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Editor :

Prof. Dr. W. VERRAES

Rijksuniversiteit Gent

Laboratorium voor Morfologie en Systematiek der Dieren

Ledeganckstraat 35, B-9000 Gent (Belgium)

The *Belgian Journal of Zoology* publishes original manuscripts in the field of zoology. The manuscripts are evaluated by the editorial board.

The opinions of the authors published in this journal remain, nevertheless, their own responsibility.

Manuscripts are to be sent to : Prof. Dr. Walter VERRAES, Laboratorium voor Morfologie en Systematiek der Dieren, Ledeganckstraat 35, B-9000 Gent (Belgium).

FROM THE EDITOR

I take the pleasure to present here the first issue of the « BELGIAN JOURNAL OF ZOOLOGY ». It continues the series of the « ANNALES DE LA SOCIETE ROYALE ZOOLOGIQUE DE BELGIQUE » - « ANNALEN DER KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE ». During the past years, the Administrative Board of the Royal Belgian Zoological Society has made efforts to internationalize and to modernize the journal. Impulses were especially given by the consecutive Presidents Prof. Dr. O. Vanderborght, Prof. Dr. J. M. Pasteels and Dr. J. Van Goethem.

The internationalization of the journal started already from 1989 (volume 119) on, by enlarging the Editorial Board, including foreign members, and by working effectively with referees from different countries, as well as by accepting without problems the submission for publication of manuscripts of foreign zoologists non-members. Furthermore, there are no restrictions concerning the fields of research.

The renewal has become a fact from the present first issue of volume 120 by giving a shorter, perhaps more attractive name to the journal and also by considering a modern lay-out and nice colour. The quality of the type has been improved. The quality of printing the figures and the photographs remains high, thanks to the care of the Printing Office « Michiels » at Tongeren. I wish to thank all persons who concentrated on this renewal.

We do hope that the members will enjoy the metamorphosis of our journal and will not hesitate to submit their papers of quality. Our journal is covered already for years in Current Contents and in Science Citation Index. It is distributed in more than 42 countries over all continents.

The way from research to published manuscript is quite long. I am grateful to the associate editors Prof. Dr. E. Schockaert and Prof. Dr. M. Chardon, for their efficient editorial assistance.

Prof. Dr. W. Verraes,
Editor

KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE V.Z.W.

SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE A.S.B.L.

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Nieuwe leden 1989 — Nouveaux membres 1989

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RAAD VAN BEHEER — CONSEIL D'ADMINISTRATION VERGADERINGEN — RÉUNIONS

Er werden vijf vergaderingen gehouden : 21 januari, 12 april, 24 mei, 21 september en 13 december 1989.

Le conseil d'administration s'est réuni à cinq reprises, les 21 janvier, 12 avril, 24 mai, 21 septembre et 13 décembre 1989.

WETENSCHAPPELIJKE VERGADERINGEN — RÉUNIONS SCIENTIFIQUES

Trois réunions scientifiques ont été organisées en 1989, un colloque de malacologie à Bruxelles le 20 mai, la journée des jeunes organisée à Bruxelles le 28 novembre et enfin, la journée sur le thème de la biologie cellulaire à Namur le 16 décembre 1989.

Vergadering van 20 mei 1989 — Réunion du 20 mai 1989 Colloquium — Colloque Malacologie

La réunion, organisée en association avec la Société belge de Malacologie et la « Belgische Vereniging voor Conchyliologie », se tient à l'Institut royal des Sciences naturelles de Belgique.

Bienvenue par J. Van Goethem, président de la S.R.Z.B.

Verwelkoming door J. Van Goethem, voorzitter van de K.B.V.D.

Session — Zitting 1 : Président — Voorzitter Cl. Massin (I.R.S.N.B.)

A. V. DHONDT (K.B.I.N.) — La Société Malacologique de Belgique, 1863-1902.

Th. BACKELJAU (K.B.I.N.) — On the original diagnoses of *Arion simrothi* KUNKEL and *Arion magnus* TORRES-MINGUEZ (Gastropoda, Pulmonata).

Ph. BOUCHET (Muséum national d'Histoire naturelle, Paris). — La protoconque des Gastéropodes, marqueur biologique.

Zitting — Session 2 : Voorzitter — Président A. Delsaerdt (Voorzitter B.V.C.)

R. HOUART (S.B.M.), La protoconque des Muricidae et son importance pour la détermination des espèces.

W. SLEURS (K.B.I.N. & K.U.L.). — A biogeographical analysis of the rissoinid fauna of the eastern Pacific (Mollusca : Gastropoda).

A. Verhecken (B.V.C.). — De indol-pigmenten van de mollusken.

R. DUCHAMPS (Président S.B.M.). — Ph. Dautzenberg, son oeuvre, sa collection.

Zitting — Session 3 : Voorzitter — Président Annie Dhondt (K.B.I.N.)

M. POULICEK (U.Lg.). — Mécanismes de la biodégradation des coquilles de Mollusques dans les sédiments marins.

A. SIMON & M. POULICEK (U.Lg.). — Biodégradation anaérobique des coquilles de Mollusques : approche expérimentale.

Y. FINET (Muséum d'Histoire naturelle de Genève). — La faune malacologique des Iles Galapagos : composition faunistique et facteurs influençant les affinités biogéographiques.

Th. WHITEHEAD & K. LAMPRELL (Queensland Museum, Brisbane, Australia). — Research on Australian Bivalves.

Bezoek aan de invertebratenzaal : voorstelling van twee nieuwe toonkasten over Belgische mollusken en van twee nieuwe toonkasten over Ph. Dautzenberg (1849-1935).

Visite de la salle des Invertébrés : présentation de deux nouvelles vitrines sur les mollusques de Belgique et de deux nouvelles vitrines sur Ph. Dautzenberg (1849-1935).

Posters :

R. DUCHAMPS (Président S.B.M.) — Ph. Dautzenberg (1849-1935).

J. VAN GOETHEM (K.B.I.N.). — W. Adam (1909-1988).

A. SIMON, M. MULDERS, G. GOFFINET & M. POULICEK (U.Lg.). — Aspects ultrastructuraux de la biodégradation des trames organiques des coquilles de Mollusques.

W. H. DE SMET & E. A. VAN ROMPU (R.U.C.A.). — Thrush predation and shell polymorphism in *Cepaea nemoralis* (L.) (Helicidae) along the Belgian coastal dunes.

W. H. DE SMET & E. A. VAN ROMPU (R.U.C.A.). — Area effects and clines for shell polymorphism in *Cepaea nemoralis* (L.) (Helicidae) along the Belgian coastal dunes.

F. DELOOR, E. ROLAND & R. SHERIDAN (U.E.M.). — Relation entre la vitesse, la durée du déplacement et la morphologie pédieuse chez quelques Pulmonés Stylommatophores.

E. ROLAND, F. DELOOR & R. SHERIDAN (U.E.M.). — Mise en évidence du pouvoir attractant exercé par le globule muqueux d'*Arion distinctus*.

N. DELAUNOIS & R. SHERIDAN (U.E.M.). — Répartition des Turridae en baie de Morlaix.

L. HIERNAUX. — François-Xavier Roffiaen, membre fondateur et ancien président de la Société Malacologique de Belgique (1820-1898).

Vergadering van 28 october 1989 — Réunion du 28 octobre 1989
Dag der Jongeren — Journée des jeunes

De vergadering gaat door in het Koninklijk Belgisch Instituut voor Natuurwetenschappen.

Organisatie : A. Huysseune en M. Baguette.

BAILLIEUL, M. (R.U.C.A.). — Invloed van complexatie op de biologische beschikbaarheid van cadmium voor het pekelkreeftje *Artemia franciscana*.

MARNEFFE, Y. (U.Lg.). — Modalités et voies de transfert de micropolluants organochlorés (PCB) dans le plancton de l'écosystème mosan.

BAUWENS, J. (U.I.A.). — Invloed van een zuiveringsstation op de invertebraten-gemeenschappen van een Kempische laaglandbeek.

MULDERS, M. (U.Lg.). — Aspects ultrastructuraux de la biodégradation aérobie des trames organiques des coquilles de mollusques.

SIMON, A. (U.Lg.). — Biodégradation et préservation des vertèbres de poisson en milieu marin et en estuaire.

BOGAERTS, K. (S.C.K.). — De invloed van verzuring en aluminium op de fysiologie van de Amerikaanse dwergmeerval, *Ictalurus nebulosus* (Le Sueur).

BEULLENS, K. (K.U.L.). — De relatie tussen het genetisch geslacht en groottedimorfisme bij jonge Europese paling.

KEMPENAERS, B. (U.I.A.). — Competitie tussen de koolmees en de pimpelmees voor slaapplaatsen in de winter.

LENS, L. (U.I.A.). — Interspecific competition in group territorial tits : a study of individual niche shifts.

COLAZZO, S. (U.I.A.). — Beschrijving en funktie van de zang bij de huiskrekel *Acheta domesticus* (Orthoptera: Gryllidae).

CLAESSEN, C. (U.I.A.). — Systematics of the African bat genus Epomophorus (Mammalia : Megachiroptera).

BARBIER, Y. (F.S.A. Gx — U.E.M.). — Entomofaune comparée des terrils d'Hensies et St-Antoine.

MARÉCHAL, B. (U.C.L.). — Impact de l'acarien phytophage Tetranychus urticae sur les cultures de tomates en serre.

MOENS, N. (K.U.L. en L.U.C.). — Chemische en ultrastructurele ontogenie van de mandibulaire klier bij *Formica sanguinea* (Latr.).

JANSSENS DE BISTHOVEN, L. (K.U.L.). — Buccal deformities en Chironomus group thummi larvae (Diptera, Chironomidae) description and quantification.

HENDRICKX, K. (K.U.L.). — Werking van delta-endotoxines van *Bacillus thuringiensis* in de middendarm van Lepidoptera.

PLASMAN, N. (U.L.B.). — Relations entre sous-populations de macrophages et *Trypanosoma cruzi*.

LARDON, F. (S.C.K.). — Effect van 241-Americium op bloedvormende en stromale stamcellen na foetale en perinatale radioactieve besmetting.

Posters :

BOURDON, S. (V.U.B.). — Isometrie criteria.

BYAMUNGU, N., K. MOL & E. KUHN (K.U.L.). — Somatostatin increases plasma T3 concentrations and stimulates in vitro T4-5' deiosination in *Tilapia nilotica* in the presence of high T4 levels.

CLEUREN, J. & DE VREE, F. (U.I.A.). — The use of the tongue and hyoïd apparatus during feeding in *Caiman crocodilus*.

COLFS, C. (V.U.B.). — De invloed van veranderingen van sociale factoren en omgevingsfactoren in gevangenschap bij de tuimelaar, *Tursiops truncatus* (Montagu, 1821).

DAES, G., SCHOETERS, G. & VANDERBORGHT, O. (S.C.K.). — Mineralization of adult mouse bone narrow in vitro.

DE CLERCK, G. (R.U.G.). — Intraspecific variation of the mushroom coral Fungia (Fungia) fungites.

GOFFLOT, F., MAZY, C., VAN MAELE-FABRY, G. & Picard, J.J. (U.C.L.). — Mixtures of chemically defined medium and serum as culture media for 8.5 days mouse embryos.

PACOLET, W., PECTOR, R. & OLLEVIER F. (K.U.L.). — The effect of cattle blood in formulated starter diets on the growth and survival of European glasseels (*Anguilla anguilla* L.).

SMETS, H., NOTERDAEME L. & OLLEVIER F. (K.U.L.). — Serological identification of Aeromonas strains isolated from fish.

TIPS, A. (K.U.L.). — Search for the function(s) of recently discovered myotropic peptides of the locust, *Locusta migratoria migratorioides*.

R. & F. TISSON, F. VAN DAMME, P., OLLEVIER, F. & DE SCHRIJVER, R. (K.U.L.). — Linoleic acid requirement of the African catfish (*Clarias gariepinus Burchell*, 1822) larvae.

VALCKENBORGH, F. (V.U.B.). — Ekologische modellering : populatie dynamiek van levendbarende hagedis, *Lacerta vivipara Jacquin*.

VERDYCK, P. (R.U.C.A.). — The flea beetles (Coleoptera : Chrysomelidae : Alticinae) of a dune wood vegetation.

VOLCKAERT, F. (K.U.L.). — Biochemische genetika van het zwemmen van de Amerikaanse jakobsschelp *Plecopecten magellanicus*.

VOLCKAERT, F. & ZOUROS, E. (K.U.L.). — Biochemical genetics of valve snapping of the scallop *Placopecten magellanicus* (Gmelin).

WIAUX, B., J. DELIGNE & L. DE VOS (ULB). — Dimorphisme antennaire de *Phocarantha semipunctata* (Coleoptera Cerambycidae).

Vergadering van 16 december 1989 — Réunion du 16 décembre 1989
Vergelijkende Celleer — Cytologie comparée

La réunion est organisée aux Facultés Universitaires de Namur, à la mémoire des Professeurs J. Naisse (U.L.B.) et A. Bauchau (F.U.N.).

Organisation : P. De Vos.

Accueil et évocation du Professeur J. Naisse par M^me^ M. LECLERCQ-SMEKENS et M. J. DELIGNE et du Professeur A. Bauchau par M. P. DE VOS.

COMPÈRE, PH. (U.Lg). — Cytologie comparée de l'épithélium branchial de trois crabes : Cancer pagurus, Carcinus maenas et Eriocheir sinensis en relation avec leur aptitude ionorégulatrice.

WELCOMME (F.U.N.). — Source d'énergie pour le transport d'ions dans les branchies du crabe euryhalin Eriocheir sinensis.

DANGUY, A. (U.L.B.). — Lectin Histochemistry of glycoconjugates in secretory cells of fish epidermis.

JANS Y. & JANGOUX, M. (U.E.M.). — Induction expérimentale « in vivo » de la formation de corps mous intracoelomiques chez Holothuria tubulosa.

LORET , S. (F.U.N.). — Caractères morphologiques et biochimiques des types cellulaires de la glande digestive du crabe Carcinus maenas.

JANGOUX, M. & L. LAMBERT, D. (U.E.M.). — Nature mésothéliale de la musculature tégumentaire chez les Astéries (Echinodermata).

VAN HERP (Katholieke Universiteit Nijmegen). — Neuropeptides and neurotransmitters in the organ X-sinus gland complex, an important neuro-endocrine integration center in the eyestalks of crustaceans.

KESTEMONT, PH. (F.U.N.). — Etude comparative de la croissance ovocytaire chez les Poissons à ovogenèse synchrone et asynchrone.

HUYSSEUNE, A. (R.U.G.). — Chondroid bone in cichlid Fish : actual state of knowledge.

CLOPTENS, F. (K.U.L.). — Het voorkomen van « adipokineticlike » hormonen bij enkele Insekten.

HEMPTINNE, J.-L. (U.L.B.). — Les glandes exocrines des genitalia des femelles d'Adalia bipunctata.

SANDERSON, F., THIBAUT-VERCRUYSSEN, R. & RONVEAUX-DUPAL, M.-F. (F.U.N.). — Oxidative stress and bledding phenomena in relation with the cytoskeleton.

DEPELCHIN, S., LECLERCQ-SMEKENS, M., DEGEN, A. & LELOUP, R. (F.U.N.). — Etude morphologique comparative de la prolifération et de la différentiation de kératinocytes humains cultivés sur divers substrats.

RASMONT , M. (U.L.B.). — Différenciation cellulaire des Eponges d'eau douce.

Posters :

H. RAES, H. (R.U.G.). — Two types of endocrine cells in the midgut of the Honeybee.

RAES, H. & DE COSTER, W. (R.U.G.). — Subcellular effects of mercury on the columnar cells of the Honeybee ventriculum.

Rapport de l'assemblée générale du 20 janvier 1990
Verslag van de algemene vergadering van 20 januari 1990

1. Approbation du procès-verbal de l'assemblée générale du 21 janvier 1989.
   Goedkeuring van het verslag van de algemene vergadering van 21 januari 1989.

Le rapport de l'assemblée générale du 21 janvier 1989 publié dans les Annales 119 (1) : 101-103 est approuvé à l'unanimité.

Het verslag van de algemene vergadering van 21 januari 1989, zoals verschenen in de Annalen 119 (1) : 101-103, wordt eenparig goedgekeurd.

2. Activiteitsverslag 1989 door J. Van Goethem, voorzitter.
   Rapport d'activités 1989 par J. Van Goethem, président.

De Vereniging organiseerde drie bijeenkomsten. De eerste was een colloquium met als thema « Malacologie », dat plaats had op het K.B.I.N. Het was bedoeld als een herdenking van het 125-jarig bestaan van onze Vereniging, die in 1863 gesticht werd onder de benaming « Société malacologique de Belgique ». Daarom traden naast onze eigen Vereniging nog als coorganisatoren op : de « Société belge de Malacologie » en de « Belgische Vereniging voor Conchyliologie ». Twee firma's hebben in ruime mate deze manifestatie gesponsord.

De tweede bijeenkomst was de jaarlijkse « Jongerendag », eveneens op het K.B.I.N. De jongerenafgevaardigden, Ann Huysseune en Michel Baguette, die tijdelijk Thierry Hance vervangt, mogen van harte gelukgewenst worden voor de puike organisatie en de grote opkomst van voordrachtgevers.

La troisième réunion a eu lieu aux « Facultés Universitaires de Namur » à l'invitation du Prof. P. De Vos. La réunion avait pour thème la Cytologie comparée ; elle était organisée à la mémoire des professeurs J. Naisse (U.L.B.) et A. Bauchau (F.U.N.).

La participation à chacune de ces trois réunions était très satisfaisante. Au total 46 communications et 32 affiches ont été présentées. La qualité scientifique était excellente (voir aussi ci-dessus).

Le Conseil d'Administration s'est réuni cinq fois.

Krachtlijnen van het beleid zijn :

— het moderniseren en verder internationaliseren van het wetenschappelijk tijdschrift van onze Vereniging,

— de inspanningen i.v.m. de wetenschappelijke vergaderingen concentreren m.a.w. minder vergaderingen houden per jaar maar ze doen winnen aan belang,

— vooral de jonge vorsers aanspreken en hen een forum bieden.

3. Verslag over de Annalen door W. Verraes, hoofdredakteur.

Rapport sur la publication des Annales, par W. Verraes, rédacteur en Chef.

Ons tijdschrift is bezig aan een metamorfose, terwijl het ondertussen evenwel volwaardig funktioneel-operationeel moet blijven. Dit is noch voor organismen, noch voor tijdschriften een eenvoudige zaak.

Jaargang 119 bestond zoals gebruikelijk uit de resp. in juni en in december 1989 verschenen twee afleveringen, maar daarnaast hebben wij nog een supplement in augustus 1989 kunnen laten verschijnen.

Even een formeel overzicht :

— Aflevering 1 telde 103 pp. en bevatte naast het « In Memoriam Emeritus Prof. Dr. L. De Coninck » nog 5 gewone artikels en twee reeksen Abstracts, resp. rond aktiviteiten der Vereniging in Louvain-la-Neuve (22.10.88) en in Brussel (10.12.88), alsook boekbesprekingen en de publikatie van het Administratief Rapport 1988, verzorgd door onze sekretaris, de heer Henri André.

— Aflevering 2 telde 127 pp. en was naast de publikatie van twee gewone artikels helemaal gewijd aan het Colloquium Malacologie dat doorging in Brussel (20.05.89). Een extra-inspanning werd geleverd om de geschreven neerslag van dit colloquium nog in december 1989 het licht te laten zien.

— Tenslotte werd nog een supplement van 127 pp. uitgegeven, waarin de 218 Abstracts van het Third International Congress of Vertebrate Morphology, dat doorging van 20 tot 25 augustus 1989 in Antwerpen, werden opgenomen.

De redaktionele verwerking van de ingezonden stukken neemt alsmaar meer tijd in beslag. Ik waardeer dan ook zeer de onbaatzuchtige hulp van mijn kollega's E. Schockaert en

M. Chardon, die elk een deel van het redaktioneel werk op zich nemen met eigen verantwoordelijkheid, net zoals ik onbezoldigd en in de z.g. vrije tijd, wat niet te onderschatten is. Daarenboven wordt de taal der manuskripten thans in regel onder de Engelse loupe genomen door Mr. R. Kime en onder de Franse loupe door M. Chardon, wat eveneens zeer gewaardeerd wordt. Tenslotte is sinds 1989 de Editorial Board effektief geïnternationaliseerd : naast 7 Belgische leden maken nu ook 10 buitenlandse leden deel uit van deze Board.

Dit zijn allemaal signalen die wijzen in de richting van een kwaliteitsverhoging van het tijdschrift én die het tot een internationaal, niet tot één discipline beperkt, tijdschrift maken. Maar wij willen doorheen deze geruisloze metamorfose ook het visueel imago van ons tijdschrift aantrekkelijker maken. Een werkgroep en de Raad van Beheer bespreken de wijziging van het omslagblad en enkele andere vereenvoudigingen, alsook de mogelijkheid tot het insturen van de manuskripten op schijf, dit alles binnen de ruimte die de Penningmeester ons kàn toestaan en ruggespraak met de drukkerij Michiels in Tongeren.

Uiteraard is de voorbereiding van de publikatie der eerste aflevering van volume 120, die in juni 1990 het levenslicht moet zien, volop aan de gang. De aangekondigde vorm-transformaties zullen dit jaar verder worden uitgetest vooraleer in omloop te worden gebracht.

Bij dit alles konden en kunnen wij rekenen op de vlotte en sympatieke medewerking en inzet van de heer M. Toppets en zijn medewerkers, die er altijd in slagen om een verzorgde uitgave uit het niets te voorschijn te toveren, én op tijd. Ik waardeer dit zeer en wens hier opnieuw mijn dank hiervoor uit te spreken. Wij wéten dat er in het heelal niets « vanzelf » gebeurt, en die onwrikbare wet geldt niet alleen voor de opbouw van wetenschappelijke kennis, maar ook voor de publikatie ervan, net zoals dit geldt voor het léven der Vereniging.

Tot slot mijn welgemeende dank aan de auteurs, die ons hun geesteskinderen willen toevertrouwen, en aan de anonieme referees, die « voor de goede zaak » een kwaliteitskontrole van de aangeboden produkten doen.

4. Rapport sur la gestion de la bibliothèque par J. Deligne, bibliothécaire.
   Verslag over de werking van de bibliotheek door J. Deligne, bibliothecaris.

Expédition des Annales. — Les fascicules 118(2) et 119(1) ont été adressés à tous les correspondants étrangers qui nous envoient en échange une ou plusieurs revues et à tous nos clients ainsi qu'à deux revues bibliographiques. Les fascicules 119(2) et 119 (suppl. 1) sont en cours d'expédition. Les réclamations relatives à des numéros plus anciens ont été satisfaites.

Gestions des collections. — Suite à une réorganisation des Services de l'Université Libre de Bruxelles qui gérent notre bibliothèque, les ouvrages les plus intéressants et les plus récents de nos collections ont été regroupés dans la « Bibliothèque des Sciences et Techniques » (B.S.T.) de l'Université, sise au 30 av. Depage à 1050 Bruxelles. Sur la présentation de leur carte, les membres de notre Société peuvent avoir accés non seulement à nos collections mais aussi à l'ensemble des ouvrages de la B.S.T. Pour toute information complémentaire ou pour tout problème éventuel, le bibliothécaire invite les membres à prendre contact avec lui.

Remerciements. — Nous remercions vivement M^me^ Cantraine et M. De Cock ainsi que l'ensemble du personnel de la B.S.T. pour leur précieuse collaboration.

5. Financieel verslag door F. Fiers, penningmeester.
   Rapport financier par F. Fiers, trésorier.

Het boekjaar 1989 wordt afgesloten met een batig saldo van 31.101 fr. De factuur voor de aflevering 119 (2) moet evenwel nog betaald worden. Deze som wordt gedekt door de toegekende subsidie van het Ministerie van Onderwijs (subs. '89) en de achterstallige facturen (verkoop en auteurs-bijdragen).

De inkomsten van het voorbije jaar zijn vooral gedrukt door de afwezigheid van de subsidie van het « Ministère de l'Education nationale ». Daartegenover blijken de verkoop van het volume 117 suppl en de publiciteitsbijdragen belangrijke inkomsten die het verlies van hogervermelde subsidie enigszins compenseren.

Het vooropgesteld budget voor het boekjaar 1990 voorziet een volume 120 met maximaal 200 blz. Deze noodzakelijke beperking volgt uit de noden voor het verzenden van het tijdschrift (119/2, 119/suppl en 120/1) die dit jaar zullen oplopen tot een bedrag van 70.000 fr.

Tot slot wil ik hier de heer J. Van Goethem danken voor het waarnemen van het financieel beheer tijdens mijn langdurige afwezigheid in het buitenland.

6. Aanduiding van twee commissarissen ter nazicht van de rekeningen.

Désignation de deux commissaires aux comptes.

M[lle] A.-M. Leloup et M. A. Ovaere sont désignés comme commissaires aux comptes. Après vérification décharge est donnée aux administrateurs. De algemene vergadering verleent ontheffing aan de beheerders.

7. Elections statutaires — Statutaire verkiezingen.

Le mandat des administrateurs suivants est unanimement renouvelé pour deux ans : H. M. André, J. Deligne, P. De Vos, F. Fiers, G. Goffinet et E. Schockaert.

8. Aktiviteiten 1990 — Activités 1990.

Het volgende programma voor 1990 wordt goedgekeurd :

14 maart 1990 : Vergadering over aquatische organismen (zoete en mariene waters), in de K.U. Leuven. Organisatie : J. Billen & F. Ollevier.

16-17 november 1990 : Mechanismen van biologische herkenning, te Antwerpen. Organisatie : F. De Vree & J. Hulselmans (U.I.A.— R.U.C.A.). Vergadering in combinatie met de dag der jongeren. Organisatie : A. Huysseune en M. Baguette.

9. Divers — Varia.

M[lle] A.-M. Leloup propose la création d'un groupe de travail « Pédagogie de la Zoologie ». Un résumé d'une page de ce projet peut être envoyé lors d'un prochain courrier.

M. J. Godeaux signale que le prochain prix Edouard Van Beneden, prix quinquennal de la Société royale des Sciences de Liège, sera attribué en 1990. Tout renseignement complémentaire, s'adresser au Pr J. Godeaux, à l'Institut de Zoologie de l'Université de Liège.

Zeven nieuwe geassocieerde leden worden aanvaard : Ph. Berbiers, K. Bogaert, G. De Boeck, Y. Quinet, E. Schoeters, J. Tits, C. d'Udekem d'Acoz.

De volgende algemene vergadering zal plaats hebben op 19 januari 1991.

H. M. André,
Secrétaire — Secretaris

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MODÉLISATION DE LA CROISSANCE D'UNE POPULATION EN PHASE COLONISATRICE : LE CAS DE *APHIS FABAE* SCOPOLI (HOMOPTERA : APHIDIDAE)

par

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RÉSUMÉ

Lorsque la distribution d'âges d'une population est constante, LOTKA (1925) a montré que sa croissance peut être décrite par l'équation exponentielle : $N_t = N_0 e^{rt}$ dans laquelle N_0 est l'effectif initial de la population, N_t l'effectif au temps t et r le taux intrinsèque d'accroissement naturel.

Cependant, si cette équation peut prédire, dans des conditions théoriques définies, la croissance d'une population établie depuis longtemps, elle ne peut s'appliquer à une population colonisatrice.

En effet, dans le cas d'*Aphis fabae*, la colonisation d'une nouvelle plante-hôte se fait généralement au départ de quelques jeunes femelles, c'est-à-dire que la distribution d'âges est éloignée de celle prédite par la table de survie et de fécondité. De plus, cette distribution sera modifiée chaque fois qu'une nouvelle larve sera produite ou qu'un individu mourra.

Pour résoudre ce problème, l'évolution du taux de natalité d'une population créée par une femelle virginipare adulte âgée de 1 jour a été étudiée. A partir de cette observation, un nouveau modèle a été établi. Il montre que la natalité évolue de manière sinusoïdale amortie et atteint après 50 jours la valeur du taux intrinsèque de natalité. En conséquence, la croissance de la population est plus rapide et le nombre d'individus plus élevé dans le cas d'une population en phase colonisatrice par rapport à ce que le modèle exponentiel prévoit.

Modelisation of the population increase during a colonizing phase : The case of *Aphis fabae* Scopoli (Homoptera : Aphididae)

SUMMARY

LOTKA (1925) showed that, when the age distribution within a population is constant, the growth can be described by the exponential equation $N_t = N_0 e^{rt}$, where N_0 is the initial size of the population, N_t the size at time t, and r the intrinsic rate of natural increase.

Yet, if this equation may predict the growth of a long standing population in defined conditions, it cannot be applied to colonizing populations.

Indeed, in the case of *Aphis fabae,* the colonization of the host plant starts generally with a few young females, meaning that a population at an age distribution is far away from that predicted by life and fertility tables. Moreover, this distribution will be modified according to births and deaths.

To solve this problem, we have studied the evolution of the birth rate in a population created by only one newly-hatched adult virginiparous female. A new model has been established, which shows that the birth rate evolves in a damped sinusoidal way and reaches the intrinsic birth rate only after 50 days.

Consequently, the population appears to grow faster in a colonizing population than it is predicted by the exponential equation.

INTRODUCTION

L'établissement de la table de survie et de fécondité d'une espèce est un des éléments essentiels des études de dynamique de population. Elle contient entre autre, les probabilités de survie pour chaque âge, la fécondité âge-spécifique et prédit la distribution d'âges de la population (DEEVEY, 1947 ; ANDREWARTHA et BIRCH, 1954 ; LEBRUN, 1973 ; SOUTHWOOD, 1976).

Le taux d'accroissement de la population (r) s'obtient en résolvant l'équation de LOTKA (1925) :

$$\frac{dN}{dt} = bN - \mu N = (b - \mu)N = rN \qquad (1)$$

où (b) est le taux instantané de natalité, (μ) le taux instantané de mortalité et (dN) l'accroissement de l'effectif N au cours de l'intervalle de temps infinitésimal dt. L'intégration de l'équation (1) est le modèle exponentiel bien connu :

$$N_t = N_0 e^{rt} \quad (2)$$

Si la distribution d'âges de la population considérée est stable, c'est-à-dire que la proportion des différentes classes d'âges reste constante au cours du temps, on peut démontrer que les taux de natalité (b) et de mortalité (μ), et par conséquent le taux d'accroissement (r) sont des constantes. Celui-ci reçoit alors le nom de « taux intrinsèque d'accroissement naturel » et peut se calculer grâce à l'équation suivante :

$$1 = \sum_{0}^{k} e^{-r_m x} \, l_x m_x \quad (3)$$

où x est l'âge, k est la valeur maximale de x, l_x la probalité de survie et m_x la fécondité âge-spécifique (LOTKA, 1925 ; PIELOU, 1969).

Ce taux constant de croissance permet de prédire l'évolution des effectifs d'une population installée depuis suffisamment longtemps pour avoir atteint une distribution stable des différentes classes d'âge.

Par contre, il ne donne aucun renseignement sur l'évolution des effectifs d'une population en phase colonisatrice. C'est-à-dire, par exemple, ce qui se passe lorsqu'un puceron colonise une nouvelle plante hôte et y fonde une population. Dans ce cas, chaque fois qu'une larve est déposée, la distribution d'âges est en effet modifiée et dès lors le taux d'accroissement devient variable. La question se pose alors de savoir comment évolue ce taux d'accroissement pendant la phase colonisatrice.

Pour résoudre ce problème, le cheminement suivi comprend deux étapes. Dans un premier temps la table de survie et de fécondité de *Aphis fabae* a été construite. Les résultats utiles ici seront brièvement exposés. Ensuite, sur base des données expérimentales obtenues, l'évolution de la natalité d'une population créée au départ d'une seule femelle adulte âgée de 1 jour a été analysée et un nouveau modèle de croissance a été élaboré.

TABLE DE SURVIE DE *APHIS FABAE*

Matériel et méthodes

La table de survie de *Aphis fabae* a été établie en suivant une cohorte de 56 femelles virginipares de la naissance à la mort du dernier individu.

Chaque jour, les événements concernant chaque femelle ont été consignés dans un tableau, à savoir, la mort, la mue et le nombre de larves déposées.

L'expérience a été réalisée dans des conditions constantes de température, d'humidité et de luminosité. Une description exhaustive de ces conditions est entreprise dans HANCE (1988). En outre, on s'est efforcé à ce qu'aucun facteur ne soit limitant : ni la nourriture, ni l'espace et qu'aucune prédation ne soit possible. La

méthode de calcul des différents paramètres de la population est également décrite dans HANCE (1988).

Résultats

Le Tableau 1 présente la table de survie et de fécondité. La première colonne contient les valeurs de probabilité d'être encore en vie à l'âge x (l_x), et la seconde colonne la fécondité journalière moyenne, m_x.

TABLEAU 1

*Table de survie et de fécondité d'*Aphis fabae. x : *âge en jour,* l_x : *probabilité d'atteindre l'âge* x, m_x : *fécondité de l'âge* x, r_m : *taux d'accroissement naturel,* c_x : *propotion des individus d'âge* x

x	l_x	m_x	$l_x m_x$	$e^{-r_m x} l_x m_x$	$e^{-r_m x} l_x$	C_x	C_x %
0	1,000	0,000	0,000	0,0000	1,0000	0,3666	
1	0,974	0,000	0,000	0,0000	0,6285	0,2304	
2	0,956	0,000	0,000	0,0000	0,3981	0,1460	89,50 %
3	0,938	0,000	0,000	0,0000	0,2521	0,0924	
4	0,938	0,000	0,000	0,0000	0,1627	0,0596	
5	0,938	0,057	0,053	0,0060	0,1050	0,0385	
6	0,920	2,767	2,546	0,1839	0,0664	0,0244	
7	0,903	6,818	6,157	0,2869	0,0421	0,0154	
8	0,903	6,860	6,195	0,1863	0,0272	0,0100	
9	0,850	7,717	6,559	0,1273	0,0165	0,0060	10,50 %
10	0,850	8,019	6,816	0,0854	0,0106	0,0039	
11	0,850	7.906	6,720	0,0543	0,0069	0,0025	
12	0,832	6,863	5,710	0,0298	0,0043	0,0016	
13	0,814	6,102	4,967	0,0167	0,0027	0.0010	
14	0,797	5,313	4,234	0,0092	0,0017	0,0006	
15	0,761	4,778	3,636	0,0051	0,0011	0,0004	
16	0,726	4,049	2,940	0,0027	0,0007	0,0002	
17	0,690	3,461	2,388	0,0014	0,0004	0,0001	
18	0,690	2,333	1,610	0,0006	0,0003	0,0001	
19	0,637	2,083	1,327	0,0003	0,0002	0,0001	
20	0,602	1,647	0,991	0,0002	0,0001	0,0000	
21	0,566	0,781	0,442	0,0000	0,0001	0,0000	
22	0,513	0,690	0,354	0,0000	0,0000	0,0000	
23	0,478	0,222	0,106	0,0000	0,0000	0,0000	
24	0,460	0,231	0,106	0,0000	0,0000	0,0000	
25	0,460	0,038	0,017	0,0000	0,0000	0,0000	

TABLEAU 1 *(suite)*

x	l_x	m_x	$l_x m_x$	$e^{-r_m x} l_x m_x$	$e^{-r_m x} l_x$	C_x	C_x %
26	0,425	0,000	0,000	0,0000	0,0000	0,0000	
27	0,372	0,000	0,000	0,0000	0,0000	0,0000	
28	0,319	0,000	0,000	0,0000	0,0000	0,0000	
29	0,301	0,000	0,000	0,0000	0,0000	0,0000	
30	0,230	0,000	0,000	0,0000	0,0000	0,0000	
31	0,124	0,000	0,000	0,0000	0,0000	0,0000	
32	0,106	0,000	0,000	0,0000	0,0000	0,0000	
33	0,071	0,000	0,000	0,0000	0,0000	0,0000	
34	0,035	0,000	0,000	0,0000	0,0000	0,0000	
35	0,035	0,000	0,000	0,0000	0,0000	0,0000	
36	0,035	0,000	0,000	0,0000	0,0000	0,0000	
37	0,035	0,000	0,000	0,0000	0,0000	0,0000	
38	0,000	0,000	0,000	0,0000	0,0000	0,0000	
TOTAL		78,735	63,875	0,9961	2,728	1,0000	100,00 %

En appliquant la formule (3), le calcul du taux intrinsèque d'accroissement naturel vaut 0,438, ce qui revient à dire que dans ces conditions, la population double tous les 1,58 jours. Les taux intrinsèques de natalité et de mortalité valent respectivement 0,461 et 0,023.

Ce sont ces données expérimentales qui serviront de base au raisonnement suivant.

ÉTAPES DE LA CONCEPTION D'UN NOUVEAU MODÈLE

Modélisation de l'évolution de la natalité

La question posée peut se résumer de la façon suivante : dans le cas d'une population créée au départ d'une seule femelle virginipare adulte âgée de 1 jour, comment les paramètres de la population vont-ils évoluer et quel temps faudra-t-il avant d'atteindre une distribution d'âges stable ?

Plusieurs éléments sont à considérer. Le taux d'accroissement d'une population est la différence entre le taux de natalité et le taux de mortalité. Il a été montré par LOTKA (1925) que lorsque la distribution d'âges est stable, ce taux est constant. Cependant, la distribution d'âges d'une population qui se constitue au départ d'une seule femelle va fortement se modifier à chaque intervalle de temps, avec la ponte de nouveaux individus, avant d'atteindre une valeur fixe. Dès lors, le taux de natalité b devient un paramètre variable qui va conditionner les valeurs du taux d'accroissement pour chaque unité de temps.

Le taux de natalité s'exprime comme étant le rapport du nombre total de naissances dans la population au temps t et du nombre total d'individus, soit $B_t/N_t = b$.

Or, lorsque la population est créée par une femelle âgée de 1 jour, le nombre total de naissances le premier jour est assimilable à la valeur de la fécondité m_x de ce jour, c'est-à-dire m_5 dans le cas de la femelle virginipare de *Aphis fabae*. Les jours suivants, ce nombre restera la valeur de m_x correspondant à l'âge de la femelle et ce jusqu'au moment où les larves déposées le premier jour se mettront également à pondre, ce qui pour *Aphis fabae* est le sixième jour. Donc, pendant les cinq premiers jours, le nombre de naissances dans la population est équivalent à la fécondité journalière de la femelle de départ. De même, la valeur de N_t le premier jour est égale à 1, étant donné qu'une seule femelle constitue alors toute la population. Ensuite, la valeur de N_t s'accroîtra du nombre de larves produites la veille, à condition que, pour faciliter le raisonnement, la mortalité de ces larves soit considérée comme nulle. Dans le cas exemplatif de *Aphis fabae*, l'évolution de la natalité pour les cinq premiers jours calculée sur base des valeurs de la table de survie s'exprime comme suit :

$$b_1 = B_{t1}/N_1 = m_5/1 = 0{,}057$$
$$b_2 = B_{t2}/N_2 = m_6/(1 + m_5) = 2{,}767/1{,}057 = 2{,}618$$
$$b_3 = B_{t3}/N_3 = m_6/(1 + m_5 + m_6) = 1{,}783$$
$$b_4 = B_{t4}/N_4 = m_6/(1 + m_5 + m_6 + m_7) = 0{,}645$$
$$b_5 = B_{t5}/N_5 = m_6/(1 + m_5 + m_6 + m_7 + m_8) = 0{,}441$$

On remarque immédiatement que les valeurs de b pour chaque unité de temps sont différentes.

Sur base des valeurs précédentes, il est possible de calculer :

$$\frac{b_1 - b_2}{t_1 - t_2} = \frac{\Delta b}{\Delta t}$$

Si l'intervalle de temps tend vers zéro, alors la limite de Δb est la dérivée de b, soit db.

Dans le cadre de ce travail, on appellera la quantité infinitésimale db/dt : « vitesse instantanée de natalité, v_t ». De nouveau, grâce aux données expérimentales on détermine :

$$v_2 = \frac{b_2 - b_1}{t_2 - t_1} = 2{,}561$$

$$v_3 = \frac{b_3 - b_2}{t_3 - t_2} = 0{,}835$$

$$v_4 = \frac{b_4 - b_3}{t_4 - t_3} = -1{,}138$$

$$v_5 = \frac{b_5 - b_4}{t_5 - t_4} = -0{,}204$$

On constate que les vitesses ne sont pas égales entre elles et que la quantité,

$$\frac{v_2 - v_1}{t_2 - t_1} = \frac{\Delta_v}{\Delta_t}$$

peut également être calculée ; elle exprime alors la variation de la vitesse de natalité entre deux intervalles de temps. Lorsque l'intervalle de temps tend vers zéro, alors : Δv tend vers dv et $dv/dt = a_t$ ou l'accélération instantanée de natalité. Sachant que $v = db/dt$, dv/dt sera égal à d^2b/dt^2. Comme pour la vitesse, l'accélération peut être calculée sur base des données expérimentales et on obtient les valeurs du Tableau 2.

TABLEAU 2

Valeurs de b_t, v_t *et* a_t *pendant les cinq premiers jours de croissance d'une population au départ d'une femelle âgée de 1 jour, de* Aphis fabae

Jour	*b*	*v*	*a*
1	0,057	—	—
2	2,618	2,561	—
3	1,783	− 0,835	− 3,581
4	0,645	− 1,138	− 0,303
5	0,441	− 0,204	0,934

Trois éléments vont donc influencer l'évolution du taux d'accroissement : le taux de natalité instantané (b_t), la vitesse instantanée de natalité, et l'accélération instantanée de natalité. Il est donc nécessaire d'établir la relation existant entre ces trois éléments. Si l'accélération était nulle, la vitesse serait constante et le taux de natalité serait incrémenté, à chaque unité de temps, d'un nombre constant. De même, une accélération constante ou variable déterminera de manière différente l'évolution de la natalité. Il est donc intéressant de trouver la relation qui existe entre l'accélération et la natalité.

Le Tableau 2 laisse supposer qu'il existe une relation linéaire entre l'accélération et la natalité du type :

$$b = C_1 - C_2 a \qquad (4)$$

Le calcul de régression linéaire sur ces valeurs permet, en effet, d'obtenir un coefficient de détermination de 0,9977. Il reste à découvrir la signification des

constantes qui permettent d'exprimer cette relation linéaire existant entre l'accélération et la natalité.

Un premier facteur qui peut modifier, de manière constante, l'évolution de la natalité, est la durée du temps de développement (D). Il est facile de s'en rendre compte en imaginant deux espèces qui possèdent la même fécondité, mais dont l'une commence à pondre un jour plus tôt. Une population de cette espèce fondée au départ d'un seul œuf aura une descendance un jour plus tôt. Cette avance d'un jour, se répercutera aussi sur le moment où les premières larves de la seconde génération apparaîtront à leur tour, cette fois deux jours avant celles de la première population. Cet effet cumulatif représente une accélération supérieure. Il en découle que plus le temps de développement sera long, plus la population devra fournir un grand effort de natalité pour obtenir une même accélération.

Le même raisonnement est applicable à la fécondité. A savoir que deux populations dont les espèces respectives sont dotées du même temps de développement mais non de la même fécondité n'auront pas le même faciès d'évolution quant à leur natalité. Deux éléments interviennent dans la fécondité : d'une part, le nombre d'œufs (n) qu'une femelle est capable de pondre au maximum durant sa vie (taux brut de reproduction : G.R.R.) et d'autre part, la durée de la vie adulte (I). Plus celle-ci sera longue pour un même nombre d'œufs pondus, moins les accélérations de natalités seront contrastées. C'est donc du rapport G.R.R./I = F, dont il faut tenir compte.

L'équation 4 peut donc s'exprimer sous la forme :

$$b = C_1 - (D/F)a$$

ou encore :

$$b = C_1 - \frac{D}{F}\frac{d^2b}{dt^2} \qquad (5)$$

sachant que

$$a = \frac{d^2b}{dt^2}$$

Cependant, cette équation ne tient pas compte de la mortalité possible des femelles au cours de leur période d'oviposition. Cette mortalité est un effet frein qui s'oppose à la natalité et qui s'applique sur la vitesse en la diminuant. Elle s'exprime mathématiquement comme $- Sv$ (où S est la survie). L'équation (4) devient alors :

$$b = C_1 - \frac{D}{F}a - Sv$$

ou encore :

$$b = C_1 - \frac{D}{F}\frac{d^2b}{dt^2} - S\frac{db}{dt} \qquad (6)$$

Recherche des solutions de l'équation (6)

Il s'agit d'une équation différentielle linéaire du second degré à coefficients constants. Selon le théorème d'existence et d'unicité des solutions, elle possède une solution unique (ROUCHE et MAWHIN, 1973). Pour notre part, la méthode de résolution adoptée est celle des approximations successives. Elle consiste à chercher l'ensemble des solutions particulières dont la somme constitue la solution générale de l'équation (DEFARES et SNEDDON, 1960). Cette méthode offre l'avantage de faciliter la compréhension du devenir des constantes lors des développements mathématiques.

a) *Recherche des solutions particulières de*

$$D\frac{d^2b}{dt^2} + S\frac{db}{dt} + Fb = C_1 \qquad (6)$$

1) C_1 étant une constante, C_1/F est une solution particulière de l'équation et toutes les solutions de (6) tendent vers C_1/F lorsque t tend vers l'infini. On peut vérifier cela en remplaçant b par sa valeur C_1/F dans (6) et on obtient

$$F\frac{C_1}{F} = C_1$$

la dérivée première et donc la dérivée seconde d'une constante étant nulle.

En pratique, C_1/F doit être égal au taux de natalité lorsque la distribution d'âges est stable. En effet, lorsque le temps tend vers l'infini, la distribution d'âges se stabilise et la natalité tend vers le b prévu par la table de survie.

2) Il reste à trouver une solution générale de l'équation sans second membre.

$$D\frac{d^2b}{dt^2} + S\frac{db}{dt} + Fb = 0 \qquad (7)$$

Dans ce cas on peut chercher une solution particulière de la forme $b = e^{at}$. Ce qui implique que

$$\frac{db}{dt} = a e^{at} \text{ et } \frac{d^2b}{dt^2} = a^2 e^{at}$$

En substituant dans (7)

$$Da^2e^{at} + Sae^{at} + Fe^{at} = 0$$

en mettant e^{at} en évidence

$$e^{at}(Da^2 + Sa + E) = 0$$

et il apparaît que $b = e^{at}$ est une solution de l'équation différentielle et seulement si a est une solution de $Da^2 + Sa + F = 0$ (8) appelée équation caractéristique (en effet e^{at} sera toujours différent de 0).

L'équation 8, comme toute équation du second ordre, admet deux racines (qui peuvent être confondues) de la forme :

$$a_1 = \frac{-S + \sqrt{S^2 - 4DF}}{2D}$$

et

$$a_2 = \frac{-S - \sqrt{S^2 - 4DF}}{2D}$$

Sa résolution dépend donc de la valeur du terme $S^2 - 4DF$.

Or S étant la valeur de la survie, D le temps de développement et F la fécondité journalière, $S^2 - 4DF < 0$.

L'équation (7) ne peut donc avoir que deux racines complexes du type

$$a = p \pm qi \tag{9}$$

où

$$p + qi = \frac{-S + \sqrt{S^2 - 4DF}}{2D}$$

$$= \frac{-S}{2D} + \frac{\sqrt{S^2 - 4DF}}{2D}$$

$$= \frac{-S}{2D} + \sqrt{\frac{(S^2 - 4DF)}{4D^2}(-i^2)}$$

$-i^2$ par définition des nombres complexes étant égal à 1 et donc

$$p + qi = -\frac{S}{2D} - \sqrt{\frac{-S^2}{4D^2} + \frac{F}{D}}\,i$$

on en déduit que

$$p = -\frac{S}{2D} \text{ et } q = \sqrt{\frac{-S^2}{4D^2} + \frac{F}{D}}$$

La solution générale de (7) sera donc la somme des deux solutions particulières,

$$b = K_1 e^{a_1 t} + K_2 e^{a_2 1}$$

$$b = K_1 e^{(p + qi)t} + K_2 e^{(p - qi)t} \quad (10)$$

Or, tout nombre complexe peut s'écrire sous forme trigonométrique en passant des coordonnées cartésiennes aux coordonnées polaires. En effet, un nombre complexe est la somme d'un nombre réel et d'un nombre imaginaire (voir Fig. 1).

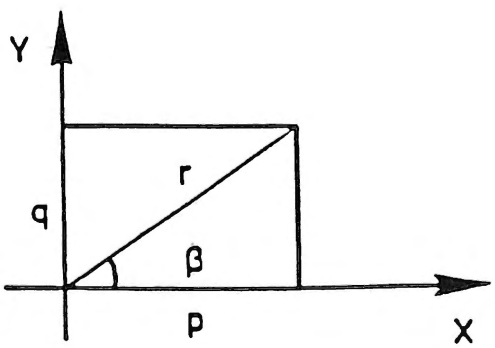

Fig. 1. — Relation entre la forme cartésienne et la forme trigonométrique d'un nombre complexe.

$$z = p + qi$$

ou

$$z = r \cos \beta + i \sin \beta$$

et donc $z = r (\cos \beta + i \sin \beta)$

en développant les valeurs sin β et cos β en série on obtient

$$z = r\left[1 - \frac{\beta^2}{2!} + \frac{\beta^4}{4!} - \frac{\beta^6}{6!} \ldots + i\left(\beta - \frac{\beta^3}{3!} + \frac{\beta^5}{5!} \ldots\right)\right]$$

$$z = r\left[1 + i\beta - \frac{\beta^2}{2!} - \frac{i\beta^3}{3!} - \ldots\right] \text{ et } i^2 = -1$$

$$z = r\left[1 + i\beta + \frac{(i\beta)^2}{2!} + \frac{(i\beta)^2}{3!} + \ldots\right]$$

$$z = re^{i\beta}$$

et donc

$$e^{i\beta} = \cos\beta + i\sin\beta$$

Dès lors en remplaçant dans (7) on obtient

$$\begin{aligned} b &= e^{pt}\,[\mathrm{K1}\,(\cos q\,t + i\sin q\,t) + \mathrm{K2}\,(\cos q\,t - i\sin q\,t)] \\ &= e^{pt}\,[\cos q\,t\,(\mathrm{K1} + \mathrm{K2}) + i\sin q\,t\,(\mathrm{K1} - \mathrm{K2})] \\ &= e^{pt}\,(\mathrm{C1}\cos q\,t + \mathrm{C2}\sin q\,t) \end{aligned}$$

posons

$$\frac{\mathrm{C1}}{\sqrt{\mathrm{C1}^2 + \mathrm{C2}^2}} = \sin\omega$$

et

$$\frac{\mathrm{C1}}{\sqrt{\mathrm{C1}^2 + \mathrm{C2}^2}} = \cos\omega$$

alors

$$b = e^{pt}\sqrt{\mathrm{C1}^2 + \mathrm{C2}^2}\,(\sin\omega\cos qt + \cos\omega\sin qt)$$

$$= e^{pt}\,\mathrm{A}\,(\sin qt + \Omega)$$

b) *Solution générale de* (6)

La solution finale de

$$\mathrm{D}\frac{d^2b}{dt} + \mathrm{S}\frac{db}{dt} + \mathrm{F}b = \mathrm{C1}$$

est la somme des solutions trouvées aux points 1 et 2, soit

$$b = \frac{C1}{F} + e_{pt}\, A\, (\sin qt + \Omega) \quad (11)$$

or il a été montré précédemment que

$$p = -\frac{S}{2D}$$

et

$$q = \sqrt{\frac{F}{D} - \frac{S^2}{4D^2}}$$

on en déduit

$$b = \frac{C1}{F} + e^{(-S/2D)t}\, A \left(\sin \sqrt{\frac{F}{D} - \frac{S^2}{4D^2}}\, t + \Omega\right) \quad (12)$$

où A est l'amplitude de l'onde non amortie et Ω la phase de départ. Selon cette équation, le taux de natalité d'une population créée au départ d'une femelle évolue comme une sinusoïde amortie et tend vers C1/F (qui correspond au taux intrinsèque de natalité) lorsque le temps tend vers l'infini.

Calcul de la solution de l'équation (12) pour *Aphis fabae*

Les constantes de l'équation :

$$b = \frac{C1}{F} + e^{(-S/2D)t}\, A \sin \left(\sqrt{\frac{F}{D} - \frac{S^2}{4D^2}}\, t + \Omega\right) \quad (13)$$

sont connues expérimentalement et peuvent être extraites de la table de survie. La durée du temps de développement, la fécondité journalière moyenne et la survie ont respectivement pour valeur : D = 5 j, F = 2,38 larves/jour et S = 1 − d = 0,977.

La valeur de la natalité à l'état stationnaire (C1/F) a été déterminée grâce à la table de survie : b = 0,461. De plus, la phase initiale peut être considérée comme nulle. Il reste à connaître la valeur de A. A représente l'amplitude maximale, c'est-à-dire la valeur maximale que peut prendre b. Or, il a été montré que b vaut 2,618 (Tableau 2), le deuxième jour de la croissance de la population. Il suffit alors d'égaler cette valeur de b à l'équation (12), dans laquelle on remplace t par 2 et où A demeure la seule inconnue :

On en déduit que A = 2,679.

Une fois la valeur journalière de b connue, l'étape suivante consiste à trouver la relation unissant le taux d'accroissement et le taux de natalité. En effet, vu l'évolution sinusoïdale de b, la relation exponentielle qui dit que

$$N_t = N_0 e^{r_m t} \text{ et } \lambda = e^{r_m}$$

ne peut plus s'appliquer tant que la stabilisation n'est pas survenue. Par contre, on sait que, à chaque unité de temps, la population s'accroît du nombre des naissances (B) et est diminuée du nombre de morts (M), soit :

$$N_t = N_{(t-1)} + B_{(t-1)} - M_{(t-1)}$$

En égalant cette équation à la suivante $N_t = \lambda N_{(t-1)}$ alors il revient

$$\lambda = \frac{N_{(t-1)}}{N_{(t-1)}} + \frac{B_{(t-1)}}{N_{(t-1)}} - \frac{M_{(t-1)}}{N_{(t-1)}}$$

Après simplification $\lambda = 1 + b_{(t-1)} - \mu_{(t-1)}$.

La croissance des effectifs s'obtient par $N_t = \lambda N_{(t-1)}$.

Vérification de la validité du modèle

Afin de vérifier la validité du modèle, 3 types de contrôle ont été effectués. D'une part en condition de laboratoire, le suivi d'une population de pucerons en phase colonisatrice a bien montré que la natalité évolue comme une sinusoidale amortie.

Ensuite un programme de simulation a été mis au point par VAN IMPE sur base du travail qu'il a effectué en 1985 pour *Tetranychus urticae*. Il correspond au développement théorique de NISBET et GURNEY (1982). Il permet de connaître l'évolution journalière des effectifs de la population de pucerons, de calculer la répartition en classes d'âge et le taux d'accroissement pour chaque unité de temps. Il est principalement constitué de deux équations :

1) le nombre de femelles (F) d'âge x au temps t est égal au nombre de femelles d'âge $(x - 1)$ au temps $(t - 1)$, multiplié par la survie (S) propre à cet âge $(x - 1)$:

$$F_{x,t} = F_{x-1,t-1} S_{x-1}$$

2) le nombre d'œufs pondus au temps t égale la somme des œufs pondus par les femelles des différents âges. Ces œufs donnant toujours naissance à des femelles, leur symbole est F_0, soit les femelles d'âge 0 :

$$F_{0,t} = \sum_{0}^{x} F_{x,t} m_x$$

La population au temps t sera donc :

$$N_t = \sum_{0}^{x} F_{x,t}$$

La matrice de données de départ est constituée des fécondités âge-spécifiques et des valeurs de survie, cette dernière étant posée constante. Les résultats obtenus donnent l'évolution de la natalité (b), la valeur par laquelle la population est multipliée à chaque unité de temps (λ), et les effectifs correspondants.

Ce programme a mis en évidence l'adéquation entre le modèle différentiel présenté ici et la simulation sur ordinateur. La Fig. 2 montre en effet, que non seulement l'évolution des taux de croissance au cours du temps est similaire, mais que de plus la période de la sinusoïde amortie est identique. En outre de cette façon, il a été possible de confirmer l'existence d'une relation linéaire entre la natalité et son accélération, relation qui est à la base de l'élaboration du modèle. La Fig. 3 illustre bien ce caractère linéaire de la droite obtenue dont l'équation est $b = 0{,}481 - 1{,}364\,a$ où b est la natalité et a son accélération ($r^2 = 0{,}741$).

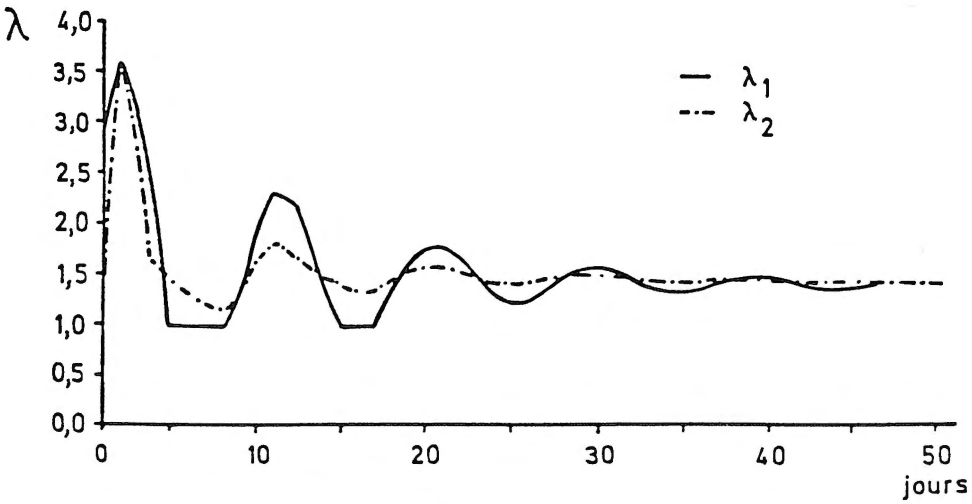

Fig. 2. — Comparaison de l'évolution des taux d'accroissement d'une population d'*Aphis fabae*. Valeurs prévues 1) par la sinusoïdale amortie, 2) par le programme de simulation.

Enfin, en troisième lieu, la comparaison entre l'évolution des effectifs générée par le modèle élaboré ici par le modèle exponentiel de LOTKA (1924) a été réalisée. Pour le modèle exponentiel, le taux intrinsèque d'accroissement naturel de *Aphis fabae* a été appliqué (0,438).

La Fig. 4 montre que la croissance est plus rapide lorsque l'on tient compte de l'évolution sinusoïdale de la natalité par rapport au taux constant du modèle exponentiel. Cette observation tient son origine dans le fait que 1) dans le modèle sinusoïdal, ce sont d'abord les amplitudes qui interviennent et 2) celles-ci sont dès lors

moins amorties que les amplitudes négatives. La résultante des deux donne donc une valeur de natalité en moyenne supérieure à la valeur de stabilisation.

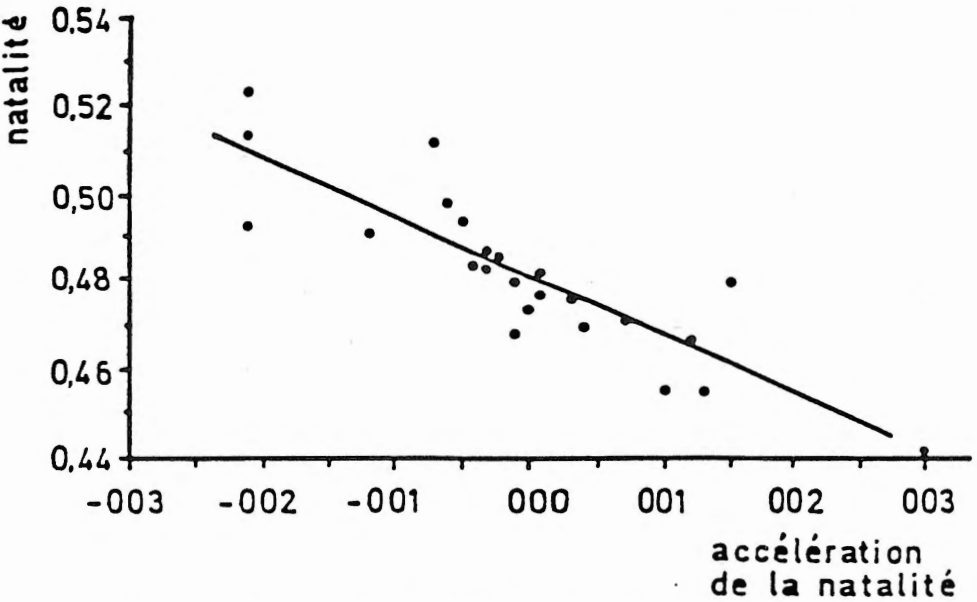

Fig. 3. — Relation entre la natalité de la population de *Aphis fabae* et son accélération, sur base des valeurs obtenues par le programme de simulation. La vitesse (larves/jours) est en ordonnée et l'accélération (larves/jours^2) en abscisse.

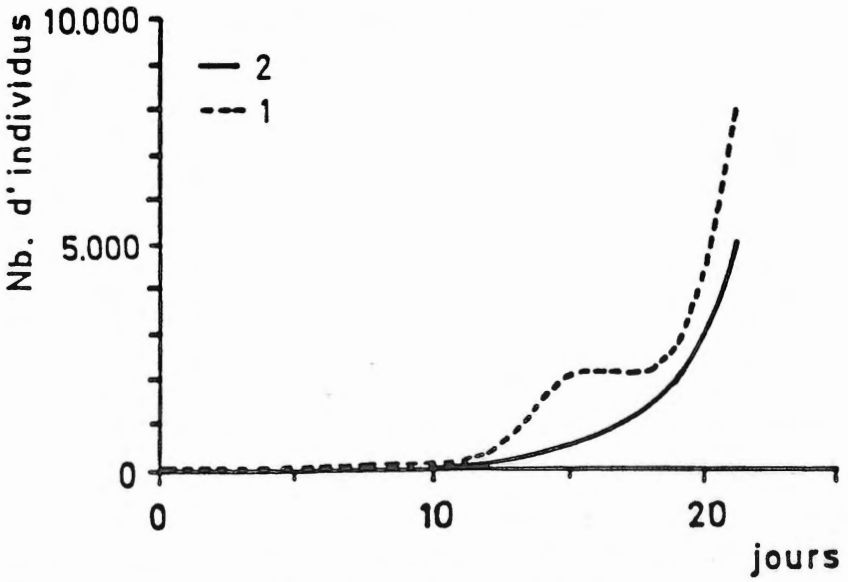

Fig. 4. — Comparaison de la croissance des effectifs de *Aphis fabae*. Valeurs prévues 1) par la sinusoïde amortie, 2) par le modèle exponentiel.

Il ressort de ces différentes comparaisons, que l'évolution sinusoïdale de la natalité prédite par l'équation (12) permet de modéliser l'évolution d'une population créée au départ d'une femelle adulte et donc extrêmement éloignée d'une distribution d'âges stable. L'introduction dans ce modèle de l'étude de l'évolution de la mortalité conduira à un ajustement plus précis par rapport aux données expérimentales.

CONCLUSIONS

Plus de cinquante jours sont nécessaires avant d'obtenir une stabilisation de la natalité de la population. A ce moment, les effectifs atteints sont de l'ordre de 400 millions d'individus, c'est-à-dire bien plus que ce qui est supportable par la plante hôte. Or, la colonisation d'une nouvelle plante se fait en général chez *Aphis fabae* par de jeunes femelles adultes, ailées ou non, soit une population toujours éloignée de la distribution d'âges stable. Dès lors, les effectifs de la population seront toujours plus élevés que ceux qui seraient observés dans l'hypothèse d'une croissance purement exponentielle ! Cette particularité est donc une explication de plus au prodigieux pouvoir de multiplication des pucerons.

Trois facteurs jouent un rôle primordial dans ce modèle, le temps de développement (D), la fécondité journalière moyenne (n/t) et la survie (S). Ce sont ces éléments qui concernent l'individu qui détermineront le temps nécessaire à l'amortissement, et la période de la sinusoïde exprimant la natalité de la population. Le taux de natalité de la population à l'état stationnaire reste un facteur déterminant, auquel il faut ajouter l'amplitude maximale de la première oscillation.

Ce modèle montre qu'il existe une régulation du taux de natalité qui est la conséquence mathématique du type de croissance de la population. En effet, le taux de natalité est maximum en début de phase colonisatrice et puis tend progressivement vers une valeur de stabilisation à mesure que s'élève l'effectif de la population. Il ne s'agit donc pas de densité dépendance à proprement parler mais d'un phénomène naturel lié à la stabilisation au cours du temps de la distribution d'âges de la population colonisatrice. Deux causes distinctes peuvent donc être invoquées pour expliquer la diminution de la natalité avec l'augmentation des effectifs : 1) un effet mathématique de stabilisation que l'on nommera « amortissement », 2) un second effet à caractère adaptif plus net connu sous le nom de « densité dépendance ». Il est évident qu'en conditions naturelles voire expérimentales, il est pratiquement impossible de distinguer la part de ces deux effets.

L'application de ce nouveau modèle à toute espèce qui montre une stratégie démographique similaire, c'est-à-dire un grand nombre d'Arthropodes préjudiciables aux plantes cultivées, est envisageable. D'autant que le développement mathématique rend possible des comparaisons entre espèces distinctes ou entre conditions expérimentales différentes. L'influence de l'augmentation du temps de développement ou de mortalité, due par exemple à une diminution de la température ou même à une application d'un traitement insecticide, pourra donc être aisément quantifiée.

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EVALUATION OF METHODS FOR FIBROBLAST ELIMINATION IN MIXED CULTURES

by

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SUMMARY

The usefulness of inhibitors of fibroblast growth in cultures for the selective culture of neoplastic epithelial cells was assessed using a model system. Two cell populations, permanent neoplastic epithelial HeLa cells and finite fibroblastic FLOW-4000 cells, were mixed, and cultured in 24 well plates. A fibroblast elimination treatment was performed for one set of 24 well plates, the other 24 well plates were used as controls. While the total cell population was determined by counting, the measurement of alkaline phosphate activity of HeLa cells was used for the estimation of the fraction of HeLa cells in the mixed culture ; in this way cell growth kinetics could be determined for the two subpopulations. The percentage of HeLa cells in the mixed cultures was evaluated every day until confluency of the culture. Using this model, several schedules for the inhibition of fibroblast growth were tested : Falcon Primaria culture plates, D-valine, putrescine, cytosine arabinoside, geneticin, and an anti-fibroblast antibody. Geneticin was useful for the selective elimination of fibroblasts in a mixed culture as well as for a series of primary cultures.

Key words : cell culture, epithelial cells, fibroblasts.

INTRODUCTION

The selective elimination of fibroblasts from primary cultures of normal or neoplastic tissues has been an exasperating problem for scientists involved in experiments using epithelial cells from normal or neoplastic tissues. Usually primary cultures start off nicely as mixed cultures of fibroblasts and epithelial cells, but after a few passages one ends up with a culture of solely fibroblasts. Several

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reports have described methods for the selective culture of epithelial cells from normal or neoplastic tissues (OWENS, 1975, FOGH, 1975, BASHOR, 1979). These procedures do not completely eliminate fibroblasts, while others employ complex technical procedures (e.g. organ perfusion, cloning, alternate animal passage and tissue culture, mechanical destruction of undesired cell types, enzymatic treatments, selection by antibodies) which limit general usefullness. As a result, success of a fibroblast elimination schedule depends largely on the skill of the technician.

There is great need for simple and accurate methods for the selection of epithelial or other cell types out of cultures. In fundamental research and in clinical studies, the use of laboratory animals is becoming more stringent. For the oncologist and the clinician, cell culture of neplastic cells is useful only in practice if within a short time span the neoplastic cells can be selected out of the culture (WELANDER, 1983). These cells can then be used for the selection of therapeutics, antibodies to tumor-associated antigens, or even for more complicated procedures such as the production of antigenic tumor cell variants (BOON, 1983).

Techniques for the selection of epithelial cells have been tested on primary cultures with as the main measurement the visual observation of the elimination of fibroblasts. It was our goal to develop a quantitative method for the measurement of fibroblast elimination from mixed cultures. We describe a method to tests the usefulness of potential inhibitors of fibroblast growth in a culture of a permanent neoplastic cell line and a finite fibroblast line.

METHODS AND MATERIALS

Cell culture

A permanent neoplastic cell line, HeLa (epithelial cancer, human cervix), and a finite fibropblastic line FLOW-4000 (human kidney, FLOW inc.) were cultures in Earle's modified Eagle's medium supplemented with 10 % heat inactivated fetal calf serum (FCS) and 1 % non-essential amino acids. Antibiotics were not added to the growth medium. Subculture of confluent cultures at split ratios of 1:10 (FLOW-4000) and 1:30 (HeLa) was accomplished with a calcium and magnesium free phosphate buffered saline which contained trypsin (0.25 %, w/v) and EDTA (0.04 %, w/v). The subculture interval was usually 7 days.

Fibroblast inhibition assay

After trypsinization, epithelial HeLa and fibroblastic FLOW-4000 cells were mixed at a concentration of 1×10^4 cells/ml each or 5×10^3 cells/ml for HeLa cells and 1.5×10^4 cells/ml for FLOW-4000 cells. The final cellular concentration was 2×10^4 cells/ml. One ml cell suspension aliquots were plated in each well of 24 well plates (Costar, Mark II 3424). The cells were allowed to adhere to the plastic surface. The second day the fibroblast inhibitor was added to the culture wells and left in the well for a selected time.

Daily, treated and non-treated culture wells ($n = 4$) were trypsinized and the total cellular population was measured by a direct enumeration of cells employing a hemocytometer. This procedure was repeated until the cultures were confluent. The HeLa subpopulation was estimated in an indirect way by a measurement of the alkaline phophatase (AP) activity of HeLa cells using the formula (the method for the determination of the AP activity is given below):

$$\frac{AP_m - (\text{total cell count}) \times AP_{FLOW}}{AP_{HeLa} - AP_{FLOW}} = \text{HeLa cell count } (\times 10^4)$$

AP_m, AP activity of the mixed culture;

AP_{FLOW}, AP activity of a FLOW-4000 culture expressed per 10^4 cells;

AP_{HeLa}, AP activity of a HeLa culture expressed per 10^4 cells.

Control plates of HeLa and of FLOW-4000 cells were used to measure daily the AP activity of the HeLa cells and the background activity of the FLOW-4000 cells. FLOW-4000 cells were used for the experiments only between passage 2 and 5; therefore new batches were regularly established from cryopreserved cells.

Determination of the statistical significance between treated and non-treated cultures was analyzed by the parametric Students't test and the non-parametric MANN-WITNEY method.

Morphology

Cells growing in tissue culture containers were examined by standard light microscopy. Alternatively, cells were grown on tissue culture chamber slides (Miles Lab.; LABTEK 4808), fixed, and stained by standard histological staining procedures.

Determination of AP activity

The cells of the mixed or control cultures were suspended in isotonic saline after trypsinization. The AP activity was measured for intact cell suspensions (5×10^3 to 1×10^4 cells/ml). The total AP activity was determined kinetically by the method of VAN BELLE *et al.* (1977). One unit of enzyme activity is defined as the liberation of 1 µmol nitrophenol ($E_{405\,nm} = 14{,}600$ liters $mol^{-1}\,cm^{-1}$) from 4-nitrophenyl phosphate per min at 37° C when incubated in a solution containing 5 *m*M 4-nitrophenyl phosphate in 0.1 M N-ethylamino ethanol buffer (pH 10.2).

Products and treatments

Primaria culture plates (24 well plate, Becton Dickinson, Falcon 3847) with a surface modified polystyrene that inhibits fibroblast growth was used instead of Costar 24 well plates. Putrescine (1,4 diaminobutane, Janssen Chimica 23-1400-1; 34 and 56 mg/L) and cytosine arabinoside (Janssen Chimica 22-718-20; 3 mg/L) were added to the 24 well plates on day 1, and left in the growth medium for the duration of the experiment. For D-valine medium (Gibco BRL, 041-2570), the normal

growth medium was changed on day 1 by D-valine medium supplemented with 5 % FCS, dialysed for 2 days at 4° C against an Earle's salt solution ; the growth medium of the control plate was also supplemented with 5 % FCS. Geneticin (G418 sulfate, Sigma G5013) was added on day 1 at a concentration of 1 mg/L growth medium and left for two to three days ; then the wells were washed and normal growth medium was replaced. The monoclonal antibody to fibroblasts (LICR LON/FIB 86.3) was a gift from Dr. Edwards (University of Cambridge, Department of Pathology). Anti-fibroblast containing ascites was diluted in Earle's modefied Eagle's medium (1:50 to 1:1000). The cells were washed and the anti-fibroblast containing medium was added. After 1 hr at 37° C, the cells were rinsed and rabbit complement put on, diluted 1:5 to 1:50 in medium. The plate was again incubated for 1 hr at 37° C. Finally, the complement was removed, the cells rinsed with medium and growth medium replaced.

RESULTS

AP activity of the cell lines

The neoplastic epithelial cell line, HeLa, contained a large amount of AP activity (3.01 ± 1.02 U AP/mg protein). This AP activity was mainly membranous and was found on the plasma membrane (this was verified using histochemistry for total AP on electron microscopical level, results not shown). The AP activity of HeLa cells was 266 ± 10 *n*U/cell. In the fibroblast line there was only a negligeable amount present on the plasma membrane, 4.7 ± 1.1 *n*U/cell. This difference was used to estimate the amount of HeLa cells in culture.

To be able to use the measurement of AP activity of HeLa cells for the estimation of the amount of HeLa cells in the mixed culture, it was essential to evaluate the effect of the dilution of the cell suspension on the total AP activity (Fig. 1A), and to test the effect of the cell density during the culturing period on the total AP activity (Fig. 1B). There was a linear correlation between the dilution of the cell suspension and the AP activity measured. The dilution used in the experimental setup was always around 5×10^3 to 1×10^4 cells/ml. The increasing cell density during the culturing period had only a small effect on the total AP activity (slope = − 0.0011). For that reason, HeLa cells were seeded in 24 well plates at the same concentration as in the experimental setup, and AP activity was determined daily.

Elimination of fibroblasts out of mixed cultures

In the assay, presented in Fig. 2, two plastic growth surfaces where compared for their effect on the growth of epithelial cells and fibroblasts in mixed cultures. For the total populations as well as for HeLa cells and FLOW-4000 cells, the growth

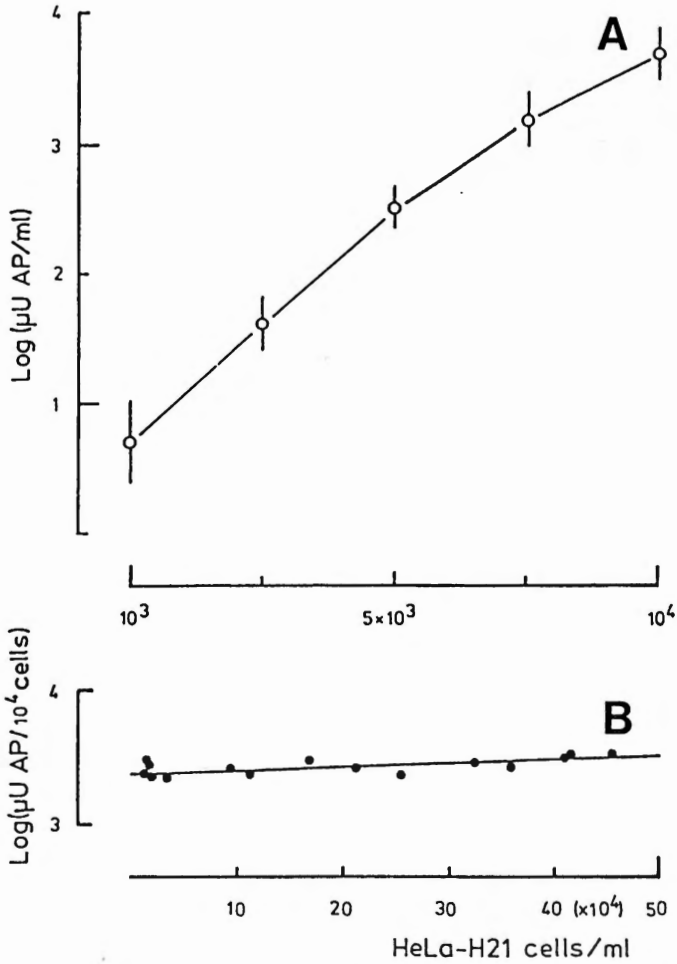

Fig. 1. — (A) Kinetic measurement of the total alkaline phosphatase activity of different HeLa cell dilutions (Mean ± SD, $n = 4$). (B) Kinetic measurement of the total alkaline phosphatase activity in function of cell density of HeLa cells. Cell density is expressed as the amount of HeLa cells of one well in one ml.
Linear regression : $y = 3.4 - 0.0011X$, $R = 0.93$.

curves had a normal sigmoidal pattern. Falcon Primaria plates with a modified polystyrene surface were found to be not superior to Costar plates for the inhibition of the growth rate of FLOW-4000. On the contrary, proliferation of FLOW-4000 cells was more pronounced in the Falcon Primaria, although this increase was not significant. This is also clearly depicted in Fig. 3.

Fig. 3 summarizes the results of experiments in which several methods were compared : adding putrescine (two concentrations were tested : 34 mg/L and 56 mg/L)

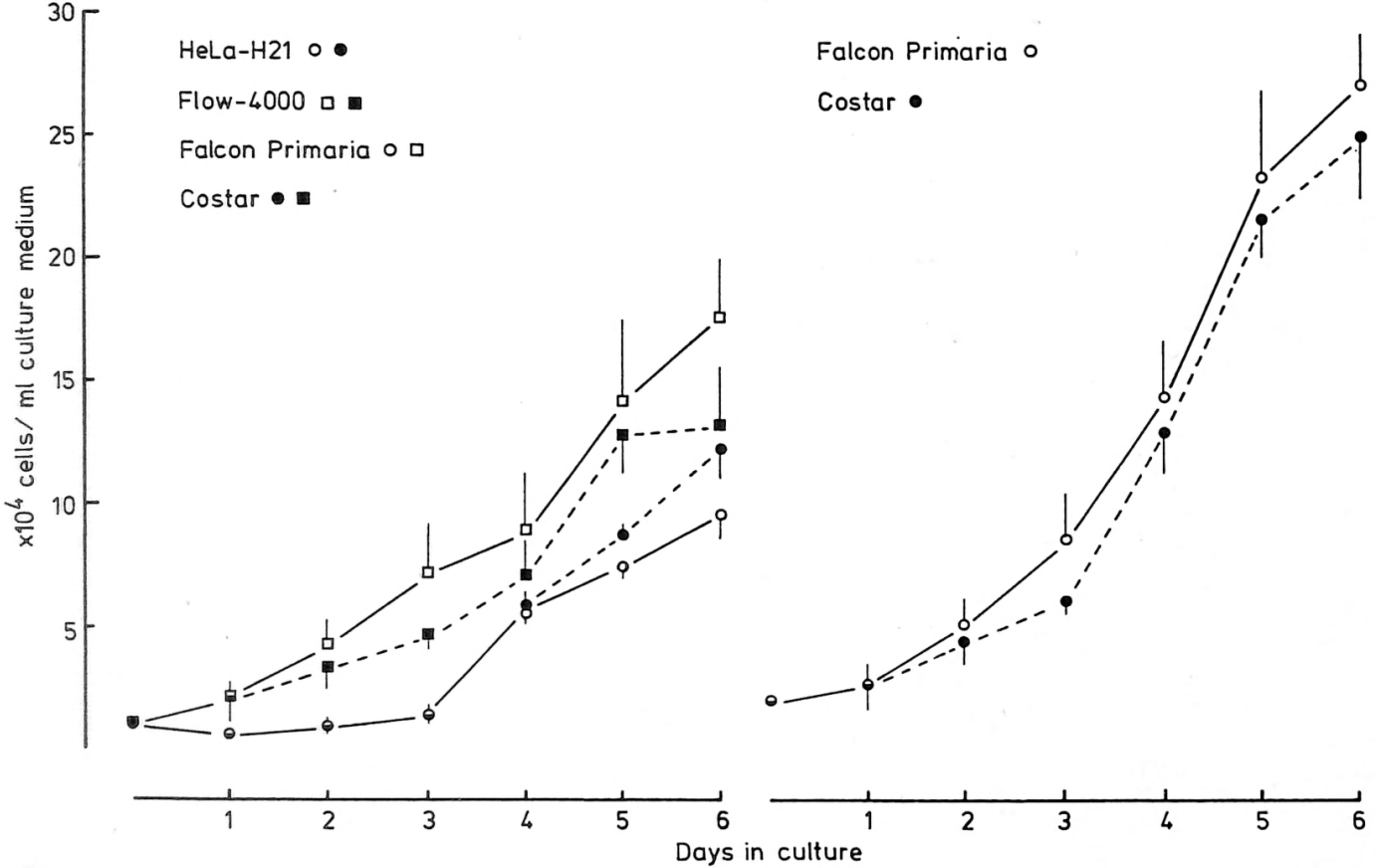

Fig. 2. — Comparison of the cell growth of a mixed culture of epithelial cells, HeLa, and fibroblasts, FLOW-4000, for two different plastic surfaces : the 24-well plate of Costar and the 24-well plate of Falcon primaria (inhibits fibroblast growth). HeLa and FLOW-4000 were each seeded at a concentration of 1×10^4 cells/well. (A) cell counts per day per cell type ; (B) total cell counts per day (mean ± SD, $n = 4$).

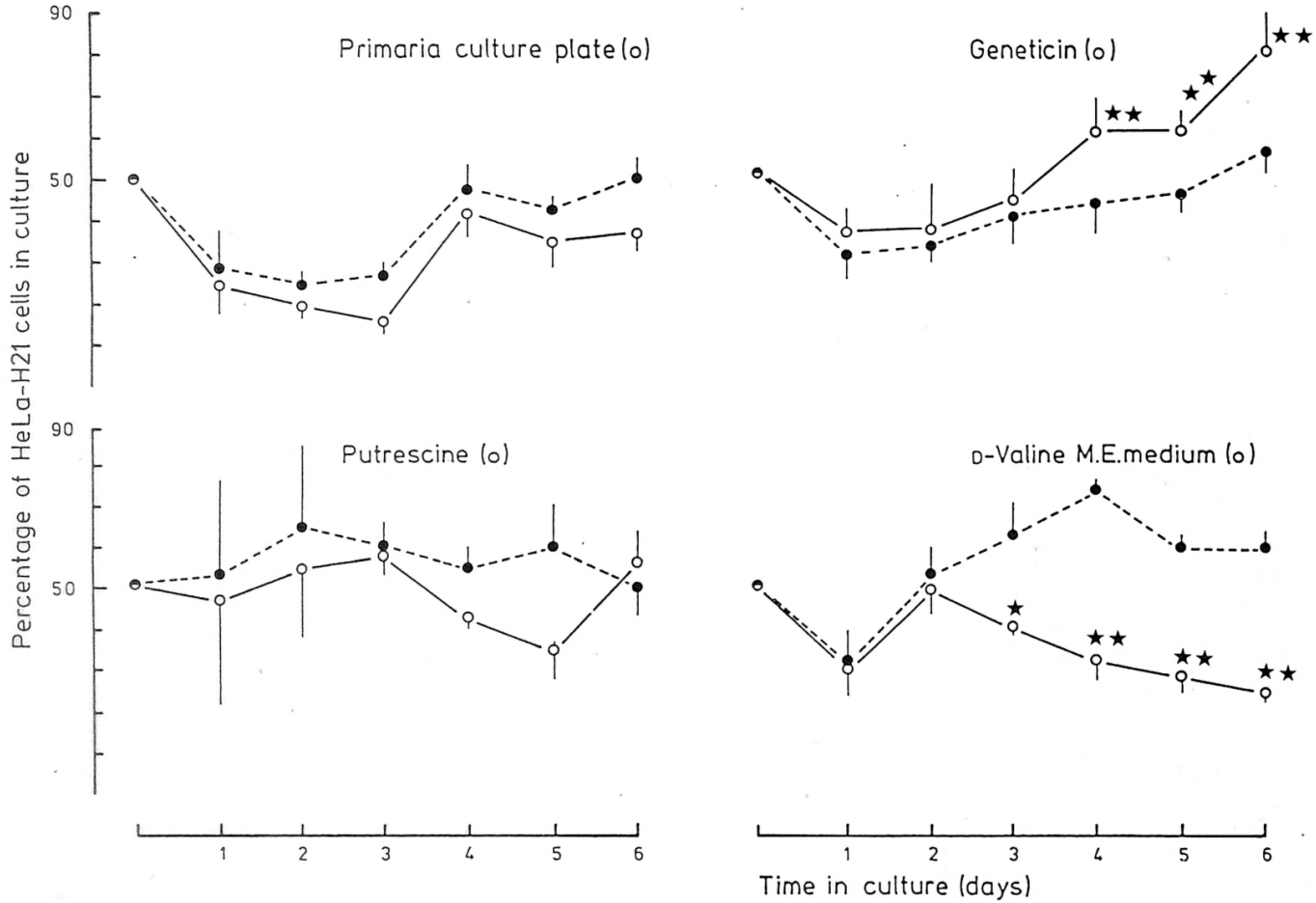

Fig. 3. — Percentage of proliferating HeLa cells in a mixed culture of HeLa and FLOW-4000 cells for different treatments (○) as compared with non-treated control cultures (●). Cells were seeded at a concentration of 1×10^4 cells per well for each of the two cell types. Putrescine (56 mg/L) and D-Valine growth medium were added for the whole duration of the experiment. Geneticin (1 mg/L) was added on day 1, and was discarded on day 3 (mean ± SD, n = 4 to 8 ; * $p < 0.05$, ** $p < 0.025$).

to the growth medium, replacing L-valine in the growth medium by its D-enantiomer, and adding geneticin to the medium for a few days. The data are presented as the percentage of HeLa cells in the mixed culture per day. Geneticin was the only product able to reduce significantly the growth of fibroblasts. When geneticin was added for two days to a mixed culture of HeLa and FLOW-4000 cells (1×10^4 cells/ml for the two cell types), the percentage of HeLa cells was significantly ($p < 0.025$) higher from day 4 on when compared with the control cultures. Growth medium that contained D-valine instead of L-valine had an adverse effect, since it reduced the growth of both subpopulations (for HeLa cells from 3 on and for FLOW-4000 cells from day 4 on). For the total populations this effect became measurable from day 4 on (for D-valine cultures, day 3 [9.31 ± 0.45] × 10^4 cells and day 4 [12.81 ± 2.00] × 10^4 cells ; for control cultures, day 3 [11.69 ± 1.95] × 10^4 cells and day 4 [27.94 ± 3.37] × 10^4 cells). However, this reduced growth rate was more pronounced for the HeLa subpopulation (Fig. 3).

Cytosine arabinoside at the concentration of 3 mg/L was toxic for both cell types (for the total cell populations, on day 1 [1.19 ± 0.12] × 10^4 cells *versus* the control [2.05 ± 0.51] × 10^4 cells and on day 2 [2.03 ± 0.45] × 10^4 cells *versus* the control [8.64 ± 0.37] × 10^4 cells). The treated cultures were extinct after 4 to 5 days. The other treatments had no or little adverse effects on the cell growth kinetics. The curves of treated and control cultures were quite comparable (as for the results shown in Fig. 2) and the elimination of part of one subpopulation resulted in an increase of the other.

As geneticin was able to inhibit the growth rate of fibroblasts in mixed cultures, we investigated if this was also true for cultures where the percentage of epithelial cells was lower than 50 %. Therefore, we reduced the amount of HeLa cells to 25 % of the total cell population (2×10^4 cells/well, or 5×10^3 HeLa cells/well), and tested this aminoglycoside for two different time spans, two and three days (Fig. 4). There was a significant increase of the HeLa population in the treated cultures for both incubation times when compared with the non-treated controls. However, prolongation of the treatment did not benefit the increase of epithelial cells. Proliferation of the HeLa population only increased after a lag period of one day when the geneticin treatment was ended. Before treatment, the epithelial HeLa cells appeared as small colonies, while the fibroblasts occupied most of the growth surface. After treatment, the fibroblasts could only be found sparingly as small clusters of cells that did not grow well between large colonies of proliferating HeLa cells.

Selective antibodies to undesired cell types would be the perfect tool to select cells in a rapid and non-toxic way. Using tissue culture chamber slides, we tested several parameters for the elimination of fibroblasts by an anti-fibroblast antibody (LICR LON/FIB 86.3). Different dilutions of the anti-fibroblast antibody containing ascites (1:50 to 1:1000), and of the complement (1:5 to 1:50) were tried. The combination of 1:200 for LICR LON/FIB 86.3 and of 1:10 for the complement resulted in maximal lysis of fibroblasts. A minimal incubation period of 30 min. at 37° C was needed for an optimal effect. Treatments of cultures on days 1, 2 or 4 gave

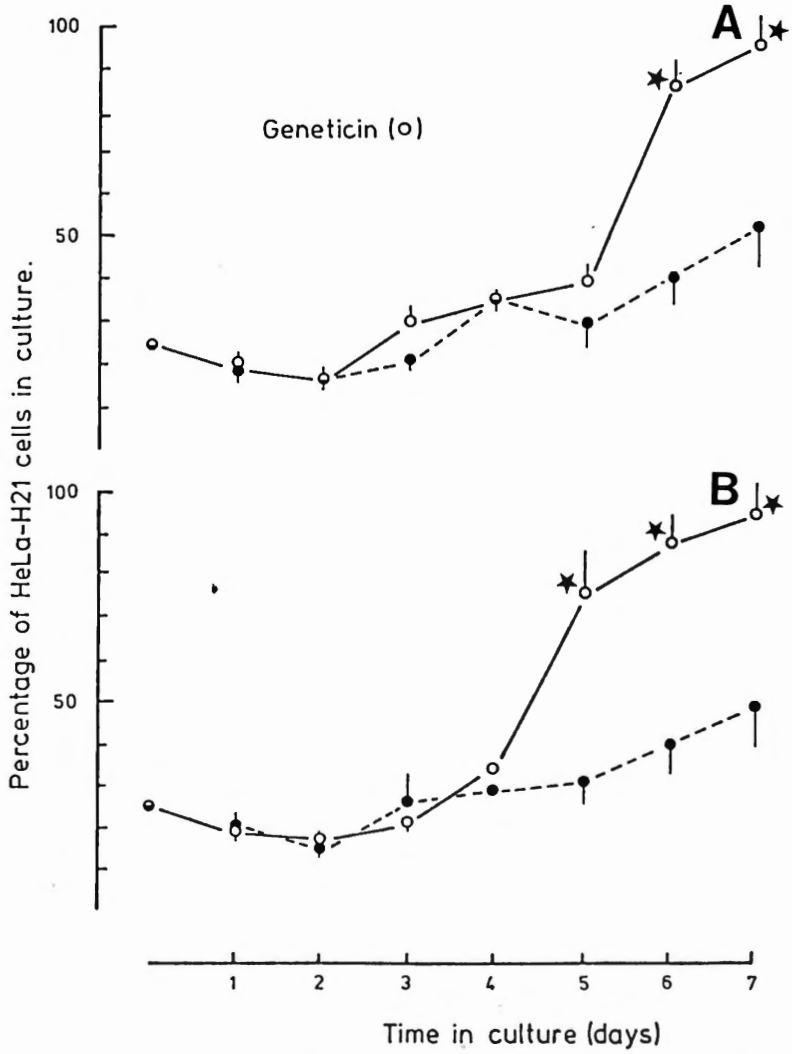

Fig. 4. — Percentage of proliferating HeLa cells in a mixed culture of HeLa and FLOW-4000 cells for a treatment of Geneticin (o) as compared with non treated control cultures (•). Cells were seeded at a concentration of 0.5×10^4 HeLa cells/well and 1.5×10^4 FLOW-4000 cells/well. Geneticin (1 mg/L) was added on day 1 and was discared on day 4 (A), or day 3 (B) (mean ± SD, $n = 4$; * $p < 0.025$).

comparable results. LICR LON/FIB 86.3 was clearly able to destroy a large part of the fibroblast population out of mixed cultures. The HeLa cells remained as polygonal cells, while most of the elongated, often spindle shaped fibroblasts disappeared from the bottom of the culture well leaving empty spaces. However, the whole FLOW-4000 population was never completely eliminated. Consequently,

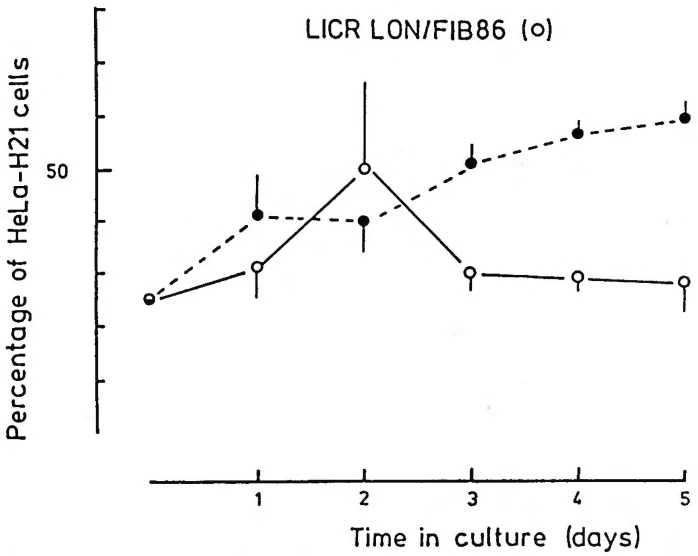

Fig. 5. — Percentage of proliferating HeLa cells in a mixed culture of HeLa and FLOW-4000 cells, treated with anti-fibroblast antibody and complement (○) as compared with non-treated control cultures (●). Cells were seeded at a concentration of 5×10^3 HeLa and 1.5×10^4 FLOW-4000 cells/well. Treatment was performed on day 1 : anti-fibroblast Moab (LICR LON/FIB 86.3) for 1 hr at 37° C followed by rabbit serum 1:10 diluted in Earle's modified Eagle's medium for 1 hr at 37° C (mean ± SD).

when we tried the antibody on our model with a treatment on day 1, we were not able to show a continuous inhibitory effect on FLOW-4000 cells after an additional four days of culture (Fig. 5). On the contrary, the surviving FLOW-4000 cells, which were clearly reduced in number the day after the treatment, proliferated again after a lag period of one day (for the treated cultures, on day 1 $[4.37 \pm 0.47] \times 10^4$ cells, on day 2 $[2.42 \pm 0.27] \times 10^4$ cells and on day 3 $[5.71 \pm 1.65] \times 10^4$ cells ; control cultures, on day 1 $[3.74 \pm 0.60] \times 10^4$ cells, on day 2 $[7.10 \pm 1.93] \times 10^4$ cells and on day 3 $[9.65 \pm 2.19] \times 10^4$ cells), and overgrew the HeLa population quite rapidly.

Elimination of fibroblast-like cells out of primary cultures

Geneticin has been used routinely for the selection of epithelial cells (neoplastic and normal ; ovary, testis, kidney, lung ; Tab. 1). In contrast to collagenase and putrescine each treatment of geneticin resulted in a clear elimination of most of the fibroblast-like population present in the culture flasks. Two treatments were often needed, and the whole duration of the selection took from 2 to 4 weeks. The

cultures could usually be kept for about 3 months but storage in liquid nitrogen was only possible in some casses (3) where the amount of cells was sufficient.

TABLE 1

Elimination of fibroblast-like cells out of primary cultures of human tissues

Tissue (1)	Treatment (2)	Number of experiments	Result (3)	Days in culture
Ovary CA	collagenase	2	F + E islands	200, 150
	geneticin	2	mainly E islands	120, 90
Parathyroid AD	collagenase	2	F + small E islands	100, 60
	putrescine	2	F + E islands	50, 60
Testes CA	geneticin	1	only E	110 (ST) (4)
Kidney CA	geneticin	3	only E	120, 160 (ST) 100 (ST)
Lung	geneticin	6	only E islands	150, 103, 120, 130, 90, 100

(1) Tissue, CA, carcinoma, AD, adenoma.

(2) Treatment, collagenase, 0.5 g/L added to the growth medium when fibroblast-like cells started to dominate and was continued until they started to die, putrescine, 0.56 mg/L growth medium, geneticin, 1 mg/L growth medium, treatments of two days.

(3) Results, F, fibroblast-like cells, E. Epithelial-like cells.

(4) ST, Stored in liquid nitrogen.

DISCUSSION

Short-term cultures of neoplastic human cells contain an abundance of stromal cells which may disturb refined studies. It is very peculiar that in the majority of primary cultures set up from human tumors, both stromal and neoplastic cells die after a lilited number of cell generations, resulting in eventual extinction of these cultures. The success rate of establishment of neoplastic cell cultures has been low (e.g. IOACHUM *et al.*, 1974 ; LEIBOVITZ *et al.*, 1976 ; MCBAIN *et al.*, 1984 ; KIRKLAND and BAILEY, 1986). This can largely be explained by an overgrowth of fibroblasts, or by the finite life of neoplastic cells.

The state of the art of the establishment of neoplastic epithelial cell cultures involves cloning, alternate animal passage and tissue culture, enzymatic treatments (collagenase, trypsin), substrate modification, mechanical destruction of undesired cell types, density gradients, etc. (FOGH, 1975 ; OWENS, 1975 , BASHOR, 1979 ; FRESHNEY, 1983), or combinations of these techniques (KEDAR *et al.*, 1982 ; DANES, 1985 ; KIRKLAND and BAILEY, 1986 ; etc.).

An apparently elegant method was developed by GILBERT and MIGEON (1975). They exchanged L-valine by D-valine in the growth medium resulting in a survival of cells that contained D-amino acid oxidase, an enzyme that only occurs in specialized epithelium of many mammals but not in fibroblasts (GILBERT and MIGEON, 1975 ; ORGEBIN-CRIST *et al.*, 1984). In our model system, growth medium that contained D-valine reduced the growth of the HeLa population more than the growth of FLOW-4000 cells. Apparently, the neoplastic epithelial line HeLa does not produce D-amino acid oxidase, and is therefore more susceptible to growth media without L-valine. This may also be true for other neoplastic epithelial cell types. Therefore, the application of D-valine containing media for the selection of epithelial cells is only useful for epithelia of specific origin. This may also be true for putrescine and cytosine arabinoside products being used for the isolation of bovine parathyroid cells (BRANDI *et al.*, 1986) and the culture of rat and rabbit epididymal cells (ORGEBIN-CRIST *et al.*, 1984), respectively.

Another method was introduced by HALABAN and ALFANO (1984), who selectively eliminated fibroblasts from cultures of normal human melanocytes with geneticin. Geneticin (G-418 sulphate) is an aminoglycoside related to gentamicin and is used as a selective agent in molecular genetics. In our model as well as in primary cultures of cells of different tissues, geneticin was a reliable tool for the elimination of fibroblasts. The fibroblasts are possibly eliminated because geneticin is taken up, what is accompanied by an increase of lysosomal activity (intracellular staining for acid phosphatase) and a deterioration of the cell by bursting of lysosomes. The analogue gentamicin has been shown to be taken up by fibroblasts in culture and this uptake is accompanied by a marked increase in the total volume of lysosomes (AUBERT-TULKENS *et al.*, 1979). This increase may be an indication of an early lysosomal change, a phenomenon which has been correlated with nephrotoxic effects of aminoglycosides (GIULIANO, 1986).

Antibodies to (un)desired cells may in the future prove to be an ideal tool for the selection of certain cell types. The main problems will be the development of monoclonal antibodies with sufficient affinity and specificity, and of accurate methods to perform the separation (complement activation, magnetic beads, chromatography, growth surface coating, e.g.). In some cases the production of suitable antibodies may prove not to be to difficult as for the immune elimination of host fibroblasts, when the goal is the cultivation of human tumor cells transplanted in nude mice (OKABE *et al.*, 1983). It is also possible to select cell types that express a specific antigen on their cell surface, like brush border enzymes of proximal tubular cells of the kidney (SMITH and GARCIA-PEREZ, 1985), or tumor-associated antigens (TRELEAVEN *et al.*, 1984). The use of tumor-associated antigens for the selection of neoplastic cells has the disadvantage that interesting cell types may be lost, because of the heterogeneity of most tumors for the expression of such antigens. Therefore, the idea of eliminating the fibroblast is very attractive.

Using the monoclonal antibody LICR LON/FIB 86 EDWARDS *et al.* (1980) and GUSTERSON *et al.* (1981) were able to selectively culture epthelial cells from human squamous carcinoma and keratinocytes. We were also able to show morphologically the elimination of a part of the fibroblast fraction out of mixed cultures on

tissue culture chamber slides. This result is in agreement with the findings of EDWARDS *et al.* (1980), who could only kill part of the fibroblast population after each treatment. In our experiments, the remaining FLOW-4000 cells seemed to recover rapidly. A second reason for the failure may be the short time interval between seeding and treatment (24 hrs), as not sufficient antigen may be present on the FLOW-4000 plasma membrane. This short time after seeding was used (1) to be able to compare the results with previous experiments and (2) to allow the antibody an easy access to the fibroblasts present. GUSTERSON *et al.* (1981) pointed out that the use of reseeded populations of cells facilitated access of the antibody to fibroblasts otherwise trapped beneath the epithelial cells. The complement used may also be a reason for the uncomplete elimination of the fibroblast population. The antibody LICR LON/FIB 86 only killed well with rabbit serum, but this serum was somewhat toxic on its own, and the optimal dilution used by these authors was 1:20 to 1:40. The serum dilution that we used for complement activation was 1:10. The toxic effect of the rabbit serum on HeLa cells may have reduced its proliferation, although no toxic effect was observed on control cultures.

ACKNOWLEDGEMENTS

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ABREVIATIONS USED

FCS, fetal calf serum.

AP, alkaline phosphatase.

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THE FOOD OF *HAPLOCHROMIS BURTONI* (PISCES : CICHLIDAE) OF LAKE MUGESERA (RWANDA)

by

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SUMMARY

In view of a better comprehension of the trophic relationships of Lake Mugesera (a lake of the Akagera system, Rwanda), a stomach and gut content analysis has been carried out on 38 adult specimens of *Haplochromis burtoni* (GÜNTHER, 1893), a cichlid which was up to now only known from the Lake Tanganyika system. *H. burtoni* is a benthic insectivore and detritivore and shows a wide range of prey species. The main animal prey are Chironominae larvae. Rotifera are numerically abundant in the food contents, but volumetrically insignificant.

INTRODUCTION

Lake Mugesera is a shallow dentritic lake, one of the water bodies located in the valleys adjacent to the Nyabarongo river, which is part of the Akagera watershed (Rwanda) (Fig. 1). The hydrobiological aspects of Lake Mugesera are discussed by DAMAS (1953, 1954) and NTAKIMAZI (1985).

In order to arrive at a better understanding of the trophic relationships that occur in Lake Mugesera, a study has been carried out on the food of *Haplochromis burtoni* (GÜNTHER, 1893), a small cichlid species, one of the 16 fish species actually recorded in this lake.

Two questions were investigated : (1) on which food items feeds this population (qualitative aspect) and (2) in which proportions are the various food items ingested (semi-quantitative aspect).

MATERIALS

The fishes (n = 38) were caught between the 27th of July and the 12th of August 1985 on the Western shore of Lake Mugesera at a location called « Bac » (02°08'S 30°19'E, Fig. 1). They were collected with a beach seine of fine mesh size or with gill nets at different periods of the day : 6h-7h (5 females (f), 4 males (m), 9h-10h (2f, 5m), 14h-15h (2f, 3m), 15h-16h (4m) and 17h-18h (5f, 8m).

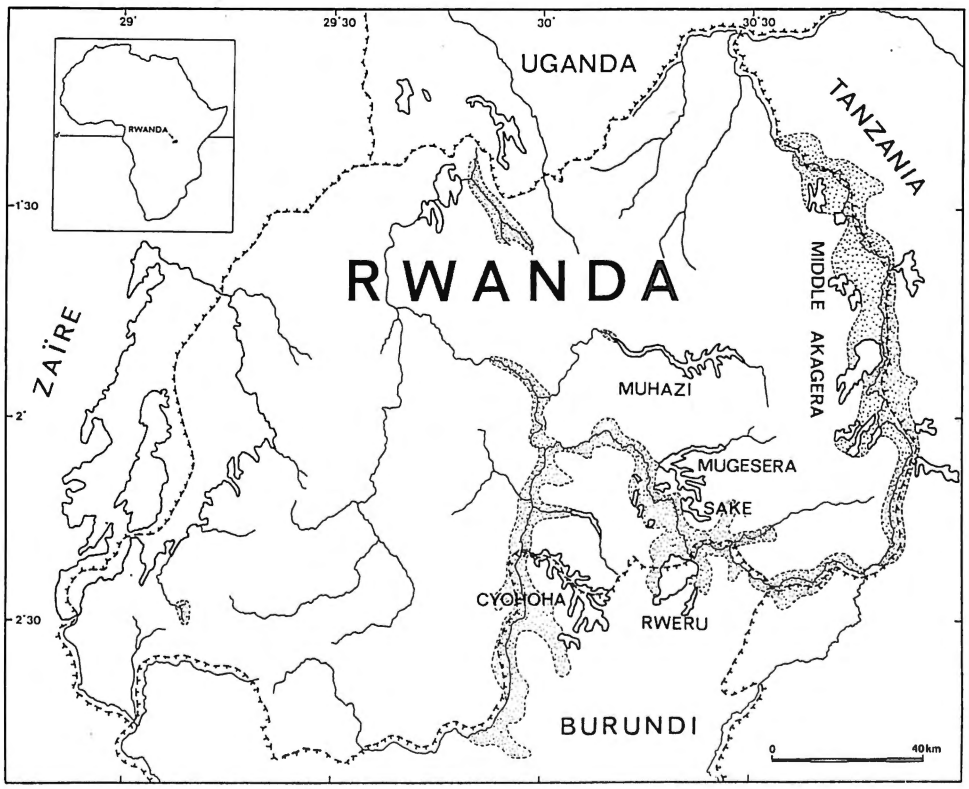

Fig. 1. — Map of Rwanda : the lakes of the Bugesera-depression. Dotted areas indicate swampy depressions.

After capture, they were immediately fixed in 4 % formaldehyde to stop digestion and transferred later into 70 % ethanol for preservation.

In this study no distinction is made between sexes nor catch periods : male as well female fishes of the five periods are pooled in one analysis in order to provide a general idea of the food of the species.

The chosen sample is homogeneous for total fish length (mean total length = 70 ± 0.3 mm), weight (mean weigth = 4.8 ± 0.1 g) and gut length (mean

gut length = 157 ± 2.8 mm). Therefore there is no need to standardize the quantitative data generated by the numerical and the point methods in function of the gut lengths.

The captured fishes proved to be conspecific with *H. burtoni*. This is rather surprising as, up to now, this species was supposed to be endemic to the Lake Tanganyika area. In this area it inhabits shallow coastal waters, swamps and rivers. Nevertheless, after comparison with the reference material from this area, housed in the Koninklijk Museum voor Midden-Afrika in Tervuren, the Mugesera species showed to be identical with *H. burtoni* in all morphological aspects. It also exhibited two colour types, a blue and a yellow one in adult males, as is reported for the Tanganyika populations (see *e.g.* LOISELLE, 1975 ; FERNALD and HIRATA, 1979 ; CRAPON DE CAPRONA and FRITZSCH, 1985).

MATERIAL AND METHODS

We preferred to study both stomach and gut contents, because the data thus obtained are to a large extent complementary : (1) some food items are found only in the stomach because of their high digestibility (WINDELL and BOWEN, 1978) ; (2) the analysis of both zones offers the advantage of avoiding a possible bias of the results due to mechanical selective accumulation processes that may occur during the digestion (JANSSENS DE BISTHOVEN, 1987).

After their removal, the stomach and gut contents were transferred to microtubes in 70 % ethanol and stained with Rose of Bengal to provide an easy recognition of animal tissues. The samples were counted and identified under a light microscope in wet preparations.

The stomach and the gut of each specimen were kept apart and examined separately. For the quantification of each food item, data of all fishes were taken into account.

In order to quantify the food items, the numerical method (HYNES, 1950 ; BERG, 1979 ; HYSLOP, 1980), the method of percentage of occurrence (*e.g.*, used by BISHAI, 1977) and the method of points, proposed by OLATUNDE (1978) were used. The first method was applied to whole animal preys and hard fish structures : the items were counted and the summation over the entire sample was then divided by the total number of fishes. Thus for each food item a mean value per fish is obtained. The method of occurrences was applied to all the food items encountered, including uncounted items such as Protozoa, sand particles, minute setae of Oligochaeta, and Phytoplankton ; the occurrence values represent percentages of fishes containing these food items. The point method of OLATUNDE (1978) was modified to our purposes. We awarded each food item a defined number of points, depending on its estimated volume. These point values are therefore not a function of the degree of fullness of the stomach or the gut. Although the use of point methods in the analysis of stomach and gut contents was qualified by WINDELL (1968) as « a subjective allotment of points », we applied such method because no other valuable, easy, and fast alternative was available to quantify the food items

in a volumetrical way. The method is applied to all food items, including those items, such as soft animal detritus, hard invertebrate remnants and plant material, that could not be numerically quantified. The results generated by the point method are afterwards condensed in seven food categories, in order to provide a general view of the food bulk. The (modified) point method of OLATUNDE (1978) allows to express the volumetrical significance of the food items in the total food bulk and is complementary to the numerical method.

FRYER and ILES (1972) found a relation between the relative gut length of cichlid species of Lake Tanganyika and their trophic niche : species with a relatively long intestinal tube showed a pronounced herbivorous or microphageous feeding habit, while species with a relatively short intestinal tube were rather carnivorous. ZIHLER (1982) qualified the so-called relative gut length (intestinal length/standard or total fish length) as a quite inappropriate value for comparison of the relative gut length between cichlid species, because of the occurrence of different body forms and thus of different allometric relations in the cichlid family. Therefore, this author suggested to use the « intestinal weight length » (IWL = intestinal length/$\sqrt[3]{\text{body volume or weigth}}$) because the volume or the weigth of fishes is less dependent on the body form. We nevertheless used the value generated by the mean gut length/mean total fish length, called « the Relative Gut Index » (RGI) by FRYER and ILES (1972), because the obtained value can be compared with RGI values of other cichlids, mentioned in FYER and ILES (1972). By using the RGI, a rough estimation of the trophic niche of *H. burtoni* can be made.

RESULTS

A. Data presentation :

The stomach and gut contents of *H. burtoni* quantified by the numerical method and the method of occurrence are condensed in Table 1. Only those food items scoring an occurrence percentage > 10 % in either stomach or gut, were taken into consideration. The other food items and the encountered Nematoda are mentioned in the text.

The following list discusses the food items found in *H. burtoni* and displayed in Table 1.

— Organisms : counted on the basis of entire animals or heads.

1. Copepoda : both nauplii and adults.
2. Rotifera : Brachionidae and other families.
3. Undetermined worms : wormlike structures.
4. Trichoptera : Leptoceridae. The cases are also ingested.
5. Hydracarina : only juvenile Hydrachnellae (A. FAIN, pers. comm.).
6. Chironomidae pupae : whole pupae or (paired) respiratory organs.

7. cf. *Tanypus* spp. larvae.
8. cf. *Pentaneura* spp. larvae.
9. cf. *Procladius* spp. larvae.
10. cf. *Glyptotendipes* spp. 4th instar. Only the fourth instar larvae of cf. *Glyptotendipes* are mentioned in Table 1 at the generic level. The other Chironominae growth stages are mentioned under the heading « undetermined Chironominae ».
11. Chironominae 4th instar : includes cf. *Glyptotendipes* larvae, *Chironomus* group *plumosus* larvae as well as 4th instar undetermined Chironominae larvae.
12. Undetermined Chironominae of 3rd instar.
13. Undetermined Chironominae of 2nd instar.
14. Undetermined Chironominae of 1st instar.

(15.) Total Chironominae : items 11-12-13-14.

— « Subunits » : parts of fish skeleton and other organic structures, individually counted.

16. Fish scales.
17. Plant seeds : Cyperaceae.
18. Invertebrate eggs.

— Uncounted items due to their microscopical size. Only the frequency of occurrence (%) is given.

19. Oligochaeta minute setae.
20. Phytoplankton : *Trachelomonas* sp., *Oscillatoria* sp., *Melosira* sp., *Microcystis* sp., *Merismopodia* sp., *Spirulina* sp., *Phacus* sp., *Pediastrum* sp., *Tetraëdon* sp., *Kirchneriella* sp., *Navicula* sp., *Cymbella* sp., *Pinnularia* sp. and *Nitzchia* sp. (N. PODOOR, pers. comm.).
21. Protozoa.
22. Mineral particles.

— Organic remnants expressed as occurrence percentages.

23. Soft animal remnants : weak structureless animal material having a fish or an invertebrate origin, half-digested remnants.
24. Hard insect remnants : hard chitinous invertebrate structures such as wings, mandibulae, maxillae, legs, antennae, etc.
25. Plant material.

Fig. 2 gives the relative importance of 7 food categories, representing the total food bulk, according to the point method. Both stomach and gut data are represented.

1. Zooplankton : Cladocera and Copepoda.
2. Ostracoda, Rotifera, Oligochaeta, undetermined larvae.
3. Diptera (pupae as well as larvae).
4. Ephemeroptera, Trichoptera, Hydracarina.
5. Animal remnants : soft animal remnants and hard chitinous structures. The soft animal remnants were quantified by counting light microscopical fields, which were then converted into point values. The chitinous structures, such as legs, mouth parts and antennae, were counted before converting them in point values. The remnants of insect carapaces were counted per encountered aggregation, before conversion into points.
6. Plant material : Macrophyta remnants and seeds were individually counted and computed in point values, according to their estimated volume.
7. Parts of fish skeleton : scales.

B. Results :

In 95 % of the guts, Chironominae larvae were found with a mean value of 44.5 larvae per fish. In the gut a high occurrence is reached by cf. *Glyptotendipes* spp. larvae (occ. % of 4th instar larvae = 21 %), chironomid pupae (occ. % = 55 %), and 1st, 2nd, 3rd and 4th instar undetermined Chironominae.

TABLE 1

Stomach and gut contents of H. burtoni (n = 38) : N = data according to the numerical method in absolute values. OCC % = data according to the method of percentages of occurrence

	FOOD ITEM	STOMACH		GUT	
		N	OCC %	N	OCC %
01	Copepoda	0.1	13	0.4	18
02	Rotifera	3.0	21	21.0	55
03	Undeterm. worms	0.1	3	0.4	13
04	Trichoptera	0.1	3	0.4	18
05	Hydracarina	1.5	3	1.0	28
06	Chironom. pupae	0.4	21	1.8	55
07	cf. *Tanypus* spp.	0.1	13	0.2	18
08	cf. *Pentaneura* spp.	0.03	3	0.6	13
09	cf. *Procladius* spp.	0.0	0	0.5	28
10	cf. *Glyptotendipes* spp. 4th instar	0.5	24	1.2	21
11	Chiron. 4th instar	1.2	42	2.0	58
12	3rd	2.3	47	10.0	87
13	2nd	2.5	53	21.2	89
14	1st	6.7	55	11.3	92
15	Total Chironominae	12.7	92	44.5	95
16	Fish scales	0.1	30	0.1	10
17	Plant seeds	0.2	5	3.0	28
18	Invertebr. eggs	0.2	8	0.7	13
19	Oligoch. setae	—	3	—	21
20	Phytoplankton	—	5	—	37
21	Protozoa	—	present	—	present
22	Mineral particles	—	60	—	84
23	Anim. soft remnants	—	58	—	92
24	Ins. hard remnants	—	16	—	32
25	Plant remnants	—	87	—	100

Less than 10 % of the fishes contained *Chironomus* group *plumosus*, and this at very low rates. Therefore it is probable that the majority of the undetermined Chironominae larvae are also belonging to the genus cf. *Glyptotendipes*.

13 to 28 % of the guts contained cf. *Tanypus* spp., *Pentaneura* spp. and cf. *Procladius* spp. larvae (Chironomidae, Tanypodinae). However, Chironominae are more abundant than Tanypodinae larvae.

Among the non-Diptera organisms, the Rotifera are numerically the dominant prey. Their mean numerical value is relatively high in the gut (n = 21) (stomach occ. % = 21 %, gut occ. % = 55 %) (Table 1).

The occurrence of the following food items varies in stomach or gut between 13 % and 30 % : Hydracarina, Trichoptera larvae, Copepoda, Oligochaeta (« minute » setae), plant seeds and phytoplankton (predominantly *Microcystis* sp.) and undetermined wormlike structures. A number of prey are only rarely present in the digestive tract and are not given in Table 1. Their mean values do not exceed n = 1 and the occurrence percentage lies below 10 %. These prey are : Cladocera, *Chaoborus* larvae and pupae, *Chironomus* group *plumosus* larvae, Ostracoda, Ephemeroptera nymphs, case structures of unknown origin, Lepidoptera scales and Tardigrada.

The only fish remnants encountered are ctenoid fish scales. No attempt was undertaken to clarify the origin of the scales, since only a very small number was found.

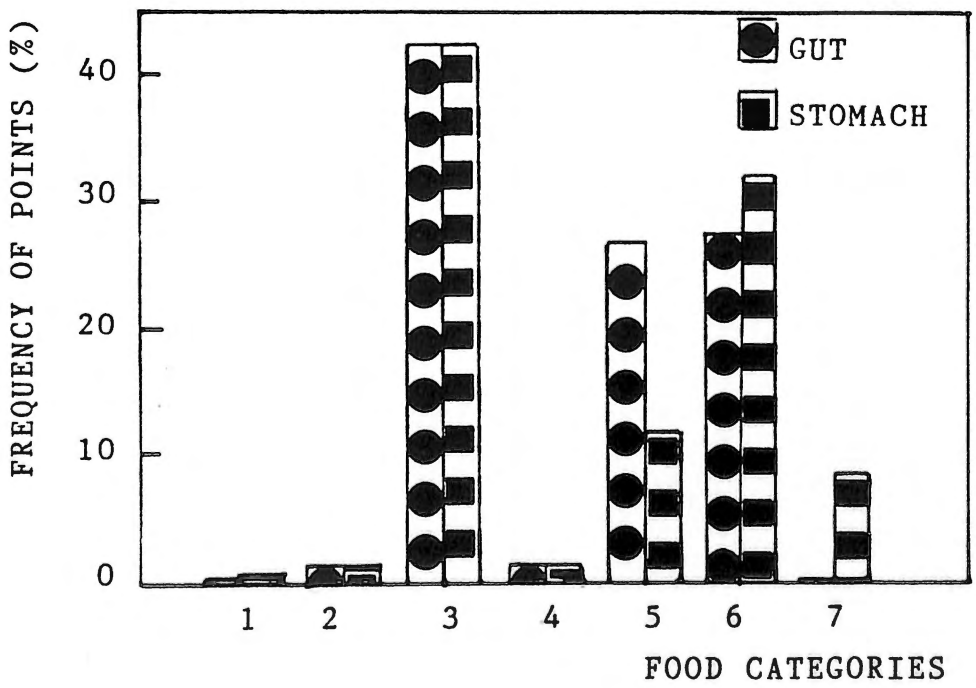

Fig. 2. — Stomach and gut contents of *H. burtoni*. Relative importance of the food items represented in 7 food categories and expressed as volume % of the food bulk in the respective zones, according to the method of points. Food category 1 = zooplankton ; 2 = Ostracoda-Rotifera- Oligochaeta- undetermined larvae ; 3 = Diptera ; 4 = non-Diptera Arthropoda ; 5 = animal remnants ; 6 = plant material ; 7 = fish scales.

When looking at the relative volume of the food items in the total food bulk (Fig. 2), macrophytes and animal remnants (soft animal remnants as well as hard invertebrate structures, cfr. *supra*) represent each 25 % of the food volume in the gut.

Diptera are the main prey types, scoring 43 % of the food volume in both zones, while the other animal prey each represent less than 3 % of the total food volume.

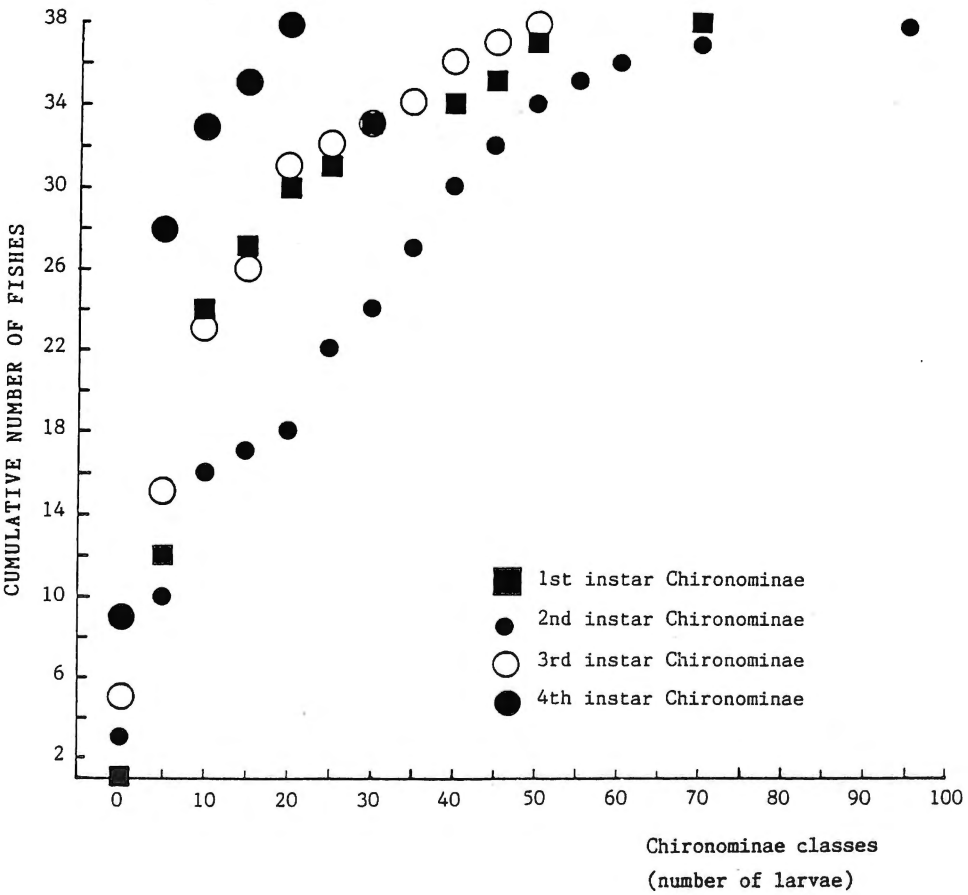

Fig. 3. — Graphical representation of the ingestion frequency of the four Chironominae growth stages in *H. burtoni* (illustration of the non-parametrical Smirnov test). The abcissa generates classes of each 5 midge larvae, from 0 (first class) to 96-100 larvae (last class). In the ordinate are given the 38 fishes. The graph represents the number of fishes (in a cumulative way) containing consecutively 0 larvae of each growth stage, 1 to 5 larvae of each growth stage, 6 to 10 larvae etc. until the maximum is reached.

Although the percentages of occurrence of the four Chironominae larval stages are higher in the gut than in the stomach (which is a general trend in our data, due to the larger gut volume), their mutual order is similar in both zones. In order to assess if the fishes do predate more frequently on one or another growth stage, all Chironominae larvae of stomach and gut are taken into account in the non-parametrical one-way Smirnov test (CONOVER, 1980). The one-way Smirnov test showed that second instar larvae are consumed significantly more ($P < 0.025$) than the other growth stages and the fourth instar larvae less (Fig. 3).

Finally the presence of parasitical Nematoda in both stomach ($n = 1.5$; occ. = 32 %) and guts ($n = 0.5$; occ. = 28 %) is noted. Two forms are encountered : the adult stage of *Rhabdochona (?) pasci* BAYLIS, 1928 and the juvenile stage of Dioctophimatidae cf. *Eustrongylides* JÄGIRSKIÖLB, 1909 (F. PUYLAERT, pers. comm.).

The Relative Gut Index of the analysed population of *H. burtoni* is 2.24.

DISCUSSION

H. burtoni is considered to be a species endemic to Lake Tanganyika and the rivers associated with this lake (GREENWOOD, 1979).

Therefore it is rather surprising to find this species in Lake Mugesera (Rwanda), a part of the Akagera system, which is clearly separated from the Tanganyika system.

In this area, *H. burtoni* has also been collected in Lake Cyohoha, Sake, Rweru and the smaller lakes in between. However, the species is not found upstream in Lake Muhazi, nor downstream in the depression of the Middle-Akagera river (see Fig. 1).

Two hypotheses could be postulated to explain this distribution. Firstly, the presence of *H. burtoni* could be regarded as a remnant of an earlier local connection between the two systems (Tanganyika and Akagera). However, there is no other ichthyogeographical evidence to confirm this thesis. Secondly, more likely, the presence of *H. burtoni* in the Akagera system could be a byproduct of the introduction of an allochtonous fish species. Unregistered introductions and transfers, mainly involving *Tilapia*-species, have been made quite frequently in Rwanda (see *e.g.* DE VOS *et al.*, in press).

Concerning the food of *H. burtoni,* until now almost no data were reported, except for some data from POLL (1953), who characterized the species as omnivorous, with as stomach and gut contents fish bones, insect larvae, Diatomeae, filamentous algae, undetermined plant detritus and sand particles. We found all these items in our study too. The fact that we found a high occurrence of mineral particles in the digestive tract indicates that the fish is a benthic feeder.

Following the Relative Gut Index (RGI) scale applied to the cichlid fishes of Lake Tanganyika (FRYER and ILES, 1972), *H. burtoni* has a RGI value of 2.24 which

lies between the typical values found in omnivorous cichlids (RGI = 0.8-3.2) and those of herbivorous cichlids (RGI = 1.7-8.0) of Lake Tanganyika.

H. burtoni shows a wide prey range. The numerically dominant prey are Chironominae larvae and pupae (especially cf. *Glyptotendipes* spp.), and Rotifera. Since a large part of the food bulk is composed of plant material, it seems quite probable that some prey species (*e.g.* Rotifera and Chironomini larvae) are associated with macrophytes and thus ingested at the same time. Therefore we may assume that *H. burtoni* is feeding as well on a benthic (mineral) substrate as on an organic substrate (plants in a detrital or living form). Since Diptera larvae represent more than 40 % of the mean food volume in stomach and gut, *H. burtoni* can be characterized as a predominantly insectivorous fish.

A large amount of animal and vegetal detritus in the food bulk also indicates a detritivorous behaviour.

Finally, the presence of zooplankton, such as Cladocera, Copepoda and *Chaoborus* larvae (partially planktonic), as well as the presence of plant seeds in the food bulk, emphazises again as well the omnivorous, as the detritivorous character of the species.

As IVLEV (1961) pointed out, more investigations on the abundance and the availability of the prey, and on the use of space and time by the fishes is needed to achieve a better understanding of their trophic ecology. A factor analysis on the same data (JANSSENS DE BISTHOVEN and OLLEVIER, 1988) suggests a day-time dependent feeding behaviour of the fishes and a possible trophic niche difference between male and female fishes.

In order to assess whether the prevalence in uptake of second instar Chironominae larvae over the other growth stages and of Chironominae over Tanypodinae would be a result of selective feeding or of a higher availability of these larvae in the environment, a quantitative study of the benthos is needed.

CONCLUSIONS

Haplochromis burtoni (GÜNTHER, 1893) is an omnivore : it is a benthic insectivore as well as a detritivore. Its volumetrically dominant food items are Chironominae larvae (mostly larvae of cf. *Glyptotendipes* spp.), and macrophytes. Rotifera are numerically abundant, but their relative volume in the food bulk is not large. The presence of plant seeds and zooplankton emphasizes the omnivorous character of the species.

The capture of *H. burtoni,* a species believed to be endemic to the Lake Tanganyika system, in the Akagera system may be explained as a by product of introductions of *Tilapia* species in the area.

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ACCELERATION OF THE WEANING PROCESS OF YOUNGER RHESUS MONKEY INFANTS BEFORE THE ANNUAL MATING SEASON STARTS

by

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SUMMARY

Part of the weaning process of young captive rhesus monkeys (*Macaca mulatta*) was studied during the weeks preceding the onset of the annual mating season of autumn-winter. The delay in the emancipation of the youngest infant and this of the infants of subordinate mothers was most conspicuous in the beginning of the study period. Even though the maximal age difference between the infants was relatively large, only slight differences between their degrees of independence remained at the end of the study period. The results suggest that the mothers accelerate the emancipation of their late infants as the following mating season approaches. This catching up phenomenon could have an adaptive value for the mothers.

Key-words : primatology, *Macaca mulatta,* weaning.

INTRODUCTION

The relationship between a rhesus monkey (*Macaca mulatta*) mother and her infant changes markedly in the weeks and months following birth (in captivity : DIENSKE and METZ, 1977 ; SIMPSON *et al.,* 1986 ; on Cayo Santiago : BERMAN, 1980 ; in India and Nepal : JOHNSON and SOUTHWICK, 1984). It was possible to build up a picture of this relationship by analysing the type, the quality and the pattern of interactions that take place between them (HINDE, 1976). A certain consistency in the gradual nature of the development of the mother-infant relationship does appear (HINDE and SPENCER-BOOTH, 1967). Nevertheless it became clear that various factors may induce differences in mother-infant relationships (SIMPSON and HOWE, 1980). Some of them result from the individual properties of the mothers

and infants themselves. For example : the sex of the infant, the status and seniority of the mother in the colony, the number of infants the mother had before and the characteristics of infant or mother (BERMAN, 1980 ; HINDE and HERRMANN, 1977 ; HOOLEY and SIMPSON, 1981 ; TARTABINI and SIMPSON, 1986 ; STEVENSON-HINDE and SIMPSON, 1981 ; SEAY, 1971 ; WHITE and HINDE, 1975). Other differences are determined by social factors as the group composition, the presence of playmates or of other females coveting the infant (DE JONGE *et al.*, 1981 ; SIMPSON and HOWE, 1986 ; SPENCER-BOOTH, 1968). Of course these factors may interact in a complicated way. In this study, we analysed the influence of the dominant versus subordinate status of the mother on the development of rhesus mother-infant interaction. During the weaning period the developing organism must make the major transition from a state of complete dependence on parental care to one of independence. The concept of weaning is of central importance in the study of behavioural development. However it is still a term with many shades of meaning (MARTIN. 1984). It may sometimes retain its narrow reference to nutritional self-sufficiency from mother's milk and self-sufficiency that is imposed rather than voluntary (ALTMANN, 1980). In this restricted sense it refers to a relatively brief preriod during mammalian ontogeny. However, in a broad sense the word weaning covers the whole process of loosening the mother-infant bond, resulting in an independent adult existence (COLLINGE, 1987). This constitutes a major transformation which may span an extensive period in ontogeny (GALEF, 1981). Here, we use the term weaning in a broad sense and chose to study an area of this last aspect, expressed in some weaning-related behaviour of both mothers and infants. We think it is also important to regard weaning as part of the whole reproductive strategy and not only as an isolated process of ontogeny. Rhesus monkeys, as many other primates, are considered as seasonally polyoestrous as conception occurs only in particular seasons during which females cycle again, if they do not conceive at once (RICHARD, 1985). In practice, seasonal acyclicity may often be difficult to distinguish from postpartum amenorrhea, but the two have quite different implications for the interbirth interval. For example, after the death of their suckling infant non-seasonal primates exhibiting a postpartum amenorrhea begin to cycle again shortly after the infant's death (as for *Presbitys entellus* : see HRDY, 1980, or for *Papio cynocephalus* : see ALTMANN, 1980). Females that are seasonally polyoestrous do not resume their cycle before the next mating season. In the long run it is the timing of gestation and lactation, not the sexual cycle as such, that is important for reproductive success. In seasonally breeding primates the interbirth interval depends on the number of months in the year during which a female can cycle and conceive.

The present study bears on a colony that has one mating season per year, lasting from autumn through winter, like the wild rhesus monkeys of North India (LINDBURG, 1971).

MATERIAL AND METHODS

Material

Eight mother-infant pairs, living in four social groups were studied. Six infants were females, two males. At the beginning of the study the youngest infant was 7 weeks, the oldest 18 weeks (Fig. 1). Each group consisted of five to eight individuals including two mothers with their infants.

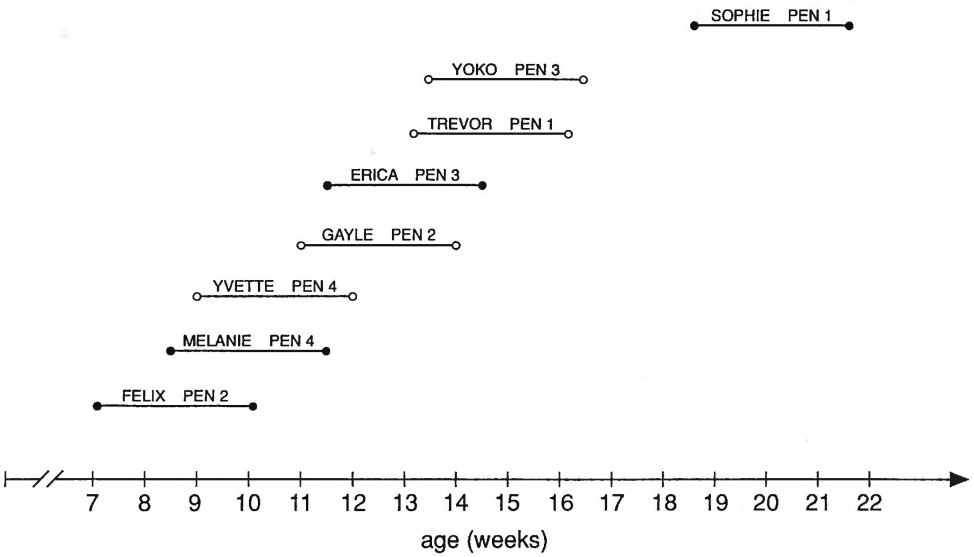

Fig. 1. — The ages of the infants during the observation period.

Infants of		
● dominant mother :	pen	○ subordinate mother :
Sophie °06.04.82	1	Trevor °13.05.82
Felix °27.06.82	2	Gayle °31.05.82
Erica °27.05.82	3	Yoko °13.05.82
Melanie °16.06.82	4	Yvette °12.06.82

The composition of the groups did not change during the study period. Each of them inhabited an outdoor pen (floor : 8 × 3 m, height : 4 m) communicating with an indoor room (floor : 2.5 × 1.5 m, height : 2 m). Indoor temperatures were kept above 13°C. Members of one group could hear, see and smell those of the other groups, but could not make physical contact. Every morning, monkey pellets, peanuts and sunflower seeds were distributed on the floor. The monkeys immediately started to eat and stopped spontaneously after a while, thus leaving a certain amount of food available during the whole day until the next morning. At the beginning of the afternoon a supplement of fresh fruit and vegetables was added. This was completely consumed in the next two hours. Further details of the history and management of the colony are given by ANDERSON and SIMPSON, (1979).

Many different ethologists used to make observations on the colony, almost daily, for many years.

Method

All observations were made from an inside room with one window to the monkeys' inside room and one window to their outer part. The monkeys were accustomed to observers, who behaved as neutrally as possible. A microphone transmitted the sounds from the cage to the observation room.

Quantitative data on mother and infant were separately collected on checksheets in a way that allowed continuous and sequential notes and also a two minutes scan method (HINDE, 1973). At any time, during the observation session, any situation or change of situation was recorded in respect to four classes of distance : on nipple, all mother-infant contact except infant on nipple, without contact but within arm's reach (within 60 cm) and at a distance greater than 60 cm. Any change from one class of distance to the other was recorded as either approach or leaving by mother or/and infant. Every two minutes a mark was written down in order to use those sequential and uninterrupted notes also as an instantaneous scan method with a two minutes interval. In addition, the following interactions were also noted whenever they occured :

Mother-infant interactions :

Mother rejects her infant (R) : occasions on which the mother hits the infant (including pulling, grabbing and biting), passively prevents or breaks contact within 5 s of the infant making contact with or taking the nipple. A mother passively prevents access to her nipple by putting her arm up as the infant's mouth approaches her chest and/or by pulling her nipples up and/or by twisting her torso away (SIMPSON and Howe, 1986).

Mother restrains her infant (r) : occasions when the mother holds the infant tightly as it tries to move or squirm away, or when she pulls an infant that has already broken contact, closer to herself again.

Mother-mother interactions :

Attacking, threatening, chasing and *supplanting* directed by mother A at mother B, and *avoiding, fear-grinning* and *presenting* (in non-grooming context) directed by mother B at mother A, were regarded as evidence for dominance of mother A over mother B (SIMPSON and HOWE, 1986). In every group one mother was regarded as dominant if the above described interactions always occured in the same direction. For this study we used the observations made between august 12th and september 6th, 1982. Most of these were done between 12 a.m. and 1.30 p.m. or between 4 and 7 p.m., while mother and infant were not sleeping nor involved in an eating session. The two mother-infant pairs of the same social group were each observed on the same day during 62 to 107 min. The principle was to change from group

every day. Therefore, every four days the two mother-infant pairs of the same group were reobserved. Only a few times this rythm could not be respected due to external circumstances. But the time interval between two following sessions was always comprised between a minimum of three and a maximum of five days, as shown on the graphs (Figs. 2 to 6).

From an other study we made on the social context of this four groups, it became obvious that group 4 showed a particular terrorizing adult male. He often bit the members of his group in a context that could not predict his agression. This influenced the agonistic, grooming and play interactions of all members of the group. Therefore, the results of group 4 are sometimes considered separately.

RESULTS

Six relative frequencies were used to analyze the data. Two were measures of mother-infant proximity. Three of the other measures concerned relative roles of mother and infant in determining the mother infant proximity as described by HINDE and SPENCER-BOOTH (1967) and HINDE (1974).

1. Percentages of mother-infant contacts (Fig. 2)

The number of instantaneous observations, made at a two minutes' interval on the same day, when a mother was in contact with her infant (N*c*) as proportion of the total (N*t*) watched, is expressed in :

$$Nc \times 100 / Nt$$

This measure is inspired from the index described by HINDE and SPENCER-BOOTH (1967), but we used a scan method rather than a one-zero sampling. The decreasing percentages of contacts reflect the increasing independence of the infant.

In the beginning of the study, the highest percentages of contacts in groups 1, 2 and 3 occured for all subordinate mothers and the dominant mother of the youngest infant. At the end the situation looked quite different as the higher percentages decreased faster. Consequently, the differences between the means of the dominant mothers and the subordinate ones seem to diminish with time. The status of the mother and even the age of the infant would act less and less upon their independence as time went on. It looked as if the degrees of independence of the offspring tended to level out. This suggests a « catching up » phenomenon. This is most strongly supported by the fact that, at their infant's 14 weeks, the subordinate mother-infant pair of pen 1 (1 SMI) and 3 SMI rate higher than 2 SMI and the dominant mother-infant pair of pen 3 (3 DMI), but then they do their « catching up » in the next 2-3 weeks. Moreover, the fact that the ranges of the observations, at 13-14 weeks, for 1 SMI and 3 SMI hardly overlap with those for 2 SMI and 3 DMI is important because it suggests that the differences are not merely due to sampling error in the observations. The infant of the subordinate mother of group 4 would be the only exception. Hence, at this stage her independence would remain

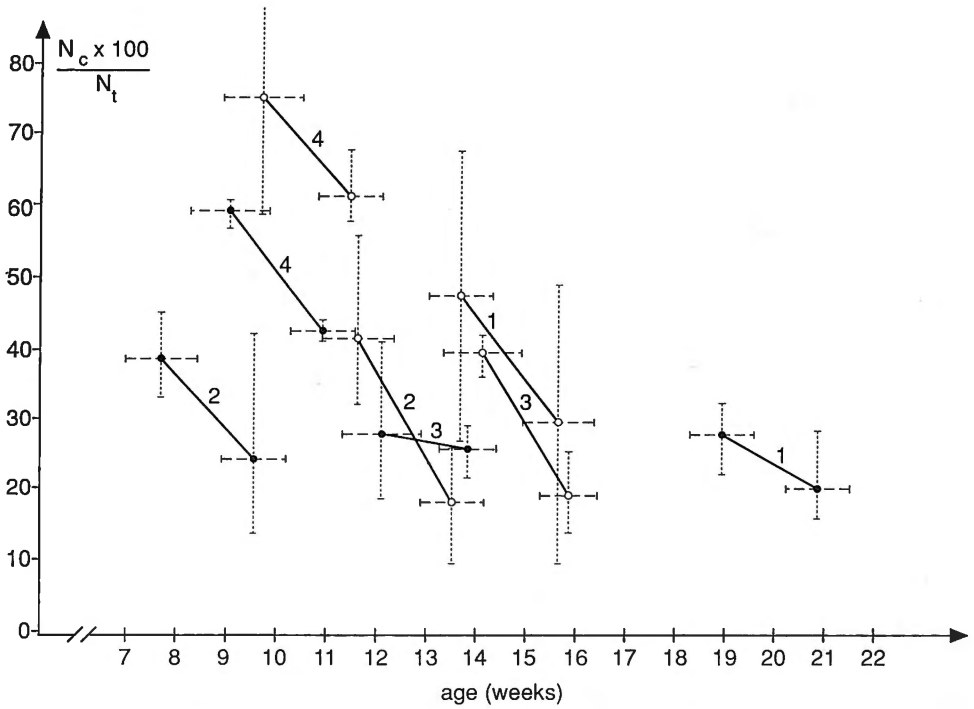

Fig. 2. — Proportion of time in contact : number of two minutes' observations during which the infant was in contact with the mother as proportion of the total number of observations. The means of the index and the extreme values (limits of vertical dotted lines) are put in the middle of each observation period represented by the horizontal dotted line. The mean of the first three and that of the last three observation sessions are joined by a plain line. They are plotted according to the age of the infant in the middle of the period including the three sessions.

- ● Infant with dominant mother,
- ○ Infant with subordinate mother

more related to its mother's rank. Nevertheless two short observation sessions (of 22 and 33 minutes during the same two observation periods of three weeks) of the subordinate mother-infant pair of group 4, made in the « absence » of the dominant male (*i.e.* while he was sitting in the other part of the cage or sleeping) showed much lower percentages of contacts (57 % and 55 %) than those on the graphs (76 % and 62 %).

2. **Relative frequency of rejections** (Fig. 3)

This measure was given by the ratio of the number of times the infant attempted to get on its mother and was rejected (R) to the total number of contacts, either

on the infant's initiative (MK*i*), or on the mother's (MK*m*) and of the infant's unsuccessful attempts to contact (R), *i.e.* :

$$R \times 100 / MKi + MKm + R$$

Since the frequency with which the infant gains contact on the mother's initiative (MK*m*) decreases rapidly over the first weeks of life, the relative frequency of rejections during the study period approximates to the failure rate of the infant's attempts (HINDE, 1974).

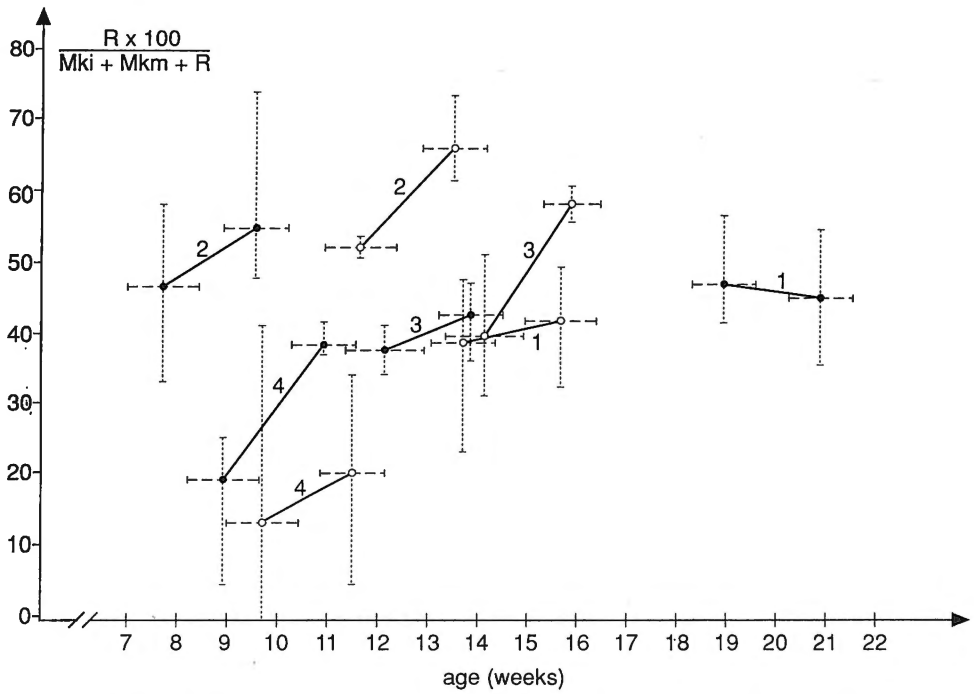

Fig. 3. — Relative frequency of rejections : ratio of the number of occasions on which the infant attempted to gain contact and was rejected by the mother (R) to the number of occasions on which it made contact on the mother's initiative (M*km*), made contact on its own initiative (M*ki*) or attempted unsuccessfully to gain contact (R). The means of the index and the extreme values (limits of vertical dotted lines) are put in the middle of each observation period represented by the horizontal dotted line. The mean of the first three and that of the last three observation sessions are joined by a plain line. They are plotted according to the age of the infant in the middle of the period including the three sessions.

- ● Infant with dominant mother,
- ○ Infant with subordinate mother

All mothers increased their relative frequency of rejections except the dominant mother of the oldest offspring, who tended to decrease it.

Those measures remained lower for the mothers of group 4, especially for the subordinate mother. Nevertheless, as for the preceding index, her relative frequencies of rejections were higher in the « absence » of the dominant male (36 and 41 % instead of the 13 and 20 % on the graphs).

3. Infant's role in making mother-infant contact (Fig. 4)

This index is the difference between the percentage of contacts (on nipple or other) made on infant's initiative and the percentage of contacts broken by the infant, *i.e.* :

$$(MKi \times 100 / MKi + MKm) - (BKi \times 100 / BKi + BKm)$$

abbreviated to % MK*i* − % BK*i* (HINDE, 1974 and HINDE and ATKINSON, 1970).

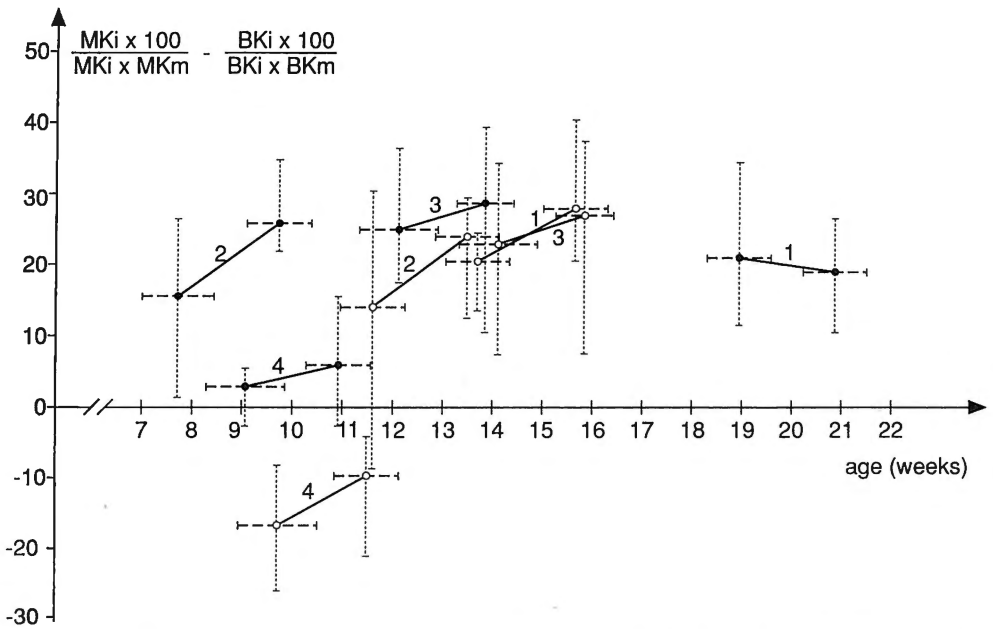

Fig. 4. — Infant's role in seeking contact : number of contacts made on the infant's initiative, as a percentage of the total number made by mother and infant, minus number of contacts broken off by the infant, as percentage of the total number broken. The means of the index and the extreme values (limits of vertical dotted lines) are put in the middle of each observation period represented by the horizontal dotted line. The mean of the first three and that of the last three observation sessions are joined by a plain line. They are plotted according to the age of the infant in the middle of the period including the three sessions.

- ● Infant with dominant mother,
- ○ Infant with subordinate mother

If the infant were responsible for all makes and no breaks this index would have a value of + 100 %, and if the mother were responsible for all makes and no breaks it would be − 100 %. A positive value means that the infant is primarily responsible for the contacts.

From Fig. 4 it is obvious that all infants, except one, were more responsible than their mothers for seeking contacts. As for the frequencies of rejections, the infant's role increased with time, except for the oldest infant where it rather decreased.

In group 4, it is the subordinate mother who is primarily responsible for seeking contacts. But here too, as in the previous measures, the results were markedly different and even positive in the « absence » of the adult male (+ 8 and + 14 % instead of − 15 and − 19 % on the graphs). On these occasions the subordinate mother was not seeking infant's contact more than mothers in other groups.

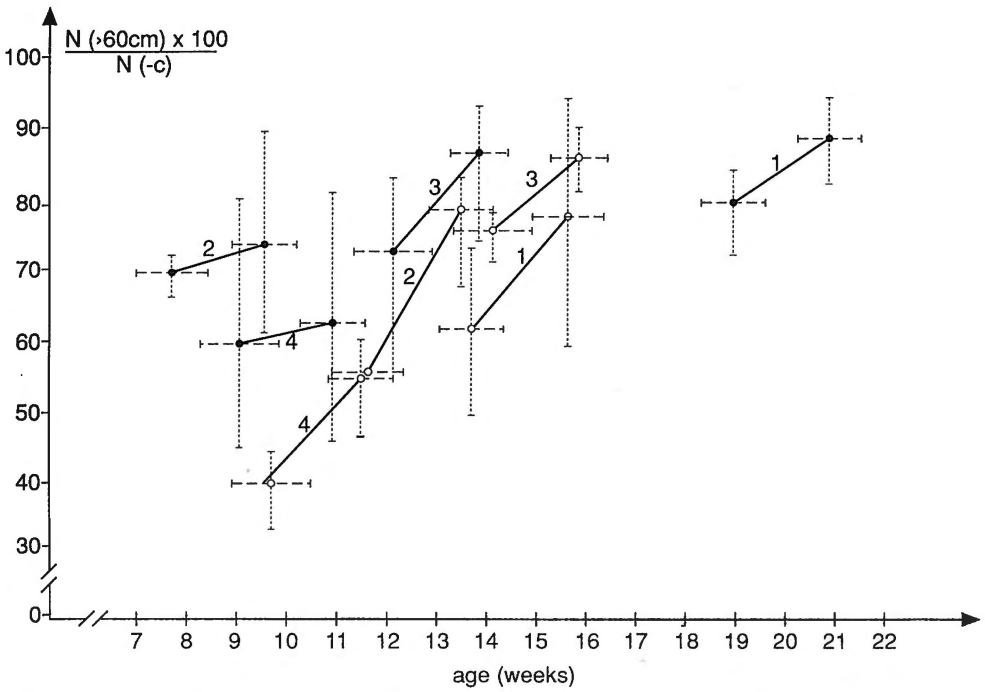

Fig. 5. — Proportion of time out of arm's reach of mother : number of two minutes' observations during which the infant was more than 60 cm from the mother (N > *60 cm*) as proportion of the number for which it was off her (N − *c*). The means of the index and the extreme values (limits of vertical dotted lines) are put in the middle of each observation period represented by the horizontal dotted line. The mean of the first three and that of the last three observation sessions are joined by a plain line. They are plotted according to the age of the infant in the middle of the period including the three sessions.

- ● Infant with dominant mother,
- ○ Infant with subordinate mother

4. The proportion of time spent at a distance of more than 60 cm (Fig. 5)

This measure resulted from the number of instantaneous observations made at a two minutes' interval on the same day, when the infant was at more than 60 cm from its mother as a proportion of the total number when it was off her, *i.e.* :

$$N(> 60\ cm) \times 100 / N(-c) \quad N(-c) = Nt - Nc$$

The distance of 60 cm was chosen because it is considered as the limit of a rhesus mother's arm reach.

This index is inspired from that described by HINDE and SPENCER-BOOTH (1967), but we used a scan method rather than a one-zero sampling.

The increasing proportion of time the infant spent at a distance of more than 60 cm also shows some kind of increasing independence of the infant. The values of the younger offspring of the subordinate mother of groups 1, 2 and 4 who at first spent least time at a distance, increased the fastest. The degrees of independence of the infants of groups 1, 2 and 3 seemed to level out with time. The same could be said for the babies of group 4. In the last group the status of the mother kept acting most upon the emancipation of the offspring. From the beginning to the end of the observation period the infant of the subordinate mother remained markedly more dependent.

5. Infant's role in seeking proximity less than 60 cm (Fig. 6)

The infant's role in proximity was assessed by counting the number of approaches (the distance decreases from > 60 cm to < 60 cm) and leavings (the distance increases from < 60 cm to > 60 cm), and by calculating the difference between the percentage of approaches due to the infant's movement (AP*i*) and the percentage of leavings due to the infant's movement (L*i*), *i.e.* :

$$(APi \times 100 / APi + APm) - (Li \times 100 / Li + Lm)$$

A positive result would indicate that the maintaining of proximity is mostly due to the mother and a negative result that it is mostly due to the infant (HINDE and ATKINSON, 1970 ; HINDE, 1974).

All measures were positive except in the beginning for the infant of the subordinate mother of group 4. This sustains the fact that almost all infants were mostly responsible for seeking the proximity of the mother. The subordinate mother of group 4 was the only mother, who was primarily responsible for seeking proximity and this solely at the beginning of the observation period. As time went on, she drastically decreased her responsibility and at the end the baby became even more responsible than herself.

6. Restraints by the mother

The last measure is the percentage of restraints by the mother. It is defined as the ratio of the number of times the mother restrained (r) her infant to the total

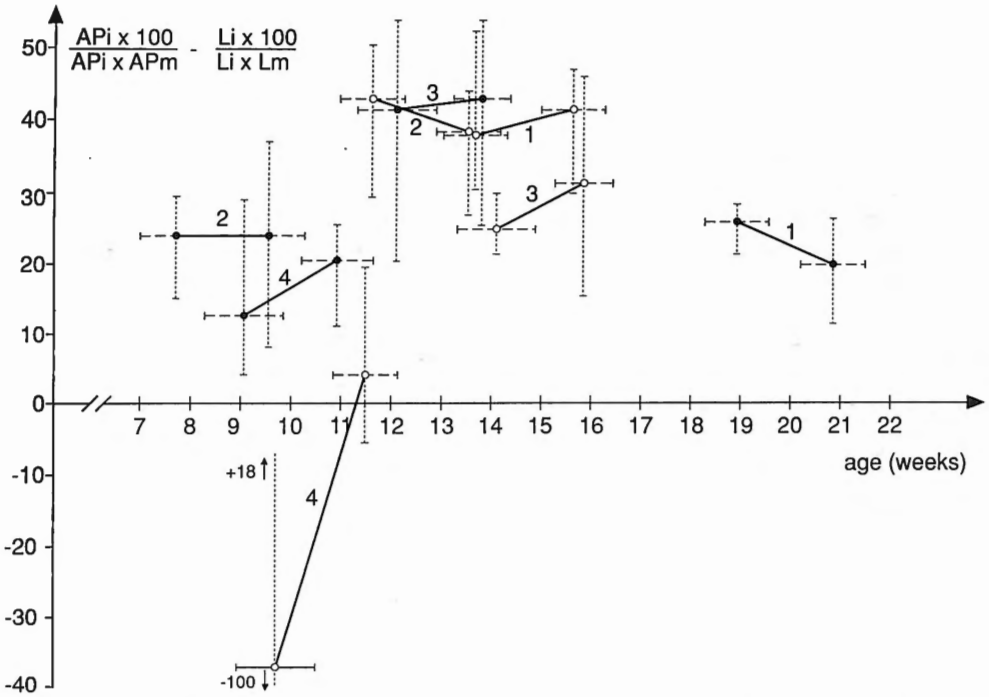

Fig. 6. — Infant's role in seeking proximity : number of approaches made by the infant, as percentage of the total number made by the mother and the infant, minus the number of leavings made by the infant, as percentage of the total number made. The means of the index and the extreme values (limits of vertical dotted lines) are put in the middle of each observation period represented by the horizontal dotted line. The mean of the first three and that of the last three observation sessions are joined by a plain line. They are plotted according to the age of the infant in the middle of the period including the three sessions.

- ● Infant with dominant mother,
- ○ Infant with subordinate mother

number of times the mother broke contact and the infant's either successful or unsuccessful attempts to break contact, *i.e.* :

$$r \times 100 / BKi + BKm + r$$

All mothers restrained extremely rarely, except the subordinate mother of group 4. Of all dominant mothers of groups 1, 2 and 3 only two restrained their infant (once each). The subordinate mothers of the same groups scored a mean of 10 times in absolute value (*i.e.* 1 %) in the beginning and restrained only twice at the end.

In group 4, the dominant mother restrained 4 times in the beginning and only once at the end. While the subordinate mother of this group, counted 24 % of restrainings in the beginning and 19 % at the end. As for other measures, the situation was markedly different in the « absence » of the adult male, when she restrained only once in 64 attempts by her infant to leave her.

Although the values of this index were too low to be considered alone, they confirm the general trend of the other results, *i.e.* the active involvement of the subordinate mother of group 4 which delayed the emancipation of her infant.

Temporal evolution of the measures

The age-changes in the mother-infant interactions could depend on changes in the infant's behaviour, changes in the mother's behaviour, or both (HINDE, 1974). If the proportion of time in contact (Fig. 2) decreases with increasing percentages of rejections (Fig. 3) and increasing infant's role in contact (Fig. 4), the emancipation of the infant must be primarily due to changes with the mother. This was the case for each mother-infant pair, except the one with the oldest infant (group 1). Here the correlation is positive and the emancipation could be primarily due to changes with the infant.

The situation is different, if we consider the proportion of time at a distance of more than 60 cm (Fig. 5). The changes in the infant's role in maintaining proximity was only clear for the subordinate mother-infant pair of group 4. For this pair we can tell that, as both indices increased with time, the mother is primarily responsible for this kind of increasing independence too.

In sum, for most pairs, the mother's changes were primarily responsible for the decreasing proportion of time in contact, but when contact was broken, changes in mother and infant had a similar role in the increasing proportion of time spent beyond arm's reach. This could be due to the fact that the mother's most important point is to get free from « too much contact » with her infant. Once the contact is broken, she may concede a bigger part of initiative to the infant to augment its proportion of time out of arm's reach.

DISCUSSION

In four captive groups of rhesus monkeys, part of the weaning process of eight infants was observed in detail during the last weeks preceding the onset of the annual mating season. Several factors seem to influence the rate of increasing independence of the infants. The results suggest that this independence may be delayed in three circumstances. First, the younger age of the infant, as described by DIENSKE and METZ (1977) and HINDE (1974). Second, the lower status of the mother, consistent with SIMPSON and HOWE (1986). Finally, the particular social context of the presence of a feared adult male. SIMPSON and HOWE (1986) observed that the mothers, submitted to higher rates of aggression from other adults, played a more important role in maintaining proximity with their 8-week-old infants.

Furthermore, two particular points appear in our observations, most obviously in groups 1, 2 and 3. On the one hand, the differences between the dominant and the subordinate mothers were more noticeable at the beginning than at the end of the study. On the other hand, even the expected differences between the degrees of independence in relation to the infants' ages only, became surprisingly smaller at

the end of the study. This is an amazing finding if one expects a gradual development of the mother-infant relationship only in function of the infants' age. Facts tend to give evidence of a « catching up » phenomenon : the emancipation going faster for offspring that, in the beginning, are more dependent of their mother. As the correlations between the indices suggest, this catching up phenomenon would be primarily due to the behaviour of the mother, who would fasten the emancipation of her « late » infant. This is not in contradiction with TRIVERS' argument (1974) that the infant must be selected to elicit more parental investment than the parent is selected to provide. Another major factor, different from infants' age, could interfere. At the approach of the annual mating season, the emancipation of the « late » infants would be accelerated (in regard of the more precocious infants) and reach an optimal degree at the onset of the mating season. In terms of inclusive fitness, this could have an adaptive value for the mother : too much contact between mother and infant could make it difficult for the mother to invest in her sexual activities with males or a delay in the infants' weaning could delay the mother in resuming her sexual cycles. SIMPSON *et al.* (1981) observed that mothers proving quicker to conceive their next infants tend to reject them more often.

This emphasizes that there are many factors that may influence the reproductive strategy and that these likely interact in a complex fashion. It could be worthwhile to reconsider the evolution of mother-infant relationship, in seasonally polyoestrous species, by gathering the observations of infants grouped in age-classes relative to the onset of the following mating season, as well as pooled in the usual age-classes only in function of their birth date. This could give some insight in the question : is the rate of emancipation of young seasonally polyoestrus primates, a function of the proximity to the following mating season ?

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ABSTRACTS

Assembly at the K.B.I.N./I.R.B.S.N. on 18 October 1989

THE EFFECTS OF COMPLEXATION ON THE BIOAVAILABILITY OF CADMIUM TO THE BRINE SHRIMP, *ARTEMIA FRANCISCANA*.

by

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Metals in solution exist as a number of chemical species (inorganic and organic complexes, colloids, ...). The biologically available fraction of a particular metal is the fraction of the total concentration that is available for biological uptake, and is dependent on the chemical form of the element in solution. The bioavailable species of cadmium for aquatic biota is the free hydrated cation Cd^{2+}. The free metal ion concentration is decreased by ligand complexation, which will reduce the availability of cadmium.

Within this context, it is important to know the effect of metalligand binding strength on the bioavailability of the metal. For this purpose we have studied the effect of complexation with organic ligands with different affinities for cadmium on biological uptake. The free Cd^{2+} concentration was calculated by a computer model. Accumulation experiments were conducted with brine shrimp, *Artemia franciscana*.

For all ligands tested, accumulation of cadmium decreased with complexation. However, a significant accumulation remained when the free Cd^{2+} concentration was virtually zero. Bioavailability was independent of the binding strength of the cadmium-ligand complexes formed.

* R. B. is a senior research assistant of the N.F.S.R.

ENTOMOFAUNE COMPARÉE DES TERRILS D'HENSIES ET ST-ANTOINE

par

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Au cours de la saison 1988, une campagne de piégeage (bacs colorés, « pit fall trap ») et de récoltes au filet a été menée sur deux terrils houillers du Hainaut occidental (Belgique). Le premier terril (St-Antoine) est âgé de 30 ans, élevé (80 m), formé d'éléments grossiers et couvert d'une végétation très diversifiée. Le second terril (Hensies) est récent (13 ans), tabulaire, formé d'éléments fins (1 mm de diamètre moyen) et grossiers et enfin, couvert d'une végétation éparse et peu diversifiée. Il est constitué d'anciens bassins à schlamms. Les taxons étudiés sont les Coléoptères Carabidae, les Hyménoptères Vespiformes (Scolioidea, Vespoidea, Pompilidae, Sphecidae) et les Hyménoptères Apoidea (Andrenidae, Halictidae, Megachilidae, Anthophoridae, Apidae). 8262 spécimens de 214 espèces ont été capturés (1948 spécimens de 129 espèces au Terril St-Antoine et 6314 spécimens de 151 espèces au Terril d'Hensies). Les faunes des deux terrils sont très riches et diversifiées mais différentes. La faune du Terril St-Antoine est calcicole alors que la faune du Terril d'Hensies est plutôt psammophile. Cette dernière est exceptionnelle en Belgique avec une nouvelle espèce pour la faune belge : *Dienoplus exiguus* (Handlirsch) dont 361 spécimens ont été récoltés. Cette étude a permis de montrer que diversités floristique et faunique ne sont pas toujours corrélées. D'autre part, on voit que des terrains industriels, totalement artificiels, peuvent abriter une faune extrêmement riche et diversifiée. Les résultats d'une telle étude peuvent servir à imaginer des aménagements de terrains industriels.

THE INFLUENCES OF A WASTE WATER TREATMENT PLANT ON THE MACRO-INVERTEBRATE COMMUNITIES OF A LOWLAND RIVER IN THE CAMPINE (BELGIUM)

by

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The combined effects of purified and unpurified sewage on the macro-invertebrates of the Witte Nete, a Campine lowland river, have been studied.

In ten localities, eight of which were downstream a waste water treatment plant, the benthic fauna on 9.6 m^2 of the bottom has been collected with a handnet and later on identified and counted, The interpretation of the data was done with the aid of statistical techniques and biological water assessment methods.

Immediately downstream the treatment plant the benthic fauna consists of oxygen-stress tolerant organisms, whilst species from the upstream communities are less oxygen-stress tolerant. As an effect of self-purification the biological water-quality becomes a little better further downstream. No visible effect on the recovery of the macro-invertebrate community of the Witte Nete could been proved after the confluence with a smaller unpolluted lowland river. Further on, after a strong aeration by a water-mill, the benthic fauna again consists of oxygen-stress (pollution) sensitive organisms, although the organic pollution has not yet disappeared, as shown by the chemical water analysis. The high oxygen concentration stimulates the bacterial activity, with the result that a few km further downstream the invertebrate community is again oxygen-stress tolerant. This lowland river is not long enough to recover from the pollution. More aeration could have a positive effect on the biological quality of this watercourse by stimulating the process of selfpurification.

RELATIONSHIP BETWEEN GENOTYPE SEX AND SIZE-DIMORPHISM OF YOUNG EUROPEAN EEL (*ANGUILLA ANGUILLA* L.)

by

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It is a wellknown fact that eels (*Anguilla anguilla* L.), larger than 40 cm, show a size-dimorphism which is related with the phenotypic sex of the fishes and that, in general, phenotypic females are larger than phenotypic males of the same age. Size-dimorphism however is also observed before eels reach the stage of gonadal differentiation, which starts at a body-length between 14 and 35 cm, independently of the fish-age.

In this paper the relationship between size-dimorphism and sexual *genotype* of eels smaller than 40 cm was studied using an immunological technique (the indirect enzyme (= peroxidase)-conjugated antibodymethod) to demonstrate the H-Y antigen. In all animal species examined thusfar, the sexual genotype is associated with the presence or absence of the H-Y antigen in somatic tissue. This antigen is present on the cells of the heterogametic sex (XY or ZW) and absent in the homogametic sex (XX or ZZ). The European eel is believed to have a ZZ/ZW sex-determining mechanism.

The 50 largest eels (23.9-32.4 cm ; 16.5-49.0 g) and the 50 smallest eels (6.7-13.2 cm ; 0.2-2.5 g) from one age-class (11 months) population were selected and examined. Testing of somatic tissue for the presence of the H-Y antigen revealed an overbalance (69 %) of genetic female ($H\text{-}Y^+$) animals in the group of the largest eels. The group of the smallest eels on the contrary consisted mainly (87 %) of genetic male ($H\text{-}Y^-$) animals.

We therefore conclude that the size-dimorphism of eels, smaller than 40 cm, is correlated with their genotypic sex.

pH AND ALUMINIUM EFFECTS ON SOME OSMOREGULATORY AND HAEMATOLOGICAL PARAMETERS OF THE ACID-RESISTANT AMERICAN BULLHEAD *ICTALURUS NEBULOSUS* (LE SUEUR) *

by

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The influence of low pH (pH 4.0) and several Al-concentrations (0.007 ; 0.04 ; 0.11 mmol/l) was examined on some ionoregulatory and haematological parameters of *I. nebulosus*. A striking result was that the bullhead, besides increasing its Na^+-efflux, also increased its Na^+-influx after exposure to pH 4.0 for 84 hours. Acute exposure to Al at pH 4.0 gave similar results. Most acid-sensitive fishes show an increased Na^+-efflux and a decreased Na^+-influx at pH- and Al-stress, which then leads to a net whole body Na^+ loss. The bullhead thus seems to be able to compensate Na^+ losses. This might be a physiological mechanism, responsible for its acid-resistant nature.

Literature studies show that high external Ca^{++}-concentrations sometimes can exercise an ameliorating influence on the physiology of freshwater fishes at low pH and high Al-concentrations. After a pre-exposure to pH 4.0 for 4 days, we examined the effect of 0.025 and 1 mmol Ca^{++}/l at this pH, with and without addition of 0.04 mmol Al/l, on *I. nebulosus*. Neither at low pH, nor at low pH with Al, did the external Ca^{++}-concentration of 1 mmol/l have an ameliorating effect on pH- and Al-effects.

Finally we investigated whether populations of *I. nebulosus* from an acid lake are physiologically adapted to low pH and therefore exhibit physiological differences to bullheads from a neutral lake. Two populations of bullheads, from an acid and a neutral lake, showed significant differences in ionoregulatory and haematological parameters. Most of these differences, however, disappeared after an acclimation of two populations to pH 6.8 for 5 weeks. The physiological response of both populations to a subsequent acidification (pH 4.3) remained comparable during 14 days. Our results thus indicate that pH differences of natural waters have not yet given rise to physiological strains of *I. nebulosus*.

* Partly supported by a CEC-contract, EV4V-0116B, Environmental Research Programmes.

SOMATOSTATIN INCREASES PLASMA T3 CONCENTRATIONS AND STIMULATES IN VITRO T4 5'-DEIODINATION ACTIVITY IN *TILAPIA* IN THE PRESENCE OF HIGH T4 LEVELS

by

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In teleost fishes, the conversion of thyroxine (T4) into 3, 5, 3'-triiodothyronine (T3) has been confirmed in many tissues and in the eel and rainbow trout, this peripheral conversion is under growth hormone or steroid control.

The involvement of hypothalamic factors in thyroid hormone regulation is poorly understood in fishes. Among them, somatostatin (SRIF) which is known to have an influence on hypophyseal function in several teleosts including *Tilapia,* was used in this study to enhance T4 or T3 conversion in *Tilapia nilotica.*

SRIF was injected into *Tilapia* with high or low T4 levels and followed by blood sampling at 0, 4, 8 and 24 hours after injection.

T4, T3 and 3, 3', 5'-triiodothyronine (reverse T3, rT3) were assayed in the plasma by radioimmunoassay.

An injection of ovine growth hormone (oGH), porcine follicle stimulating hormone (pFSH) and bovine thyrotrophin stimulating hormone (bTSH) increased plasma concentrations of T4 and rT3 after 4 and 8 hours, whereas plasma T3 was unaffected.

An injection of SRIF alone did not influence thyroid hormone levels, but enhanced T4 5'-deiodination in the liver.

If however SRIF was injected together with these hormones which raised plasma T4 or with T4 itself, an increase in plasma concentrations of T3 could be observed, whereas the increase of rT3 levels was less pronounced.

It is concluded that SRIF may switch the normal 5-deiodination activity and increased rT3 during hyperthyroxinemia into a 5'-deiodination activity and a raise of T3 in *Tilapia nilotica.*

SYSTEMATICS OF THE AFRICAN BAT GENUS *EPOMOPHORUS* BENNETT, 1836 (MAMMALIA : MEGACHIROPTERA) *

by

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Clinal size variation, biometric differences and possible overlap between the different groups were studied by means of univariate as well as multivariate analyses. About 4 000 specimens were processed, using their skull, wing and tooth measurements.

Based on the configuration of the palatal ridges, two groups can be distinguished : the *E. wahlbergi* group characterized by the presence of only one postdental palatal ridge and represented by one species ; and the so-called *E. gambianus* complex characterized by two postdental palatal ridges and including five closely related species, namely *E. gambianus, E. crypturus, E. angolensis, E. labiatus* and *E. sp. n.* The new species from Kenia, Ethiopia and Somalia, represented by the smallest *Epomophorus* specimens, is being described. *E. pousarguesi* and *E. reii* are junior synonyms of *E. gambianus ; E. minor* and *E. anurus* are junior synonyms of *E. labiatus. E. wahlbergi* is largely sympatric with the *E. gambianus* complex. Within the *E. gambianus* complex, however, the species are allopatric or only slightly sympatric over a very limited area of their distributional range.

A remarkable variation in body measurements is noticed within *E. wahlbergi.* Smaller specimens are distributed along a west-east orientated axis, larger specimens along a north-south axis. Specimens of all sizes are present in Kenya.

THE USE OF THE TONGUE AND HYOID APPARATUS DURING FEEDING IN *CAIMAN CROCODILUS* *

by

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To gain insight in the use and function of the hyolingual apparatus during inertial feeding in crocodiles the feeding mechanism of *Caiman crocodilus* was studied by cineradiography.

Analysis of the cineradiographic records reveals two cycletypes : inertial bites (reposition, kill/crush and transport) and swallowing cycles. All these cycle-types can be characterized by their gape profile and the displacement of the neck, cranium and hyolingual apparatus. Inertial bites are initiated by an elevation of the neck and cranium which results in a retraction of the head and a backward acceleration of the prey.

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Simultaneously the prey is lifted by the hyolingual apparatus. As soon as the lower jaw is depressed, the prey is rapidly pushed upward by the hyolingual apparatus. During fast mouth-closure the neck and cranium are abruptly depressed, the lower jaw is elevated and the hyolingual apparatus is rapidly retracted ventrally. Depression of the neck and cranium thrusts the head forward and impacts the backward moving prey more posteriorly in the buccal cavity. During swallowing the hyoid is first moved in front of the prey and then rapidly retracted posteroventrad, forcing the prey into the oesophagus during the opening and closing of the mouth. After mouth-closure, the hyoid apparatus is again protracted.

The tongue and the hyoid apparatus play an active role during inertial feeding in crocodiles. In the beginning of the feeding sequence the movement of the hyolingual apparatus is mainly a dorsoventral one, whereas, the anteroposterior displacement gains importance towards the end.

DESCRIPTIVE AND FUNCTIONAL STUDY OF THE SONG OF THE HOUSE CRICKET, *ACHETA DOMESTICUS* (ORTHOPTERA : GRYLLIDAE)

by

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The acoustic communication of the house cricket, *Acheta domesticus,* was studied in the laboratory. In order to establish the repertory of the cricket, individuals were put together in couples and observed. Analysis of the sounds produced revealed that three different acoustical signals could be distinguished. By setting up a series of playback experiments with both sexes, the function of these signals was determined.

1) The aggression song is produced mainly during encounters of conspecific males. The playback experiments also revealed that the reception of this song keeps neighbouring males at a distance.

2) The courtship song is a display of the male, set off by a stimulus of the female. In *Acheta domesticus* it seems that the emission of the courtship song is necessary to initiate the mating ritual.

3) The calling song is produced by isolated males and attracts conspecific females, while it keeps rival males away.

Further research was carried out on the calling song. The attractiveness of a few isolated song characteristics (number of chirps per second, number of pulses per chirp, frequency) to females was tested. Their correlation with body size and dominance rank of the males was tested. Positive phonotaxis by the females was induced only by the number of pulses per chirp. Furthermore this was the only characteristic which was significantly correlated with body size. No correlation was found between dominance rank and any of the song characteristics.

We may conclude that females of *Acheta domesticus* orientate towards the song of large males and probably use the number of pulses per chirp to assess the size of the calling males.

MINERALIZATION OF ADULT MOUSE BONE MARROW IN VITRO

by

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Starting from adult bone marrow cells we have set up and improved a model for bone formation in vitro. Mineralization was used as an in vitro model for bone formation. We have investigated the effects of the composition of the tissue culture medium (TCM), the addition of serum components and the addition of bone growth regulating hormones.

The three cell culture systems used are : (1) adult bone marrow, cultivated as an intact organ ; (2) stromal cells, obtained by selective cultivation of adult bone marrow cells ; (3) immortalized stromal cells, obtained by infection of adult bone marrow with a c-fos oncogen virus (E. Mathieu, Biochemistry, UIA). The mineralization is studied by ^{85}Sr-uptake, which is used as a calcium tracer. Effects of the bone growth regulating hormones are tested by measuring the alkaline phosphatase activity.

The mineralization process has a shorter latency period in the presence of serum containing TCM as compared to a serum free TCM. The serum probably contains growth factors for the primary cultures. Serum components in the TCM influence the physiology of the bone formation in vitro. Stromal cells start mineralization in serum containing TCM, they contain the cells responsible for mineralization. The immortalized cells provoked mineralization immediately and with the same course in serum containing TCM and serum free TCM. The immortalized cells are probably differentiated cells with a self-regulating capacity for the mineralization. Effects of the bone growth regulating hormones, parathyroid hormone and vitamin D_3 on the mineralization are not measurable by the ^{85}Sr uptake. This can be caused by the insensibility of the ^{85} Sr technique or by the separation of the ^{85}Sr metabolism and the cellular process in which parathyroid hormone and vitamin D_3 act. Parathyroid hormone and vitamin D_3 modified the alkaline phosphatase activity of the immortalized cells. This provides evidence for the presence of receptors for the hormones in the immortalized cells.

INTRASPECIFIC VARIATION OF THE MUSHROOM CORAL *FUNGIA (FUNGIA) FUNGITES*

by

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In order to establish the current value of the 13 varieties described by DÖDERLEIN in 1902, 202 specimens of the mushroom coral *Fungia (Fungia) fungites* were submitted to multivariate analysis.

Nine different parameters were measured but after the Kolmogorov-Smirnov (K.S.) test only the parameters « area » and « number of teeth/cm^2 aboral surface » proved to be not

normally distributed. Only they served as data for the clustering programs PAM and AGNES. The K.S. test also showed that, during growth, the regularity of the perimeter changes and that this change has a significant influence on the area which, until now, was always considered to be a continuously varying parameter. The clustering programs proved that, on basis of the used parameters, the 13 varieties do *not* exist but that the intraspecific variability is not as could be expected in a really homogeneous group. We may be dealing with two isolated groups which, after complementary histological/biochemical research, perhaps could be elevated to subspecies level.

TOXICITY OF DELTA-ENDOTOXINS FROM *BACILLUS THURINGIENSIS* TO LARVAE OF THE LEPIDOPTERAN ORDER

by

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The spores of various *Bacillus thuringiensis* strains are used as a safe microbiological insecticide. Upon sporulation this bacterium produces crystalline inclusions which contain the proteinaceous delta-endotoxin protoxin. At present, there are specific toxins known for Lepidoptera, Coleoptera and Diptera. The expression of the toxin in plants gave new dimensions to the application of the *B. thuringiensis* delta-endotoxins. However, the mode of action of these toxins is not fully understood. Three hypotheses were postulated during several years of investigation. The first hypothesis suggests the active potassium transporter as the target for the delta-endotoxin (1). In the second hypothesis the delta-endotoxin is assumed to alter specifically the permeability for potassium (2). In the last hypothesis the toxin is believed to create non-specific pores whereby the osmotic pressure contributes to cell lysis (colloid osmotic lysis) (3).

Using the ion-amino acid cotransporter to determine the ion permeability, an effect of the delta-endotoxin on the passive level of ion-transport was demonstrated. Furthermore the permeability change was not restricted to potassium. Even the permeability to bigger molecules was altered. These results give full credit to the colloid osmotic lysis hypothesis. The use of toxins with a different specificity spectrum, demonstrated the presence of a receptor molecule in the midgut of the target insect. In the future the further characterisation of the receptor molecule will be undertaken.

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BUCCAL DEFORMITIES IN *CHIRONOMUS* GROUP *THUMMI* LARVAE (DIPTERA, CHIRONOMIDAE) OF A NATURAL POPULATION IN THE DIJLE WATERSHED AS A SIGNAL FOR TOXIC STRESS : QUANTIFICATION

by

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Morphological deformities of the heavily sclerotized mandibles and mentum are assessed in *Chironomus* gr. *thummi* larvae of the polluted Dijle watershed. It is expected that such deformities can be used as an accurate biological signal for heavy metal and pesticide contamination in aquatic ecosystems. A quantification system for deformities is worked out and optimized for an adequate description of the deformities. This system is also used to compute correlations between the deformities and the substrate-bound contaminant concentrations. Five zonations of mentum and mandibles are arbitrarily chosen in function of their structural homogeneity and two types of structural aberrations are defined. Values for ten deformity variables are allocated to the larvae of 8 locations.

The locations which are most polluted with heavy metals and pesticides contain the highest percentages of deformed larvae and the highest mean deformity scores per larva. A principal component- and a factor analysis were performed : the larvae could not be clearly discriminated by their sampling locations and three essential groups of correlated deformity variables are extracted. Most deformed larvae share deformities quantified by one or more variables of the three groups, independently of their sampling location. The data-analysis provides new elements for the elaboration of an optimal quantification system : (1) a number of variables can be discarded, (2) some variables need more accentuation in order to stress the aberrant character of new, twisted, splitted or fused teeth structures, (3) additional variables must be defined in order to make a better discrimination of the different deformity types.

COMPETITION BETWEEN THE GREAT TIT (*Parus major*) AND THE BLUE TIT (*P. caeruleus*) FOR NEST-BOXES AS ROOSTING SITES IN WINTER : an aviary experiment

by

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Great and Blue Tits use nest-boxes for roosting in winter. In our study area, two types of nest-boxes are available, differing in diameter of the entrance hole. Large-holed (32 mm) boxes can be used by both Great and Blue Tit, whereas small-holed (26 mm) boxes can only

be used by the smaller Blue Tit. When both types of boxes are available most Blue Tits roost in the small-holed boxes. We argue that Blue Tits are forced to use small-holed boxes because of competition with the dominant Great Tit. To test this hypothesis we performed an aviary experiment.

We showed that in absence of the Great Tit, 80 % of the Blue Tits preferred the large-holed boxes. However, in the presence of a Great Tit, 56 % (of the 80 %) of the Blue Tits changed to roosting in small-holed boxes (the same individuals were tested twice). These results suggest that Blue Tits do not use the large-holed nest-boxes, because these boxes are claimed by the dominant Great Tits.

EFFECTS OF ^{241}Am ON HAEMOPOIETIC AND STROMAL STEM CELLS IN MICE AFTER FOETAL AND PERINATAL RADIOACTIVE CONTAMINATION

by

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Previous results demonstrated that radioactive contamination causes radiation damage to bone-marrow cells at lower dose if contamination occurred *in utero* or soon after birth.

In this study we investigated the radiosensitivity to ^{241}Am of bone-marrow haemopoietic and stromal cells in mice, contamined *in utero* and/or during lactation. Different contamination procedures were compared : (1) single intravenous injection of the pregnant mother at the 14th day of gestation, (2) contamination during lactation and (3) continuous contamination *in utero* between the 7th and 14th day of gestation and between the 14th and 19th day of gestation. To ensure a permanent radioactive contamination *in utero*, an osmotic pump was implanted subcutaneously in the pregnant mother during a defined period of gestation, and served as a constant source for ^{241}Am-citrate delivery.

14 and 25 weeks after radioactive contamination, the radiation effect on bone-marrow cells in the offspring was estimated.

Changes in quantity of haemopoietic and stromal stem cells were measured by means of short-term cultures (CFU-GM and CFU-f). Using long-term cultures, we also tested whether the capacity of the stromal adherent layer to sustain haemopoiesis *in vitro* was changed.

14 weeks after radioactive contamination, the number of granulocyte-macrophage progenitor cells was decreased, except in the experiment with continuous contamination between the 14th and 19th of the embryonic development. The capacity of the stromal adherent layer to sustain *in vitro* haemopoiesis in long-term cultures was decreased after radioactive injection at the 14th day of gestation.

At 25 weeks postcontamination, we were still able to detect radiation effects. After reseeding haemopoietic cells on the stromal layer derived from contaminated mice, the capacity of the stroma to maintain CFU-GM proliferation now seemed to be increased.

These results suggest a changed regulation of the blood formation after radioactive contamination *in utero* and/or during lactation.

A STUDY OF INDIVIDUAL NICHE SHIFTS DEMONSTRATING INTERSPECIFIC COMPETITION AMONG TITS (PARUS SPP.) DURING WINTER *

by

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We studied niche shifts to examine the existence of interspecific competition in two sympatric tit species *P. cristatus* and *P. montanus*. In our study area (4°25'E, 51°25'N — Kalmthout, Belgium), seasonal variation in interspecific niche overlap was strongly related to wind velocity.

On days with low wind velocity, the foraging niche of *P. cristatus* was not affected by the presence of *P. montanus*, whereas the latter species, in presence of the congeners, slightly moved towards the inner parts of the pine trees. However, on days with high wind velocity, *P. montanus* strongly shifted away from the foraging sites occupied by *P. cristatus* towards the outermost and highest positions. In contrast, in subplots where both species lived in allopatry, they converged towards lower and more inner parts of the trees as wind stress increased.

Simultaneous observations of an increase in the number of chases of *P. montanus* by *P. cristatus* suggested that interspecific intolerance was the direct cause of the displacement of the subdominant species from the preferred parts of the trees, but only in conditions of environmental stress. Wind-induced shifst of sympatric individuals of *P. montanus* towards energetically unfavourable foraging positions differed between individuals with unequal social status : subdominant juveniles shifted more strongly towards the uppermost and outermost foraging positions, whereas similar age-dependent effects were not observed in the dominant species.

As a result, on days with high wind velocity the intraspecific (age-related) foraging patterns of sympatric populations of *P. cristatus* and *P. montanus* differed significantly, resulting in the smallest niche overlap between individuals with the largest differences in social rank.

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RED SPIDER MITE DAMAGE ASSESSMENT FOR GREENHOUSE TOMATOES

by

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The red spider mite *Tetranychus urticae* causes yield reductions by destroying the epidermal cells of the leaflets. This destruction reduces the photosynthesis, alters the stomates and causes an hydric stress. The study aims to identify when curative actions are required for infected greenhouse tomatoes. The method consists of an evaluation of the risk by an estimation of the density of mites on the leaflets. The economic injury level can be assessed by visual ranking of the domage on a specifically designed scale.

The severity of leaflet damage has been categorized into 5 classes : class 1, first incipient signs of damage (3 *T.u.*/dm^2) ; class 2, extension of the spot (10 *T.u.*/dm^2) ; class 3, new spots (30) ; class 4, beginning of drying (100) ; class 5, all the area is damaged, great drying (350 *T.u.*/dm^2).

Physiological impact of mites at each injury level has been observed by two methods : the study of the photosynthetic activity and of the stomatal resistance to water diffusion. The photosynthesis is exponentially affected by the damage. About 25 % of the photosynthetic area could be removed without affecting the yield ; this corresponds to class 1 of the scale. The stomatal resistance stays low during the first three damage classes and increases above. The increase is considered as closing of the stomatal openings.

In conclusion, treatment is required early after the first visual damage at class two.

PATHWAYS AND FATE OF POLYCHLORINATED BIPHENYLS (PCB) IN PLANKTON OF THE MEUSE RIVER

by

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A survey of the distribution and amount of persistent organochlorine toxins in the Belgian environment emphasized that the aquatic ecosystems are particularly contaminated by PCB. According to their high lipophility, their low solubility and their low biodegradability, these xenobiotics accumulate up food chains. As a consequence, the PCB concentrations in biological systems are usually higher in strength than in abiotic environments according to the bioconcentration processes. In aquatic ecosystems, phyto- and zooplankton constitute a main way of entrance of persistent toxins such as PCB into food chains.

The present work deals with the fate and the pathways of PCB in the different compartments of the Meuse river ecosystem (water, suspended matter, sediments and plankton).

It is obvious that such a study involved the analysis of the dynamic trophic relations between phyto- and zooplankton. This part of the work has been done by an *in situ* study in 6 collecting stations located at Huy, Ampsin, Ivoz-Ramet, Liège, Monsin and Lixhe.

In the Meuse river, the zooplanktonic organisms were mainly rotifers, the main species of which were *Brachionus calyciflorus, B. angularis, Keratella cochlearis* and *Synchaeta sp.*

Grazing experimental studies have been performed on rotifers collected in the Meuse river. From these experiments and the *in situ* observations, it is obvious that the dynamic relations between planktonic organisms were related, on one hand, to the zooplankton grazing pressure on phytoplankton and, on the other hand, to the physico-chemical characteristics of the ecosystem.

PCB analysis in the different compartments of the Meuse river have shown that the PCB contamination of rotifers was particularly high. As a consequence, these organisms appeared as strong bioaccumulators of these remanent xenobiotics. However, these considerations don't allow us to determine the relative importance of the two principal ways of entrance of PCB into aquatic organisms, *i.e.* :

1. — direct way of entrance of PCB in organisms through the exchange areas such as gills, epithelium, membranes...
2. — PCB absorption of contaminated food.

The relative importance of these two ways of PCB entrance in zooplankton has been measured. For that purpose, grazing measurements have been performed on *Brachionus calyciflorus* and on *Keratella cochlearis* by means of unicellular alga (*Distyosphaerium ehrenbergianum*) marked with a hard β radioactive tracer (P^{32}). The results of these experiments have allowed us to determine the phytoplankton quantity that rotifers (*i.e.* zooplankton in the Meuse river) were able to eat within a given period of time (feeding rate).

According to these results and to the PCB accumulation kinetics performed with natural algal cells (*D. ehrenbergianum*) and with rotifers (*B. calyciflorus*), the relative importance of direct entrance through the exchange surfaces and of the trophic way in the PCB contamination degree of Meuse zooplankton has been measured. Moreover, it appeared obvious that a biological dilution phenomenon of PCB occurred both in phyto- and zooplanktonic populations.

CHEMICAL AND ULTRASTRUCTURAL DEVELOPMENT OF THE MANDIBULAR GLAND IN *FORMICA SANGUINEA* (LATR.) *

by

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Formica sanguinea robs nests, especially of *F. fusca* and *F. rufibarbis*, taking the worker pupae. The latter emerge in the *sanguinea* nests and become slaves. During the raids, *F. sanguinea* repels defending opponents by a chemical secretion. In the majority of the Formicidae, the mandibular gland has been reported to be the source of alarm pheromones, that form part of the general communication system.

Ants, taken from two large laboratory colonies, were marked with paint spots immediately after hatching and prepared for analysis at determined time intervals. The contents of the glands of *F. sanguinea* workers at different ages were chemically analysed using the solid sampling technique (1) on capillary columns. Two components were recognized : 3-isopropylpentanol and methyl-3-isopropylpentanoate. The substances were identified by their mass spectrum and by comparison of their retention time with standard compounds. A considerable individual variation in quantity and composition of the secretion is observed. Pooling the individuals into age groups reveals a distinct increase of the absolute quantity of the ester and alcohol. Although the absolute quantity of methyl-3-isopropylpentanoate increases, its proportion decreases in older workers.

A study of the glandular ultrastructure in the same age groups describes the various cellular and cytoplasmatic elements of the mandibular gland. This exocrine gland includes type-3 and type-1 cells, following the definition of NOIROT and QUENNEDEY (2).

The ethological function of the secretion is so far unknown. We propose to study the behavioural effects by means of bioassays, testing pure and synthetic components.

* Supported by an « IWONL » grant.

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ULTRASTRUCTURAL FEATURES OF AEROBIC DEGRADATION PROCESSES IN THE ORGANIC MATRIX OF MOLLUSCAN SHELLS

by

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Previous experimental data concerning the degradation of molluscan shells placed at the sea water-sediment interface have shown that the biomass, the diversity and the hydrolytic activity of micro-organisms responsible for this degradation present maximal values after an immersion from 12 to 18 months.

In order to characterize the different types of aerobic microborers, the alteration profiles caused by these organisms inside the organic matrix and the relations between microborers and the organic substrate, fragments of *Nautilus pompilius* mother-of-pearl and *Pinna nobilis* prismatic layer were immersed for 13 months, at 37 m depth, in the experimental site of the Bay of Calvi (Corsica). After appropriate treatments the samples were examined with the use of transmission (TEM) and scanning (SEM) electron microscopes.

Microcoenoses are principally composed of bacteria (very numerous), blue-green algae and fungi. Diatoms and perforating bryozoans were also observed with the SEM. Examination of ultrathin sections reveals that microborers are localized inside the extracrystalline organic matrix of prismatic layers whereas they are in close contact with them in mother-of-pearl. Alterations look like lysis profiles parallel or perpendicularly oriented to the interlamellar sheets in mother-of-pearl. No preferential orientation in organic sheaths was observed in the prismatic layer. In the close vicinity of microborers the organic matter is generally completely disorganized, sometimes totally lacking so that a halo free of any material surrounds the organisms. In the prismatic layer, lysis of organic sheaths leaves a thin electron dense film, probably tanned, in close contact with the mineral. These alteration profiles are spatially very heterogeneously distributed.

THE EFFECT OF CATTLE BLOOD IN FORMULATED STARTER DIETS ON THE GROWTH AND SURVIVAL RATE OF EUROPEAN GLASS EELS (*ANGUILLA ANGUILLA* L.)

by

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The intensive rearing of European glass eels *Anguilla anguilla* L. has recently become of great interest in Europe. Data on the nutritional requirements of these glass eels during the initial feeding period are scarce.

Previous experiments indicated that cattle spleen is a very attractive starter diet.

We therefore investigated the effect of the addition of cattle blood to paste feeds on growth and mortality of glass eels.

As starter diets, six isonitrogenous and isocaloric paste feeds were formulated. Five pastes contained different percentages of cattle blood (respectively 0, 15, 30, 45 and 60 % on a wet weight basis) and one had 30 % spleen incorporated. The initial stocking density was 22 kg/m^3 ; water temperature was maintained at 25°C and glass eels were fed 4 times a day till satiation. After a 2 month feeding period, growth rate and mortality were determined.

The highest growth rate was obtained for glass eels fed on the paste containing 60 % blood (SGR (= specific growth rate) of 0.61 % body weight/day). The group receiving paste without cattle blood showed a negative SGR of − 0.70 % body weight/day. Pastes composed of resp. 15, 30, 45 % blood and of 30 % spleen resulted in SGR of resp. 0.00, 0.21, 0.45 and 0.33 % body weight/day.

The higher the blood content in the food the lower the mortality after 2 months : resp. 54, 44, 40, 37 and 33 % for the fish fed on paste with 0, 15, 30, 45 and 60 % blood incorporated. The average mortality of the group fed with a paste food composed of 30 % spleen was 38 %.

It is still a matter of discussion whether the increased growth rate and decreased mortality in the groups receiving paste containing cattle blood is due to an improved digestibility and/or attractivity of the food with this ingredient.

CARACTÉRISATION DES SOUS-POPULATIONS DE MACROPHAGES PÉRITONEAUX ET ÉTUDE DE LA VIABILITÉ CELLULAIRE LORS DE L'INFECTION A *TRYPANOSOMA CRUZI*

par

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T. cruzi, agent de la maladie de Chagas, est capable d'infecter de nombreux types cellulaires dont les macrophages. Ces cellules, connues pour leur hétérogénéité, jouent des rôles importants dans la réponse immune anti-parasitaire.

Comme on n'atteint jamais 100 % d'infection « in vitro », il devrait exister différentes sous-populations de macrophages résistantes à *T. cruzi*.

Grâce à la technique des gradients de Percoll, nous avons isolé 12 fractions de cellules de la cavité péritonéale de souris Balb/c mâles. Ensuite, nous avons caractérisé ces fractions cellulaires par :

— Marquage de 7 antigènes membranaires révélé par immunofluorescence indirecte (F4/80, 2.4G2, MAC-1, THY1.2, 21.1.1, L3T4*a*) et directe (IgM).
— Evaluation du pouvoir phagocytaire vis-à-vis de billes de latex fluorescentes et de bactéries fluorescéinées (*Micrococcus lysodeikticus*).
— Comparaison de la présentation de l'antigène de classe II (I*e*) du complexe majeur d'histocompatibilité en association avec différentes concentrations du peptide à processer.

Dans une deuxième étape, nous avons tenté de mettre en corrélation le taux d'hexosaminidase avec la parasitose des cellules de la population totale, lors de leur infection par *T. cruzi.*

L'analyse des résultats est résumée comme suit :

1) Les cellules des densités 1.050 à 1.060 g/ml appartiennent à la lignée des lymphocytes (elles sont L3T4*a* +, THY1.2 + et IgM +). De plus, le test de phagocytose est faible.
2) Les cellules des densités 1.064 à 1.096 g/ml appartiennent à la lignée des monocytes-macrophages (elles sont 2.4G2 +, F4/80 +, les tests de phagocytoses et la présentation de I*e* sont très positifs). De plus, il existerait parmi ces macrophages de la même lignée cellulaire des stades différents de maturation. En effet, les cellules des densités 1.064 à 1.082 g/ml seraient plus immatures que celles des densités 1.087 à 1.096 g/ml (hétérogénéité de positivité lors des différents tests effectués).
3) Les cellules des densités 1.104 et 1.117 g/ml appartiennent à la lignée des polymorphonucléaires et des mastocytes.

Quant à l'hexosaminidase, elle pourrait servir de marqueur de viabilité cellulaire et d'estimation de la parasitose. En effet, son taux diminue lorsque les amastigotes apparaissent (dès 48 h d'incubation) et s'annule lors de la rupture des cellules due à la multiplication massive du *T. cruzi* (96 h d'incubation).

BIODEGRADATION AND PRESERVATION OF FISH VERTEBRAE IN MARINE AND ESTUARINE CONDITIONS

by

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Biodegradation processes are of utmost importance, both from an ecological point of view (for the recycling of organic matter and avoidance of detritus accumulation) and a paleontological point of view (fossilisation phenomena). In marine sediments, very few vertebrate remains are found, so we must suppose that their biodegradation occurs rapidly. Preservation of fish bones is often explained by anoxic or estuarine conditions at the burying site. To check these hypotheses, we studied experimentally *in situ* fish (*Scorpaena porcus*) vertebrae biodegradation in marine (both in oxic and anoxic sediments) and in estuarine conditions.

Anoxic conditions were created by use of closed jars, containing sediment sampled *in situ* (organoclastic sand, $CaCO_3$-poor sand or estuarine « slikke ») in the Calvi Bay (Corsica) or Le Guillec estuary (Bretagne). When the jars were opened, enzymatic hydrolytic activity assessments were performed by use of Apizym kits. Samples were subsequently processed for S.E.M. observations.

Hydrolytic activity assessed in vertebrae undergoing biodegradation in marine oxic conditions is higher than in marine anoxic ones. This relatively good preservation of anoxically

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biodegraded vertebrae is confirmed by S.E.M. observations. Interstitial water of the same jars sediment showed low hydrolytic activity. No differences in biodegradation rate could be noticed between calcified and decalcified sediment.

It is important to note that our previous biodegradation experiments with Mollusk shells and Echinoid skeletal plates showed that their weathering rate is the same in oxic and in anoxic conditions. So we can distinguish skeletal structures with low organic content (Mollusk shells, Echinoid skeletal plates) from skeletal structures with high organic content (fish bones, crustacean cuticle) at the level of their biodegradation characteristics. Indeed, biodegradation of the first ones occurs at the same speed in oxic and anoxic conditions, while biodegradation of the second ones occurs much slower in anoxic conditions.

In estuarine conditions, vertebrae (and the other skeletal structures studied in our previous experiments) showed very low hydrolytic activity. On the contrary, interstitial water of the same jars sediment showed huge hydrolytic activity. This probably means that estuarine decomposers are merely adapted to degrade the particulate (more accessible) organic matter coming from rivers and do not attack organic matter hidden in skeletal formations, access to which is impeded by mineral compounds. Preservation is enhanced by a huge sedimentation rate, putting material away from bacterial activity.

SEROLOGICAL IDENTIFICATION OF *AEROMONAS* SPECIES ISOLATED FROM FISH

by

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A serological identification of four species of the genus *Aeromonas, Aeromonas hydrophila, Aeromonas caviae, Aeromonas sobria* and *Aeromonas salmonicida* was performed using different serological techniques such as slide and tube agglutination tests, microtiter tests and ELISA.

Polyclonal sera against these bacterial species were produced in rabbits. The optimal absorption time for the coating of the microtiter plates with bacterial Antigen and the conservation of such coated plates was investigated.

In order to obtain more specific sera the produced polyclonal sera were absorbed with the related bacterial strains. The absorbed sera were then tested using the microtiter test.

SEARCH FOR THE FUNCTION(S) OF RECENTLY DISCOVERED MYOTROPIC PEPTIDES OF THE LOCUST, *LOCUSTA MIGRATORIA MIGRATORIOIDES R. & F.* *

by

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A few years ago, 44 peptides were isolated from an extract of 10,000 brain-cc-complexes of the locust, *Locusta migratoria*. They all influenced the contractility-pattern of the cockroach hindgut (*Leucophaea maderae*) (1). Other functions remained to be investigated.

In order to obtain a reasonable amount of material, a number of the identified peptides were synthesized (Texas and Leuven ; Merrifield's method, (2)). After synthesis, decoupling and deprotection, they all were purified on Seppak-columns (C_{18}) and by RP-HPLC (μ-Bondapak phenyl).

The first 11 peptides so obtained (of which 10 stimulated and 1 inhibited the myogenic contractions) belong to 4 different famillies, according to their sequence similarity. Some of them resemble peptides of vertebrates (such as CCK, gastrin). After determining and comparing the thresholds of the natural and synthesized products on the cockroach hindgut, the effect of the peptides was studied on the locust itself.

For this purpose, the foregut of the locust was suspended in a chamber containing approximately 2 ml bioassay saline. Contractions were recorded by a transducer-recording system. Effects on other visceral muscles, such as the oviduct, were investigated in the same way.

Most of the peptides had a stimulating effect not only on the cockroach hindgut, but also on the locust foregut and oviduct. Other possible biological activities of the locust neuropeptides are being investigated, including their influence on diuresis and on secretion of digestic enzymes. Other bioassays are in preparation. In order to localize the peptides an attempt is being made to prepare homologous antibodies.

Immunocytochemical and chromatographic findings suggest that not only the central nervous system but also the intestinal tract contains peptidergic material. An attempt is being made to isolate the peptides of the Malpighian tubules, in order to compare them with those found in the brain.

* Supported by IWONL grant.

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(2) DRYLAND and SHEPPARD (1986), *J. Chem. soc. Perkin.*, Trans.: 125-137.

PRELIMINARY DATA ON LIPID REQUIREMENTS OF *CLARIAS GARIEPINUS* LARVAE

by

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Experimental feeds, with *Candida utilis* as main ingredient, were formulated in order to study lipid requirements of *Clarias gariepinus* larvae. Feeds with lipid contents ranging from 1.4 % to 23 % were given to *Clarias gariepinus*, which were reared up to a mean weight of approximately 180 mg (80-fold weight increase). It was found that all fish grew well on the experimental diets. Fish receiving the diet with the highest lipid content (23 %) reached the lowest weight (145 mg) after 12 days, while no significant differences in final weight or growth rate were found between fish fed diets with lipid contents ranging from 1.4 % to 19.4 % (average weight 179 mg). In all groups survival rate was above 90 % after 12 days. A positive relationship between the lipid content of the diet and the lipid content of the fish was found.

Lipid free diets based on casein were not digestible by *Clarias gariepinus* larvae. Casein diets to which mixtures of soybean oil, linseed oil and cocos fat were added, in order to obtain different linolenic acid : stearic acid ratios, gave low growth rates (increase of 2.5 mg to 6 mg in 8 days) and high cannibalism rates. When these diets were fed to fish above 10 mg, the mean weight of all fish larvae increased to approximately 30 mg in 20 days, and no significant differences were found between the treatment groups. The fatty acid pattern of these feeds was well reflected in the fish body. Increasing linolenic acid levels in the diets from 2.5 % to 27.2 % (in % of total lipids) resulted in linolenic acid levels in the fish body increasing from 1.10 % to 10.46 % (in % of total lipids). Although the level of (*n*-3) product fatty acids in the diets was equal to zero, the amount of (*n*-3) product fatty acids in the fish body remained almost constant (6.4 % of the total lipids).

Based on the growth data and the fatty acid patterns in the fish body, it can be assumed that *Clarias gariepinus* has very low linolenic acid requirements. Incorporation of 0.3 % linolenic acid in the diet should be sufficient to meet the linolenic acid requirement of *Clarias gariepinus* larvae.

THE FLEA BEETLES (COLEOPTERA : CHRYSOMELIDAE : ALTICINAE) OF A DUNE WOOD VEGETATION

by

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A list of the Alticinae (Coleoptera : Chrysomelidae) caught in a dune-wood vegetation in France (« Dunes de la Slack » between Wimereux and Ambleteuse) is given. The Alticinae were collected using a sweepnet and pitfalls. As a result of this sampling 79 Alticinae were caught belonging to 13 different species (of which 6 species belong to one genus). The interesting species are discussed and characterized, with special attention for *Longitarsus jacobaeae* (WATERHOUSE, 1858), a species used for biological control of tansy ragwort (*Senecio jacobaea* L.) in the U.S.A. Figures of the aedeagus and the spermatheca of two *Aphthona* species are given. For each species a list of the host plants is added.

BIOCHEMICAL GENETICS OF VALVE SNAPPING OF THE SCALLOP *PLACOPECTEN MAGELLANICUS*

by

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The correlation between phenotype (such as growth rate, viability and metabolic rate) and multiple-locus heterozygosity has been tested in a large number of bivalves. Several correlations support the genetic model of heterosis, but an equally large number of studies remain inconclusive. The superior growth of heterozygous bivalves has been related to their conservative growth metabolism, more precisely to the greater efficiency of protein synthesis. The absence of such correlation between growth and heterozygosity in pectinids suggests the importance of other processes than growth metabolism in influencing fitness.

We studied in the laboratory the swimming metabolism and genotype of 13 month old scallops (*Placopecten magellanicus* (GMELIN)). One hundred and twenty three scallops were induced to snap their valves by injecting starfish extract. Oxygen uptake was measured before and after the stimulation. The accumulation of the end metabolite octopine and the enzyme Odh (which catalyses the production and breakdown of octopine) were measured. Degree of heterozygosity was not correlated with size, oxygen uptake, swimming activity and Odh enzyme activity, but correlated with octopine content in the adductor muscle. Based on this information and the literature we conclude that traits related to activity are correlated with

the degree of multiple-locus heterozygosity in motile molluscs (such as *Placopecten magellanicus* and *Pecten maximus*), while traits related to growth metabolism are correlated with heterozygosity in sedentary molluscs (such as *Mytilus edulis, Mulinia lateralis* and *Crassostrea virginica*).

THE ANTENNAE DIMORPHISM IN *PHORACANTHA SEMIPUNCTATA* (COLEOPTERA : CERAMBYCIDAE) *

by

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In this work the antennae and their sensorial receptors are compared in the male and the female of the Eucalyptus pest *Phoracantha semipunctata.* It is intended to yield the morphological basis on which future electrophysiological and ethological studies will be grounded and it is part of a program of integrated pest management.

Measurements on the antennae are made with the help of a biometer WILD MMS 235. The sensilla are observed and counted from SEM micrographs taken with a ISI DS 130 electron microscope. TEM observations have also been made.

The antennae of the male are significantly longer than those of the female. This sexual dimorphism is more accentuated in larger individuals and for the terminal articles of the antennae.

We have observed 10 different types of sensilla belonging to 2 main groups : (1) The articulated sensilla group comprises 5 types of sensilla chaetica which are presumably mechanoreceptors and 1 type of s. trichodea with probable tactile and gustative functions ; (2) The nonarticulated sensilla group comprises 4 types of most likely olfactive sensilla basiconica.

The mechanoreceptor sensilla are definitely more numerous in the male ($\sim$ 44,000) than in the female ($\sim$ 27,000) but the sensillar density is equal in both sexes ($\sim$ 600/mm^2).

On the contrary, olfactive sensilla are equally numerous in both sexes ($\sim$ 12,000) but their density is less in the male ($\sim$ 160/mm^2) compared to the female ($\sim$ 290/mm^2).

The lengthening of the antennae of the male seems thus not to be correlated with a conspicuous development of the olfactory battery. It only allows the male to explore by touch a wider area of its near environment.

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ABSTRACTS

COMPARATIVE CYTOLOGY

Organized in memoriam of Prof. Dr. J. NAISSE (U.L.B.)
and Prof. Dr. A. BAUCHAU (F.U.N.D.P.)
(Facultés Universitaires Notre-Dame de la Paix,
Namur, 16 December 1989)

HOMMAGE A MADEMOISELLE NAISSE *

Si je prends la parole aujourd'hui, c'est avant tout pour *remercier* Jacqueline Naisse de tout ce qu'elle nous a apporté, à nous ses élèves — étudiants, mémorants, doctorants. Ils furent nombreux en effet, tous ceux qui comme moi, ont eu la chance de la connaître, de la cotoyer, voire même de travailler avec elle. L'influence et le rôle déterminant qu'elle exerça tant sur notre formation scientifique que sur notre mode de pensée, de raisonnement sont indéniables et laisseront une empreinte ineffaçable.

Sa principale qualité était, je crois sa *disponibilité*. Toujours affable, prête à rendre service, elle était avant tout à *l'écoute* des autres, des jeunes en particulier. On ne frappait jamais en vain à la porte de son bureau : celui-ci était d'ailleurs toujours ouvert. Nous avons encore tous en souvenir le défilé perpétuel qu'il y avait dans son bureau. C'était vrai à Bruxelles, mais aussi ici à Namur.

Ce que nous recherchions auprès d'elle, c'était bien sûr ses conseils avisés (en effet son érudition scientifique et sa culture générale étaient très vastes), mais c'était surtout un *réconfort*.

Elle avait avant tout à cœur d'encourager et de valoriser le travail, surtout celui des débutants.

Elle savait, pour parler simplement : « recharger les accus ». Son enthousiasme scientifique était à toute épreuve et elle savait faire passer le message.

Elève de Monsieur P. Brien à qui elle vouait une admiration sans borne et qu'elle considérait comme son « maître à penser scientifique », elle s'est dévouée toute sa vie à la Biologie au sens étymologique du mot : bios logos : étude de la vie. Elle était curieuse de tout, toujours prête à apprendre et surtout prête à apprendre aux autres à apprendre...

Elle avait, je crois, conservé jusqu'au bout cette capacité d'*émerveillement* à l'observation des choses de la vie : une antenne de papillon observée au microscope, une blatte qui traversait la pièce pendant la conversation la mettaient de bonne humeur (nous connaissions tous

*Voir aussi le texte de J. DELIGNE, *Annls Soc. r. zool. Belg.*, 1988(2), 128-129.

son amour des insectes) et lui faisait oublier tous ses soucis. Car comme chacun d'entre nous, elle avait aussi ses propres soucis tant personnels (sa santé était chancelante) que professionnels (en effet, à force d'être toujours disponible pour les autres, elle avait quelque peu négligé sa carrière). Mais je crois, qu'elle n'aurait pour rien au monde voulu changer sa vie.

Sa recherche occupait ses pensées jour et nuit (elle dormait très peu d'ailleurs). Combien de fois ne m'a-t-elle pas dit : « on entre *en recherche scientifique* comme on entre *en religion*, c'est une *vocation*, il faut avoir le *feu sacré*, ne jamais le laisser s'éteindre et cela doit mobiliser toute votre énergie ».

Aussi ne voyait-elle pas toujours d'un très bon œil survenir chez ses doctorants (et je parle ici d'expérience personnelle) un évènement tel qu'une grossesse ou un accouchement qui ne pouvait immanquablement, à ses yeux, que pertuber le bon déroulement de la recherche. Il fallait pour regagner son estime et sa confiance lui prouver que de tels incidents n'étaient qu'occasionnels et n'influenceraient en rien ni notre travail ni notre capacité à raisonner.

Une fois que l'on s'était « *engagé en recherche* » il fallait « *prendre ses responsabilités* » et poursuivre : c'étaient des locutions qu'elle affectionnait tout particulièrement.

Il est certain qu'elle a su transmettre à tous ceux qui ont travaillé avec elle, l'amour de la recherche, la curiosité scientifique et surtout une grande rigueur scientifique, ce dont nous lui serons toujours reconnaissants.

Mais si elle était, à juste titre, intransigeante quant au bon déroulement des expériences scientifiques, elle est toujours restée *profondément humaine*, soucieuse et attentive aux problèmes personnels de ses collaborateurs tant techniques que scientifiques.

Elle était aussi profondément *respectueuse des opinions d'autrui* et s'il était impossible, ou du moins très difficile, de lui faire changer ses propres opinions tant philosophiques que scientifiques, elle était cependant toujours *ouverte au dialogue*. C'est pour cette raison qu'elle s'entendait si bien avec le Père Bauchau avec qui elle partageait la même curiosité scientifique, le même goût de la recherche, de la culture en général et aussi... du tabagisme.

C'est par estime pour lui, qu'elle a tenu, à la mort de ce dernier, à assurer le cours d'Endocrinologie comparée des invertébrés à Namur, et ce malgré une santé déjà chancelante et de nombreuses autres charges professionnelles.

Il y aura bientôt deux ans que Jacqueline Naisse nous a quittés brusquement et discrètement, comme elle l'avait souhaité. Elle craignait en effet par dessus tout de vieillir, non pas par crainte de problèmes physiques (nous savons tous qu'elle se souciait fort peu de son aspect extérieur et de sa santé) mais surtout par crainte de voir ses facultés intellectuelles diminuer ainsi que de ne plus pouvoir accéder — la retraite venue — au laboratoire qui était toute sa vie. Le mot « retraite » en lui-même lui paraissait inconcevable.

Si Jacqueline Naisse était fière d'être l'élève de Monsieur Paul Brien, je suis personnellement très fière d'avoir été la sienne et le plus bel hommage que je puisse lui rendre est de continuer à respecter et appliquer ses enseignements.

Pour moi, elle restera toujours Mademoiselle Naisse. C'était une grande Dame.

M. LECLERCQ-SMEKENS

HOMMAGE AU PROFESSEUR ADRIEN BAUCHAU *

Voici deux ans que le Professeur Adrien Bauchau nous a quittés à la veille de son septantième anniversaire. Emérite depuis 1983, il éprouvait le plaisir de consacrer la majeure partie de son temps à la recherche scientifique et c'est un mois après son retour d'un voyage d'étude en Polynésie française qu'il est mort. Jusqu'au bout, il a gardé cette espérance de mener à leur terme les recherches qu'il venait d'effectuer sur le terrain. La veille de sa mort, il corrigeait encore les épreuves d'un article paru depuis dans la revue « Indo Malayan Zoology ».

D'origine anversoise, il avait en tout cas gardé un esprit quelque peu frondeur. Il entre dans la Compagnie de Jésus en 1937 et dix ans plus tard, il présente sa thèse de doctorat en Sciences. Son travail réalisé sous la direction du Professeur Koch à Leuven, concerne des recherches sur le rôle de la glande sinusaire chez le crabe chinois *Eriocheir sinensis*. En 1953, il séjourne à Boston aux Etats-Unis où il participe avec le Dr. Dorothy Bliss aux premières découvertes concernant le contrôle neurohormonal de la croissance des Crustacés. A partir de 1954, il sera essentiellement rattaché aux F.U.N.D.P. où il assure avec brio l'enseignement de la Biologie animale et de l'Endocrinologie comparée tout en continuant des travaux en relation avec la Physiologie de la croissance des Crustacés.

In 1966 publiceert hij een boek over over het leven van de Krabben. Hij interesseert zich onder andere aan hun voedingsbedrag en aan het controleren van hun bloedsuikerspiegel.

Vanaf 1973 gaat zijn belangstelling uit naar de bloedcellen van de schaaldieren. Later benadert hij het probleem van de biochemische mechanismen betreffende de hormonale controle en rond 1980 onderzoekt hij de chemoreceptie door een studie van het reproduktiegedragspatroon en de ultrastructuur van de sensorische borstels bij de schaaldieren.

Drie jaren later, vrij van alle academische en administratieve opdrachten, wijdt hij zich volledig aan het onderzoek. Gedurende de zomers 1984 en 1985 verblijft hij in het King Leopold III Biological Station van de ULB-VUB in New Guinea-Papouasia. Hij bestudeert er voor twee soorten krabben *Hippa* en *Scopimera,* de adaptatie aan het substraat waarop ze leven. In de zomer van 1987, enkele weken voor zijn dood, was hij in Tahiti-Moorea om de aanwezigheid van bepaalde krabben die in Papouasia waren ontdekt, te verifiëren.

Zijn wetenschappelijke activiteit was voor een groot deel de bron van de overdenkingen die de vorm hebben aangenomen van lezingen en publicaties. Adrien Bauchau heeft altijd de evolutie van de verschillende levensfilosofiëen ter harte genomen en er zich in gemengd met zeer persoonlijke bijdragen.

Cet homme, ce Biologiste qui a marqué plus d'un entre nous était plein de respect et d'amabilité pour ceux et celles qu'il côtoyait. Il orientait naturellement la relation vers plus de liberté, scandant ses dires d'un grand éclat de rire. Le Département de Biologie de Namur a, déjà de son vivant, souhaité créer un Prix Adrien Bauchau destiné à récompenser un licencié de Namur pour son mémoire de fin d'études. C'est maintenant chose faite et j'ai le plaisir d'annoncer qu'il sera attribué pour la première fois en 1990.

P. DEVOS

* Voir aussi le texte de P. DEVOS, *Annls. Soc. r. zool. Belg.*, 1988(2), 125-127.

THE PRESENCE OF « ADIPOKINETIC-LIKE » HORMONES IN SOME INSECT SPECIES

by

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The adipokinetic hormone is a peptide which elevates the lipid concentration in the haemolymph of locusts. This peptide belongs to a group of peptides (the AKH/RPCH peptides family) in which the primary structure is very much alike. Antisera directed against two AKH-like hormones (Cam-HrTH-II and Lom-AKH-I) were used in a comparative immunohistological study on several insect species : *Carausius morosus, Extatosoma tiaratum, Sipyloidea sipylus* (all Phasmidae) and *Sarcophaga bullata* (a grey fleshfly). In *Carausius* and *Extatosoma* only the Cam-Hr-TH-II peptide could be demonstrated in the glandular region of the corpora cardiaca. No other reaction was found in neurons in other regions of the central nervous system. In *Sipyloidea,* however, besides Cam-HrTH-II in the glandular region of the corpora cardiaca, a second peptide, which resembles more Lom-AKH-I, was located in neurons in the pars intercerebralis of the central nervous system. In *Sarcophaga* an even more complicated reaction pattern was observed. Neurons in the subœsophageal ganglion and the tritocerebrum seem to be more Cam-HrTH-II-like, while some neurons in the pars lateralis seem to be more Lom-AKH-I-like. A third AKH-like peptide, which is different from Cam-HrTH-II and Lom-AKH-I is present in the corpora cardiaca and the pars intercerebralis. This molecule might be similar to DCC-II, a AKH-like peptide isolated from different horsefly species.

CYTOLOGIE COMPARÉE DE L'ÉPITHÉLIUM BRANCHIAL DE TROIS CRABES (*Cancer pagurus, Carcinus maenas* et *Eriocheir sinensis*) EN RELATION AVEC LEUR APTITUDE IONOREGULATRICE

par

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Cancer pagurus est un crabe sténohalin exclusivement marin et non osmorégulateur. Son épithélium branchial est uniformément mince (2 μm), de type pavimenteux simple et à fonction uniquement respiratoire.

Carcinus maenas compte parmi les quelques espèces de décapodes marins modérément euryhalins. Habitant la zone des marées et les eaux saumâtres de salinité variable, il est capable d'hyperréguler son hémolymphe en eau de mer diluée jusqu'à 70 %. Ne pouvant survivre dans des milieux dont la salinité est inférieure à 25 % de celle de l'eau de mer, ses capacités osmorégulatrices paraissent donc limitées. Il est généralement considéré comme un représentant typique des osmorégulateurs faibles. Son épithélium branchial présente deux aspects distincts.

Les 6 paires de branchies antérieures sont limitées par un épithélium mince semblable à celui de *C. pagurus*. Par contre, les trois paires de branchies postérieures présentent des petites zones spécialisées, couvertes d'un épithélium plus épais (10 µm), de type prismatique et dont l'étendue ne dépasse jamais 30 % de la surface totale des lamelles. Les caractéristiques ultrastructurales de cet épithélium rappellent fortement celles des épithelia transporteurs d'ions : nombreux replis membranaires baso-latéraux et cytoplasme riche en mitochondries. En milieu dilué (eau de mer 30 %), il subit des modifications ultrastructurales qui suggèrent la participation de systèmes de transport ionique : apparition de replis membranaires apicaux, allongement des replis basaux dont la membrane s'associe étroitement à celle des mitochondries.

Eriocheir sinensis est un crabe migrateur dont le cycle de vie se divise en une phase de croissance en eau douce et une phase de reproduction en eau de mer ou en eau saumâtre. Parfaitement euryhalin, il est classé dans la catégorie des osmorégulateurs puissants. Ses 5 paires de branchies antérieures sont identiques à celles de *C. pagurus* et de *C. maenas*. Par contre, l'épithélium prismatique transporteur des branchies postérieures apparaît bien plus développé que celui de *C. maenas*, à la fois par son étendue, jusqu'à 70 % de la surface des lamelles, et par son ultrastructure. En eau douce, les replis membranaires apicaux sont plus abondants et plus longs que chez *C. maenas ;* la face interne des membranes porte de petites structures ampouliformes connues sous le nom de « portasomes ». Elles seraient impliquées dans le transport ionique au niveau apical.

En conclusion, le développement graduel d'un épithélium transporteur d'ions au niveau des branchies postérieures illustre bien les différentes performances osmorégulatrices de ces trois crabes. Ce développement semble s'exprimer aussi bien au niveau de la spécialisation des cellules épithéliales que de l'étendue de l'assise qu'elles constituent.

LECTIN HISTOCHEMISTRY OF GLUCOCONJUGATES IN SECRETORY CELLS OF FISH EPIDERMIS

by

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A lectin histochemical study was performed on paraffin embedded sections of the skin from ostariophysans to gain insight into the carbohydrate residues and oligosaccharide sequences of mucous and club cells. Thirteen biotinylated lectins (Con-A, PSA, LCA, UEA-I, DBA, SBA, SJA, RCA-I, BSL-I, WGA, *s*-WGA, PhA-E and PHA-L) were used as probes and avidin-biotin-peroxidase complex (ABC) was used to visualize the binding sites of lectins. These selectively localized the carbohydrate residues in mucous and alarm substance cells (club cells). Oligosaccharides with « identical » biochemically defined sugar compositions can be distinguished. Marked differences between the five examined species were detected. The known saccharide specificities and binding patterns of the lectins employed in the study allowed some conclusions about the nature and the distribution of the sugar residues in the oligosaccharide chains of glycoconjugates in epidermal unicellular glands. The existence of differences, even among the same zoological group, emphasizes the need for careful com-

parisons and the dangers of hasty generalization. An attempt to explain the above variations deserves further work.

Few quantitative data exist which compare the mucous cells of control and acid- or alkaline-stressed fish. Moreover, few studies using lectins have been conducted in fishes exposed to a deleterious environment. Lectin histochemistry allows us to detect precise modifications during the exposure to acid and alkaline environments. Our results demonstrate that the three species studied (*Brachydanio rerio, Xiphophorus helleri, Kryptopterus bicirrhis*) elicit different patterns of carbohydrate residues synthesis when exposed to pH stress.

In conclusion, lectin histochemistry might prove to be a valuable tool for the assessment of sugar residues in ectothermic vertebrates.

ÉTUDE MORPHOLOGIQUE COMPARATIVE DE LA PROLIFÉRATION ET DE LA DIFFÉRENCIATION DE KÉRATINOCYTES HUMAINS CULTIVÉS SUR DIVERS SUBSTRATS

par

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Ce travail est une contribution à l'étude de la prolifération et de la différenciation des kératinocytes humains *in vitro*. Dans ce but, des kératinocytes ont été ensemencés sur des substrats de complexité croissante afin de déterminer celui qui favoriserait le mieux leur développement : des gels de collagène (collagène de type I), des dermes reconstruits artificiellement ou dermes équivalents (collagène de type I + fibroblastes) et des conjonctifs naturels recouverts d'une membrane basale (amnios désépithélialisé).

Il ressort de cette étude que la *prolifération* est la plus rapide sur l'amnios et que c'est sur les dermes équivalents et les gels que la *stratification* est la plus conforme à celle observée *in vivo*. Par contre, c'est uniquement sur gels que les *relations intercellulaires* sont les plus développées. Quant à la *maturation kératosique*, elle démarre le plus vite sur les amnios mais c'est sur les dermes équivalents que son évolution est la plus conforme à celle observée *in situ*, c'est-à-dire progressive et en relation avec la stratification. Dans les amnios, les phénomènes de dyskératose sont beaucoup plus fréquents que sur les autres supports. Quel que soit le substrat utilisé, la maturation kératosique est en général incomplète : il n'y a jamais formation d'une véritable couche cornée continue.

LES GLANDES EXOCRINES DES GENITALIA DES FEMELLES DE LA COCCINELLE *Adalia bipunctata* (L.) (*Col., Coccinellidae*)

par

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Les genitalia des femelles d'*Adalia bipunctata* sont constitués de deux coxites et de deux pleurites dérivant du neuvième segment abdominal ainsi que d'un élément provenant du dixième tergite.

Cette communication décrit la morphologie des coxites qui sont situés de part et d'autre du vagin. Ils se caractérisent par une partie postérieure plane, peu épaisse, approximativement ovale qui se prolonge vers l'avant et vers le haut par une structure ressemblant à une gouttière dont la concavité est orientée vers le vagin. Une membrane chitineuse prolonge les lèvres de la gouttière de façon à entourer son côté convexe. Cette structure détermine le sac coxal qui est muni d'une ouverture au niveau de la partie plane du coxite. La face convexe de la gouttière est tapissée par un épithélium glandulaire dont chaque cellule est organisée autour d'un réservoir extracellulaire oblong, contenant une pelote de filaments enchevêtrés. Ceux-ci convergent vers un disque criblé, zone poreuse peu épaisse que traverse la sécrétion cellulaire pour gagner le sac coxal.

Les cellules glandulaires possèdent un réticulum endoplasmique lisse très développé. L'importance de cette organelle ainsi que la réaction positive de la sécrétion cellulaire au Noir Soudan indiquent que les cellules exocrines des coxites produisent, soit un hydrocarbure, soit une substance lipidique.

L'activité des cellules coxales est synchronisée avec la maturation des ovaires, c'est-à-dire que l'excrétion débute quand les premiers oocytes effectuent leur vitellogénèse et qu'elle se prolonge durant toute la période d'activité ovarienne. Sur base d'informations écologiques et de quelques expériences portant sur le comportement sexuel d'*A. bipunctata*, je propose que les glandes coxales produisent, soit une substance défensive protégeant les œufs contre les prédateurs, soit une phéromone sexuelle.

LES CORPS BRUNS COELOMIQUES CHEZ *Holothuria tubulosa* : MORPHOLOGIE ÉVOLUTIVE DES CORPS BRUNS INDUITS EXPÉRIMENTALEMENT

par

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Le coelome des holothuries abrite fréquemment des masses ovoïdes, connues sous le nom de corps bruns (CB). Les CB peuvent atteindre plusieurs mm de diamètre ; ils sont constitués par l'agrégation de coelomocytes associés ou non à des formations parasitaires et/ou des particules indésirables d'origine exogène.

Par injections intracoelomiques de suspensions de carmin (particules de forme tétraédrique caractéristique), nous avons induit chez *H. tubulosa* la formation de CB artificiels et suivi cinétiquement (O-168*h*) leur évolution en microscopie électronique à transmission (MET). Peu après l'injection (~ 90 min) des amas coelomocytaires rouges (CB induits) s'observent dans le coelome. Ces CB se composent de trois sous-unités structurales qui sont : des amibocytes chargés de particules de carmin (phagocytes), de larges plages de carmin entourées chacune par plusieurs amibocytes (encapsulation), et des sphérulocytes. Chacune des entités précitées évoluera séparément au cours du temps, entraînant *ipso facto* une évolution de l'aspect du CB.

De lâches, les CB deviennent de plus en plus compacts pour acquérir leur densité maximale après 3 jours. Cette densification résulte à la fois de l'intensification de l'enchevêtrement des pseudopodes amibocytaires et de l'apparition d'un liant extracellulaire.

Les divers phagosomes d'un même amibocyte vont progressivement fusionner en un ou deux phagosomes de grande taille. Au sein de ce dernier les particules de carmin sont compactées puis désagrégées en un matériel homogène et finement granuleux. Ce matériel est ensuite rassemblé en petits nodules entourés chacun d'une paroi peu dense aux électrons. Ces nodules se transforment enfin (entre le 3e et le 7e jour) en corps résiduels.

Les plages de carmin encapsulées évoluent comme les amas de carmin des grands phagosomes pour former (après 5 à 7 jours) des corps résiduels semblables aux précédents, mais de situation extracellulaire.

Les sphérulocytes libèrent leur contenu au sein des CB. Certains d'entre eux seraient à l'origine du liant extracellulaire ; d'autres pourraient intervenir dans la dégradation des éléments retenus dans les capsules (formations parasitaires, particules exogènes).

Après 7 jours, les CB induits ont achevé leur évolution et sont en tous points semblables aux CB naturels présents dans le coelome des *H. tubulosa* témoins. Ces observations indiquent que les CB des holothuries sont des sites actifs de dégradation des éléments indésirables présents dans le coelome.

CHONDROID BONE IN CICHLID FISH : ACTUAL STATE OF KNOWLEDGE

by

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Chondroid bone (CB) is a particular kind of mineralized skeletal tissue which supports articular facets in the head of cichlid fish.

CB on the upper pharyngeal jaw/neurocranial joint develops when osteoblasts which first deposit acellular bone lamellae become entrapped. Mature chondroid bone consists of large haphazardly dispersed chondrocyte-like cells embedded in a mineralized matrix. The latter histochemically resembles bone matrix and lacks demonstrable cartilage-specific type II collagen. Recent transmission electron microscopical observations confirm the assumed formation of CB by osteoblasts at its distal border and its breakdown by true multinuclear osteoclasts at its proximal border. The ultrastructural observations also confirm at the same time the chondrocytic phenotype of the CB cells and the bone-like nature of the matrix.

Entrapping of cells as in CB instead of their withdrawing as in normal acellular bone is seen as a mechanism to achieve a faster growth rate. There is no explanation yet as to how and why entrapped cells acquire the chondrocytic phenotype.

ÉTUDE COMPARATIVE DE LA CROISSANCE OVOCYTAIRE CHEZ LES POISSONS À OVOGENÈSE SYNCHRONE ET ASYNCHRONE

par

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Chez les Poissons, trois modèles de base du développement de l'ovaire ont été déterminés en se basant sur une approche cytologique et dynamique de l'ovogenèse : les ovaires synchrones, groupe-synchrones et asynchrones.

Dans le premier groupe se trouvent les poissons à ponte unique, comme les salmonidés, les percidés et certains cyprinidés tels que le hotu *Chondrostoma nasus*, le gardon *Rutilus rutilus* ou la vandoise *Leuciscus leuciscus*. Chez ces poissons, on ne trouve dans l'ovaire que des ovocytes protoplasmiques en début de recrudescence gonadale. De ces ovocytes va se détacher une seule cohorte de cellules qui entameront une phase de croissance trophoplasmique pour atteindre le stade vitellogénique en période de reproduction. Sur un plan quantitatif, on ne retrouve également que deux modes dans l'histogramme de répartition des tailles d'ovocytes.

Le second groupe, c-à-d les poissons à ovogenèse groupe-synchrone, se caractérise par la présence simultanée d'au moins deux populations d'ovocytes bien distinctes. C'est le cas du barbeau *Barbus barbus* dont l'histogramme de répartition des tailles d'ovocytes présente trois

modes caractéristiques. Une classe omniprésente constituée par les ovocytes de petite taille, une classe d'ovocytes en croissance trophoplasmique avancée mais qui ne participeront pas à la ponte de l'année, et enfin une classe d'ovocytes mûrs qui seront pondus très prochainement.

Enfin, le troisième groupe comprenant les poissons à ovogenèse asynchrone se caractérise extérieurement par la multiplicité des pontes au cours d'une même saison de reproduction et pour une même femelle. Tous les stades ovocytaires sont présents sans prédominance d'une classe particulière. Chez le goujon *Gobio gobio*, la classe d'ovocytes de réserve constituée par les ovocytes protoplasmiques est toutefois très abondamment représentée alors qu'on observe un étalement de la fréquence des autres classes. Cette prédominance marquée des ovocytes protoplasmiques est associée à une absence aussi marquée d'ovogonies, laissant supposer que le stock d'ovocytes de réserve est constitué très tôt et semble suffire pour plusieurs années sans qu'il y ait transformation continue des ovogonies en ovocytes.

L'appartenance à l'un ou l'autre groupe n'est cependant pas définitive et une espèce placée dans des conditions particulières (température, photopériode, abondance de nourriture) peut adopter une stratégie de reproduction différente, avec recrutement continu d'une classe d'ovocytes vers la suivante et présence de pontes répétées.

MORPHOLOGICAL AND BIOCHEMICAL FEATURES OF THE DIGESTIVE GLAND CELLS OF THE CRAB *Carcinus maenas* (CRUSTACEA, DECAPODA) *

by

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In order to elucidate the mechanisms involved in the control of the capacity of glucose storage for the Decapod digestive gland (hepatopancreas), we developed a method providing viable cell suspensions from this organ. The separation of their different cell types was performed on a 45 % preformed Percoll gradient. Beside the four types described by Jacobs (*i.e.* E, R, F and B cells (1)), we observed another having a multivesicular appearance. These cells which probably form a subpopulation of R cells enriched in calcium phophate spherules, were called R*. They are found abundantly in the pyloric stomach when the glycogen content of the digestive gland is high (January). This observation led us to attribute to them either the function of a glucose source for the hemolymph through the midgut epithelium during non-feeding periods (winter) or an excretory role because of the calcium phophate spherules that accumulate during intermolt period (also in winter).

The fact that the number of R* cells in the gastric juice seemed seasonally related to the glycogen level of the gland, suggested a seasonal control of the cell differenciation in this

* These results were partly presented at the « Journées du GABIM », Bordeaux (Oct. 88) and Nantes (Nov. 89).

(1) JACOBS W. (1928), *Z. Zellforsch.*, **8** : 1-62.

tissue. This hypothesis was verified by comparing the cell proportions of two batches of digestive glands characterized by significantly different levels of glycogen. For this purpose, the proportion of each cell fraction obtained from the Percoll gradient was estimated by determining its protein content, coupled to biochemical markers, which were glycogen for R cells, glycogen an Pi for R* cells and α-Amylase for B cells. Results seem to confirm that the seasonal fluctuations of the glycogen level in the digestive gland could result from a seasonal fluctuation in the amount of the cells involved in the storage of glycogen (R* cells).

TWO TYPES OF ENDOCRINE CELLS IN THE MIDGUT OF THE HONEYBEE

by

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Gut endocrine cells are widely distributed through the animal kingdom. Somatostatin reactive cells were localized in midgut tissue of the honeybee.

We found small endocrine-like cells dispersed between the columnar cells of all but the anterior fourth of the midgut. Like the columnar cells, they originate from the regenerative crypts. We were able to discern two different gut endocrine cell types : they are characterized by the presence of secretory granules, respectively vesicles, which are usually concentrated in the basal region of the cytoplasm.

Apart from the different morphology of their secretory product, which may correspond to a different type of peptide hormone, the ultrastructure and general appearance of the two cells is very similar. Both are relatively electrondense with a broad cell base and a tapered apex which opens into the lumen and is covered by a few relatively short microvilli.

In contrast to the columnar cells, the endocrine cells have no basal labyrinth ; the close contact between the secretion vesicles and the basal cell membrane suggests secretion towards the haemolymph by exocytosis.

In this poster a short description of the ultrastructure of both cell types is presented.

SUBCELLULAR EFFECTS OF MERCURY ON THE COLUMNAR CELLS OF THE HONEYBEE VENTRICULUM

by

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The increasing problem of environmental contamination with heavy metals has stimulated the search for suitable invertebrate biomonitors. For the system to work, the effects and the methods of detoxication and storage of each contaminant must be well known.

In invertebrates, toxic metals are either linked reversibly to specific proteins and further disposed of by the lysosomal system, or are combined irreversibly with an organic matrix by a process of biomineralisation.

By means of energy dispersive X-ray analysis, mercury could not be detected in the mineralised granules of honeybees after chronical peroral administration of $HgCl_2$.

In bees which were deprived of a protein source, this metal caused serious cellular damage in the midgut epithelium which was apparently unable to fulfil its barrier function.

In contrast to this, during the period of pollen nutrition, no cellular damage could be found. At the subcellular level, the columnar cells showed a picture which was highly suggestive of an increased protein production in the R.E.R. and the involvement of the lysosomal system in the disposal of the protein bound metal. Also striking was the apparent inhibition of spherocrystal formation.

OXIDATIVE STRESS AND BLEBBING PHENOMENA IN RELATION TO THE CYTOSKELETON

by

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It is known from previous studies that binding and internalization of LDL and of LDL-gold complexes are in endothelial cells submitted to an oxidative stress (1). Morphological changes accompany these biochemical modifications : blebbing of the cellular contour is the most frequently observed phenomenon. We performed the present study in order to check the integrity of the cytoskeleton in these injured cells. We used cultured human umbilical vein EC at confluence after the first passage. Following incubation (from 0 to 120 min) in a reactive oxygen species producing system (xanthine-xanthine oxidase), the cells were examined by

(1) POUMAY Y. and RONVEAUX-DUPAL M. F. (1988), *J. Cell. Physiol.*, **16**, 289-296.

phase microscopy, immunofluorescence staining and scanning and transmission electron microscopy.

Phase contrast microscopy revealed a progressive and reversible retraction of the cells concomitant with their rounding off and with the exhibition of many blebs at the cellular contour.

Immunofluorescence staining allowed us to visualise F-actine microfilaments (MF) using NBD-phallacidin while anti-αtubulin and anti-vimentin antibodies revealed microtubules (MT) and intermediate filaments (IF) respectively.

In EC incubated 60 min. in a xanthine-xanthine oxidase medium, we noted a severe decrease in the number of stress fibers and a progressive disappearance of the cortical network. After a 120 min. incubation actin appeared scattered in the rounded, injured cells. The fluorescence was particularly intense in the perinuclear region and under the internal border of numerous blebs.

The microtubules network seemed markedly depolymerised in the blebbed cells. Only a few microtubules radiated from the centrosomes while the fluorescence appeared intense in the perinuclear region and in the whole bleb.

The intermediate filaments network did not seem modified in the injured cells. Most of them displayed a loosely bundled arrangement of these filaments. Vimentin was apparently absent from the blebs.

Electron microscopy confirmed the rounding off, the retraction and the blebbing of the cells submitted to this oxidative treatment.

By using Scanning Electron Microscopy, the ampleness of the blebbing becomes particularly evident : numerous blebs, variable in size and able to fuse were clearly seen.

The electron microscopic observations allowed us to advance a possible mechanism for bleb formation : it could be produced by a kind of strangulation due to rope-like microfilaments which aggregated at the basis of the blebs. Moreover, tubular or vesicular structures (V) could eventually induce the bleb detachement from the cell. Elsewhere, the microfilament network has nearly disappeared. Some blebs displayed few microfilaments, lining the internal face of their plasmamembrane. Microtubules and intermediate filaments seemed to be absent from the blebs.

In summary, both photonic and electron microscopic approaches confirm the rounding off, the retraction and the blebbing of injured cells. Immunofluorescence and ultrastructural analysis indicate an alteration of MF as associated with the blebbing phenomenon.

However, divergences persist between results concerning the microtubules and others concerning the intermediate filaments networks.

NEUROPEPTIDES AND NEUROTRANSMITTERS IN THE X-ORGAN SINUS GLAND COMPLEX, AN IMPORTANT NEUROENDOCRINE INTEGRATION CENTER IN THE EYESTALK OF CRUSTACEA

by

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The neuroendocrine system of Crustacea is complex and elements of it are found throughout the central nervous system. Neurons with a putative neuroendocrine function occur in all ganglia, but three regions show remarkable clustering of them. These are the optic ganglia which contain the x-organ sinus gland complex, the pericardial organ and the post-commissural organs. Until now, the x-organ sinus gland complex has received most attention. It represents the most important source of neuroendocrine products which are involved in the regulation of a variety of biological processes such as moulting, reproduction, pigment migration and carbohydrate metabolism. Immunocytochemical application of several antisera against biologically active peptides of vertebrates and invertebrates resulted in the detection of a more extensive peptidergic system than formerly recognized. The distribution of this peptidergic material reflects the versatility of the eyestalk complex for neuromodulatory as well as neurohormonal functions. This view is also supported by the occurrence of serotoninergic and dopaminergic cells in the same regions, illustrating their neurotransmittory functions. The organization of both systems postulates their importance for the regulation of synthesis, transport, storage and release of typical crustacean neuropeptides such as MIH, GIH, RPCH and CHH synthesized in the x-organ of the last optic ganglion and recently localized by immunocytochemistry.

The aforementioned relationship is demonstrated by studying the functional aspects of the hyperglycemic hormone-producing system in the eyestalk complex. The Crustacean Hyperglycemic Hormone (CHH) can be visualized in a group of neuroendocrine cells of the last optic ganglion, in fibers forming part of a tractus to the neurohemal region and in a considerable part of the axonterminals composing the sinus gland. Studies on the secretory dynamics of the CHH-system revealed a daily rhythmicity in the synthetic activity of the perikarya, in the transport of CHH-material and in the release of CHH into the hemolymph resulting in a 24-hours rhythm of the blood glucose level and the locomotory activity of the animal. Recent investigations indicate that the endogenous clock modulating this rhythmicity is located within the optic lobes. Informations about the external light/dark schedule have to be transmitted to the CHH-cell system. Detailed cytological and physiological experiments dealing with the neuronal imput on this system indicate that serotonine and, at a lower scale, also dopamine stimulate the CHH-cell system and that met-enkephalin inhibits the dynamics of the system. As methods such as pulse chase labeling of neuropeptide precursors and molecular biological methods using DNA and *m*RNA probes, are recently introduced in the study of crustacean neuroendocrinology, their results will contribute to elucidate the complexity of the neuroendocrine integration activities in the x-organ sinus gland complex.

SOURCE ÉNERGÉTIQUE POUR LE TRANSPORT D'IONS DANS LES BRANCHIES DU CRABE EURYHALIN *Eriocheir sinensis* ADAPTÉ À UNE FAIBLE SALINITÉ

par

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Les branchies postérieures (ayant un épithélium transporteur d'ions) et antérieures (ayant un épithélium respiratoire) du crabe *Eriocheir sinensis* adapté à l'eau douce contiennent respectivement 3,0 ± 1,1 % et 5,0 ± 0,9 % de glycogène (n = 12).

La majorité de ce glycogène ne se trouve pas dans les cellules épithéliales mais sous forme de particules alpha, dans des cellules formant une travée transépithéliale.

Les branchies de ces crabes ont été perfusées dans un milieu hypotonique avec un Ringer de 550 mosmoles.

Dans ces branchies, après perfusion avec de l'[U $^{-14}$C] glucose, on a estimé une vitesse maximale d'entrée de ce glucose dans les cellules plus importante dans les branchies postérieures : 0,04 nmoles/s/mg de protéines que dans les branchies antérieures : 0,03 nmoles/s/mg de protéines. Le pourcentage de radioactivité retrouvé dans le glycogène par rapport à celui retrouvé dans l'ensemble du tissu est sensiblement le même pour les branchies antérieures et postérieures : 8 ± 2 % (n = 12).

Si on perfuse ces branchies avec un Ringer dépourvu de glucose, on observe que les branchies postérieures et antérieures consomment respectivement 11,3 ± 4,0 et 3,1 ± 1,5 µg de glycogène par minute par g de tissu (n = 20).

Si les branchies de ces crabes sont incubées en milieu plus concentré (eau de mer) avec un Ringer de 1100 mosmoles (conditions dans lesquelles il a déjà été montré que les pompes ioniques voient leur activité diminuer) les branchies postérieures réduisent de moitié la consommation de glycogène tandis qu'aucune variation ne s'observe dans les branchies antérieures.

Ces observations sont compréhensibles du fait que l'activité de transport ionique est plus élevée dans les branchies postérieures que dans les antérieures ; les branchies postérieures consomment de ce fait plus d'énergie que les branchies antérieures.

L'isolement par élutriation de fractions enrichies en cellules de la travée (contenant la majorité du glycogène branchial) est en cours et servira à rechercher les différents stimuli (hormonaux,...) susceptibles de modifier la synthèse et la dégradation du glycogène.

KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE V.Z.W.

SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE A.S.B.L.

ERE-VOORZITTERS — PRÉSIDENTS D'HONNEUR

P^r M. POLL (U.L.B.) ; Prof. Dr. H. KOCH (K.U.L.).

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Dr. A. HUYSSEUNE (R.U.G.), D^r Th. HANCE (U.C.L.).

Nieuwe leden 1989 — Nouveaux membres 1989

Overeenkomstig art. 6 van de statuten werden volgende personen aanvaard als geassocieerde leden :

Selon l'article 6 des statuts, les personnes suivantes sont acceptées comme membres associés :

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M. BRUHWYLER Jacques (De Vos P. & Micha Cl.)
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de Hr. DE LEY Paul (Verraes W. & Geraert E.)
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de Hr. VOLCKAERT Filip (Billen J. & Fiers F.)
M. WIAUX Bernard (Deligne J. & Van Mol J.-J.)
Mej. ZWIJSEN An (Moens L. & De Vree F.)

RAAD VAN BEHEER — CONSEIL D'ADMINISTRATION VERGADERINGEN — RÉUNIONS

Er werden vijf vergaderingen gehouden : 21 januari, 12 april, 24 mei, 21 september en 13 december 1989.

Le conseil d'administration s'est réuni à cinq reprises, les 21 janvier, 12 avril, 24 mai, 21 septembre et 13 décembre 1989.

WETENSCHAPPELIJKE VERGADERINGEN — RÉUNIONS SCIENTIFIQUES

Trois réunions scientifiques ont été organisées en 1989, un colloque de malacologie à Bruxelles le 20 mai, la journée des jeunes organisée à Bruxelles le 28 novembre et enfin, la journée sur le thème de la biologie cellulaire à Namur le 16 décembre 1989.

Vergadering van 20 mei 1989 — Réunion du 20 mai 1989 Colloquium — Colloque Malacologie

La réunion, organisée en association avec la Société belge de Malacologie et la « Belgische Vereniging voor Conchyliologie », se tient à l'Institut royal des Sciences naturelles de Belgique.

Bienvenue par J. Van Goethem, président de la S.R.Z.B.

Verwelkoming door J. Van Goethem, voorzitter van de K.B.V.D.

Session — Zitting 1 : Président — Voorzitter Cl. Massin (I.R.S.N.B.)

A. V. DHONDT (K.B.I.N.) — La Société Malacologique de Belgique, 1863-1902.

Th. BACKELJAU (K.B.I.N.) — On the original diagnoses of *Arion simrothi* KUNKEL and *Arion magnus* TORRES-MINGUEZ (Gastropoda, Pulmonata).

Ph. BOUCHET (Muséum national d'Histoire naturelle, Paris). — La protoconque des Gastéropodes, marqueur biologique.

Zitting — Session 2 : Voorzitter — Président A. Delsaerdt (Voorzitter B.V.C.)

R. HOUART (S.B.M.), La protoconque des Muricidae et son importance pour la détermination des espèces.

W. SLEURS (K.B.I.N. & K.U.L.). — A biogeographical analysis of the rissoinid fauna of the eastern Pacific (Mollusca : Gastropoda).

A. Verhecken (B.V.C.). — De indol-pigmenten van de mollusken.

R. DUCHAMPS (Président S.B.M.). — Ph. Dautzenberg, son oeuvre, sa collection.

Zitting — Session 3 : Voorzitter — Président Annie Dhondt (K.B.I.N.)

M. POULICEK (U.Lg.). — Mécanismes de la biodégradation des coquilles de Mollusques dans les sédiments marins.

A. SIMON & M. POULICEK (U.Lg.). — Biodégradation anaérobique des coquilles de Mollusques : approche expérimentale.

Y. FINET (Muséum d'Histoire naturelle de Genève). — La faune malacologique des Iles Galapagos : composition faunistique et facteurs influençant les affinités biogéographiques.

Th. WHITEHEAD & K. LAMPRELL (Queensland Museum, Brisbane, Australia). — Research on Australian Bivalves.

Bezoek aan de invertebratenzaal : voorstelling van twee nieuwe toonkasten over Belgische mollusken en van twee nieuwe toonkasten over Ph. Dautzenberg (1849-1935).

Visite de la salle des Invertébrés : présentation de deux nouvelles vitrines sur les mollusques de Belgique et de deux nouvelles vitrines sur Ph. Dautzenberg (1849-1935).

Posters :

R. DUCHAMPS (Président S.B.M.) — Ph. Dautzenberg (1849-1935).

J. VAN GOETHEM (K.B.I.N.). — W. Adam (1909-1988).

A. SIMON, M. MULDERS, G. GOFFINET & M. POULICEK (U.Lg.). — Aspects ultrastructuraux de la biodégradation des trames organiques des coquilles de Mollusques.

W. H. DE SMET & E. A. VAN ROMPU (R.U.C.A.). — Thrush predation and shell polymorphism in *Cepaea nemoralis* (L.) (Helicidae) along the Belgian coastal dunes.

W. H. DE SMET & E. A. VAN ROMPU (R.U.C.A.). — Area effects and clines for shell polymorphism in *Cepaea nemoralis* (L.) (Helicidae) along the Belgian coastal dunes.

F. DELOOR, E. ROLAND & R. SHERIDAN (U.E.M.). — Relation entre la vitesse, la durée du déplacement et la morphologie pédieuse chez quelques Pulmonés Stylommatophores.

E. ROLAND, F. DELOOR & R. SHERIDAN (U.E.M.). — Mise en évidence du pouvoir attractant exercé par le globule muqueux d'*Arion distinctus*.

N. DELAUNOIS & R. SHERIDAN (U.E.M.). — Répartition des Turridae en baie de Morlaix.

L. HIERNAUX. — François-Xavier Roffiaen, membre fondateur et ancien président de la Société Malacologique de Belgique (1820-1898).

Vergadering van 28 october 1989 — Réunion du 28 octobre 1989
Dag der Jongeren — Journée des jeunes

De vergadering gaat door in het Koninklijk Belgisch Instituut voor Natuurwetenschappen.

Organisatie : A. Huysseune en M. Baguette.

BAILLIEUL, M. (R.U.C.A.). — Invloed van complexatie op de biologische beschikbaarheid van cadmium voor het pekelkreeftje *Artemia franciscana*.

MARNEFFE, Y. (U.Lg.). — Modalités et voies de transfert de micropolluants organochlorés (PCB) dans le plancton de l'écosystème mosan.

BAUWENS, J. (U.I.A.). — Invloed van een zuiveringsstation op de invertebraten-gemeenschappen van een Kempische laaglandbeek.

MULDERS, M. (U.Lg.). — Aspects ultrastructuraux de la biodégradation aérobie des trames organiques des coquilles de mollusques.

SIMON, A. (U.Lg.). — Biodégradation et préservation des vertèbres de poisson en milieu marin et en estuaire.

BOGAERTS, K. (S.C.K.). — De invloed van verzuring en aluminium op de fysiologie van de Amerikaanse dwergmeerval, *Ictalurus nebulosus* (Le Sueur).

BEULLENS, K. (K.U.L.). — De relatie tussen het genetisch geslacht en groottedimorfisme bij jonge Europese paling.

KEMPENAERS, B. (U.I.A.). — Competitie tussen de koolmees en de pimpelmees voor slaapplaatsen in de winter.

LENS, L. (U.I.A.). — Interspecific competition in group territorial tits : a study of individual niche shifts.

COLAZZO, S. (U.I.A.). — Beschrijving en funktie van de zang bij de huiskrekel *Acheta domesticus* (Orthoptera: Gryllidae).

CLAESSEN, C. (U.I.A.). — Systematics of the African bat genus Epomophorus (Mammalia : Megachiroptera).

BARBIER, Y. (F.S.A. Gx — U.E.M.). — Entomofaune comparée des terrils d'Hensies et St-Antoine.

MARÉCHAL, B. (U.C.L.). — Impact de l'acarien phytophage Tetranychus urticae sur les cultures de tomates en serre.

MOENS, N. (K.U.L. en L.U.C.). — Chemische en ultrastructurele ontogenie van de mandibulaire klier bij *Formica sanguinea* (Latr.).

JANSSENS DE BISTHOVEN, L. (K.U.L.). — Buccal deformities en Chironomus group thummi larvae (Diptera, Chironomidae) description and quantification.

HENDRICKX, K. (K.U.L.). — Werking van delta-endotoxines van *Bacillus thuringiensis* in de middendarm van Lepidoptera.

PLASMAN, N. (U.L.B.). — Relations entre sous-populations de macrophages et *Trypanosoma cruzi*.

LARDON, F. (S.C.K.). — Effect van 241-Americium op bloedvormende en stromale stamcellen na foetale en perinatale radioactieve besmetting.

Posters :

BOURDON, S. (V.U.B.). — Isometrie criteria.

BYAMUNGU, N., K. MOL & E. KUHN (K.U.L.). — Somatostatin increases plasma T3 concentrations and stimulates in vitro T4-5' deiosination in *Tilapia nilotica* in the presence of high T4 levels.

CLEUREN, J. & DE VREE, F. (U.I.A.). — The use of the tongue and hyoïd apparatus during feeding in *Caiman crocodilus*.

COLFS, C. (V.U.B.). — De invloed van veranderingen van sociale factoren en omgevingsfactoren in gevangenschap bij de tuimelaar, *Tursiops truncatus* (Montagu, 1821).

DAES, G., SCHOETERS, G. & VANDERBORGHT, O. (S.C.K.). — Mineralization of adult mouse bone narrow in vitro.

DE CLERCK, G. (R.U.G.). — Intraspecific variation of the mushroom coral Fungia (Fungia) fungites.

GOFFLOT, F., MAZY, C., VAN MAELE-FABRY, G. & Picard, J.J. (U.C.L.). — Mixtures of chemically defined medium and serum as culture media for 8.5 days mouse embryos.

PACOLET, W., PECTOR, R. & OLLEVIER F. (K.U.L.). — The effect of cattle blood in formulated starter diets on the growth and survival of European glasseels (*Anguilla anguilla* L.).

SMETS, H., NOTERDAEME L. & OLLEVIER F. (K.U.L.). — Serological identification of Aeromonas strains isolated from fish.

TIPS, A. (K.U.L.). — Search for the function(s) of recently discovered myotropic peptides of the locust, *Locusta migratoria migratorioides*.

R. & F. TISSON, F. VAN DAMME, P., OLLEVIER, F. & DE SCHRIJVER, R. (K.U.L.). — Linoleic acid requirement of the African catfish (*Clarias gariepinus Burchell*, 1822) larvae.

VALCKENBORGH, F. (V.U.B.). — Ekologische modellering : populatie dynamiek van levendbarende hagedis, *Lacerta vivipara Jacquin*.

VERDYCK, P. (R.U.C.A.). — The flea beetles (Coleoptera : Chrysomelidae : Alticinae) of a dune wood vegetation.

VOLCKAERT, F. (K.U.L.). — Biochemische genetika van het zwemmen van de Amerikaanse jakobsschelp *Plecopecten magellanicus*.

VOLCKAERT, F. & ZOUROS, E. (K.U.L.). — Biochemical genetics of valve snapping of the scallop *Placopecten magellanicus* (Gmelin).

WIAUX, B., J. DELIGNE & L. DE VOS (ULB). — Dimorphisme antennaire de *Phocarantha semipunctata* (Coleoptera Cerambycidae).

Vergadering van 16 december 1989 — Réunion du 16 décembre 1989
Vergelijkende Celleer — Cytologie comparée

La réunion est organisée aux Facultés Universitaires de Namur, à la mémoire des Professeurs J. Naisse (U.L.B.) et A. Bauchau (F.U.N.).

Organisation : P. De Vos.

Accueil et évocation du Professeur J. Naisse par M^me^ M. LECLERCQ-SMEKENS et M. J. DELIGNE et du Professeur A. Bauchau par M. P. DE VOS.

COMPÈRE, PH. (U.Lg). — Cytologie comparée de l'épithélium branchial de trois crabes : Cancer pagurus, Carcinus maenas et Eriocheir sinensis en relation avec leur aptitude ionorégulatrice.

WELCOMME (F.U.N.). — Source d'énergie pour le transport d'ions dans les branchies du crabe euryhalin Eriocheir sinensis.

DANGUY, A. (U.L.B.). — Lectin Histochemistry of glycoconjugates in secretory cells of fish epidermis.

JANS Y. & JANGOUX, M. (U.E.M.). — Induction expérimentale « in vivo » de la formation de corps mous intracoelomiques chez Holothuria tubulosa.

LORET , S. (F.U.N.). — Caractères morphologiques et biochimiques des types cellulaires de la glande digestive du crabe Carcinus maenas.

JANGOUX, M. & L. LAMBERT, D. (U.E.M.). — Nature mésothéliale de la musculature tégumentaire chez les Astéries (Echinodermata).

VAN HERP (Katholieke Universiteit Nijmegen). — Neuropeptides and neurotransmitters in the organ X-sinus gland complex, an important neuro-endocrine integration center in the eyestalks of crustaceans.

KESTEMONT, PH. (F.U.N.). — Etude comparative de la croissance ovocytaire chez les Poissons à ovogenèse synchrone et asynchrone.

HUYSSEUNE, A. (R.U.G.). — Chondroid bone in cichlid Fish : actual state of knowledge.

CLOPTENS, F. (K.U.L.). — Het voorkomen van « adipokineticlike » hormonen bij enkele Insekten.

HEMPTINNE, J.-L. (U.L.B.). — Les glandes exocrines des genitalia des femelles d'Adalia bipunctata.

SANDERSON, F., THIBAUT-VERCRUYSSEN, R. & RONVEAUX-DUPAL, M.-F. (F.U.N.). — Oxidative stress and bledding phenomena in relation with the cytoskeleton.

DEPELCHIN, S., LECLERCQ-SMEKENS, M., DEGEN, A. & LELOUP, R. (F.U.N.). — Etude morphologique comparative de la prolifération et de la différentiation de kératinocytes humains cultivés sur divers substrats.

RASMONT , M. (U.L.B.). — Différenciation cellulaire des Eponges d'eau douce.

Posters :

H. RAES, H. (R.U.G.). — Two types of endocrine cells in the midgut of the Honeybee.

RAES, H. & DE COSTER, W. (R.U.G.). — Subcellular effects of mercury on the columnar cells of the Honeybee ventriculum.

Rapport de l'assemblée générale du 20 janvier 1990
Verslag van de algemene vergadering van 20 januari 1990

1. Approbation du procès-verbal de l'assemblée générale du 21 janvier 1989.
   Goedkeuring van het verslag van de algemene vergadering van 21 januari 1989.

Le rapport de l'assemblée générale du 21 janvier 1989 publié dans les Annales 119 (1) : 101-103 est approuvé à l'unanimité.

Het verslag van de algemene vergadering van 21 januari 1989, zoals verschenen in de Annalen 119 (1) : 101-103, wordt eenparig goedgekeurd.

2. Activiteitsverslag 1989 door J. Van Goethem, voorzitter.
   Rapport d'activités 1989 par J. Van Goethem, président.

De Vereniging organiseerde drie bijeenkomsten. De eerste was een colloquium met als thema « Malacologie », dat plaats had op het K.B.I.N. Het was bedoeld als een herdenking van het 125-jarig bestaan van onze Vereniging, die in 1863 gesticht werd onder de benaming « Société malacologique de Belgique ». Daarom traden naast onze eigen Vereniging nog als coorganisatoren op : de « Société belge de Malacologie » en de « Belgische Vereniging voor Conchyliologie ». Twee firma's hebben in ruime mate deze manifestatie gesponsord.

De tweede bijeenkomst was de jaarlijkse « Jongerendag », eveneens op het K.B.I.N. De jongerenafgevaardigden, Ann Huysseune en Michel Baguette, die tijdelijk Thierry Hance vervangt, mogen van harte gelukgewenst worden voor de puike organisatie en de grote opkomst van voordrachtgevers.

La troisième réunion a eu lieu aux « Facultés Universitaires de Namur » à l'invitation du Prof. P. De Vos. La réunion avait pour thème la Cytologie comparée ; elle était organisée à la mémoire des professeurs J. Naisse (U.L.B.) et A. Bauchau (F.U.N.).

La participation à chacune de ces trois réunions était très satisfaisante. Au total 46 communications et 32 affiches ont été présentées. La qualité scientifique était excellente (voir aussi ci-dessus).

Le Conseil d'Administration s'est réuni cinq fois.

Krachtlijnen van het beleid zijn :

— het moderniseren en verder internationaliseren van het wetenschappelijk tijdschrift van onze Vereniging,

— de inspanningen i.v.m. de wetenschappelijke vergaderingen concentreren m.a.w. minder vergaderingen houden per jaar maar ze doen winnen aan belang,

— vooral de jonge vorsers aanspreken en hen een forum bieden.

3. Verslag over de Annalen door W. Verraes, hoofdredakteur.

Rapport sur la publication des Annales, par W. Verraes, rédacteur en Chef.

Ons tijdschrift is bezig aan een metamorfose, terwijl het ondertussen evenwel volwaardig funktioneel-operationeel moet blijven. Dit is noch voor organismen, noch voor tijdschriften een eenvoudige zaak.

Jaargang 119 bestond zoals gebruikelijk uit de resp. in juni en in december 1989 verschenen twee afleveringen, maar daarnaast hebben wij nog een supplement in augustus 1989 kunnen laten verschijnen.

Even een formeel overzicht :

— Aflevering 1 telde 103 pp. en bevatte naast het « In Memoriam Emeritus Prof. Dr. L. De Coninck » nog 5 gewone artikels en twee reeksen Abstracts, resp. rond aktiviteiten der Vereniging in Louvain-la-Neuve (22.10.88) en in Brussel (10.12.88), alsook boekbesprekingen en de publikatie van het Administratief Rapport 1988, verzorgd door onze sekretaris, de heer Henri André.

— Aflevering 2 telde 127 pp. en was naast de publikatie van twee gewone artikels helemaal gewijd aan het Colloquium Malacologie dat doorging in Brussel (20.05.89). Een extra-inspanning werd geleverd om de geschreven neerslag van dit colloquium nog in december 1989 het licht te laten zien.

— Tenslotte werd nog een supplement van 127 pp. uitgegeven, waarin de 218 Abstracts van het Third International Congress of Vertebrate Morphology, dat doorging van 20 tot 25 augustus 1989 in Antwerpen, werden opgenomen.

De redaktionele verwerking van de ingezonden stukken neemt alsmaar meer tijd in beslag. Ik waardeer dan ook zeer de onbaatzuchtige hulp van mijn kollega's E. Schockaert en

M. Chardon, die elk een deel van het redaktioneel werk op zich nemen met eigen verantwoordelijkheid, net zoals ik onbezoldigd en in de z.g. vrije tijd, wat niet te onderschatten is. Daarenboven wordt de taal der manuskripten thans in regel onder de Engelse loupe genomen door Mr. R. Kime en onder de Franse loupe door M. Chardon, wat eveneens zeer gewaardeerd wordt. Tenslotte is sinds 1989 de Editorial Board effektief geïnternationaliseerd : naast 7 Belgische leden maken nu ook 10 buitenlandse leden deel uit van deze Board.

Dit zijn allemaal signalen die wijzen in de richting van een kwaliteitsverhoging van het tijdschrift én die het tot een internationaal, niet tot één discipline beperkt, tijdschrift maken. Maar wij willen doorheen deze geruisloze metamorfose ook het visueel imago van ons tijdschrift aantrekkelijker maken. Een werkgroep en de Raad van Beheer bespreken de wijziging van het omslagblad en enkele andere vereenvoudigingen, alsook de mogelijkheid tot het insturen van de manuskripten op schijf, dit alles binnen de ruimte die de Penningmeester ons kàn toestaan en ruggespraak met de drukkerij Michiels in Tongeren.

Uiteraard is de voorbereiding van de publikatie der eerste aflevering van volume 120, die in juni 1990 het levenslicht moet zien, volop aan de gang. De aangekondigde vorm-transformaties zullen dit jaar verder worden uitgetest vooraleer in omloop te worden gebracht.

Bij dit alles konden en kunnen wij rekenen op de vlotte en sympatieke medewerking en inzet van de heer M. Toppets en zijn medewerkers, die er altijd in slagen om een verzorgde uitgave uit het niets te voorschijn te toveren, én op tijd. Ik waardeer dit zeer en wens hier opnieuw mijn dank hiervoor uit te spreken. Wij wéten dat er in het heelal niets « vanzelf » gebeurt, en die onwrikbare wet geldt niet alleen voor de opbouw van wetenschappelijke kennis, maar ook voor de publikatie ervan, net zoals dit geldt voor het léven der Vereniging.

Tot slot mijn welgemeende dank aan de auteurs, die ons hun geesteskinderen willen toevertrouwen, en aan de anonieme referees, die « voor de goede zaak » een kwaliteitskontrole van de aangeboden produkten doen.

4. Rapport sur la gestion de la bibliothèque par J. Deligne, bibliothécaire.
   Verslag over de werking van de bibliotheek door J. Deligne, bibliothecaris.

Expédition des Annales. — Les fascicules 118(2) et 119(1) ont été adressés à tous les correspondants étrangers qui nous envoient en échange une ou plusieurs revues et à tous nos clients ainsi qu'à deux revues bibliographiques. Les fascicules 119(2) et 119 (suppl. 1) sont en cours d'expédition. Les réclamations relatives à des numéros plus anciens ont été satisfaites.

Gestions des collections. — Suite à une réorganisation des Services de l'Université Libre de Bruxelles qui gérent notre bibliothèque, les ouvrages les plus intéressants et les plus récents de nos collections ont été regroupés dans la « Bibliothèque des Sciences et Techniques » (B.S.T.) de l'Université, sise au 30 av. Depage à 1050 Bruxelles. Sur la présentation de leur carte, les membres de notre Société peuvent avoir accés non seulement à nos collections mais aussi à l'ensemble des ouvrages de la B.S.T. Pour toute information complémentaire ou pour tout problème éventuel, le bibliothécaire invite les membres à prendre contact avec lui.

Remerciements. — Nous remercions vivement M^{me} Cantraine et M. De Cock ainsi que l'ensemble du personnel de la B.S.T. pour leur précieuse collaboration.

5. Financieel verslag door F. Fiers, penningmeester.
   Rapport financier par F. Fiers, trésorier.

Het boekjaar 1989 wordt afgesloten met een batig saldo van 31.101 fr. De factuur voor de aflevering 119 (2) moet evenwel nog betaald worden. Deze som wordt gedekt door de toegekende subsidie van het Ministerie van Onderwijs (subs. '89) en de achterstallige facturen (verkoop en auteurs-bijdragen).

De inkomsten van het voorbije jaar zijn vooral gedrukt door de afwezigheid van de subsidie van het « Ministère de l'Education nationale ». Daartegenover blijken de verkoop van het volume 117 suppl en de publiciteitsbijdragen belangrijke inkomsten die het verlies van hogervermelde subsidie enigszins compenseren.

Het vooropgesteld budget voor het boekjaar 1990 voorziet een volume 120 met maximaal 200 blz. Deze noodzakelijke beperking volgt uit de noden voor het verzenden van het tijdschrift (119/2, 119/suppl en 120/1) die dit jaar zullen oplopen tot een bedrag van 70.000 fr.

Tot slot wil ik hier de heer J. Van Goethem danken voor het waarnemen van het financieel beheer tijdens mijn langdurige afwezigheid in het buitenland.

6. Aanduiding van twee commissarissen ter nazicht van de rekeningen.

Désignation de deux commissaires aux comptes.

M[lle] A.-M. Leloup et M. A. Ovaere sont désignés comme commissaires aux comptes. Après vérification décharge est donnée aux administrateurs. De algemene vergadering verleent ontheffing aan de beheerders.

7. Elections statutaires — Statutaire verkiezingen.

Le mandat des administrateurs suivants est unanimement renouvelé pour deux ans : H. M. André, J. Deligne, P. De Vos, F. Fiers, G. Goffinet et E. Schockaert.

8. Aktiviteiten 1990 — Activités 1990.

Het volgende programma voor 1990 wordt goedgekeurd :

14 maart 1990 : Vergadering over aquatische organismen (zoete en mariene waters), in de K.U. Leuven. Organisatie : J. Billen & F. Ollevier.

16-17 november 1990 : Mechanismen van biologische herkenning, te Antwerpen. Organisatie : F. De Vree & J. Hulselmans (U.I.A.— R.U.C.A.). Vergadering in combinatie met de dag der jongeren. Organisatie : A. Huysseune en M. Baguette.

9. Divers — Varia.

M[lle] A.-M. Leloup propose la création d'un groupe de travail « Pédagogie de la Zoologie ». Un résumé d'une page de ce projet peut être envoyé lors d'un prochain courrier.

M. J. Godeaux signale que le prochain prix Edouard Van Beneden, prix quinquennal de la Société royale des Sciences de Liège, sera attribué en 1990. Tout renseignement complémentaire, s'adresser au Pr J. Godeaux, à l'Institut de Zoologie de l'Université de Liège.

Zeven nieuwe geassocieerde leden worden aanvaard : Ph. Berbiers, K. Bogaert, G. De Boeck, Y. Quinet, E. Schoeters, J. Tits, C. d'Udekem d'Acoz.

De volgende algemene vergadering zal plaats hebben op 19 januari 1991.

H. M. ANDRÉ,
Secrétaire — Secretaris

GUIDE TO THE AUTHORS

1. For publication, manuscripts (original + two copies, including illustrations) must be sent to the Redaction Secretary : Prof. Dr. Walter VERRAES, State University of Gent, Laboratorium voor Morfologie en Systematiek der Dieren, Ledeganckstraat 35, B-9000 Gent (Belgium) (Tel. : 091-22.78.21, extension 459).
2. Members of the Society, as well as non-members, can publish in the journal. Only members benifit from a waiver of page charges, including four figures or two pages with plates. This waiver is subject to annual consideration by the Council of the Society. Non-members must pay for all pages at cost price (± 1.500 BE.F. per printed page, inclusive illustrations).
3. The manuscript is to be written by preference in English or French. If written in French, it must include a correct English translation of the title and the summary. Each manuscript is submitted to the editorial board. After acceptance, the authors remain fully responsible for their article. Short notes, not exceeding three printed pages, including illustrations, are also accepted. Notes have to be prepared in the same form as manuscripts, including a summary, introduction, material and methods, results, discussion, and references. Articles resulting from communications during the meetings of the Society have priority for publication. Abstracts of oral communications and posters are also published.
4. (a) For preparing the manuscript, the authors must follow rigorously the lay-out as shown in articles of a recent issue. Manuscripts must be typed doubly spaced on one side of consecutively numbered pages. All figures, single or composite (i.e. anatomical drawings, graphics, histograms, schemes, etc.) must be prepared on separate sheets and drawn with black ink. Such figures must account for readability after reduction. Scales are to be indicated where possible. The number of the figures, the name(s) of the author(s) and the intended degree of reduction are to be indicated in pencil on the reverse side of each figure. Idem for photographic illustrations and for tables. In the left margin of the manuscript, the author should indicate where tables or figures are to be inserted. Legends of figures, plates, and tables are to be typed in sequence, beginning on a separate sheet.

   (b) The authors can submit the final version of their article on floppy disc. It is strongly recommended to prepare it in ASCII FILE MS-DOS (PRO-DOS or McINTOSH FILES are also accepted) without any lay-out instruction in the text. The system used must be indicated on the floppy disc. All footnotes as well as the legends to the figures, the plates and the tables must be put on the disc, but distinctly after the running text. Tables as well as their headings must obligatorily be typed in the desired lay-out on a separate paper (not on the floppy disc). In addition, three offprints of the article, as well as three copies of all illustrations and their legends must be submitted, including the indications by hand for the final lay-out, including the places where the figures, plates and tables must be inserted (in left margin). For other details : see also sub 4 (a).
5. The authors will receive 50 reprints of their article free of charge. Additional reprints (25 or multiple) can be ordered on a form which is sent together with the first galley proof. Reprints of abstracts are not made.
6. Submitted manuscripts may not be presented to another journal.

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