge addressed a wider landscape. On the other hand, the less successful search for nests in the near Dembeni showed that densities in parts of the island under intense cultivation were lower. The density in unbroken forest remains unknown.

With the density of the study area extrapolated to the whole island, we estimate respectively 385, 606 and 1141 territories on Mayotte, for the three methods of density estimation. Given the methodological discussion above, we estimate the total population to be between 500 and 1000 pairs, possibly near 750 pairs.

Breeding density

In western Europe, where grassland habitats tend to be fragmented, average breeding densities of between 1 and 10 breeding pairs (bp)/50 km^2 are the norm. They rarely exceed 12-25 bp/50 km^2 (Osieck & Shawyer, 1997). Cramp (1985) listed some high densities in parts of Europe. In lowland Scotland 23 pairs breed per 50 km^2. In central Europe one pair per village is the rule. Exceptionally, in Switzerland four pairs were found in one area of one km^2. Indeed, locally the Barn Owl can breed in high densities, but over large areas this density flattens off. The European values are 50-100 times lower than those attained on nestbox-rich oil-palm estates in Malaysia where prey-saturated habitats are vast and contiguous (Taylor, 1994). It is therefore possible that the breeding density on Mayotte could have been higher previously as suggested by our informants. Nevertheless, a population density of ca. 2 bp/km^2 over a large surface on Mayotte is high compared with data in the literature, which mostly relates to continents and moderate cli-

mates. However, on another group of tropical islands, the Galapagos, densities of comparable magnitude have been found (De Groot, 1983). In general, population density seems to be regulated by the number of nesting or roosting sites (Cramp, 1985 ; Osieck & Shawyer, 1997). This is decidedly not the case in our study area. By 1998, Barn Owls had not started to use a single one of the 11 nestboxes placed, in 1996, in the central area of Mayotte (Louette, 1998). Territorial activity was the most obvious limiting factor in the investigated part of Mayotte. The high density may be due to one or more of the following factors : the near-absence of other avian predators, and the presence of abundant food supplies (commensal rats) (Louette, 1999). There is no systematic destruction of Barn Owls on the island, although localised disturbance may affect the population (in villages and urban areas). Over the whole study area enough nesting sites seem to be available, but the lower density in regions under intense cultivation could be due to localised shortage of nesting sites.

ACKNOWLEDGEMENTS

Werner Plompen, Pieter Stevens, Diane Tonnoeyr, Alain Pibot and Nathalie Van Moeffaert were helpful with some of the counts. Ludo Smets assisted in working out the count methodology. Alain Reygel prepared the figures and Wim Tavernier typed the table. This study became possible thanks to Jacques Fourmy and Jean-Yves Cousin, successive directors of the « Service de l'Environnement et de la Forêt (Direction de l'Agriculture) de Mayotte ». The 1996 campaign was financed by the DAF/SEF, the 1997/1998 campaign by the European Union (PIN-Mayotte, FED).

REFERENCES

Archer, G. & E.M. Godman (1961) – *The birds of British Somaliland and the Gulf of Aden. Vol. 3.* Gurney & Jackson, London : 627-1042.

Basri Wahid, M., S. Ismail & N. Kamarudin (1986) – The extent of biological control of rats with Barn Owls, *Tyto alba javanica* in Malaysian oil palm plantations. *The Planter Kuala Lumpur*, **72** : 5-18.

Battistini, R & P. Vérin (1984) – *Géographie des Comores*. Agence de Cooperation culturelle et technique, Editions Fernand Nathan, Paris. 142 pp.

Baudvin, H. (1975) – Biologie de reproduction de la chouette effraie (Tyto alba) en Côte d'Or : premiers résultats. *Le Jean le Blanc*, **14** : 1-51.

Benson, C.W. (1960) – The birds of the Comoro islands : results of the British Ornithologists' Union Centenary Expedition, 1958. *Ibis*, **103B** : 5-106.

Bezzel, E. (1985) – *Kompendium der Vögel Mitteleuropas. Nonpasseriformes.* Aula-Verlag Wiesbaden. 792 pp.

Bibby, C.J., N.D. Burgess & D.A. Hill (1992) – *Bird Census Techniques.* Academic Press, Harcourt Brace & Company, London. 257 pp.

Bole, J. & C. Cibard (1994) – *Découvrons Mayotte : Géographie*. Direction de l'enseignement, Mayotte. Hatier, Paris. 96 pp.

Braaksma, S. & O. de Bruijn (1976) – De Kerkuilstand in Nederland. *Limosa,* **49** : 135-187.

BRUIJN DE, O. (1994) – Population ecology and conservation of the Barn Owl, *Tyto alba* in farmland habitats in Liemers and Achterhoek. *Ardea,* **82** : 1-109.

BUNN, D.S., A.B. WARBURTON & R.D.S. WILSON. (1992) – *The Barn Owl*. T & AD Poyser, London. 264 pp.

CRAMP, S., (ed). (1985) – *Handbook of the birds of Europe, the Middle East and North -Africa*. *Vol 4.* Oxford University Press, Oxford. 960 pp.

DE GROOT, R.S. (1983) – Origin, status and ecology of the owls in Galapagos. *Ardea*, **71** : 167-182.

DE JONG, J. (1995) – *De Kerkuil.* Friese Pers Boekerij, Leeuwarden. 136 pp.

DUCKETT, J.E. (1991) – Management of the Barn Owl (*Tyto alba javanica*) as a predator of rats in oil palm (*Elaeis guineensis*) plantations in Malaysia. *Birds of Prey Bulletin*, **4** : 11-21.

FORBES-WATSON, A.D. (1969) – Notes on birds observed in the Comoro islands on behalf of the Smithsonian Institution. *Atoll Res. Bull*., **128** : 1-23.

GOODMAN, S. M., O. LANGRAND & C.J. RAXWORTHY (1993) – The food habits of the Barn Owl *Tyto alba* at three sites on Madagascar. *Ostrich*, **64** : 160-171.

HEIM DE BALSAC, H. (1965) – Quelques enseignements d'ordre faunistique tirés de l'étude alimentaire de *Tyto alba* dans l'ouest de l'Afrique. *Alauda,* **33** : 309-322.

HUSTINGS, M.F.H., R.G.M. KWAK, P.G.M. OPDAM & M.J.S.M. REYNEN (1985) - *Vogelinventarisatie. Achtergronden, richtlijnen en verslaggeving*, PUDOC, Wageningen. 495 pp.

KOWALSKI, M., T. LIPPOMAN & P. OGLECHI. (1991) – Census of owls Strigiformes in the eastern part of Kampinos National Park (Central Poland). *Acta Ornithologica (Warsaw)*, **26** : 23-29.

LENTON, G.M. (1984) – The feeding and breeding ecology of barn owls *Tyto alba* in peninsular Malaysia. *Ibis*, **126 :** 551-575.

LOUETTE, M. (1988) – Les oiseaux des Comores. *Annls. Mus. R. Afr. Centrale*. *(Zool.)*, **255** : 1-192.

LOUETTE, M. (ed) (1996) – *Lutte biologique contre les rats sur Mayotte*. Rapport : Musée Royal de l'Afrique Centrale, Tervuren, Belgique. 50 pp.

LOUETTE, M. (ed) (1998) – *Lutte biologique contre les rats sur Mayotte*. Deuxième Rapport : Musée Royal de l'Afrique Centrale, Tervuren, Belgique. 42 pp.

LOUETTE, M. (ed) (1999 in press) – La Faune terrestre de Mayotte. *Annls. Mus. R. Afr. Centrale*. *(Zool.).*

LYNCH, P.J. & D.G. SMITH. (1984) – Census of eastern screech-owls (*Otus asio*) in urban open-space areas using tape-recorded song. *American Birds*, **38** : 388-391.

MENDELSOHN, J.M. (1997) – Barn Owl. In : *The Atlas Southern African Birds. Vol. 1,* J.A. HARRISON, D.G. ALLAN, L.G. UNDERHILL, M. HERREMANS, A.J. TREE, V. PARKER & C.J. BROWN (eds). BirdLife South Africa, Johannesburg : 574-575.

OSIECK, E & C. SHAWYER (1997) – *Tyto alba* Barn Owl . In : *The EBCC Atlas of European Breeding Birds : their distribution and abundance,* E.J.M. HAGEMEIJER & M.J. BLAIR (eds). T & AD Poyser, London : 398-399.

PALMER, S. & J. BOSWALL (s.d.) – *A field guide to the Bird Songs of Britain and Europe*. Swedish Radio Company, Stockholm. 16 cassettes.

PENNY, M. (1974) – *The birds of Seychelles and the outlying islands*. Collins, London. 160 pp.

RAUNET, M. (1992) – *Ile de Mayotte (Archipel des Comores, Océan Indien). Les facteurs de l'érosion des terres et de l'envasement du lagon*. CIRAD, Université de la Réunion. 68 pp.

SERLE, W., G.J. MOREL & W. HARTWIG (1977) – *A field guide to the birds of West Africa*. Collins, London. 351 pp.

SHAWYER, C.R. (1987) – *The Barn Owl in the British Isles. Its past, present and future*. The Hawk Trust, London. 112 pp.

SOLHEIM, R. (1986) – Provocation of Ural owls *Strix uralensis* by song imitation in a sparse population in east Norway. *Fauna (Oslo)*, **39** : 81-84.

TAYLOR, I. (1994) – *Barn Owls : Predator-prey relationships and conservation*. Cambridge University Press, Cambridge. 304 pp.

VAN NIEUWENHUYSE, D. & F. NOLLET. (1990) – Een onderzoek naar het verspreidingspatroon van de Steenuil *Athene noctua* in relatie met enkele landschappelijke kenmerken binnen de gemeente Meulebeke (West-Vlaanderen). *Oriolus*, **56** : 50-55.

VERHEYDEN, C. (1991) – Une nouvelle méthode pour évaluer les densités de rapaces nocturnes et leur utilisation de l'habitat. *L'Oiseau et R.F.O.*, **61** : 18-26.

WILLIAMS, J.G. (1963). – *A field guide to the birds of East and Central Africa*. Collins, London. 288 pp.

WILSON, R.J., M.P. WILSON & C.H. FRY (1988) - Tytonidae. In : *The birds of Africa. Vol. 3,* C.H. FRY, S. KEITH & E. URBAN (eds). Academic Press, London : 105-110.

WRATTEN, S.D. & G.L.A. FRY (1980) – *Field and laboratory exercises in Ecology*. Edward Arnold, Scotland, 227 pp.

Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 421-438 - Brussels 1999

Received : 25 November 1998

RÉVISION DE *ZANCLITES XENURUS*, TÉLÉOSTÉEN (PISCES, TSELFATIIFORMES) MARIN DU SANTONIEN (CRÉTACÉ SUPÉRIEUR) DU KANSAS (ÉTATS-UNIS)

LOUIS TAVERNE

Université Libre de Bruxelles (U. L. B.), Faculté des Sciences,
Département de Biologie animale, Laboratoire de Morphologie fonctionnelle (C. P. 160/11),
avenue F. D. Roosevelt 50, B-1050 Bruxelles, Belgique

Résumé. L'auteur étudie l'ostéologie de *Zanclites xenurus*, un téléostéen fossile du Crétacé supérieur marin du Kansas. Il redéfinit le genre, établit ses autapomorphies, confirme son appartenance aux Tselfatiiformes (= Bananogmiiformes) et discute de ses affinités au sein de ce taxon.

Mots clefs : *Zanclites xenurus*, Teleostei, Tselfatiiformes, Santonien marin, Kansas, ostéologie.

Revision of *Zanclites xenurus*, marine teleost (Pisces, Tselfatiiformes) from the Santonian (Upper Cretaceous) of Kansas (United States)

Abstract. The author studies the osteology of *Zanclites xenurus*, a fossil teleost from the marine Upper Cretaceous of Kansas. He redefines the genus, shows its autapomorphies, confirms its belonging to the Tselfatiiformes (= Bananogmiiformes) and discusses about its relationships within this taxon.

Key words : *Zanclites xenurus*, Teleostei, Tselfatiiformes, marine Santonian, Kansas, osteology.

INTRODUCTION

Zanclites xenurus Jordan, 1924 est un genre monospécifique provenant des gisements du Santonien (Crétacé supérieur) marin du Kansas, Etats-Unis (SCHULTZE *et al.*, 1982 : 35). Il a été décrit sur la base d'un seul exemplaire presque complet et relativement bien conservé (JORDAN, 1924 : 224-225). Il a été initialement rapporté à une nouvelle famille, les Niobraridae, ainsi que quatre autres genres monospécifiques du Crétacé supérieur du Kansas, *Niobrara encarsia* JORDAN, 1924, *Kansanus martini* JORDAN, 1924, *Luxilites striolatus* JORDAN, 1924 et *Ferrifrons rugosus* JORDAN, 1924. Aujourd'hui, les Niobraridae sont considérés comme synonymes des Plethodidae, une famille de poissons téléostéens appartenant à l'ordre fossile des Tselfatiiformes ou Bananogmiiformes (PATTERSON, 1993 : 627; NELSON, 1994 : 90). Remarquons cependant que, si *Niobrara encarsia* et *Luxilites striolatus* sont bien des Plethodidae (obs. pers.; ils feront l'objet d'une révision ultérieure), *Kansanus martini* et *Ferrifrons rugosus* n'appartiennent pas à cette famille. Le premier est un représentant des Pachyrhizodontidae (SCHULTZE *et al.*, 1982 : 35; obs. pers.). Le second est en par-

tie faux. Seules la tête et la portion antérieure du corps sont de vrais éléments fossiles, alors que le reste du corps et la queue ont été sculptés dans le substrat (obs. pers.). ARRATIA & CHORN (1998) ont montré que *Ferrifrons rugosus* était un acanthomorphe archaïque.

Dans sa description originale, JORDAN (1924 : 225) écrit que « (the) head (is) so crushed that few separate bones can be traced », ce qui explique qu'il ne dit quasi rien du squelette céphalique du poisson. De plus, cet auteur identifie erronément les os ptérygoïdes comme les mâchoires de l'exemplaire concerné, les vrais mâchoires étant perdues. Or, ma propre observation a montré que les portions préservées de la tête en permettent, au contraire, une bonne compréhension, même si le museau et une grande part de la moitié droite du toit crânien ne sont pas conservés. JORDAN (1924) ne dit rien non plus du complexe urophore du fossile bien que celui-ci soit en parfait état.

Ces éléments rendent donc nécessaire la révision de *Zanclites xenurus* et la discussion de ses relations phylogénétiques. C'est là l'objet du présent article qui s'inscrit également dans la série de publications que je consacre à l'étude de l'ordre des Tselfatiiformes (TAVERNE, 1975, 1983, sous presse a, b, c et divers travaux en préparation).

Rappelons que les Tselfatiiformes sont, avec les Ichthyodectiformes et les Crossognathiformes, l'un des trois grands ordres de téléostéens marins du Crétacé qui n'ont plus de descendants dans les ichthyofaunes modernes. L'ordre renferme une douzaine de genres répartis en deux familles, les Plethodidae et les Tselfatiidae, pour les uns (NELSON, 1994) ou regroupés en une seule famille des Plethodidae pour les autres (PATTERSON, 1993). C'étaient des poissons de taille moyenne à grande, certains dépassant largement le mètre de longueur. Ils habitaient les eaux de la Mésogée eurafricaine, de la mer intérieure nord-américaine et du Paléoatlantique.

Leur morphologie rappelle celle des Coryphaenidae et des Scombridae actuels et indique des formes à nage rapide. Leur corps est comprimé latéralement, allongé et plus ou moins élevé selon les cas. Les nageoires dorsale et anale sont hautes et longues, la dorsale s'étendant même sur toute la longueur du corps. Les nageoires pectorales sont longues et insérées haut sur les flancs. Les nageoires pelviennes sont en position abdominale et fréquemment réduites. La nageoire caudale est grande, bilobée et montre une forte hypurostégie. Le crâne est médio-pariétal, avec de grands pariétaux. La fosse temporale est couverte latéralement par le ptérotique. Le supratemporal est réduit. Le rétroarticulaire est soudé à l'angulaire mais l'articulaire demeure autogène. Des ligaments ossifiés relient la région symphysaire de la mandibule aux deux cleithra. Les dents sont très petites, pointues et regroupées en plages. Les os dentés des mâchoires et du palais sont traversés par de petits canalicules qui leur donnent un aspect ponctué tout à fait caractéristique. Les arcs neuraux et hémaux sont autogènes tout au long du squelette axial. Le sommet des ptérygophores dorsaux et anaux s'épanouit en un petit plateau. La dichotomie et la segmentation des lépidotriches sont inexistantes ou ne se marquent qu'à l'extrémité distale de ces derniers. Il n'y a ni épuraux ni uroneuraux. Le parhypural est perdu. Les vertèbres urales I et II sont soudées. Les quatre premiers hypuraux fusionnent en une large plaque, elle-même soudée à la vertèbre terminale.

La position systématique des Tselfatiiformes a toujours posé problème. Ils ont tour à tour été rangés dans les Osteoglossiformes, les Albuliformes, les Clupeiformes, les Atheriniformes, les Scombriformes ou tenus en position *incertae sedis* (NELSON, 1994).

Récemment, TAVERNE (sous presse a) a montré par une analyse cladistique détaillée que les Tselfatiiformes étaient des Clupeocephala archaïques dont les caractères étaient apomorphes par rapport à ceux des Crossognathiformes et plésiomorphes par rapport à ceux d'un clade groupant les Otocephala (Clupeomorpha + Ostariophysi) et les Euteleostei.

Pour davantage d'informations concernant les Tselfatiiformes et leur position systématique, je renvoie le lecteur à TAVERNE (sous presse a).

MATÉRIEL ET MÉTHODES

L'holotype et unique spécimen de *Zanclites xenurus* est conservé dans les collections paléontologiques de l'Université du Kansas à Lawrence (Kansas, U. S. A.) où il porte le N° KUVP 52. Il a été mis au jour à $^{1}/_{2}$ mille au nord-est de Gove City, dans le comté de Gove, au Kansas. Il provient du Smoky Hill Chalk Member de la Niobrara Formation et est d'âge santonien.

Le matériel a été examiné à l'aide d'un stéréomicroscope Wild M 5. L'observation de certains détails du crâne a été facilitée par une immersion dans l'éthanol. Les dessins ont été réalisés par l'auteur grâce à une chambre claire (camera lucida).

ÉTUDE DU MATÉRIEL

Généralités (Fig. 1)

L'unique spécimen de *Zanclites xenurus* mesure 45,5 cm de longueur standard et 58 cm de longueur totale. La hauteur maximale du corps est de 14 cm mais elle ne peut être estimée qu'approximativement car le sommet du dos manque dans la région antérieure du poisson. La tête seule, région operculaire comprise, est longue de 12 cm mais le museau n'est pas conservé. Le corps est allongé et modérément élevé. Le pédoncule caudal est court et mince. La forme générale elliptique du poisson rappelle beaucoup celle des Scombridae.

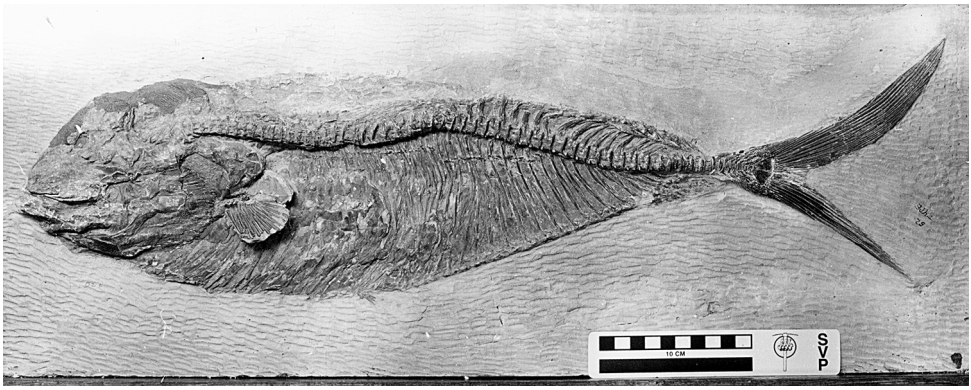


Fig. 1a. – *Zanclites xenurus* JORDAN, 1924. – Le spécimen holotype N° KUVP 52 (photographie due à la courtoisie de John CHORN de l'Université du Kansas à Lawrence).

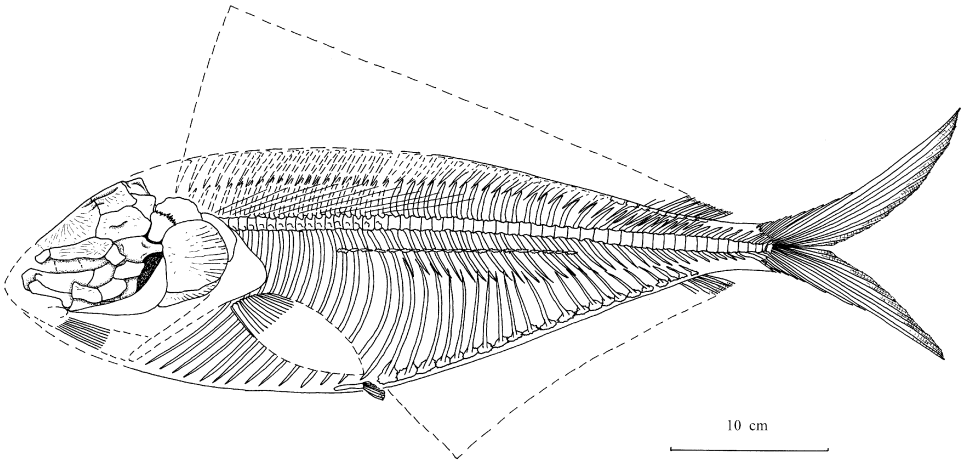


Fig. 1b. – Reconstitution du squelette complet.

Le crâne (Fig. 2)

La tête est écrasée. La région voméro-ethmoïdienne, les nasaux, l'extrémité antérieure du frontal gauche et la plus grande partie du frontal droit manquent. Toutefois, le substrat a été sculpté au-dessus des quelques restes de ce frontal droit pour donner l'illusion d'un profil crânien supérieur complet.

Le toit crânien est relativement plat et très large. Cette largeur est maximale dans la région postorbitaire. Les frontaux sont vastes, larges mais, vers l'arrière, ils ne dépassent pas le niveau de l'extrémité postérieure de l'orbite. Les pariétaux, à peu près quadrangulaires et de très grande taille, sont jointifs en arrière des frontaux, déterminant ainsi un crâne de type médio-pariétal. Près de son bord médian, chaque pariétal porte une forte crête parallèle à l'axe du poisson. Ces deux crêtes délimitent entre elles une dépression allongée qui, vers l'avant, se prolonge légèrement sur les frontaux et, vers l'arrière, atteint presque le niveau du supraoccipital. Plus latéralement, une autre crête, très courte, se remarque à la limite du frontal et du pariétal. Seul le ptérotique gauche est conservé. Il est très développé, plus long que large, plus large à l'arrière qu'à l'avant et porte en son milieu une forte protubérance qui surplombe une *dilatator fossa* très peu marquée. La surface des frontaux et des pariétaux est ornée de ridules. Les canaux sensoriels supraorbitaire et postorbitaire (= otique) sont entièrement fermés et n'ont pas laissé de traces visibles à la surface du crâne. L'extrémité postérieure du toit crânien est formée par le supraoccipital et les épiotiques (= épioccipitaux). Le supraoccipital est large, orné d'une petite crête médiane et d'un petit bourrelet transversal situé juste à la limite avec les pariétaux. L'épiotique gauche est le seul préservé. Il est bien développé, à peu près aussi long que large et forme une sorte de gros ergot postérieur. La fosse temporale (= posttemporale) n'est pas visible à l'arrière de la face latérale du crâne car le ptérotique la recouvre entièrement. Le supratemporal (= extrascapulaire) gauche est préservé. C'est un os de taille modérée, situé latéralement derrière le ptérotique et l'épiotique auxquels il s'applique fermement. Son composant membranodermique est bien développé. Postérieurement, il est articulé par une

suture dentelée avec le posttemporal. Il ne rejoint pas son homologue sur la ligne médiane du crâne et ne représente, en fait, que la partie latérale d'un supratemporal primitif. La commissure sensorielle extrascapulaire n'a laissé aucune trace visible à la surface de l'os.

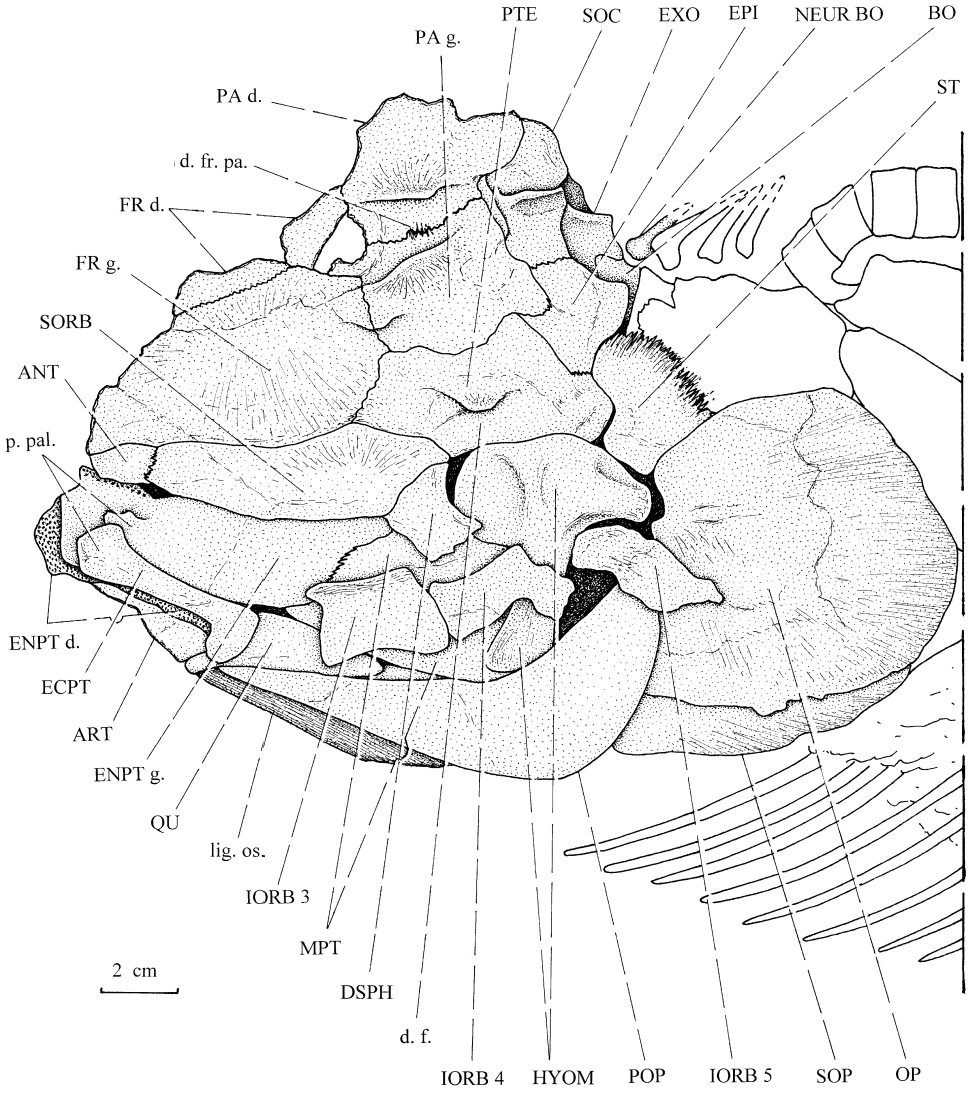


Fig. 2. – *Zanclites xenurus* JORDAN, 1924. La tête du spécimen holotype N° KUVP 52 en vue latérale gauche. Le toit crânien a été rabattu sur le neurocrâne par la fossilisation. Les pièces du squelette axial et de la ceinture scapulaire reprises telles quelles sur la figure 3 ne sont pas ombrées ni légendées.

Le toit crânien, rabattu sur le substrat, couvre le neurocrâne dont quasi rien n'est connu. La faible hauteur de l'hyomandibulaire, du carré et de la branche dorsale du pré-

operculaire font penser que ce neurocrâne devait être peu élevé. Une partie de l'exoccipital droit et du basioccipital sont visibles derrière le supraoccipital et l'épiotique. Le basioccipital forme une légère saillie en arrière du neurocrâne et réalise seul le condyle articulaire pour la première vertèbre. Un petit arc neural surmonte le basioccipital.

De la série circumorbitaire gauche, six os sont conservés : l'antorbitaire, le supraorbitaire, les trois infraorbitaires postérieurs et le dermosphénotique. Le premier (= lacrimal) et le deuxième (= jugal) infraorbitaires manquent. L'antorbitaire, le supraorbitaire et le dermosphénotique sont des os épais, articulés entre eux, comme chez presque tous les Tselfatiiformes (obs. pers.). L'antorbitaire est petit et son bord antéro-dorsal est articulé avec le bord latéral du frontal. Le supraorbitaire est large, extrêmement allongé, orné de ridules et articulé avec le frontal et la partie antérieure du ptérotique. Le dermosphénotique est modérement développé et presque quadrangulaire. Contrairement au cas de nombreux Tselfatiiformes (obs. pers.), il ne s'articule pas avec le frontal et le ptérotique; cela est assurément dû à l'allongement très marqué du supraorbitaire dont la partie postérieure envahit la place normalement occupée par le dermosphénotique. Les troisième, quatrième et cinquième infraorbitaires ont été déplacés par la fossilisation et se présentent l'un derrière l'autre depuis le niveau du métaptérygoïde jusqu'au bord antérieur de l'operculaire. Ces trois os sont bien développés, avec un composant membranodermique important, mais ils n'atteignent cependant pas l'ampleur que les trois infraorbitaires postérieurs prennent chez de nombreux Tselfatiiformes (obs. pers.). Des traces du canal sensoriel infraorbitaire se voient le long du bord antérieur du troisième infraorbitaire. On n'observe pas d'anneau osseux sclérotique.

Les mâchoires ne sont pas conservées. Seul s'observe un fragment de l'articulaire gauche, au niveau de la jonction avec le carré. Les mâchoires signalées par JORDAN (1924 : 225) sont les os ptérygoïdes. La position avancée du condyle articulaire du carré et les longueurs importantes de l'entoptérygoïde, du métaptérygoïde et de la branche ventrale du préoperculaire amènent à penser que ces mâchoires étaient courtes, à la manière des Gonorhynchoidei ou des Argentinoidei .

Le palatin n'est pas conservé. Les autres pièces de l'arc palato-carré sont visibles. L'entoptérygoïde (= endoptérygoïde, mésoptérygoïde) est long et large, aussi large vers l'avant qu'à l'arrière. Sa face interne est criblée de minuscules puits, restes de l'implantation de denticules et typiques des Tselfatiiformes. Une série de ces alvéoles dentaires se remarque aussi sur la face externe de l'os, le long de la partie la plus antérieure de son bord dorsal. Près de son extrémité antérieure, la face externe de l'os porte un processus bien marqué. L'ectoptérygoïde est édenté, épaissi en une sorte de gros bâtonnet osseux assez court, quelque peu élargi au niveau son extrémité postérieure qui contacte le carré et renflé antérieurement en un gros nodule osseux qui touche le processus de la face externe de l'entoptérygoïde, formant ainsi une sorte de vaste contrefort osseux. Il est probable que ce contrefort servait de soutien à un gros palatin. Le métaptérygoïde est très étendu mais l'essentiel de sa surface est couvert par les troisième et quatrième infraorbitaires; vers l'avant, il touche l'entoptérygoïde et, vers l'arrière, l'hyomandibulaire. Le carré est très petit, en forme de triangle écrasé et situé sous l'entoptérygoïde et le métaptérygoïde. Son condyle articulaire effilé touche l'articulaire.

La série operculaire droite est bien conservée. Le préoperculaire offre une branche ventrale très allongée et une branche dorsale, au contraire, peu élevée et qui ne rejoint pas

le niveau du ptérotique. Juste en arrière du carré, le bord supérieur de la branche ventrale du préoperculaire se dédouble et forme une petite gouttière qui recevait probablement le symplectique, lequel n'est toutefois pas visible. Le canal sensoriel préoperculaire n'a pas laissé de trace à la surface de l'os. L'operculaire est arrondi et d'assez grande taille. Son bord supérieur remonte jusqu'au supratemporal et au posttemporal. Son coin antéro-dorsal est creusé d'une encoche pour le *processus opercularis* de l'hyomandibulaire. Le sous-operculaire est étroit et allongé. La surface du préoperculaire, de l'operculaire et du sous-operculaire est ornée de ridules. On n'observe ni l'interoperculaire ni les rayons branchiostèges.

Le squelette hyoïdeo-branchial (Fig. 2)

L'hyomandibulaire est le seul os visible de l'arc hyoïdien. Sa partie dorsale est large, s'articule contre le bord latéral du ptérotique, juste en dessous de la très faible *dilatator fossa,* et surplombe partiellement le métaptérygoïde. La branche ventrale de l'hyomandibulaire est courte et épaisse. Le *processus opercularis* est long et large. Rien n'est connu des autres pièces hyoïdiennes ni du squelette branchial.

Sous le préoperculaire, on distingue les restes partiels d'un amas de ligaments ossifiés. Des ligaments de ce type sont connus chez *Tselfatia formosa* ARAMBOURG, 1943 où ils relient la région symphysaire de la mandibule à la branche ventrale du cleithrum (ARAMBOURG, 1954 : fig. 66, pl. XV, fig. 3; TAVERNE, 1983 : fig. 2). J'en ai observé également chez la plupart des autres Tselfatiiformes.

Les ceintures (Figs 1, 3)

La ceinture scapulaire est très développée. Le posttemporal est vaste, aliforme, articulé antérieurement avec le supratemporal et il surplombe l'operculaire. L'hypercleithrum (= supracleithrum) est long et large. La surface du posttemporal et surtout celle de l'hypercleithrum sont ornées de ridules. Le cleithrum est de grande taille. Il a été légèrement déplacé vers le haut par la fossilisation, de telle sorte que la plus grande partie de sa branche ventrale se retrouve cachée sous l'operculaire et le sous-operculaire. La branche dorsale du cleithrum est très large, surtout vers le bas, et porte une grosse ride qui servait sans doute d'attache musculaire. On n'observe pas de postcleithrum. La scapula (= hypercoracoïde), le coracoïde (= hypocoracoïde), le mésocoracoïde et les ptérygophores pectoraux ne sont pas connus. Seule la base de la nageoire pectorale est conservée, ce qui a amené JORDAN (1924 : 224-225) à conclure erronément qu'elle était courte. On ignore en fait sa longueur réelle. Elle compte 13 rayons, le premier de la série étant le plus épais. JORDAN (1924 : 224) en mentionne seulement 12. On n'observe pas de petite épine impaire initiale sur cette nageoire.

La ceinture pelvienne et ses nageoires sont minuscules et occupent une position abdominale, juste en avant du premier ptérygophore anal. Les nageoires pelviennes comptent chacune 7 rayons. JORDAN (1924 : 224) en signale 6. L'origine des nageoires pelviennes est située au niveau de la jonction entre les vingt et unième et vingt-deuxième vertèbres.

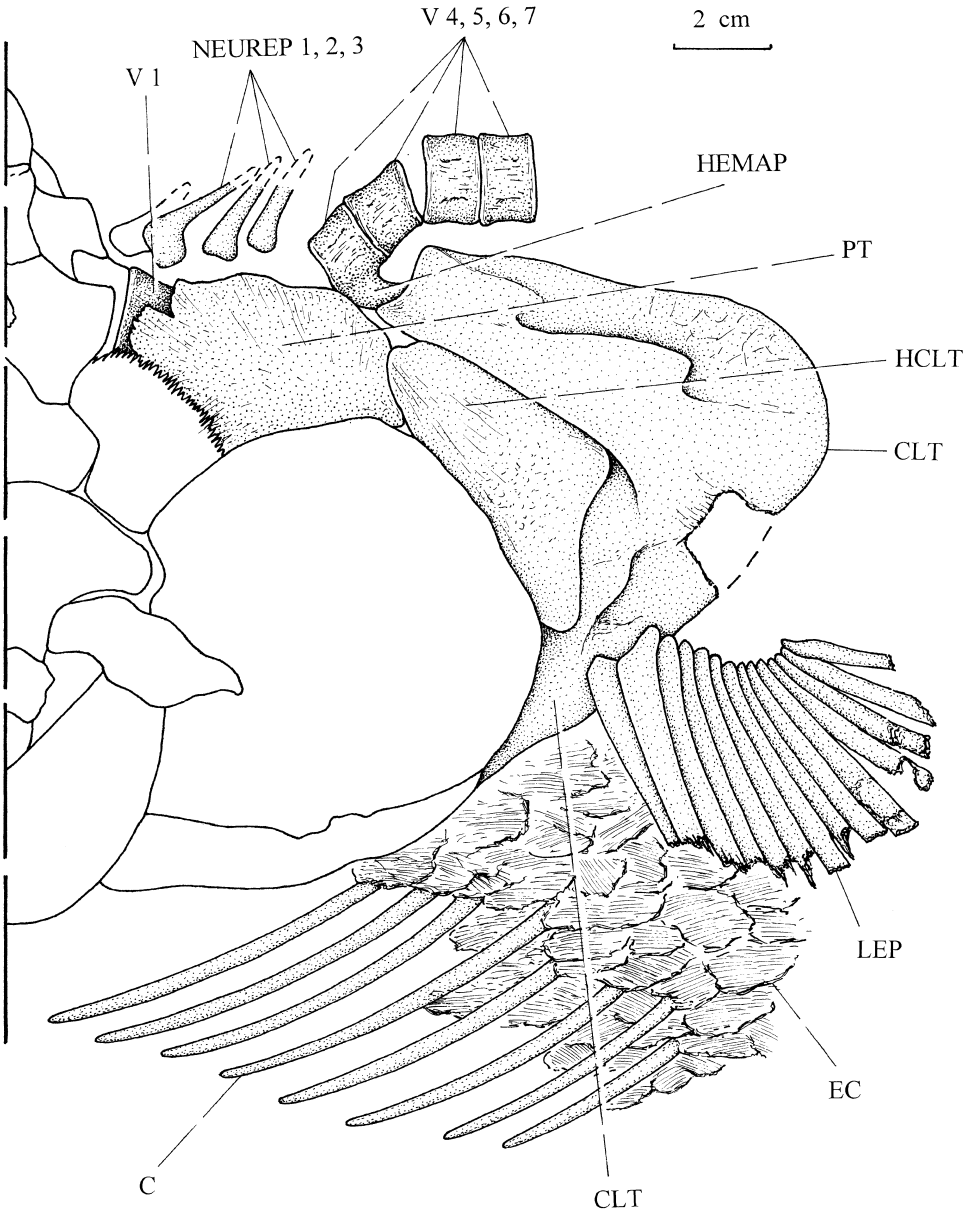


Fig. 3. – *Zanclites xenurus* JORDAN, 1924. La ceinture scapulaire et le début du squelette axial du spécimen holotype N° KUVP 52. Les pièces du crâne reprises telles quelles de la figure 2 ne sont pas ombrées ni légendées.

Le squelette axial (Fig. 1, 4)

Un fragment de la première vertèbre s'observe accolé au basioccipital et surmonté d'un petit arc neural et d'une courte neurépine. Les deux vertèbres suivantes sont recouvertes par le posttemporal et seuls sont visibles les arcs neuraux et les neurépines correspondantes. La quatrième vertèbre est la première qui se voit en entier. En tenant compte de ces faits, on relève un total de 62 vertèbres dont 26 abdominales et 36 caudales, y compris la vertèbre préurale I réduite et le petit centre terminal ural I et II. JORDAN (1924 : 225) compte 56 vertèbres dont 18 abdominales et 38 caudales. Il est vraisemblable que les premières vertèbres ainsi que le petit centre terminal ont échappé à son observation.

Les vertèbres sont fortes et plus hautes que longues. Celles de la région abdominale sont un peu plus hautes et un peu plus étroites que celles de la région caudale. Les vertèbres situées au niveau du court pédoncule caudal sont légèrement plus massives que les vertèbres caudales qui précèdent. Les faces latérales des vertèbres sont ornées de très fines rides horizontales et, parfois, de très petites alvéoles.

Les arcs neuraux (= neurarcuaux) sont autogènes sur toute la longueur du squelette axial. Les neurépines (= neuracanthes) sont courtes mais épaisses. Elles demeurent à peu près verticales jusqu'au niveau de la quarantième vertèbre. Au-delà, les neurépines s'allongent quelque peu et s'inclinent davantage vers l'arrière.

Dans la région abdominale, l'arc hémal (= hémarcual) se présente sous forme d'hémapophyses qui ne se referment pas sur elles-mêmes en une arche. L'arc hémal des trois premières vertèbres n'est pas connu. Celui de la quatrième vertèbre est bien développé et soudé au corps vertébral qu'il dépasse nettement vers le bas. Plus en arrière dans la région abdominale, l'arc hémal devient une sorte de gros processus (ce que JORDAN (1924 : 225) appelle « blunt process ») soudé à la région antéro-ventrale du corps vertébral. Cette soudure n'est cependant pas complète car une ligne de suture s'observe dorsalement, à la limite de l'hémapophyse et du corps vertébral. Dans la région caudale, l'arc hémal se referme sur lui-même en une arche et devient autogène puisqu'il s'appuie sur la vertèbre mais ne s'y soude pas. Dès la première vertèbre caudale, l'arc hémal se prolonge par une hémépine (= hémacanthe). Celles-ci sont longues et fortes. Leur longueur diminue au fur et à mesure que l'on s'approche de la queue, de même que s'accroît leur inclinaison vers l'arrière.

Les côtes sont longues, fortes, incurvées, souvent ornées d'un sillon médian, appliquées contre les hémapophyses correspondantes. Leur incurvation est très marquée au niveau des premières côtes mais elle diminue au niveau des côtes ultérieures. Ventralement, les côtes descendent jusqu'au bas du *situs viscerum*.

Des fragments d'épineuraux longs et fins s'observent dans la région abdominale où ils s'articulent sur les arcs neuraux mais sans s'y souder. On remarque aussi de petits épipleuraux courts, larges, à bords déchiquetés, plus ou moins articulés entre eux qui sont associés aux dernières côtes et aux premières hémépines.

Les nageoires dorsale et anale (Figs 1, 4B, C)

Le sommet du dos manque dans la première moitié du corps du poisson. On ignore donc où débutait la nageoire dorsale. On peut penser qu'elle était très longue et commençait un

peu en arrière de la tête, comme chez tous les autres Tselfatiiformes (obs. pers.), puisqu'on trouve quelques fragments de longs ptérygophores dorsaux dans cette région. Seuls les derniers ptérygophores dorsaux et quelques fragments des ultimes rayons sont bien conservés. Ces ptérygophores et les neurépines correspondantes sont dans un rapport 1/1 et, d'autre part, la nageoire dorsale se termine au niveau de la cinquante-quatrième vertèbre. On peut en déduire que la nageoire dorsale devait comporter une cinquantaine de rayons. Chaque ptérygophore est formé d'une tige osseuse (= axonoste) surmontée d'un plateau élargi,

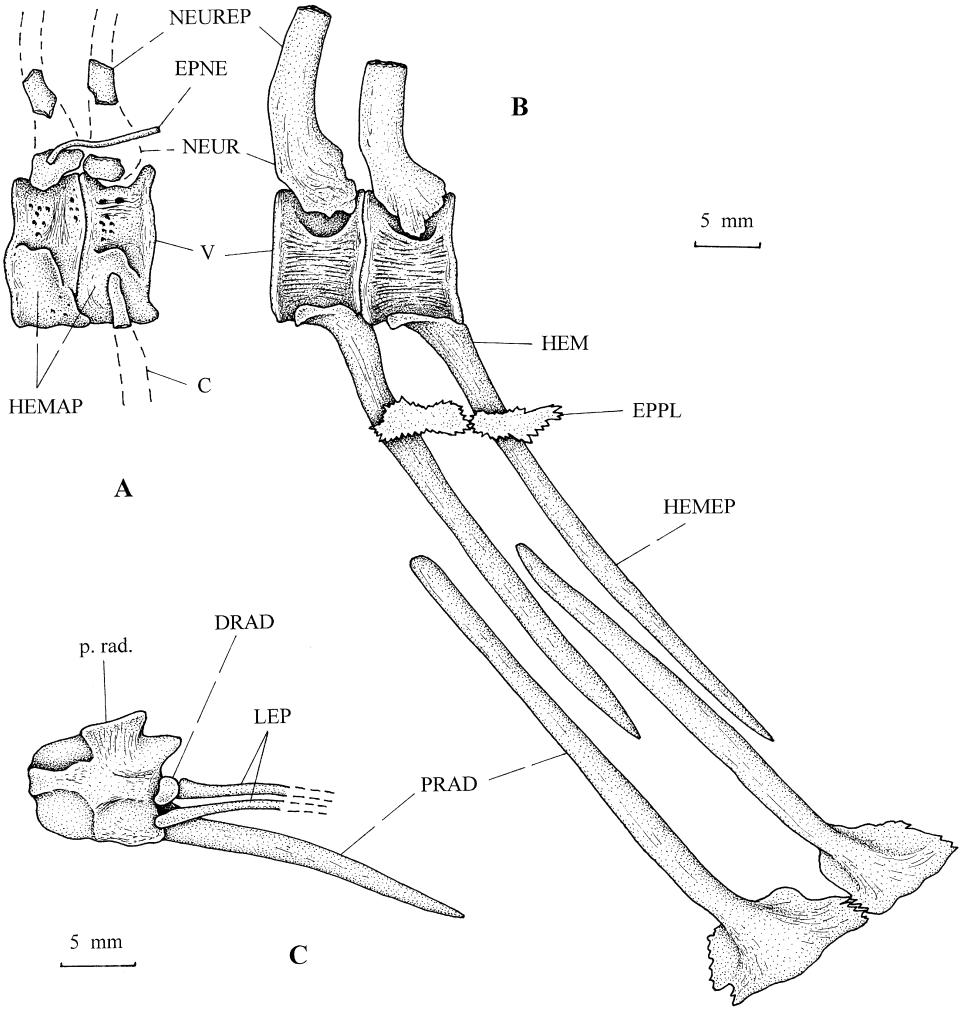


Fig. 4. – *Zanclites xenurus* JORDAN, 1924. Parties du squelette postcrânien du spécimen holotype N° KUVP 52. (A) les dix-neuvième et vingtième vertèbres, dans la région abdominale ; (B) les trente-septième et trente-huitième vertèbres, dans la région caudale, et les ptérygophores anaux associés ; (C) un ptérygophore dorsal postérieur en position oblique, avec son plateau supérieur aplati sur le substrat et en vue dorsale.

comme chez *Tselfatia formosa* (TAVERNE, 1975 : fig. 1). Il y a aussi une toute petite pièce distale (= baséoste) qui s'intercale entre les bases des deux hémi-lépidotriches de chaque rayon. Les plateaux des ptérygophores successifs s'articulent entre eux.

Seuls les derniers rayons de la nageoire anale sont préservés mais tous les ptérygophores anaux sont présents. On en compte 22. On peut donc estimer que la nageoire anale comportait 24 ou 25 rayons, y compris les petits rayons épineux initiaux. Le premier ptérygophore anal est le plus long et le plus épais de la série. Il longe le bord antérieur de la première hémépine tout en s'incurvant, de telle sorte que son extrémité ventrale se recourbe vers l'avant, déterminant ainsi ce que BLOT (1968 : fig. 1 III) a dénommé un complexe hémaxanal de type 3 et qui se retrouve chez la plupart des Tselfatiiformes (obs. pers.). Cette incurvation s'estompe très vite sur les ptérygophores suivants qui redeviennent alors rectilignes, acquièrent peu à peu une orientation qui les incline de l'avant vers l'arrière et dont la longueur diminue progressivement en s'approchant de la queue. Les extrémités ventrales des ptérygophores anaux forment des expansions osseuses aliformes qui s'imbriquent les unes dans les autres. La correspondance entre les ptérygophores anaux et les hémépines se fait dans un rapport 1/1.

Le squelette caudal (Fig. 5)

Les ultimes vertèbres caudales deviennent progressivement de plus en plus petites et montrent des faces latérales peu ornementées. La vertèbre préurale I est très réduite. Les vertèbres urales I et II sont fusionnées en un petit centre terminal soudé à une plaque hypurale. Les dernières neurépines et hémépines sont larges, épaisses et en contact les unes avec les autres. Les quatre dernières hémépines sont plus allongées que les précédentes. Les neurépines préurales I et II sont complètes, toutefois la neurépine préurale I est beaucoup plus fine que celles qui la précèdent. Les derniers arcs neuraux et hémaux sont articulés mais pas soudés aux vertèbres correspondantes dont ils recouvrent une partie importante des faces latérales. L'arc neural préural I fait exception; il est soudé au centre préural I. L'arc hémal préural I et son parhypural ont disparu comme chez la plupart des Tselfatiiformes (obs. pers.). Certains Tselfatiiformes primitifs possèdent encore un petit arc hémal préural I mais plus de parhypural (obs. pers.). Les deux hypuraux ventraux et les premiers hypuraux dorsaux sont fusionnés en une vaste plaque hypurale, elle-même soudée au petit centre terminal ural I et II. Le bord ventral de cette plaque est accolé à l'hémépine préurale II. Une légère rainure marque encore, au milieu de la plaque, la limite entre les hypuraux ventraux et dorsaux. Ce sont très probablement les troisième et quatrième hypuraux qui entrent dans la composition de la moitié dorsale de la plaque hypurale chez les Tselfatiiformes, à l'exclusion du cinquième et à l'inverse de ce que pensait TAVERNE (1983 : fig. 5), puisque BARDACK & TELLER-MARSHALL (1980 : fig. 3) figurent un exemplaire de *Tselfatia formosa* chez lequel la région distale de la moitié supérieure de la plaque hypurale se divise encore clairement en deux hypuraux distincts. Le cinquième hypural est autogène mais très réduit. Son extrémité antérieure effilée n'atteint pas le niveau du petit centre terminal. Il n'y a pas d'épural ni d'uroneuraux, ce qui est le cas chez tous les Tselfatiiformes que j'ai pu examiner. Les possibles uroneuraux signalés par NELSON (1973 : fig. 6C) chez un *Bananogmius* sp. sont respectivement une courte neurépine préurale I et le cinquième hypural autogène (obs. pers.).

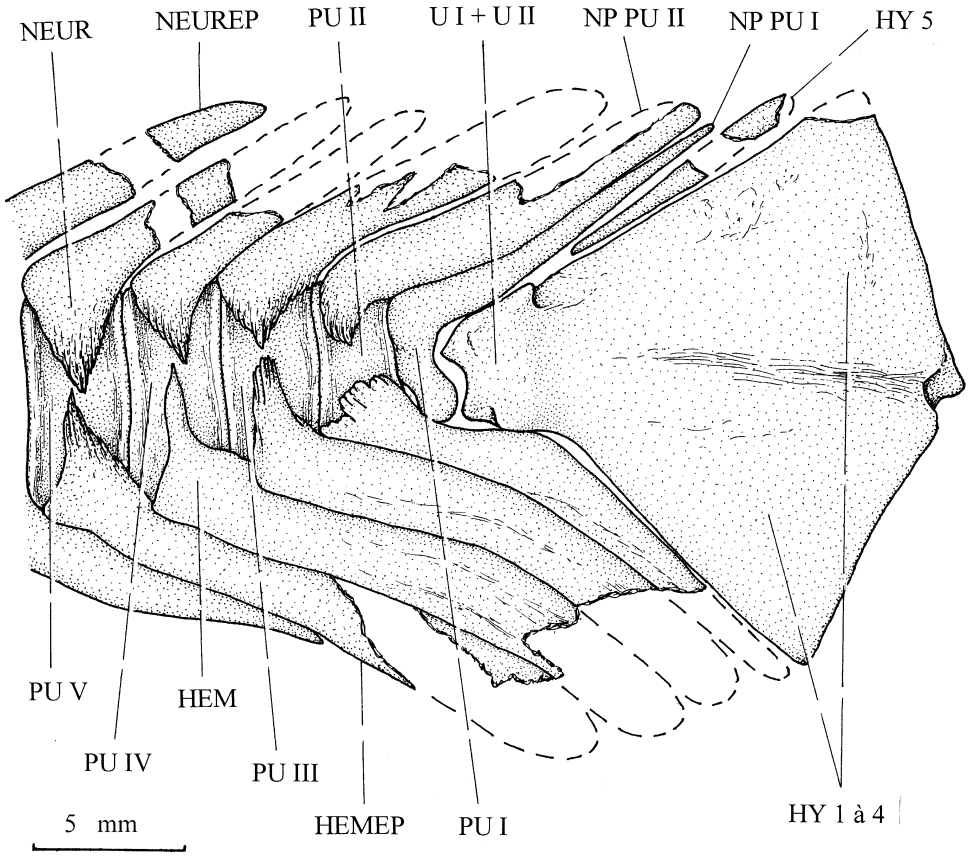


Fig. 5. – *Zanclites xenurus* JORDAN, 1924. Le squelette caudal du spécimen holotype N° KUVP 52.

Une autre interprétation du complexe urophore des Tselfatiiformes a été avancée par BARDACK & TELLER-MARSHALL (1980). Pour ces auteurs, le centre terminal ural I et II serait la vertèbre urale II, la vertèbre préurale I atrophiée serait le centre ural I, les deux premiers hypuraux auraient reculé jusqu'au niveau du centre ural II, la vertèbre préurale II serait la préurale I et la dernière hémépine serait le parhypural. Toutefois, l'existence d'un petit arc hémal appendu à la vertèbre préurale I (*sensu* TAVERNE, 1975, 1983; urale I *sensu* BARDACK & TELLER-MARSHALL, 1980) atrophiée chez certains Tselfatiiformes primitifs (TAVERNE, sous presse a : fig. 8) montre que mon interprétation est la seule correcte puisque, par définition, il n'y a plus d'arc hémal associé à la vertèbre urale I.

La nageoire caudale est grande et bilobée. Chacun des deux lobes est long et étroit, le lobe dorsal étant encore un peu plus long que le ventral. L'hypurostégie est marquée. On compte 19 rayons principaux segmentés dont 17 branchus, avec 9 rayons branchus dans le lobe dorsal et 8 dans le lobe ventral. La segmentation et la division en branches des rayons principaux ne se réalisent qu'aux extrémités distales de ces derniers. Sur la plus grande partie de leur longueur, les rayons principaux restent donc indivis. Dorsalement, en avant

du premier rayon principal, on relève encore 13 petits rayons insegmentés et non-branchus, tandis que ventralement, on en dénombre au moins 8.

La squamation

L'écaillure est très mal conservée. On observe des fragments d'écailles au niveau de la nuque ainsi qu'en dessous et en arrière de la nageoire pectorale. Ces écailles sont grandes, cycloïdes et portent des raies de croissance concentriques ainsi que, par endroits, de fins *circuli* serrés les uns contre les autres et à disposition à peu près horizontale. Je n'ai pas observé de *radii* mais c'est peut-être dû au mauvais état de préservation des écailles.

DISCUSSION

Zanclites xenurus et les Tselfatiiformes

La morphologie générale, les proportions, le pédoncule caudal aminci, la grande nageoire caudale bilobée et la forte hypurostégie de *Zanclites xenurus* correspondent à ceux de la plupart des Tselfatiiformes (HAY, 1903 : pl. II; JORDAN, 1924 : pl. XIV, XV; obs. pers.).

Plusieurs caractères ostéologiques de *Zanclites xenurus* sont également typiques des Tselfatiiformes et attestent que ce poisson appartient bien à cet ordre :

(1) le toit crânien est assez plat, avec de grands pariétaux médians et une dépression fronto-pariétale bien marquée. D'autres téléostéens primitifs du Crétacé montrent aussi une dépression frontale médiane, tels certains Pachyrhizodontidae ou le salmoniforme *Kermichthys daguini* (ARAMBOURG, 1954), mais ce sont toujours des formes à petits pariétaux latéraux (TAVERNE, 1991 : fig. 2, 1993 : fig. 2).

(2) l'antorbitaire, le supraorbitaire et le dermosphénotique sont articulés entre eux et avec le frontal (LOOMIS, 1900 : pl. XXI, fig. 2; STEWART, 1900 : pl. LXIV; HAY, 1903 : fig. 23; obs. pers.).

(3) des petits puits, restes de l'implantation de denticules, parsèment la face interne de l'entoptérygoïde (DIXON, 1850 : pl. XXXIII, fig. 2, 2a; LOOMIS, 1900 : pl. XXI, fig. 3-5, 7, 8, pl. XXII, fig. 1-7, 10, 11; STEWART, 1900 : pl. LXVII; HAY, 1903 : fig. 32, 34).

(4) des ligaments ossifiés mandibulo-cleithraux sont présents (TAVERNE, 1983 : fig. 2).

(5) la vertèbre préurale I est réduite, dépourvue de parhypural mais supporte une neurépine; il n'y a pas d'uroneuraux et ni d'épuraux; les vertèbres urales I et II sont fusionnées en un petit centre terminal; les quatre premiers hypuraux sont soudés en une large plaque, elle-même fusionnée à la vertèbre terminale; les derniers arcs neuraux et hémaux autogènes. Certains de ces caractères caudaux spécialisés existent dans diverses lignées téléostéennes mais leur conjugaison ne se réalise qu'au sein des Tselfatiiformes, à l'exclusion de tout autre ordre (NELSON, 1973 : fig. 8B et C; TAVERNE, 1975 : fig. 3, 1983 : fig. 5; obs. pers.).

La fosse temporale couverte dorso-latéralement par le ptérotique, le supratemporal large mais réduit à sa portion latérale, le fort cleithrum, la position haute de la nageoire pectorale, l'atrophie de la ceinture et des nageoires pelviennes ainsi que les longues

Ces quelques caractères justifient amplement le statut générique particulier de notre poisson au sein de l'ordre des Tselfatiiformes.

La diagnose émendée de *Zanclites*

La présente étude permet également de proposer une diagnose de *Zanclites* beaucoup plus complète que celle donnée par JORDAN (1924 : 224) : tselfatiiforme de taille moyenne; corps allongé, modérément élevé et comprimé; toit crânien large et assez plat; grands pariétaux jointifs et presque quadrangulaires; forte dépression médiane fronto-pariétale bordée latéralement par une petite crête osseuse; petite crête supraoccipitale; vaste ptérotique orné d'un renflement qui surplombe une *dilatator fossa* estompée; fosse temporale couverte dorso-latéralement; antorbitaire, surpraorbitaire et dermosphénotique articulés entre eux; supraorbitaire très allongé, articulé avec le frontal et le ptérotique et séparant le dermosphénotique du ptérotique; les trois infraorbitaires postérieurs modérément développés; basioccipital formant seul le condyle articulaire pour la première vertèbre et surmonté d'un petit arc neural; supratemporal réduit à sa partie latérale mais gardant un composant membranodermique étendu; vastes entoptérygoïde et métaptérygoïde; entoptérygoïde denticulé sur toute sa surface interne; ectoptérygoïde court, épais, renflé aux deux extrémités et édenté; très petit carré; tête de l'ectoptérygoïde accolée à un processus de la surface externe de l'entoptérygoïde et formant avec ce dernier un gros contrefort; préoperculaire à longue branche ventrale et courte branche dorsale; operculaire et sous-operculaire bien développés; hyomandibulaire large dorsalement, à branche ventrale courte et à *processus opercularis* allongé; ligaments ossifiés mandibulocleithraux présents; postemporal, hypercleithrum et cleithrum très développés; nageoire pectorale insérée haut sur les flancs et comptant 13 rayons; ceinture et nageoires pelviennes très atrophiées et situées en position abdominale juste devant l'origine de la nageoire anale; nageoire pelvienne comptant 7 rayons; nageoire dorsale comptant une cinquantaine de rayons et s'étendant sur la plus grande partie du dos; ptérygophores dorsaux élargis en plateaux à leur sommet; nageoire anale comptant 24 ou 25 rayons; complexe hémaxanal de type 3; ptérygophores anaux élargis en palettes à leur base; 62 vertèbres dont 26 abdominales et 36 caudales; vertèbres plus hautes que larges; grandes côtes incurvées dans la région abdominale; arcs neuraux autogènes; arcs hémaux complètement ou partiellement soudés aux centres vertébraux dans la région abdominale mais autogènes dans la région caudale; épineuraux et épipleuraux présents; pédoncule caudal court, mince mais renforcé par des vertèbres un peu plus grosses que les autres; vertèbre préurale I réduite, soudée à son arc neural, portant une neurépine complète mais dépourvue d'arc hémal et de parhypural; vertèbres urales I et II fusionnées en un petit centre terminal; les quatre premiers hypuraux soudés en une vaste plaque, elle-même fusionnée au petit centre terminal; cinquième hypural réduit et n'atteignant pas le centre terminal; pas de sixième hypural; pas d'épural; pas d'uroneural; nageoire caudale grande, bilobée, à lobes longs et étroits, à forte hypurostégie et comptant 19 rayons principaux dont 17 segmentés et branchus; grandes écailles cycloïdes.

La position de *Zanclites* au sein des Tselfatiiformes

Aucune étude des relations phylogénétiques au sein de l'ordre n'a été publiée jusqu'ici. Le problème de l'existence d'une seule (les Plethodidae) ou de deux familles (les

Plethodidae et les Tselfatiidae) dans l'ordre reste toujours controversé (PATTERSON, 1993 : 627 ; NELSON, 1994 : 90). Ces deux questions pendantes ainsi que l'étude ostéologique de divers autres Tselfatiiformes font actuellement l'objet de mes recherches (TAVERNE, sous presse a, b, c et plusieurs travaux en préparation). Cette situation ne permet guère pour le moment de situer *Zanclites* de façon vraiment précise parmi les autres genres de l'ordre. Quelques constatations peuvent être émises néanmoins.

Il est clair que *Zanclites* appartient au groupe qui représente la majorité des Tselfatiiformes, ceux à toit crânien plus ou moins plat et à dépression médiane fronto-pariétale plus ou moins prononcée. A ce groupe s'opposent les quelques genres qui, comme *Enischnorhynchus* BARDACK, 1965, *Tselfatia* et les formes apparentées, possèdent un toit crânien très incurvé le long de la ligne médiane et sont dépourvus de dépression fronto-pariétale (ARAMBOURG, 1954; BARDACK, 1965; BARDACK et TELLER-MARSHALL, 1980; TAVERNE, 1983, sous presse a, b).

Parmi les représentants de son groupe, *Zanclites* paraît un genre très évolué. Il montre, en effet, un état apomorphe pour plusieurs caractères connus à l'état plésiomorphe chez d'autres espèces de ce groupe. La ceinture et les nageoires pelviennes de *Zanclites* sont atrophiées et son complexe hémaxanal est de type 3, alors que les formes les plus primitives de Tselfatiiformes, comme *Bananogmius aratus* (COPE, 1877), présentent une ceinture et des nageoires pelviennes normalement développées ainsi qu'un complexe hémaxanal de type 2 (HAY, 1903, pl. II; JORDAN, 1924 : pl. XIV, fig. 1; obs. pers.). De même, l'ectoptérygoïde de *Zanclites* est édenté, alors que la plupart des membres de l'ordre exhibent encore un ectoptérygoïde denticulé (obs. pers.). Le squelette caudal de *Zanclites* est, lui aussi, le plus évolué du groupe, avec un arc neural préural I soudé à sa vertèbre, pas d'arc hémal préural I et un cinquième hypural réduit, tandis que les espèces les plus archaïques offrent un arc neural préural I autogène, un petit reste d'arc hémal préural I, un cinquième hypural bien développé et même un sixième hypural (NELSON, 1973 : fig. 8B; TAVERNE, sous presse a : fig. 8) .

REMERCIEMENTS

J'exprime ma vive gratitude au Dr. J. Chorn, au Prof. Dr. L. Martin et au Dr. D. Miao du Département de Paléontologie des Vertébrés de l'Université du Kansas à Lawrence pour l'accueil chaleureux que j'ai reçu dans leur laboratoire et pour la permission qui m'a été accordée d'étudier le poisson fossile qui fait l'objet du présent article. Je remercie également le Prof. Dr. M. Chardon de l'Université de Liège et le Dr. M. Gayet de l'Université Claude-Bernard Lyon I qui ont bien voulu lire et commenter le présent article.

LISTE DES ABREVIATIONS DES FIGURES

ANT :	antorbitaire
ART	articulaire
BO :	basioccipital
C :	côtes
CLT :	cleithrum
DRAD :	pièce distale du ptérygopgore (= baséoste)
DSPH :	dermosphénotique
EC :	écailles
ECPT :	ectoptérygoïde
ENPT (d., g.) :	entoptérygoïde (= endoptérygoïde, mésoptérygoïde) (droit, gauche)
EPI :	épiotique (= épioccipital)
EPNE :	épineuraux (= arêtes dorsales)
EPPL :	épipleuraux (= arêtes ventrales)
EXO :	exoccipital
FR (d., g.) :	frontal (droit, gauche)
HCLT :	hypercleithrum (= supracleithrum)
HEM :	arc hémal (= hémarcual)
HEMAP :	hémapophyses
HEMEP :	hémépine (= hémacanthe)
HY 1 à 5 :	hypuraux 1 à 5
HYOM :	hyomandibulaire
IORB 3 à 5 :	infraorbitaires 3 à 5
LEP :	lépidotriches
MPT :	métaptérygoïde
NEUR :	arc neural (= neurarcual)
NEUREP :	neurépine (= neuracanthe)
NEUREP 1 à 3 :	les trois premières neurépines
NEUR BO :	arc neural associé au basioccipital
NP PU I et II :	neurépines préurales I et II
OP :	operculaire
PA (d., g.) :	pariétal (droit, gauche)
POP :	préoperculaire
PRAD :	pièce proximale du ptérygophore (= axonoste)
PT :	posttemporal
PTE :	ptérotique
PU I à V :	vertèbres préurales I à V
QU :	carré (= quadratique)
SOC :	supraoccipital
SOP :	sous-operculaire
SORB :	supraorbitaire
ST :	supratemporal (= extrascapulaire)
U I + U II :	petit centre vertébral terminal complexe ural I et II
V :	corps vertébral
V 1, 4 à 7 :	la première et les quatrième à septième vertèbres
f. :	*dilatator fossa*
d. fr. pa. :	dépression médiane fronto-pariétale
lig. os. :	ligaments ossifiés mandibulo-cleithraux
p. pal. :	processus palatin formé par l'entoptérygoïde et l'ectoptérygoïde

BIBLIOGRAPHIE

ARAMBOURG, C. (1954) – Les poissons crétacés du Jebel Tselfat (Maroc). *Ed. Serv. Géol. Maroc*, *Notes et Mém.*, Rabat, **118 :** 1-188.

ARRATIA, G. & J. CHORN (1998) – A new primitive acanthomorph fish from the Greenhorn Formation (Late Cretaceous) of Nebraska. *Journ. Vertebr. Paleontol.*, Lawrence, **18** (2) : 301-314.

BARDACK, D. (1965) – New Upper Cretaceous teleost fish from Texas. *Univ. Kansas*, *Paleont. Contrib.*, **1 :** 1-9.

BARDACK, D. & S. TELLER-MARSHALL (1980) – *Tselfatia* new first records from North America and Yugoslavia. *Soc. Econ. Paleont. Mineralog.*, **1980 :** 1075-10830.

BLOT, J. (1968) – Le squelette interne de la nageoire anale et ses relations avec le squelette axial. *C. R. Acad. Sci. Paris*, sér. D, **266** : 1943-1946.

DIXON, F. (1850) – *The Geology and Fossils of the Tertiary and Cretaceous Formations of Sussex*.

LONGMAN, BROWN, GREEN & LONGMANS (eds.), London : XVI + 422 pp.

HAY, O. P. (1903) – On certain genera and species of North American Cretaceous actinopterous fishes. *Bull. Amer. Mus. Nat. Hist.*, New York, **19** (1) : 1-95.

JORDAN, D. S. (1924) – A Collection of Fossil Fishes in the University of Kansas, from the Niobrara Formation of the Cretaceous. *Kansas Univ. Sci. Bull.*, **15** (2) : 219-234.

LOOMIS, F. B. (1900) – Die Anatomie und die Verwandtschaft der Ganoid- und Knochen-Fische aus der Kreide-Formation von Kansas. *Palaeontographica*, Stuttgart, **46 :** 213-286.

NELSON, G. J. (1973) – Notes on the Structure and Relationships of Certain Cretaceous and Eocene Teleostean Fishes. *Amer. Mus. Novit.*, New York, **2524 :** 1-31.

NELSON, J. S. (1994) – *Fishes of the World*. J. Wiley & Sons, Inc., New York, 3d edit., XVII + 600 pp.

PATTERSON, C. (1993) – Osteichthyes : Teleostei. In : *The Fossil Record 2*. BENTON, M. J. (Ed.), Chapman & Hall, London : 621-656.

SCHULTZE, H.-P., J.D. STEWART, A.M. NEUNER & R.W. COLDIRON (1982) – Type and Figured Specimens of Fossil Vertebrates in the Collection of the University of Kansas Museum of Natural History. Part I. Fossil Fishes. *Univ. Kansas, Mus. Nat. Hist., Miscell. Publ.*, Lawrence, **73** : 1-53.

STEWART, A. (1900) – Teleosts of the Upper Cretaceous. *Univ. Geol. Surv. Kansas, **6**, Paleont.*, 2, *Carboniferous and Cretaceous*, S. W. WILLISTON (Ed.), W. Y. Morgan print., Topeka : 257-402.

TAVERNE, L. (1975) – A propos de *Tselfatia* Arambourg, C., 1943 (Cénomanien du Maroc), de *Protobrama* Woodward, A. S., 1942 (Cénomanien du Liban) et du rapport éventuel des tselfatioïdes fossiles (Crétacé) et des téléostéens du super-ordre des ostéoglossomorphes. *Ann. Soc. Roy. Zool. Belg.*, Bruxelles, **105** (1-2) : 53-72.

TAVERNE, L. (1983) – Ostéologie et affinités systématiques de *Tselfatia formosa*, téléostéen fossile du Crétacé supérieur de la Mésogée eurafricaine. *Ann. Soc. Roy. Zool. Belg.*, Bruxelles, **113** (2) : 165-181.

TAVERNE, L. (1991) – *Greenwoodella tockensis* Taverne and Ross, 1973 from the Marine Lower Aptian (Töck) of Helgoland (West Germany), the Oldest Known Pachyrhizodontid Fish (Teleostei, Crossognathiformes). *Geol. Jahrb.*, Hannover, A, **120 :** 305-309.

TAVERNE, L. (1993) – Révision de *Kermichthys daguini* (Arambourg, 1954) nov. gen., téléostéen salmoniforme du Crétacé de la Mésogée eurafricaine. *Biol. Jaarb. Dodonaea*, Gent, **60** (1992) : 76-95.

TAVERNE, L. (sous presse a) – Note complémentaire sur *Tselfatia formosa* et position systématique des Tselfatiiformes ou Bananogmiiformes, téléostéens marins du Crétacé (Pisces, Actinopterygii). *Geodiversitas*, Paris.

TAVERNE, L. (sous presse b) – Ostéologie du genre *Plethodus* et des nouveaux genres *Dixonanogmius* et *Pentanogmius*, poissons marins du Crétacé (Teleostei, Tselfatiiformes). *Biol. Jaarb. Dodonaea.*, Gent.

TAVERNE, L. (sous presse c) – Révision du genre *Martinichthys*, poisson marin (Teleostei, Tselfatiiformes) du Crétacé supérieur du Kansas (Etats-Unis). *Geobios*, Lyon.

Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 439-447 - Brussels 1999

Received: 24 December 1998

ARE EGGSHELLS AND EGG CONTENTS OF GREAT AND BLUE TITS SUITABLE AS INDICATORS OF HEAVY METAL POLLUTION?

TOM DAUWE([1]), LIEVEN BERVOETS([2]), RONNY BLUST([2]), RIANNE PINXTEN([1]) AND MARCEL EENS([1])

([1]) Department of Biology, University of Antwerp (U.I.A.), Universiteitsplein 1, B-2610 Wilrijk, Belgium

([2]) Department of Biology, University of Antwerp (R.U.C.A.), Groenenborgerlaan 171, B-2020 Antwerp, Belgium

e-mail: eens@uia.ua.ac.be

Abstract. We examined whether eggs of the Great Tit (*Parus major*) could be used as indicators for lead, arsenic, cadmium, copper and zinc pollution. We collected eggs from two sites with different pollution levels, and measured heavy metal levels in the egg content and eggshell separately. At the polluted site, situated near a metallurgic factory in Hoboken (Belgium), eggshells contained significantly higher concentrations of arsenic, cadmium and lead than did eggshells at the reference site on the campus of the University of Antwerp. Egg contents also contained significantly higher concentrations of lead at the polluted site than at the reference site. Both at the polluted and reference site egg contents contained higher concentrations of zinc and copper than did the eggshell. The eggshell contained higher arsenic and lead concentrations than did the egg content at the polluted site but not at the reference site. There was a clear discrepancy between essential and non-essential elements. Copper and zinc, two essential elements, were highest in the egg content, while arsenic and lead were higher in the eggshell. Moreover, the concentrations of essential elements did not differ significantly between the two sites. We also collected eggs from Blue Tits (*Parus caeruleus*) at the polluted site, and compared metal levels between the eggshell and egg content. Differences between the shell and content were similar to those for the Great Tit. At the polluted site we found no significant differences in metal levels between the two species. Our study indicates that Great and Blue Tits sequester non-essential heavy metals in their eggs, especially in the eggshell. Therefore the eggshell is suitable as an indicator for heavy metal pollution.

Key words: Heavy metals, bioindicators, eggs, Great Tit, Blue Tit, *Parus major*, *Parus caeruleus.*

INTRODUCTION

Terrestrial birds are exposed to heavy metals through air, water and their food. Once a metal has entered the body it can be stored or accumulated, or it can be excreted (BURGER, 1993). The accumulation of metals in organs causes levels to increase with age of the organism and with each succeeding step in the food chain (VAN STRAALEN & ERNST, 1991). Concentrations in long-lived carnivores can reach high levels, and may reach toxic or even lethal levels. Birds can rid the body of heavy metals through the faeces or by depositing

them in the uropygial gland, salt gland (BURGER & GOCHFELD, 1985) and feathers (BURGER, 1993). Females can also eliminate heavy metals by sequestering them in their eggs, which may jeopardise the developing embryo. Although some studies failed to detect elevated levels in the eggs of experimentally dosed females (PATTEE, 1984), others have shown elevated levels of lead and cadmium (MAEDGEN *et al.*, 1982).

Assessing ecosystem health is a daunting task, requiring the selection of indicator species that are representative of the system (BURGER & GOCHFELD, 1996). Because many contaminants can bioaccumulate, there has been a tendency to evaluate toxins in species that are high in the foodchain (BURGER & GIBBONS, 1998). Small passerines such as the Great Tit (*Parus major* L.) and the Blue Tit (*Parus caeruleus* L.) may be useful bioindicators. They are primarily insectivorous during the breeding season, and high in the foodchain. They live in many different habitats and often in large densities. They are territorial in the breeding season, and non-migratory in many populations (CRAMP & PERRINS, 1993). They nest in holes, and use nestboxes, so they are easy to study. These characteristics make them very suitable for monitoring point source contamination (EENS *et al*., 1999).

Biomonitoring requires effective determination of the load of pollutants in the indicator species. Most studies have used internal organs, but demand for non-invasive monitoring techniques has led to the introduction of feathers, faeces and eggs as bioindicators (BURGER, 1993). Eggs are suitable as bioindicators because 1) they come from a specific fragment of the population; 2) they are only formed in a specific period; 3) they have a consistent composition; 4) they are easily sampled and 5) the removal of one egg from a nest has a minor effect on population parameters (FURNESS, 1993). Although eggs have distinct advantages as bioindicators they have scarcely been used to determine heavy metal pollution. Furthermore, most studies have focused on seabirds and waterfowl, while songbirds have received little attention (MORERA *et al*., 1997; NYHOLM, 1998). NYHOLM (1998) recently stressed that there is almost no information on the transfer of heavy metals into eggs of passerine birds.

The objective of this study was to determine to what extent Great and Blue Tits sequester heavy metals in their eggs. BURGER (1994) showed that in marine birds there is a discrepancy between the egg content and the eggshell in heavy metal concentration (see also MORERA *et al*., 1997). Therefore we also analysed the egg content and the eggshell separately. For Great Tits we collected eggs from two sites: at a heavily polluted site in Hoboken close to a metallurgic factory, and at the campus of the University of Antwerp (UIA) about four kilometres to the east of the polluted site. The latter study area is considered as a reference site in this study. Environmental pollution by trace metals is important in the vicinity of the smelter of Hoboken (VAN GRIEKEN, 1996). Dust deposition is the major source of pollution, and affects the immediate surroundings of the factory (VERBRUGGEN, 1994).

MATERIAL AND METHODS

In this study we examined metal levels in eggs coming from a polluted and a reference site. The polluted site at Hoboken was bounded on the west by a metallurgic factory. This

nageoires dorsale et anale et le complexe hémaxanal de type 3 sont des traits qui peuvent se manifester dans de multiples familles de téléostéens mais qui se retrouvent chez tous ou chez beaucoup de Tselfatiiformes (obs. pers.). Cela renforce donc encore l'idée que *Zanclites xenurus* appartient bien à cet ordre.

Zanclites et les autres genres de Tselfatiiformes

Zanclites JORDAN, 1924 se distingue de tous les autres genres connus de Tselfatiiformes par au moins onze caractères particuliers :

(1) Le supraorbitaire s'allonge fortement et s'articule non seulement avec le frontal mais aussi avec le ptérotique dont il éloigne le dermosphénotique. Chez les autres Tselfatiiformes, le supraorbitaire est plus court et ne longe que le frontal, permettant ainsi au dermosphénotique de s'articuler avec le ptérotique (obs. pers.).

(2) la tête de de l'ectoptérygoïde se renfle et s'accole contre un processus osseux de la face externe de l'entoptérygoïde, formant ainsi un puissant contrefort contre lequel s'appuyait, sans doute, un gros palatin. Une telle structure n'existe pas chez les autres Tselfatiiformes (obs. pers.).

(3) L'ectoptérygoïde prend la forme d'une épaisse baguette osseuse édentée. L'ectoptérygoïde montre la forme plus classique d'un os plat, relativement étroit, effilé à ses deux extrémités et presque toujours denticulé chez les autres membres de l'ordre (obs. pers.).

(4) Le carré est réduit, de très faible hauteur et exhibe un condyle articulaire étiré. Cet os offre un développement normal chez les autres Tselfatiiformes (obs. pers.).

(5) Le préoperculaire montre une branche ventrale très allongée et une branche dorsale courte. Chez les autres genres de Tselfatiiformes, les deux branches du préoperculaire sont subégales ou bien la branche dorsale se révèle plus longue que la branche ventrale (obs. pers.).

(6) la *dilatator fossa* est estompée sur la surface du ptérotique et simplement délimitée vers le haut par un processus osseux. Chez les autres Tselfatiiformes, la *dilatator fossa* est étroite et peu profonde mais clairement marquée néanmoins (obs. pers.).

(7) les hémapophyses sont complètement ou partiellement soudées aux corps vertébraux abdominaux. Chez les autres Tselfatiiformes, les hémapophyses de la région abdominales sont autogènes (obs. pers.). Chez *Tselfatia*, néanmoins, on observe aussi une certaine tendance à la fusion des arcs avec les centres vertébraux correspondants (TAVERNE, sous presse a).

(8) les ptérygophores anaux forment des expansions aliformes qui s'imbriquent les unes dans les autres. Il n'y a pas d'élargissement distal de l'axonoste en plateau comme chez les autres Tselfatiiformes (obs. pers.).

(9) le cinquième hypural est réduit et ne touche pas la petite vertèbre terminale urale I et II. Le cinquième hypural des autres Tselfatiformes est bien développé et s'articule sur le dessus de la petite vertèbre terminale (obs. pers.).

(10) L'arc neural de la petite vertèbre préurale I est soudé à cette dernière. Cet arc est autogène chez les autres Tselfatiiformes (obs. pers.).

(11) Le supratemporal et le posttemporal sont articulés. Ce n'est pas le cas chez les autres Tselfatiiformes (obs. pers.).

site is characterised by an extremely high and localised pollution, mainly with arsenic, cadmium, copper, lead and zinc, caused by emissions and dust from ore-piles, blown up by wind. In October 1997, we added 10 Blue Tit and 21 Great Tit nestboxes to the 32 Blue Tit and 28 Great Tit nestboxes that were already present at this site. The reference site, the campus of the University of Antwerp, was situated about four kilometres east from the polluted site. At the reference site there were 43 Great Tit nestboxes, available since 1995.

We collected eggs from nests that were abandoned (either spontaneously or due to human disturbance), and non-viable eggs from Great and Blue Tit nests in Hoboken and the UIA, from April till June 1998. Since BLUS (1982) illustrated that one egg of a nest is representative for the entire clutch, we collected only one egg of a clutch (but see MORERA *et al*., 1997; FURNESS, 1993). We collected nine Great Tit eggs at the polluted site and five at the reference site. We also collected 12 Blue Tit eggs at the polluted site. The eggs were stored in the refrigerator until analysis. All eggs were analysed in the same week.

In the laboratory, eggs were opened round the airchamber with a stainless steel dissection needle. The content of the egg was transferred to an acid-washed polypropylene, 10 ml vial. The inside and outside of the eggshells were washed vigorously in deionised water in the laboratory to remove loosely adherent internal and external contamination (see BURGER, 1994). We assumed that by this procedure most of the aerial contamination from heavy metals was removed from the outside of the eggshells. The eggshell was then put in a separate acid-washed polypropylene, 10 ml vial. To determine dry weight, the samples were, before weighing, put in an oven (60°C) for 24 hours. After we determined the dry weight, we added a 1:1 mixture of HNO_3 (70%) and H_2O_2 (30%) to the egg content and eggshell. We completed the destruction with the microwave procedure described by BLUST *et al.* (1988). After microwave destruction the samples were diluted with deionised water and stored at -20°C until analysis.

We measured cadmium, copper, arsenic, lead and zinc with an axial Inductively Coupled Plasma – Atomic Emission Spectrophotometer (ICP-AES) (Varian Liberty series II). The concentrations are expressed in ppm based on dry weight. All measurements were performed on the same day. All specimens were analysed in batches, along with certified reference material of the Community Bureau of Reference (*i.e.* mussel sample, CRM 278), blanks and a standard calibration curve. Recovered concentrations of the certified samples were within 10% of the certified values, which is an acceptable margin (GOCHFELD & BURGER, 1998)

We used SPSS statistical software to perform the statistical analyses. According to the Kolmogorov-Smirnov goodness-of-fit test all data sets had normal distributions. We used student's t-tests to compare metal levels among sites and species, and paired t-tests to compare metal levels between the egg content and eggshell.

RESULTS

Intersite comparison

The metal levels in the egg content of Great Tits differed significantly between the polluted and reference site only for lead. The lead concentration in the egg content in Hoboken was

more than 15 times higher than levels found at the campus. For all other metals considered, we could not find any significant differences (Table 1). However, we should emphasise that there was a considerable difference in the mean concentrations of arsenic and cadmium between the two sites, being respectively two and 16 times higher at the polluted site in Hoboken.

TABLE 1

Mean concentrations of heavy metals (± SE) in the egg content and eggshell from Great and Blue Tits in the polluted (Hoboken) and reference (campus of the University of Antwerp) sites. Concentrations are expressed in ppm (μg g-1). Parametric student's t-tests were used to test for significant differences ($P < 0.05$) in Great Tits between sites, and in Hoboken between species. P-values are given in brackets.

	Arsenic	Cadmium	Lead	Copper	Zinc
INTERSITE COMPARISON					
Egg content					
UIA	0.22 ± 0.14	0.05 ± 0.01	0.13 ± 0.05	4.8 ± 0.8	69 ± 13
Hoboken	0.45 ± 0.25	0.8 ± 0.6	2.0 ± 0.4	5.5 ± 0.7	62 ± 3
t-test	NS	NS	4.95 (0.001)	NS	NS
Eggshell					
UIA	1.2 ± 0.6	0.08 ± 0.02	0.37 ± 0.16	1.72 ± 0.23	19 ± 6
Hoboken	4.2 ± 0.8	0.31 ± 0.08	15 ± 4	3.2 ± 0.5	28 ± 5
t-test	2.6 (0.03)	2.9 (0.02)	3.6 (0.007)	NS	NS
INTERSPECIFIC COMPARISON					
Egg content					
Blue Tit	0.33 ± 0.13	0.17 ± 0.14	2.2 ± 0.6	5.4 ± 0.7	60 ± 8
Great Tit	0.45 ± 0.25	0.8 ± 0.6	2.0 ± 0.4	5.5 ± 0.7	62 ± 3
t-test	NS	NS	NS	NS	NS
Eggshell					
Blue Tit	3.7 ± 1.2	0.15 ± 0.02	7.4 ± 1.1	2.8 ± 1.1	32 ± 8
Great Tit	4.2 ± 0.8	0.31 ± 0.08	15 ± 4	3.2 ± 0.5	28 ± 5
t-test	NS	NS	NS	NS	NS

When comparing metal levels in eggshells of Great Tits between the two sites, we found significantly higher concentrations of arsenic, cadmium and lead at the polluted site. Lead levels in Hoboken were 40 times higher than at the reference site. Arsenic and cadmium levels at the polluted site were almost four times higher than at the reference site. The essential elements, zinc and copper, did not differ significantly between the two sites (Table 1) but the mean concentrations were respectively 50 and almost 100% higher at the polluted site in Hoboken.

Comparison between eggshell and egg content

At the reference site we found a significantly higher concentration of zinc and copper in the egg content than in the eggshell of Great Tits. The other elements were higher in the eggshell but not significantly so (Fig. 1). At the polluted site we also found a significantly

higher concentration of zinc and copper in the egg content, but the eggshell also contained a significantly higher arsenic and lead concentration (Fig. 2).

We also compared metal levels in the egg content and the eggshell of Blue Tits from Hoboken. Arsenic and lead concentrations were significantly higher in the eggshell. The egg content had a significantly higher copper concentration, while for zinc a similar trend was observed (Fig. 3).

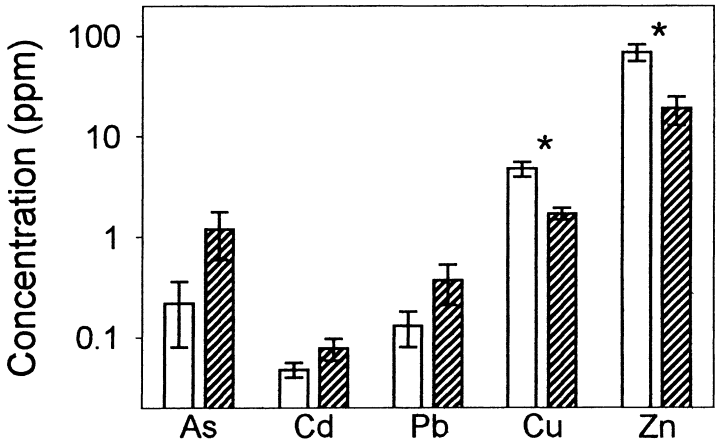


Fig. 1. – The mean concentration (± SE) of heavy metals in the egg content (open bars) and eggshell (striped bars) of Great Tits from the reference site. An asterisk denotes a significant difference between the egg content and the eggshell ($P < 0.05$) in a paired t-test. The concentration axis has a logarithmic scale.

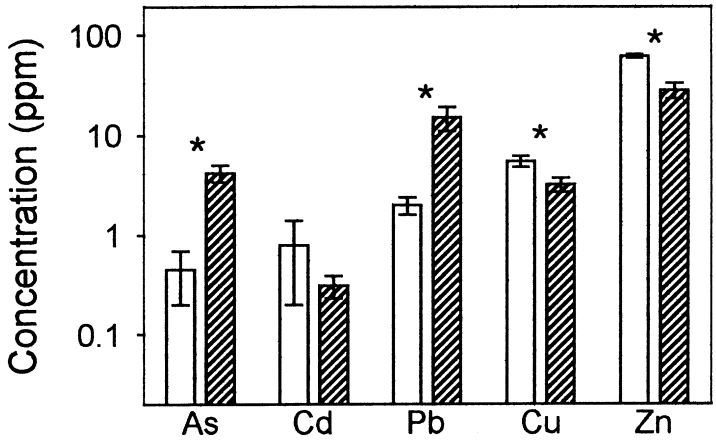


Fig. 2. – The mean concentration (± SE) of heavy metals in the egg content (open bars) and eggshell (striped bars) of Great Tits from the polluted site. An asterisk denotes a significant difference between the egg content and the eggshell ($P < 0.05$) in a paired t-test. The concentration axis has a logarithmic scale.

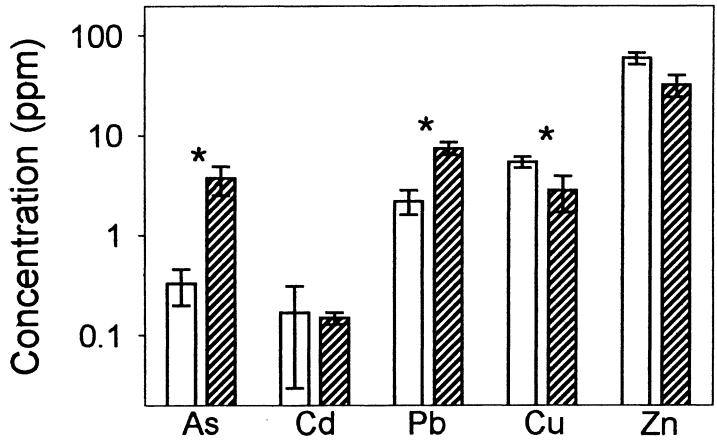


Fig. 3. – The mean concentration (± SE) of heavy metals in the egg content (open bars) and eggshell (striped bars) of Blue Tits from the polluted site. An asterisk denotes a significant difference between the egg content and the eggshell ($P < 0.05$) in a paired t-test. The concentration axis has a logarithmic scale.

Interspecific comparison

At the polluted site where both Great and Blue Tit eggs were collected, we found no significant differences between the two species in metal levels, neither in the eggshell nor in the egg content (Table 1). The mean concentrations in both species were generally similar, except for cadmium (both eggshell and egg content) and for lead (eggshell only). For these metals, concentrations were higher in Blue Tits than in Great Tits.

DISCUSSION

Our study showed marked differences in metal levels in eggshells and egg contents between the polluted and the reference site. Although some studies have failed to detect elevated levels of trace metals in experimentally dosed birds (PATTEE, 1984), our study clearly showed that Great and Blue Tits do sequester heavy metals in their eggs. Especially for lead levels, there were enormous differences between the two sites. We anticipated that lead levels would differ the most because the area round the metallurgic factory is known for its extreme lead pollution (EYLENBOSCH *et al.*, 1984). However, it is still remarkable that the differences were so extreme between the study sites, which are only four kilometres apart. Most studies that have used eggs as a bioindicator, measured organochlorines and mercury because they cause eggshell thinning (KOSTER *et al.*, 1993; OHLENDORF & HARRISON, 1986). Only a few studies have examined other heavy metals (mostly lead and cadmium) in eggs (GOCHFELD & BURGER, 1998), and even less have studied the eggshell and egg content separately (BURGER, 1993; MORERA *et al.*, 1997; NYHOLM, 1998; REID & HACKER, 1982). The levels of lead, cadmium and zinc found at the polluted site in our study are among the highest reported in the literature. Most previous studies have used

marine or piscivorous birds as indicator species (OHLENDORF & HARRISON, 1986; FASOLA *et al.*, 1998). We found only one study that examined metal levels in eggs from Great and Blue Tits. KOTH (1983) measured zinc and lead levels in whole eggs of Great and Blue Tits in urban areas. For both species, levels at the polluted site in our study were markedly higher than those detected in KOTH's study.

The separate analyses of the egg content and eggshell showed a marked difference in the accumulation of heavy metals in both samples. Moreover, our results suggested that there was also a discrepancy between essential and non-essential elements. In both the eggshell and the egg content, mean metal levels were higher at the polluted site, but the differences between the two study sites were greater in the eggshell, which resulted in significantly higher concentrations not only of lead but also of cadmium and arsenic. The mean lead, cadmium and arsenic levels were also higher in the eggshell than in the egg content. The eggshell is mainly composed of calcium, and SCHEUHAMMER (1987) found that trace metals such as cadmium and lead may interact with the metabolic pathway of calcium. Consequently, they may be incorporated more easily in the eggshell. The mean zinc and copper concentrations were, contrary to the non-essential elements, higher in the egg content. This was predictable, because zinc and copper are embedded in the quaternary structure of some proteins, and the egg content has a higher protein concentration than the eggshell. For the considered essential elements, zinc and copper, there were marked differences in the metal levels in the eggshell between the polluted and the reference site. In the egg content however, they did not differ markedly between the two sites, indicating that zinc and copper concentrations are homeostatically controlled in the egg content.

We did not find any significant differences in metal levels between the Great and Blue Tit eggs, although the mean concentrations of cadmium (both in the eggshell and in the egg content) and lead (only in the eggshell) were higher in Blue Tits. At the polluted site, we have found significantly higher concentrations of lead in the outer tail feathers of Blue Tits compared with Great Tits. In general, all heavy metals were higher in the feathers of Blue Tits (unpublished data). BURGER & GOCHFELD (1991) showed that metal levels in females were correlated with metal levels in their eggs, so we anticipated that there would be interspecific differences. However, the differences between the two species in metal levels in the outer tail feathers were not reflected in the metal levels in the eggs. Since this might be due to the small sample size in the present study, further research is necessary in this respect.

In conclusion, our data suggest that eggshells from Great and Blue Tits are suitable as bioindicators. Both essential and non-essential elements are accumulated in the eggshell. Although the egg content is less suitable as a bioindicator, because zinc and copper levels appear to be homeostatically controlled, the higher levels of non-essential elements found in our study indicate the extent to which a developing embryo may be exposed. Quite clearly, the elevated exposure of Great and Blue Tit females to lead at the polluted site caused increased amounts of lead in their eggs. Further work is, therefore, necessary to examine whether embryos or nestlings at the polluted site show an increased mortality rate. Finally, we must emphasise that our sample size was very limited, and that further research on the accumulation of heavy metals in the eggs of Great and Blue Tits is, therefore, necessary.

ACKNOWLEDGMENTS

We thank Dimitri van Pelt, Els Van Duyse, and Sylvie Hurtrez-Boussès for help with the fieldwork. This study was supported by the Fund for Scientific Research Flanders (FWO): Lieven Bervoets is supported by a Postdoctoral Fellowship, while Ronny Blust and Marcel Eens are Research Associates of the FWO. This study was also supported by the Research Council of the University of Antwerp (Rianne Pinxten) and by FWO-project G.0075.98 (Rianne Pinxten).

REFERENCES

BLUS, L.J. (1982) – Further interpretation of the relation of organochlorine residues in brown pelican eggs to reproductive success. *Environ. Pollut*., **28**: 15-33.

BLUST, R., A. VAN DER LINDEN, E. VERHEYEN & W. DECLEIR (1988) – Evaluation of microwave heating digestion and graphite furnace atomic absorption spectrometry with continuum source background correction for the determination of Fe, Cu and Cd in brine shrimp. *J. Anal. At. Spectrom.*, **3**: 387-393.

BURGER, J. (1993) – Metals in avian feathers: bioindicators of environmental pollution. *Rev. Environ. Toxicol.*, **5**: 203-311.

BURGER, J. (1994) – Heavy metals in avian eggshells: another excretion method. *J. Toxicol. Environ. Health*, **41**: 207-220.

BURGER, J. & J.W. GIBBONS (1998) – Trace elements in egg contents and egg shells of Slider Turtles (*Trachemys scripta*) from the Savannah River Site. Arch. Environ. Contam. *Toxicol.*, **34**: 382-386.

BURGER, J. & M. GOCHFELD (1985) – Comparison of nine heavy metals in salt gland and liver of Great Scaup (*Aythya marila*), Black Duck (*Anas rubripes*) and Mallard (*Anas platyrhynchos*). *Comp. Biochem. Physiol.*, **81**: 287-292.

BURGER, J. & M. GOCHFELD (1991) – Cadmium and lead in Common Terns (Aves: *Sterna hirundo*): relationship between levels in parents and eggs. *Environ. Monit. Assess*., **16**: 253-258.

BURGER, J. & M. GOCHFELD (1996) – Ecological and human health risk assessment: a comparison. In*: Interconnections between human and ecosystem health*. DI GUILIO, R.T. & E. MONOSSON (Ed.). Chapman and Hall, London: 127-148.

CRAMP, S. & C.M. PERRINS (1993) – *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic*. Volume VII Flycatchers to Shrikes. Oxford University Press, London.

EENS, M., R. PINXTEN, R.F. VERHEYEN, R. BLUST & L. BERVOETS (1999) – Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. *Ecotox. Environ. Safety*. In press.

EYLENBOSCH, W.J., M.P. VAN SPRUNGEL & R.R. CLARA (1984) – Lead pollution in Antwerp, Belgium. *Ann. Acad. Med. Singapore*, **13**: 224-230.

FASOLA, M., P.A. MOVALLI & C. GANDINI (1998) – Heavy metals, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. *Arch. Environ. Contam. Toxicol.*, **34**: 87-93.

FURNESS, R.W. (1993) – Birds as monitors of pollutants. In: *Birds as monitors of environmental change.* FURNESS, R.W. & J.J.D. GREENWOOD (Eds.). Chapman and Hall, London: 86-143.

GOCHFELD, M. & J. BURGER (1998) – Temporal trends in metal levels in eggs of the endangered Roseate Tern (*Sterna dougallii*) in New York. *Environ. Research*, **77**: 36-42.

KOSTER, M.D., D.P. RYCKMAN, D.V.C. WESELOH & J. STRUGER (1996) – Mercury levels in Great Lakes Herring Gull (*Larus argentatus*) eggs. *Environ. Pollut*., **93**: 261-270.

KOTH, T. (1983) – Zum eisen- blei- und zinkgehalt in eiern von Kohlmeisen (*Parus major*), Blaumeisen (*Parus caeruleus*) und Feldsperling (*Passer montanus*). *Luscinia*, **45**: 23-61.

MAEDGEN, J.L., C.S. HACKER, G.D. SCHRODER & F.W. WEIR (1982) – Bioaccumulation of lead and cadmium in the Royal Tern and Sandwich Tern. *Arch. Environ. Contam. Toxicol*., **11**: 99-102.

MORERA, M., C. SANPERA, S. CRESPO, L. JOVER & X. RUIZ (1997) – Inter- and intraclutch variability in heavy metals and selenium levels in Audouin's Gull eggs from the Ebro Delta, Spain. *Arch. Environ. Contam. Toxicol*., **33**: 71-75.

NYHOLM, N.E.I. (1998) – Influence of heavy metal exposure during different phases of the ontogeny on the development of Pied Flycatcher, *Ficedula hypoleuca*, in natural populations. *Arch. Environ. Contam. Toxicol*., **35**: 632-637.

OHLENDORF, H.M. & C.S. HARRISON (1986) – Mercury, selenium, cadmium and organochlorines in eggs of three Hawaiian seabird species. *Environ. Pollut.* Ser. B, **11**: 169-191.

PATTEE, O.H. (1984) - Eggshell thickness and reproduction in American Kestrels exposed to chronic dietary lead. *Arch. Environ. Contam. Toxicol*., **13**: 29-34.

REID, M. & C.S. HACKER (1982) – Spatial and temporal variation in lead and cadmium in the Laughing Gull (*Larus atricilla*). *Mar. Pollut. Bull*., **13**: 387-389.

SCHEUHAMMER, A.M. (1987) – The chronic toxicity of aluminium, cadmium, mercury and lead in birds: a review. *Environ. Pollut*., **46**: 263-295.

VERBRUGGEN, A. (1994) – *Leren om te keren: milieu- en natuurrapport Vlaanderen*. Garant, Leuven, 823 pp.

VAN GRIEKEN, R. (1996) – Milieu- en natuurrapport Vlaanderen 1996. Wetenschappelijk rapport III.3. Verspreiding van zware metalen.

VAN STRAALEN, N.M. & E. ERNST (1991) – Metal biomagnification may endanger species in critical pathways. *Oikos*, **62**: 255-256.