

ABSTRACTS

1 MUSCLE ENERGETICS AND THE MODULATION OF FEEDING KINEMATICS IN FISHES. P. Aerts and F. De Vree - University of Antwerp (UIA).

Based on its extremely fast procedure (less than 100 ms in the cichlid *Astatotilapia*), it is commonly believed that the suction feeding process in fishes is governed by a fixed central pattern generator, not subjected to any instantaneous peripheral feedback. Most likely, this interpretation of the neuro-motorics of the feeding act does not hold for *Astatotilapia* (1). Kinematic analysis shows that this fish modulates its buccal movement pattern by attuning the onset of the compression phase to the moment the prey enters the mouth. The biological significance of the evolution of such a modulating feedback system is not directly obvious from a "classical ecological" point of view. Consideration of the energetics of muscle contraction offers an alternative explanation. Based on the assumption that suction feeding relies entirely on the instantaneous phosphocreatine stores in the muscle fibres, it is postulated that an economized consumption of these stores allows the fish to perform immediate successive strenuous head part movements (new suction act, spitting ...), thus to improve the exploitation of the food resource. This hypothesis is tested experimentally by stimulating the epaxial muscles (the most important feeding muscles; (2) following the pattern deduced from EMG recordings of *Astatotilapia* feeding on crickets (*i.e.* activity periods of 70 ms at a stimulation frequency of 100 Hz, the latter coinciding with tetanization of the muscle). Force output or acceleration of the lifting skull are measured *in situ* and mutually compared for successive "feeding" stimulations. The preliminary results show a fast decrease of the muscle output when stimulated in rapid succession. This supports the premised hypothesis.

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2 OPTIMIZATION OF SKELETAL STRUCTURE IN VERTEBRATES. R. McNeill Alexander - University of Leeds, England.

Evaluation tends to optimize the structure of bones, to suit the circumstances of their use. In the studies described in this paper, hypotheses of bone optimization have been formulated and tested. The data are measurements of bone dimensions, together with the stresses that act in various activities, which have been calculated from films and force plate records, or from records obtained by other investigators from implanted strain gauges. In the long bones of vertebrate limbs, stresses due to bending moments

generally dominate over axial stresses. If bones are to be made as light as possible for given strength, they should taper in such a way as to equalize stresses along their length. The tibia of dogs tapers approximately as required (1). A bone of given strength can be lighter if it is a hollow tube, than if it is a solid rod. The optimum ratio of external to internal diameter is much lower for marrow-filled bones (because of the mass of the marrow) than for air-filled ones. The ratios for the marrow-filled bones of mammals and some birds cluster around the theoretical optima. Air-filled bird bones show much higher ratios, but it is difficult to predict optimum values for them (2). The safety factor of a structure is the ratio of design strength to expected maximum load. Safety factors are required because neither actual strength nor actual load can be predicted precisely. Increasing the safety factor reduces the probability of failure, but increases costs of construction and use. A theory predicts that optimum safety factors will be high when costs of failure are high or when loads are very variable (but may be zero if low costs of failure combine with high variability) (3). Optimum safety factors are difficult to estimate because costs of use and of failure are measured in different currencies. Observed safety factors are generally between 2 and 5. An extension of the theory takes account of fatigue failure (4). Bones may be selected for stiffness, as well as for strength. If bones bend in strenuous activities, muscles must shorten more to produce a desired movement, and larger muscles may be required. A theory of optimum stiffness predicts bone stresses similar to those observed in strenuous activities (5). The relative importance of strength and stiffness remains uncertain. Optimization models have contributed substantially to our understanding of skeletal dimensions but leave many questions unresolved.

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- (3) R.McN. ALEXANDER (1981). *Sci. Progr.*, Lond. 67 : 119-140.
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3 ALLOZYME VARIATION IN AZOREAN LEPTAXINAE (MOLLUSCA : PULMONATA). T. Backeljau, C. Brito*, R. Da Cunha*, A.M. Frias Martins* and L. De Bruyn** - Royal Belgian Institute of Natural Sciences, Brussels, *University of the Azores and **University of Antwerp (RUCA).

The helicid subfamily Leptaxinae is endemic in Macaronesia. In the Azores there are two genera (*Leptaxis* and *Helixena*) containing ten nominal species and two subspecies. Seven species are only known from their original descriptions and/or a few doubtful shells. Of the remaining species only two are typical of the Azores (*Leptaxis azorica*, *Helixena sanctae-mariae*), while the third (*Leptaxis erubescens*) was introduced from Madeira. We investigated genetic variation in the former two species using

polyacrylamide gel electrophoresis (specific enzymes in digestive glands) and isoelectric focusing (Phast-System : non-enzymatic proteins in albumen glands). The study involved 57 *Leptaxis azorica azorica* (Flores, Sao Miguel), 14 *Leptaxis azorica minor* (Santa Maria) and 31 *Helixena sanctae-mariae* (Santa Maria). The first results indicate that : 1) the genetic distance between *Leptaxis azorica azorica* from Flores and Sao Miguel is of species magnitude, 2) in Sao Miguel *Leptaxis azorica azorica* seems to consist of a western and an eastern group and 3) both subspecies of *Leptaxis azorica*, as well as the genera *Leptaxis* and *Helixena*, are genetically distinct. The subdivision of *Leptaxis azorica* in Sao Miguel may reflect the fact that this island originated from a fusion of two older, separate islands. *Leptaxis azorica* from Flores, on the other hand, is most probably a different species. This work was supported by F.K.F.O. - Grant 2.0004.91.

- 4 **FEEDING BEHAVIOUR IN THE MARINE TURTLE, *DERMO-CHELYS CORIACEA* (REPTILIA : DERMOCHELYDAE).** V. Bels and S. Renous* - University of Liège and *National Museum of Natural History of Paris, France.

The study of marine turtle provides a unique example for the functional comparison of the feeding behaviour between terrestrial and aquatic tetrapods. Feeding bouts of *Dermochelys coriacea*, have been investigated using high speed cinematography (100 frames/s). Feeding bouts involve approach to the prey, biting of the prey (=capture) and transport to the esophagus. Kinematic variables depicting the tongue, hyoid and jaw displacements were compared for biting and transport cycles. The jaw cycles are always divided in slow opening (SO), fast opening (FO) and fast closing (FC) stages. Elevation of the neurocranium is only involved for 10 to 20 % of the gape amplitude in both phases. Relative displacements of the tongue and jaws are very similar during biting and transport cycles: the tongue moves forward and upward during SO and the beginning of FO stages and then moves back- and downward. At the end of the closing stage (FC), the angular acceleration of the jaw angles decreases slightly, but a slow closing stage was not clearly observed. The kinematic pattern of the feeding cycles in *D. coriacea* does not show great difference with that described for reptiles and amphibians.

- 5 **INFLUENCE OF 17- β -ESTRADIOL ON THE GONADAL SEX OF THE EUROPEAN EEL (*ANGUILLA ANGUILLA* L.).** K. Beullens, W. Beeckman and F. Ollevier - Catholic University of Leuven (KUL).

Eels (*Anguilla anguilla* L.) show a size-dimorphism which is related to the phenotypic sex of the fish. Generally, phenotypic female eels are larger than phenotypic males of the same age. The sex ratios of these fish are uneven and vary from almost complete male populations to predominantly female populations, under natural and experimental

conditions. The majority of eel farmers in Western Europe report a high percentage of males (up to 90 %) in their stock which is, considering the size-dimorphism, less economical for the farmer. Not differentiated eels were administered orally 17- β -estradiol to influence the gonadal differentiation and the sex ratio. Concentrations of 0,1,25 and 100 mg estradiol/kg feed were used during a period of 200 days. This resulted in respectively 92.6 % males, 84.4 % males, 55.9 % females and 74.3 % females. We conclude that 17- β -estradiol influences considerably the sex ratio in favor of the females in eel populations.

6 ORIGIN OF THE TRAIL PHEROMONE IN ECITONINAE : A BEHAVIOURAL AND MORPHOLOGICAL EXAMINATION. *J. Billen* - Catholic University of Leuven (KUL).

Trail following behaviour in ants undoubtedly reaches its most spectacular development in the army ants, which form impressive raiding columns along very clear trails. Our present knowledge on the origin of army ant trails, however, is remarkably limited, and invariably goes back to a few early reports on *Eciton*, claiming the hindgut to be the source of the trail substance in these ants. In an attempt to verify and update this information, we analyzed trail following in *Eciton burchelli*, and found the hindgut to be completely inactive. Instead, the 7th abdominal sternite appeared to be the source of an extremely powerful and long-lasting trail pheromone, as could be illustrated in a bio-assay. Morphological and histological examination of this last externally visible sternite revealed the presence of a conspicuous glandular epithelium on the inner side of the sternite, that forms the ventral lining of the sting chamber. The anatomical position of this gland, which is unique among the Ecitoninae, and the absence of any other significant secretory tissue associated with the 7th sternite, make it the incontestable source of the trail pheromone in *Eciton*.

7 THE EFFECT OF PROTEIN RESTRICTION ON EPISODIC GROWTH HORMONE SECRETION OF MEAT-TYPE CHICKENS. *J. Buyse, E. Decuyper, L. Berghman, E.R. Kühn and F. Vandesande* - Catholic University of Leuven (KUL)

In this experiment, the effect of isocaloric diets containing 20 % crude protein (HP) or 15 % crude protein (LP) on the secretory growth hormone (GH) pattern of broilers was investigated. At 33 days of age, 8 HP chickens and 6 LP chickens were cannulated in the branchial artery under local anesthesia. Serial blood samples (0.25 ml) were taken at 20 minutes interval for a 5 hour period. Plasma was stored at - 20 C until GH content, by using a homologous immunoassay, was assayed. The effect of the

protein content of the diets on body weight and on GH pulsatility characteristics are presented in the table.

Body weight at 33 days of age and GH pulsatility characteristics of male broilers fed isocaloric diets containing 20 % protein or 15 % protein :

	20 % protein	15 % protein	P-value
No birds	8	6	
Body weight (g/bird)	1197 ± 29	631 ± 38	< 0.0001
Overall mean (ng/ml)	38.5 ± 4.0	125.2 ± 15.0	< 0.0001
Amplitude (ng/ml)	40.0 ± 7.2	159.3 ± 19.4	< 0.0001
Baseline (ng/ml)	21.1 ± 2.0	46.4 ± 5.8	< 0.001
Frequency (no/h)	0.68 ± 0.01	0.83 ± 0.04	< 0.01

Values are means ± standard error.

Decreasing the protein content from 20 % to 15 % decreased body weight at the same age and also markedly altered the secretory GH pattern. Broiler chickens fed the LP diet manifested a significant higher overall mean, amplitude, baseline and peak frequency compared to the HP chickens. From these results and the finding that the efficiency of utilization of dietary protein is inversely related with dietary protein content (1), the hypothesis concerning the causal relationship between the pattern of GH secretion and the efficiency of protein retention (2) is corroborated.

- (1) S. JACKSON, J.D. SUMMERS and S. LEESON (1982). *Poultry Science* 61 : 2224-2231.
- (2) E. DECUYPERE, F.R. LEENSTRA, J. BUYSE, G. BEUVING and L. BERGHMAN (1991). *Br. Poultry Sci.* 32 : 1133-1140.

8 ETHOLOGICAL APPROACH OF A GROUP OF CAPTIVE SOUTH AFRICAN (CAPE) FUR SEALS *ARCTOCEPHALUS PUSILLUS PUSILLUS* : ETHOGRAM, TIME BUDGET, SOCIAL STRUCTURE. A.J. Caudron - Université de Liège.

The behavioural elements commonly used by this species of Otariidae (Pinniped) are still poorly known. Our aim was to investigate the relations between the six members of a group housed at the Antwerpen Zoological Garden (Belgium). The objectives were to define the social organization of the group, the communicative signals used to maintain this structure and the way this social life combines with the general activity of the animals. From June 1990 to June 1991, data were collected over 300 daylight hours of observation. An ethogram was first established; the verbal description of behaviour patterns was accompanied by data about their duration, frequency of occurrence, the contexts and the individuals concerned. In addition, high-quality sound recordings were obtained for spectrographic analysis. To investigate the

notion of social structure, a quantitative study of interactions has led to the construction of sociograms concerning agonistic and affiliative behaviours. The time budget has been established for each individual before and during the breeding season. Data of reproduction in captivity were collected. The behavioural repertoire seems to show that captive Cape fur seals behave in a very similar way as all the Otariidae do. The distribution of agonistic and affiliative interactions in the group does not allow us to define any hierarchy between individuals. On the opposite, they constitute a harmonious family. In the same way, the activity budgets point out the important proportions of time spent interacting. In comparison with less equilibrated groups of Pinnipeds (1). These qualitative and quantitative descriptions of *Arctocephalus pusillus pusillus*' behaviour have never been done before. Though they were collected in confinement, we hope these elements will constitute an interesting substrate for later studies and comparative ethology between species of Otariidae.

(1) G.H. WARING (1984). *Acta Zool Fennica* 171 : 243-244.

9 **MUSCLE ACTIVITIES DURING PHARYNGEAL FOOD PROCESSING IN *OREOCHROMIS NILOTICUS* (PISCES : CICHLIDAE).** G. Claes and F. De Vree - University of Antwerp (UIA).

Pharyngeal jaw movements and muscles activities during feeding were studied in *Oreochromis niloticus* using cineradiography combined with electromyography. Food processing involves cyclic and alternating movements of the paired upper pharyngeal jaws (UPJs) and the single lower pharyngeal jaw (LPJ). Each cycle can be subdivided into four main phases: preparatory, power (compression and shearing), swallowing and recovery (1). Electromyograms were recorded from seven pharyngeal muscles: levator posterior (LP), levator externus 4 (LE4), pharyngocleithralis internus (PCI), pharyngocleithralis externus (PCE), pharyngohyoideus (PH), retractor dorsalis (RD) and levator internus (LI). The lower pharyngeal jaw is suspended in a muscular sling, that is kept in constant tension throughout the masticatory cycle. The time sequence of recruitment of the pharyngeal muscles during the power phase shows a constant pattern. During the compression phase the LP, LE4 and PCI reach their peak activity almost simultaneously, pulling the LPJ anterodorsally. This results in a powerful apposition of the LPJ against the UPJs, which are retracted by action of the retractor dorsalis. In this way high compressive forces to the dental surfaces are generated. During the shearing phase the PH and the PCE become dominant, pulling the LPJ anteroventrally, while the retractor dorsalis continues to swing the UPJs posteriorly. The pharyngeal teeth then closely pass each other in opposite directions to generate shearing of the food. Continuous low level activity of the PCI retracts the LPJ during the swallowing phase. Simultaneous LPJ depression presumably opens the oesophageal entrance, facilitating swallowing of the posteriormost food particles by clockwise rotation of the UPJs. The PCI is activated again during the recovery phase, pulling the LPJ backward and upward, while synchronous action by the LI makes the UPJs swing anteriorly

toward their initial position. The extended activity of the PCI effects a continuous fine control of the anteroposterior LPJ movements during the cycle.

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10 THE NECK OF *CAIMAN CROCODILUS*, AN IMPORTANT STRUCTURE DURING INERTIAL FEEDING. *J. Cleuren* and *F. De Vree* - University of Antwerp (UIA).

The primitive tongue of crocodylians is not suited for lingual transport as observed in most lizards. Food transport is achieved by inertial thrusts in which a back and upward movement of the craniocervical region imparts a backward acceleration to the prey during jaw opening. During mouth closure, a depression of the neck thrusts the jaws forward over the backward moving prey. All cervical muscles were studied by electromyography in combination with the use of a triaxial accelerometer. To differentiate the role of each muscle in dorsoventral and lateral movements, the activity of both muscle sides was recorded simultaneously. Bilateral contraction of all muscles of the transverso-spinalis system causes straight lifting of the head. When head elevation is accompanied by a lateral shift, some muscles show identical and simultaneous activity on both sides, while in other muscles the ipsilateral side contracts stronger and earlier. Simultaneous with cranial elevation, the neck is elevated by bilateral contraction of all cervicus muscles. Lateral flexion of the neck is caused by unilateral activity of the m. ilio-costalis cervicus only. Most muscles have multiple functions; muscles with cranial elevation as major function also assist in the lateral head flexion or in the elevation of the neck, or vice versa. At all times the occipital joint is stabilized. In dorsad movements, the immense inertial forces on the occipital joint are counteracted by the most ventral neck muscle, in lateral movements by a contraction of the contralateral muscle side. Supported by IWONL grant nr. 880217 to J.C.

11 ROLE OF DENDRITIC CELLS AND RESTING B CELLS IN THE INDUCTION OF IMMUNE RESPONSES *IN VIVO*. *G. De Becker*, *J. Urbain* and *M. Moser* - Université Libre de Bruxelles (ULB).

The initiation of an immune response requires the activation of helper T cells which in turn activate the effector cells : B cells which secrete specific antibodies recirculating into the blood and the lymph, or killer cells able to lyse infected cells or tumor cells. The helper cells appear to play a central role in the defence of the organism, since they are required for the activation of effector functions. Recently, discrete subsets of T helper cells, called Th1 and Th2, have been defined on the basis of their lymphokine pattern. These subsets have different functional properties : in particular, Th1 cells induce the production of antibodies if IgG2a isotype, whereas Th2 cells favor the

production of IgE antibodies. *In vivo*, the Th1 or Th2 responses are not always appropriate to eliminate the pathogen : for example, only the activation of Th1 cells protects the mice against the parasite *Leishmania Major*, whereas only a Th2-type response can protect the animals against some nematodes. The aim of this study is to try to modulate the Th1/Th2 responses *in vivo*. Since the activation of T helper cells requires the presentation of the antigen on specialized cells called antigen-presenting cells, we reasoned that we could immunize mice by presenting the antigen on distinct antigen-presenting cells. We have indeed shown that mice injected with antigen-pulsed dendritic cells developed a strong humoral response, characterized by high levels of IgG2a antibodies. By contrast, animals that received the antigen pulsed on resting B cells synthesized specific antibodies of IgG1 and IgE, but not IgG2a isotypes. Thus, dendritic cells induce a Th1-like response, whereas resting B cells favor the induction of a Th2-type response. Experiments are in progress to try to modify the Th1/Th2 balance in some pathological situations, in order to render more efficient the response of infected animals.

12 PATTERNS OF PARASITISM BY A GREGARIOUS PARASITOID (HYMENOPTERA : PTEROMALIDAE) ON A PATCHILY DISTRIBUTED HOST, *CRYPTONEVRA FLAVITARSIS* (DIPTERA : CHLOROPIDAE). L. De Bruyn* and J. Majer - *University of Antwerp (RUCA) and Janus Pannonius University, Hungary.

The larvae of *Cryptonevra flavitarsis* (MEIGEN) live asinquilines in the galls of *Lipara* MEIGEN species on the common reed, *Phragmites australis* Cav.Tri. ex Steud.. These galls, containing from 1 to > 30 larvae per gall, can be viewed as discrete host patches. In the present study an attempt was made to reveal spatial patterns of a primary gregarious pteromalid endoparasitoid of *C.flavitarsis*. The spatial distribution of the fly and its parasitoid were studied during the spring of 1991 in a reedbed at Pécs, Hungary. Galls were collected in the field and transported to the laboratory. Each gall was dissected and counts were made to determine the overall percent parasitation and the proportion of male parasitoids per gall and per host pupa. There was a distinct positive relation between the hosts' pupal size and the number of parasitoids emerging. On the contrary, the average parasitation per gall was low, and the response to spatial variation in host density was density-independent. The proportion of males was rather constant and independent of the number of hosts per gall and host size. Our results indicate that the pteromalid parasitoids are relative inefficient in exploiting their host patches and that the outcome of parasitation is unpredictable. *Senior research assistant of the N.F.S.R.

- 13 STRUCTURAL ADAPTATIONS FOR FORCEFUL BITING IN GOBIES (TELEOSTEI : GOBIIDAE).** *D. Decleyre, W. Verraes and P. Aerts** - University of Ghent and *University of Antwerp (UIA).

Biting is considered here as an elevation of the lower jaw against an object. The upper jaw is thereby considered to be firmly connected to the neurocranium (in reality, some protrusion of the upper jaw is possible), thus providing the necessary reaction force. Therefore, the maximal bite force of gobies is related to the development of the musculus adductor mandibulae. However, the extent of this muscle - and also the maximal bite force - is limited by spatial constraints. Some gobiid morphological features, namely the shape of the suspensorium and the partial interconnection of muscle sections in the musculus adductor mandibulae, are considered to be structural adaptations that still enhance the bite force for maximally developed cheek muscles.

- 14 SELECTION FOR LEANNESS IN POULTRY RESULTS IN A DIFFERENTIAL INFLUENCE AT THE SOMATROPHIC AND THYROTROPIC AXES ACCORDING TO THE SELECTION PARAMETERS USED.** *E. Decuyper, F. Leenstra*, B. Leclercq**, J. Buyse, L. Berghman, V. Darras, L.M. Huybrechts, T. Bartha, E. Dewil, A. Vanderpooten, H. Michels, E.R. Kühn and F. Vandesande* - Catholic University of Leuven (KUL), *Spelderholt Institute, The Netherlands and **Centre de Recherches Avicoles, INRA, France.

Selection for body weight gain up to slaughter weight resulted not only in rapidly growing modern broiler lines but also in a fat gain that increased faster than protein gain during the second part of their growing period. Selection for leanness was achieved by different selection strategies in different countries, apparently with similar results as far as fatness or leanness is concerned but resulting also in lines with a different endocrine functioning. Indeed, while the french (L) lean and fat (F) lines based on abdominal fat content by family selection, were significantly different for circulating triiodothyronine (T₃) levels but not for growth hormone (GH) levels (2), the dutch lean (FC) and fat (GL) lines, selected for respectively food conversion efficiency or growth rate (3) were not different for plasma T₃ levels during the growth period but the FC-line had a markedly increased GH-level (1). Therefore an extensive scrutiny of the thyrotropic and somatotrophic axes was performed on both genetic models for lean and fat growth in chickens during the growing period. As far as the thyrotropic axis is concerned, earlier results were confirmed while in addition a higher T₄ deiodination activity was found in the french lean line, but not in the dutch lean (FC) line compared to their respective fat lines. Similarly, liver T₃ receptor occupancy was significantly higher in the L-line but not in the FC line compared with their fat counterparts. Also for the somatotrophic axis earlier results were confirmed and while there were no significant differences in levels nor in pulsatility between the L and F lines, it was

found that the higher GH levels in the FC Line compared to the GL line could be ascribed to a greatly enhanced GH-pulsatility. GH-receptor numbers, but not the receptor affinity, was different between both lines. However, while the dutch lean line showed a low GH binding to the receptor, the french lean line had a significantly higher binding and GH receptor number compared to the fat line. GH-response to TRH was enhanced in the FC-line. Generally, lines did not differ with regard to the IGF-I concentrations. From these results it may already be concluded that selection for leanness is reaching its specific goal by changing the underlying physiological and endocrine mechanisms that determine protein and fat metabolism, but in a differential way according to the selection parameters used.

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- (2) B. LECLERCQ (1988). In : B. Leclercq and C.C. Whitehead (Eds.). *Leanness in Domestic Birds* : 295-312 (London, Butterwoth).
- (3) F.R. LEENSTRA (1988). In : B. Leclercq and C.C. Whitehead (Eds.). *Leanness in Domestic Birds* : 59-69 (London, Butterwoth).

15 BIOLOGY AND LARVAL DEVELOPMENT OF *ALLANTOGYNUS DELAMAREI*, A COPEPOD ENDOPARASITIC IN THE HOLOTHUROIDS *HOLOTHURIA TUBULOSA* AND *HOLOTHURIA MAMMATA*. V. Degouys*, D. Van den Spiegel* and M. Jangoux*&. - *Université de Mons and **Université Libre de Bruxelles (ULB).**

Out of the 120 *Holothuria tubulosa* GMELIN and *Holothuria mammata* GRUBE collected off Banyuls-sur-Mer in February 1991, 40 % were infested by the intracœlomic copepod *Allantogynus delamarei* CHANGEUX (infestation rate from 1 to 14 copepods per holothuroid). Most copepods were located in the anterior part of the host's cœlomic cavity where they were attached to cœlomic wall through their mandibles. All observed individuals were females. They each bore a large ovigerous sac in which they live and spawn. *A. delamarei* does not feed upon the host's internal organs but ingests the host's cœlomic fluid and cœlomocytes. Holothuroids react against the parasites by forming a continuous cœlomocyte envelope that completely surrounds the ovigerous sac. The ways in which the copepods infest and escape from the holothuroids are not known. Embryonic development takes place in the ovigerous sac and stops at the nauplius I stage. *In vitro* observations done in dishes containing filtered sea-water allow to follow the larval development up to the copepodite I stage. Further developmental stages and male individuals were not observed.

- 16 INHIBITION OF T-CELL ACTIVATION BY LO-CD2-a, A RAT MONOCLONAL ANTIBODY AGAINST THE HUMAN CD2 GLYCOPROTEIN.** *B. De La Parra, K. Van Den Branden, I. Smyej and H. Bazin* - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

LO-CD2-a, a rat monoclonal antibody directed against the CD2 glycoprotein, is able to modulate T-cell activation. The studies presented here show that this mAb drastically blocks T-lymphocytes activation and proliferation, presumably by inhibiting some of the processes leading to IL-2R expression during mixed lymphocyte culture. This inhibitory effect is still significant even if LO-CD2-a is added four days after the onset of mixed lymphocyte culture. Mitogen-driven T-cell activation is also significantly inhibited by the signal(s) delivered by this monoclonal antibody via the CD2 receptor. These findings indicate that a common pathway of intracellular events is shared by CD2, the TcR/CD3 complex and the lectin receptors on T-cells, and that these events are cut-out when LO-CD2-a binds to the E-receptor.

- 17 PRELIMINARY FINDINGS ON THE ULTRASTRUCTURE OF THE STOMA IN CEPHALOBIDAE (NEMATODA : RHABDITIDA).** *P. De Ley, M.C. Van de Velde and A. Coomans* - University of Ghent.

Longitudinal sections of the buccal cavity in Cephalobidae are being examined with T.E.M. for the first time, and the first results are presented here. From anteriorly to posteriorly, the stoma of *Acrobeloides nanus* can be divided into six parts, recognizable by the surrounding cells or structures : the first part is encircled by lip cuticle; the second by "arcade" cells; the third, fourth, fifth and sixth part each by a separate set of muscle cells. This structure is compared with that reported in ultrastructural studies of other bacterivorous nematodes (1,2). It is argued that the stoma of *A. nanus* does not conform to conventional interpretations of stoma structure in Cephalobidae, based on light microscopy, which distinguish five parts only. It also differs from the findings of the aforementioned studies, where it was shown that the buccal cavity can indeed be divided in only five parts in representatives of other groups. As standard nomenclature of nematode stoma parts is based largely (but not exclusively) on the five-part interpretation of the stoma of Cephalobidae, it is argued that this nomenclature as currently applied is (i) incompatible and non-homologous between Cephalobidae and other groups, and (ii) very difficult to correct without causing complete confusion. Furthermore, the exact homologies between stoma sections in Cephalobidae and in other groups are unclear, and may well be quite difficult to establish in practice. They certainly cannot be deduced from light microscopy alone, as was done in the past.

- (1) M.C. VAN DE VELDE and A. COOMANS (1991). *Revue de Nématologie* 14 : 133-146.
- (2) K.A. WRIGHT and J.N. THOMSON (1981). *Canadian Journal of Zoology* 59 : 1952-1961.

18 FUNCTIONAL ANALYSIS OF FEEDING BEHAVIOUR IN *OPLURUS CUVIERI* (REPTILIA: IGUANIDAE). V. Delheusy - University of Liège.

The feeding behaviour in *Oplurus cuvieri* is divided in four phases: capture, reduction, transport to the esophagus and cleaning. Kinematically, prey capture is a complex pattern involving a protraction-retraction cycle of the tongue outside the buccal cavity and the displacement of the jaw apparatus towards the prey. The jaw cycle is divided into slow opening (SO), fast opening (FO) and fast closing (FC) stages. SO stage is divided in SOI and SOII. During the reduction phase, the jaw cycle does not always involve a SO stage and the first reduction cycle never presents a SO stage. During transport, the duration of SO stages is highly variable. During reduction and transport of the prey, the pattern of the cyclic tongue movements are very similar. At the beginning of the gape opening (SO and beginning of FO), the tongue moves forward and upward and retracts backward at the end of FO and during FC. Gape opening during cleaning is not divided into two successive stages; the amplitude of gape angle is similar to that of SO for the other jaw cycles. Variables depicting durations and amplitudes of jaw displacements were compared by a discriminant analysis. Cycles of reduction and transport phases are largely overlapping and not well separated from cycles of cleaning and capture phases. On the contrary, the jaw cycles during the last two phases are kinematically different. Specialization in tongue displacements in these cycles may explain this difference.

19 TONGUE MORPHOLOGY OF *OPLURUS CUVIERI* (REPTILIA : IGUANIDAE). V. Delheusy, G. Toubeau* and V. Bels - University of Liège and *University of Mons.

Dissection, light microscopy and scanning electron microscopy were used to analyze tongue morphology of *Oplurus cuvieri*. The musculature of the tongue, involving extrinsic and intrinsic muscles, shows the typical pattern similar to that described in other iguanid lizards. The blood circulatory system, well developed in the tongue is connected in the fore tongue with a large venous space which is secondary divided anteriorly in two separated cavities. Dorsally, the tongue surface presents four successive regions. 1) The tongue tips are covered by flatter lingual scales, 2) the second region, following immediately the tongue tips, is covered by filamentous papillae, 3) a stratified squamous epithelium covers the third region which involves the main tongue dorsal surface, and 4) the limbs of the hind tongue are covered by flattened and pyramidal papillae. Laterally, the tongue is covered by filamentous papillae. During the feeding behaviour, the tongue is used for prey capture, reduction, transport to the esophagus and cleaning. During the capture phase, the tongue contacts the prey by the two anterior regions. In reduction and transport phases, the tongue slides below the prey at each cycle. The tongue passes over the teeth during the cleaning phase. Form-function relationships between morphology of the tongue surface and musculature are proposed for each feeding phase.

20 A SEM STUDY OF CUTICULAR STRUCTURES INVOLVED IN THE LOCKING OF THE ELYTRA IN COLEOPTERA. *J. Deligne* and *L. De Vos* - Université Libre de Bruxelles (ULB).

In order to protect efficiently the pterothorax and the abdomen, the elytra of Coleoptera must be firmly fastened. This is partly ensured by various types of groove-and-tongue joints 1) between both elytra, 2) between the elytra and thoracic or abdominal sclerites. Some authors have also reported that cuticular denticles could participate in the locking mechanism of elytra. These specialized structures were however only briefly described in a few families (1). In this study we have examined with the SEM the internal face of the elytra of 27 species belonging to 23 families of Coleoptera. In all families examined a specialized cuticular area has been detected. This area is localized in the anterior part of the lateral border of the elytra and is made of rows of thousands of spines or scales whose free edges are directed upwards. A corresponding area, the spines of which are directed downwards and obliquely, is situated on the edge of the metathoracic pleuron. In one of both areas the spines are generally more pointed narrow or spaced and more obliquely orientated, allowing easy and deep hooking with those of the opposite area. The two harrows of spines are thus strongly anchored. Unlocking of the elytra is only possible with a down and lateral double movement. The basic locking mechanism that we describe presents however specific variations in number, distribution, orientation, form and size of the spines and scales. In conclusion, it is obvious from our observations that in all species examined so far these differentiated cuticular areas are involved in the tight locking of the elytra. Their specific variations could be of some taxonomic or phylogenetic interest.

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21 THE PIVOTAL ROLE OF THE PLASMAMEMBRANE-CYTO-SKELETAL COMPLEX IN DIFFERENTIATION. *A. De Loof, P. Callaerts* and *J. Vanden Broeck* - Catholic University of Leuven (KUL).

We came to the conclusion that a universal principle underlying differentiation probably exists and that it might be a rather simple one : it seems to be a logical consequence of some of the properties of biological membranes and of the cytoskeleton. In our opinion it could be formulated as follows : "Differentiation in animals is the stepwise formation of (clusters of) cells which primarily differ at least in the properties of their plasmamembrane-cytoskeletal complex and the factors associated with it and which get mostly organised in a variety of epithelia. As a consequence of these differences in membrane-cytoskeletal properties - this causal relationship being the very point - the different cell types are able to display differential cellular morphology and physiology (e.g. protein synthesis, mitotic activity), notwithstanding the fact that they have an identical genome and similar mechanisms of protein synthesis and

processing". To put it very briefly : differences in the membrane-cytoskeletal complex are realised first, differential protein synthesis and pattern formation follow. The major "strategy" followed in differentiation seems to be to keep the genome constant but to change over and over its environment. This environment comprises two sets of constituents, ionic and macromolecular ones, both acting in a complementary way. The first one may be regarded as the coarse tuning mechanism of gene expression, the second as the fine one. An endless variability can be generated in this way.

2 2 GENETIC POLYMORPHISM, PLASTIC GENOTYPES, AND THE VARIABILITY OF VERTICAL MIGRATION PATTERNS IN ZOOPLANKTON. *L. De Meester* - University of Ghent.

The variability of diurnal vertical migration patterns in zooplankton is overwhelming. Different migration behaviour may be observed within as well as between species, and within as well as between habitats. We studied the within- and between genotype variability of phototactic responses in *Daphnia magna*. The results suggest that variability in migration patterns often will be the result of genetic differences. Genotypes are, however, not completely canalized in their phototactic responses. Well-defined and clone-dependent plasticity responses were observed in response to, for instance, the presence of predator-mediated chemicals. Specialization therefore seems to include the development of well-tuned plasticity responses. Variability in vertical migration patterns observed within a natural population can be the direct result of changes in the proximate environment (motivational factors, through modulation and/or developmental conversion), or by the succession of specialized genotypes. Due to the high capacity for local adaptation in zooplankton, especially in cyclic parthenogenetic rotifers and cladocerans, variability between populations is more likely to be due to genetic differences in responses to proximate stimuli (*i.e.* causal factors and/or motivational factors).

2 3 ROOSTING BEHAVIOUR AND WINTER DISPERSION IN BLACK-HEADED GULLS (*LARUS RIDIBUNDUS*). *G. De Schutter* - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

The function of the roost is approached by the search of acting mechanisms, rather than by the more used way of testing theoretical a priori hypothesis. Roosts of black-headed gulls are studied in central Belgium mainly for the night-time behaviours and the patterns of birds dispersion. Different results are presented : 1) the birds are not faithful to their feeding-sites; 2) they adapt to artificial cycle of food availability; 3) different roosts are hierarchically organised in a "roosts-network"; 4) population movements through this network seem to occur in periodical waves; 5) the head-

pointing of roosting and resting gulls could be involved in the dispersal system. I shall try to relate these results to each other in a first and partial approach of the links between roost functioning and winter dispersion.

24 ONTOGENY OF THE NUCLEAR T₃-RECEPTOR BINDING IN THE LIVER OF DWARF AND NON-DWARF CHICKENS. E. Dewil, L.M. Huybrechts, E. Decuyper and E.R. Kühn - Catholic University of Leuven (KUL)

During the embryonic development and postnatal growth, the binding capacity B_{max} and the affinity K_a of the nuclear T₃ binding sites have been determined in the liver of a sex-linked dwarf chicken (dw) and compared to the non-dwarf animal (Dw). The dwarfs are known to be functionally hypothyroid due to a lack of hepatic growth hormone (GH) receptors which make them unable to convert T₄ into T₃ (1). The radioreceptor assay was carried out as described previously (2), B_{max} and K_a were determined by means of Scatchard analysis. The T₄ and T₃ concentration in circulation and hepatic tissue were determined by RIA procedures. In the posthatch period the T₃ concentration in the plasma was clearly lower in the dwarf chickens. On the contrary, T₄ in plasma was higher in dw both in embryo's and in growing chicks. In liver tissue an equal difference between the two genotypes of the T₃ and T₄ concentration was found. Although the total B_{max} was only significantly higher on two points during the considered period, the occupancy of the T₃ binding sites was clearly higher in dw in embryo's as well as in chicks, and this in spite of their lower T₃ concentration. This may be linked to a slower break down of T₃ in the dwarfs compared to non-dwarf chickens. In conclusion, it can be stated that the dwarf chickens compensate their lack of T₃ in circulation and in the cell by increasing the occupancy of the T₃-receptor and therefore are still able to accomplish, to some extent at least, their metabolic functions linked to T₃.

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- (2) E. DEWIL, A. VANDERPOOTEN, E. DECUYPERE and E.R. KÜHN (1991). *IRCS Med.Sci.Res.* 19 : 393-394.

25 THE ORGANIC MATRIX OF MINERALIZATION OF *ASTERIAS RUBENS* (ASTEROIDEA : ECHINODERMATA). P. Dubois - Free University of Brussels (ULB).

The occurrence of intraskeletal organic material (IOM) has been recently demonstrated in echinoid echinoderms. Its role as an organic matrix of mineralization is only hypothesized from preliminary studies. The skeleton -or stereom- of the asteroid *Asterias rubens* contains a coherent organic material structured in radial and circular

components. This material may be extracted and separated into EDTA-soluble (0.04 % to 0.12 % w/w of the stereom) and EDTA-insoluble (0.09 % to 0.11 %) fractions. These two fractions are composed of proteins (72 to 87 %) and carbohydrates (13 to 28 %). Gel filtration HPLC of the soluble fraction shows the unusual elution profiles typical of organic matrices of mineralization. The morphology of abiotic calcite crystals grown in the presence of the soluble fraction of *A. rubens* IOM is strongly modified. Some of the modified crystals mimic crystallites naturally observed within the stereom. This suggests that the IOM of *A. rubens* may act as a true organic matrix of mineralization.

2.6 REPRODUCTION IN A POPULATION OF SHORE CRABS *CARCINUS MAENAS* (LINNAEUS, 1758) IN THE SOUTHERN NORTH SEA. *C. d'Udekem d'Acoz* - Facultés Universitaires Notre-Dame de la Paix, Namur.

The reproductive structure of a population of *Carcinus maenas* is studied at Ostend (Belgian Coast). The crabs mate from April to November but mainly from June to October. The smallest intertidal mating male was 27 mm across, but about 85 % of the copulating males were larger than 44 mm. Small copulating males seem slightly more common after July. Indirect estimations suggests that each male of 44-56 mm would mate yearly with about 3 females. Females reach the puberty (capability to mate) between 23 and 45 mm with a mean of 33 mm. Part of the females that pass through the puberty moult in early summer exuviate once again in the following months before breeding. The females probably reach puberty at a smaller size in autumn than in summer. It is estimated that about 2 % of the females of first puberal intermoult would survive up to a sixth puberal intermoult. Most females that have already bred, mate before August, while most virgin females mate from August onwards. Most large females breed in winter. Part of them have a second or a third brood in the same intermoult during the spring or in the first half of the summer (the probability to have more than one brood by intermoult sharply rises with the breadth of the female). Most small puberal females breed only once in the spring. The number of eggs by brood is proportionnal to the third power of the female breadth. Females of the third puberal intermoult would be the crab category that produces the biggest percentage of larvae. It appears that the reproductive strategies of *C. maenas* are extremely complex, and are optimised for liberating a maximum of viable larvae.

27 DRAMATICAL DECLINE IN THE NUMBER OF BREEDING STARLINGS IN THREE NESTBOX COLONIES AROUND ANTWERP. *M. Eens and R. Pinxten* - University of Antwerp (UIA).

As part of a long-term study on the reproductive behaviour of the European starling *Sturnus vulgaris*, we have monitored three populations in three nestbox colonies around Antwerp (Wilrijk, Zoersel, Kalmthout) from 1983 onwards. In all three nestbox colonies a dramatical decline in the number of breeding pairs has been observed. The number of breeding pairs during the first brood declined from 26 (1983) to 2 (1990) in the colony of Wilrijk, from 25 (1983) to 10 (1990) in the colony of Zoersel, and from 22 (1983) to 5 (1990) in the colony in Kalmthout. Declines in the number of breeding starlings have recently been found all over Europe. Although the cause(s) of this decline is (are) unknown up to now, several possibilities are discussed.

28 SONG LEARNING IN THE EUROPEAN STARLING, *STURNUS VULGARIS*. *M. Eens, R. Pinxten and R.F. Verheyen* - University of Antwerp (UIA).

We recorded the song of captive male European starlings in two successive years to see if changes occurred in song repertoire size and average song bout length. Seven out of nine males sang new song types in their second year of recording. Four out of the nine omitted one or more song types of the previous year's song repertoire. Three males which were yearlings when recorded for the first time increased both their repertoire size and average song bout length significantly while the repertoire size and average song bout length of six older males did not increase. Our results indicate that starlings are 'open-ended' learners, but also suggest that the ability to incorporate new song types into the song decreases with age. Four males that hatched in captivity copied a large proportion of their song types from adult males with whom they shared the aviary. The majority of males hatched in captivity copied song types from at least two adult males. We also present direct evidence that starlings copy heterospecific imitations from other starlings.

29 A STUDY ON THE PREDATION OF *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) (THYSANOPTERA : THRIPIDAE) BY *AMBLYSEIUS CUCUMERIS* OUDEMANS (ACARI : PHYTOSEIIDAE) AND ITS POSSIBLE FEED-BACK ON THE PLANT. *C. Gérin and T. Hance* - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

The Western Flower Thrips, *Frankliniella occidentalis*, is a new pest in glasshouses in Belgium. Few information is presently available on its biology, and particularly on a possible biological control. In this context, the prey-predator

relationships and their feed-back on the host-plant have been analysed in details. Three kinds of experiments were realized : 1) a control where bean plants were allowed to develop without the pest, 2) bean plants with *Frankliniella occidentalis* at a known starting density, 3) plants with the pest and *Amblyseius andersoni* at defined starting densities. The criteria used were 1) the evolution of the weight/area ratio of fresh bean leaves, 2) the leaf damage evolution, 3) the growth of the prey population, 4) the growth of the predator population. In these conditions, it appeared that without the pest, the weight/area ratio remained constant throughout the experiment. In the presence of *Frankliniella occidentalis*, this ratio and the leaf damage area increased linearly until the death of the plant, on day 36. The introduced predators were unable to change this evolution. Prey population growth in presence of *Amblyseius cucumeris* is rather different than without predator, but the reduction in number was not sufficient to protect the plant. This is probably the consequence of the polyphagous diet of the predator and of its poor numerical response to the prey multiplication. Nevertheless, Pelletier (1990) (1) and Van Neck (1990) (2) obtained a good protection in sweet peppers with *Amblyseius cucumeris*. In the future, we plan to study the influence of host plant and of supplementary food in the predator efficacy. But we also intend to test other predators and to deepen our knowledge on the biology of the Western Flower Thrips.

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(2) W. VAN NECK (1990). *BVA nieuws*, januari : 3.

30 ON THE SKELETAL STRUCTURES OF THE ENTEROPNEUSTA (HEMICHORDATA). J.E.A. Godeaux - University of Liège.

In Enteropneusta, the protosoma (proboscis) is bound to the mesosoma (collar) by a short and rather narrow stalk (1, 2). The complex is strengthened by the skeleton and the stomochord. (a) The skeleton, a thickening of the basal membranes underlying the epithelia, can be divided into three parts : a triangular plate set into the proboscis, a thick vertical bar in the stalk and two horns or crurae, running dorso-ventrally around the buccal cavity in the collar. The skeletal substance is well coloured by PAS, aldehyde fuchsin and acid alcian blue; the staining by aldehyde fuchsin and alcian blue is deeper after a preoxidation. Skeleton is also stained by light green and methyl blue but curiously enough toluidine blue (at pH s 3 and 4) remains practically inactive compared with the strong metachromasy exhibited by the collar ectodermal cells. The centre of the median bar, occupied by a yellow sclerified material, displays an obvious argentaffinity. The skeletal substance is made up of neutral and acid polysaccharids, mixed with -SH bearing proteins; polyphenols are present in the oldest parts. The role of the skeleton is merely the stiffening of the weak stalk and the anchoring of the strong muscular bands arising from the collar. (b) The stomochord, an endodermic caecum given off dorsally by the buccal cavity, runs above the skeletal bar and enters into the posterior part of the proboscis. The central lumen is present up to the tip of the organ and even swells in front of the skeletal plate. The pseudo-stratified epithelium is composed of vacuolated

and ciliary cells, radiating around the canal (3). The organ is wrapped in a distinct basal lamina. The stomochord is faintly stained by the different dyes, except the basal membrane, the nuclei and the apical border of the cells (cilia and microvilli) and contrary to the surrounding organs (blood, pericardial vesicle and glomerulus). This is not in agreement with Welsch and Schumacher's (4) observations. A few scattered glandular cells can be observed close to the opening of the caecum. The stomochord does not display any peculiar structure except the developed vacuolization of its cells; may be it bears the surrounding organs but thanks to its cilia, it could also be a possible disposal system for soluble wastes discharged by blood and glomerulus. It has nothing in common with the canal of the so-called neural gland of Tunicata.

- (1) C. DAWYDOFF (1948). *Traité de Zoologie* (P.P. Grassé ed.) 11 : 367-453 (Masson Paris).
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- (3) U. WELSCH and V. STORCH (1970). *Z. Zellforsch.* 107 : 234-239.
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31 DRINKING MECHANISM IN *LACERTA VIRIDIS* (REPTILIA : LACERTIDAE). V. Goosse, K. Kardong* and V. Bels - University of Liège, and *Washington State University, U.S.A.

Drinking bout in *Lacerta viridis* is divided into approach, immersion, emersion and withdrawal phases. During the immersion phase, the water is collected by the tongue. This phase ends when the tongue ceases to make regular contact with water. The emersion phase involves the elevation of the head of the lizard. Quantitative analysis of the gape and tongue cycles in immersion and emersion phases is used to emphasize the variability of the drinking pattern. High speed cinematography (100 frames/s) and cineradiography (60 frames/s) have been used to study the drinking mechanism in relationship with the morphological features of the tongue and the buccal cavity. During each immersion cycle, the water is mainly collected by the dorsal surface of the tongue and brought to a first buccal compartment situated at the Jacobson's organ opening. At the same time, the hind tongue moves the water previously in this compartment, to a second compartment situated posteriorly and laterally to the tongue. When the second compartment is full of water, it is moved to a third compartment involving all of the posterior buccal cavity situated within the throat. During emersion, all the water moves to the third compartment by gravity and then in the esophagus after its opening.

- 32 SURVIVAL RATE OF *PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT (ACARI : PHYTOSEIIDAE) WITHOUT PREY.** *T. Hance* and *G. Van Impe* - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

Phytoseiulus persimilis ATHIAS-HENRIOT is a specific predator of Tetranychid mites and is presently worldwide used in biological control programs (1). It is characterized by a strong numerical response which rapidly induces the prey population extinction (2). When its prey becomes too scarce, *P. persimilis* is forced to disperse or to die, because of this specificity. A new outbreak of the phytophagous mite is then possible and a new release of predators must be performed again. In this context, the purpose of the study was to determine the survival rate of the predator without food, in order to estimate the time during which the crop is still protected. Five cases were considered: 1) the survival of isolated individuals of unknown age; 2) the survival of individuals of unknown age, grouped per 3 (cannibalism allowed); 3) the survival of one-day old isolated individuals; 4) the survival of two-days old isolated individuals (mated individuals); 5) the survival of one-day old isolated individuals in presence of pollen. These treatments were compared with a control where eggs of *Tetranychus urticae* KOCH (Acari: Tetranychidae) were sufficiently supplied. With prey eggs, the maximum survival of *P. persimilis* was 54 days and 51 % of the individuals were still alive after 23 days. The maximum fecundity was 40 eggs per female. With pollen, no eggs were deposited but the last individual died after 32 days (the mean survival being 8.6 days). Without any food, the mean survival was only 6 days for one-day old adults, while it failed to 4 days in average for individuals of unknown age. Cannibalism changed only a little this tendency. For all treatments, the pattern of survival followed a sigmoidal curve. It appears thus that the life expectancy of *Phytoseiulus persimilis* without food is very low. This may explain the sharp sinusoidal evolution of prey and predator populations in crops.

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 (2) TH. HANCE (1988). *Annl. Soc. r. zool. Belg.* 118 : 161-170.

- 33 DEVELOPMENT OF A RADIORECEPTORASSAY FOR INSULIN-LIKE GROWTH FACTOR I (IGF-I) AND TRIIODOTHYRONINE (T₃) IN TILAPIA (*OREOCHROMIS NILOTICUS*).** *L.M. Huybrechts*, *E. Dewil*, *V. Paulissen* and *E.R. Kühn* - Catholic University of Leuven (KUL).

The presence of specific receptors for a hormone is an indication that this hormone can have a physiological function in that tissue. Since both IGF-I and T₃ are very important hormones involved in growth regulation, a better understanding of their action will be of great importance in studies concerning growth control in fish. To develop a radioreceptorassay for these hormones both liver and kidney tissue were used

because of their role in the peripheral production of IGF-I and T₃ respectively. After the tissues were homogenised, either microsomal (for IGF-I) or nucleic (for T₃) fractions were prepared by ultracentrifugation. For both receptorassays conditions concerning temperature, incubation period, pH and tracer concentration were optimized.

TABLE 1

	K _a (M ⁻¹)	B _{max}
T ₃ in liver	4.47 × 10 ⁸	160 fmol/mg DNA
T ₃ in kidney	3.08 × 10 ⁹	207 fmol/mg DNA
IGF-I in kidney	2.27 × 10 ⁸	11.89 fmol/mg protein
IGF-II in kidney	2.42 × 10 ⁸	7.31 fmol/mg protein

In these optimal conditions, displacement experiments were carried out, followed by Scatchard analysis. Table 1 summarizes the results obtained for both hormones. This is the first time that an IGF receptor has been characterized in fish. The binding of IGF-II indicates that this is probably a type I IGF receptor. The absence of IGF receptors in the liver is in agreement with previous results obtained in mammals. Results on nuclear T₃ receptors are also in agreement with others obtained in different fish species. The precise function of the observed receptors has to be investigated in future experiments.

34 SAMPLING MACROINVERTEBRATES IN PONDS : A COMPARISON BETWEEN A PONAR GRAB AND A STANDARD HAND NET. *L. Int Panis, R. Ghosh, W. Jacobs and R.F. Verheyen* - University of Antwerp (UIA).

Sampling and processing of samples is usually the most time consuming task in benthic research. It is therefore important to take samples at the right place with the best equipment. Knowledge of the distribution of communities within a pond is useful to sample as many species as possible. This distribution can be influenced by the presence of stratification, even in relatively shallow (< 4 m) ponds. Profundal benthos is usually poor in species. Littoral areas of the pond have a much higher species richness. A survey of the depth profile with an echo-sounder and the measurement of oxygen concentrations at several depths in the watercolumn before taking samples is therefore important. In a shallow part of the pond Fort VI in Wilrijk (Antwerpen) we took samples twice (january, 4 samples; march, 10 samples) on two different substrates (sand and leaves) with a Wildco Petite Ponar and a standard handnet. The Ponar has a surface of 235 cm². The surface covered with the handnet was much larger (0.3 × 5 m). After sampling, the samples were treated similarly. The Ponar samples had significantly higher numbers of taxa and individuals on both occasions and on both substrates. In the Ponar samples we found 28 and 31 taxa whereas we only collected 13 and 17 taxa with the handnet. All taxa collected with the handnet were also found in the Ponar samples.

Nine taxa (26 %) were present in both sets of Ponar samples but were not found in any of the 14 handnet samples. Six of these are Mollusca. The most abundant taxa that were not detected by the handnet (and the numbers found in the grab samples) are *Bythinella* sp. (40 ind.), Triopsidae (Crustaceae) (28) and *Valvata* sp. (9). The number of Mollusca in the Ponar samples is usually four times higher. A faunal survey of standing waters should therefore be based on Ponar samples taken mainly in the shallow oxygen-rich areas. L. Int Panis is an Aspirant of the Belgian N.F.S.R.

35 A STUDY OF THE COMMUNITIES OF BENTHIC MACRO-INVERTEBRATES IN SOME STANDING WATERS. L. Int Panis and R.F. Verheyen - University of Antwerp (UIA).

Samples of the benthic fauna were collected with a Ponar grab in two ponds in Niel and a fen in Ravels (prov. Antwerpen). For most species the spatial distribution was clumped and some species were significantly associated. Clustering and multivariate analysis were used to distinguish communities within these ponds. Graphical presentation of the main clusters on a map shows that at least four areas with a typical fauna are present in Niel. The deepest central areas are completely dominated by *Chaoborus flavicans* whereas in the other deep areas *Chironomus* sp. is the dominating taxon. In the shallow areas *Oligochaeta* are often abundant and the number of species and diversity are much higher. These areas have a homogeneous faunal composition since most species do not have a clumped spatial distribution within these areas. New biotic and abiotic samples were taken at 4 sites at one of the ponds in Niel to explain the variation as a result of abiotic influences on the fauna. Abiotic factors were correlated with the first axis of the PCA. The results show that depth, grab penetration, dry weight, Md and carbon content of the bottom could all be responsible for the biotic variation. Since many bottom characteristics are related to depth and this pond was stratified in summer, depth could be the most important factor. In the shallow (< 1m) fen, depth was the only factor that could significantly be correlated with the PCA-axes. Further research will be done to measure abiotic factors such as depth more accurately. L. Int Panis is an aspirant of the Belgian N.F.S.R.

36 A TYPOLOGY OF A FEW SMALL STREAMS IN THE FLEMISH REGION, USING MACRO-INVERTEBRATES. W. Jacobs and R.F. Verheyen - University of Antwerp (UIA).

During a one year study period, 11 small streams were sampled with a standard macrofauna-net. We sampled over a distance of 40 meters and samples were taken during two seasons (winter and summer). At the same sample-sites, 32 abiotic (chemical and structural) parameters were measured. These biotic and abiotic data were processed using

multivariate analysis techniques, hence we were able to make an ecological characterization of the sampled streams. We also tried to determine the effect of sampling-place, sampling-season and covered sampling-distance on the final result of the typology made. By comparing the results based on 20 meter- and 40 meter-samples, we were able to investigate the effect of the covered sampling-distance whereas by comparing the results based on samples taken in the winter and in the summer, the seasonal effect was investigated. By comparing different samples taken in one small stream, we hoped to examine the effect of the sampling-place on the final results of the typology. Based on the results of the classification, we could distinguish 4 different types of small streams. The most important abiotic factors responsible for the variation in macro-invertebrate composition were : stream velocity, variation on stream velocity, Ca-content, conductivity and pH. The results of this study show that the effect of the sampling-season and sampling-distance is negligible. The effect of the sampling-place could not be determined due to lack of data.

37 CONTRIBUTION TO THE STUDY OF DIAPAUSE IN *TETRANYCHUS URTICAE* KOCH (ACARI : TETRANYCHIDAE). M. Janssens and P. Nihoul - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

Photoperiodism and quality of food were analysed as environmental factors governing the onset of diapause in a laboratory strain ('White Eye') of *Tetranychus urticae* KOCH. The effectiveness of three inhibiting treatments of diapause was also tested. Experiments were achieved at 15°C on bean leaves and plants. A photoperiodic response curve was first established : the photoperiod is quite determinant and the 'Critical Night Length' (CNL) is clearly defined at 10 hours 1/2. The first post-diapause generation was also tested and the females of this progeny entered diapause too. These results are similar to those obtained with others strains of spider mites (1, 3) and demonstrate the constancy of photoperiodic response in laboratory strains. As to the nutritional factor, experiments proved its great influence at intermediate photoperiods (near the CNL'). Two of the three inhibiting treatments (light interruptions in the dark phase proved to be effective, which is in agreement with results of other authors (2, 3). Lastly, we found a strain of spider mites on glasshouse tomatoes wich appeared to unable to enter diapause. The application of control techniques of diapause like light flashes during the night is possible in glasshouse crops, but first of all depends on the strain capacity to enter diapause. So it is important to test this ability first.

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- 38 ONTOGENIC STUDY OF THE HEPATIC GROWTH HORMONE (GH) RECEPTOR IN CHICKENS SELECTED FOR A GOOD FEED CONVERSION (FC) AND A FAST GROWTH (GR).** W. Janssens, A. Vanderpooten, J. Buyse, E. Decuypere, F. Leenstra* and E.R. Kühn - Catholic University of Leuven (KUL) and *Spelderholt Centre, The Netherlands.

The influence of broiler selection for a good feed conversion and a fast growth on the hepatic growth hormone receptor was investigated during ontogenesis. The former selection resulted in lean chickens, the latter selection resulted in fat chickens. The GH receptor characteristics were determined in liver microsomal fractions (1), and plasma concentrations were measured by means of radioimmunoassay. Only at day 16 of the embryonic development the FC embryos showed a higher specific binding than the GR embryos ($P < 0.05$). On day 18 of the embryonic development, the FC line had slightly more GH receptors than the GR line ($P < 0.05$), simultaneously plasma GH concentrations were increased in the GR line. In young growing chicks of 4 weeks old the effect of selection on the hepatic growth hormone receptor was the greatest: the GR line showed a 2- to 3-fold higher percentage of specific binding than the FC line ($P < 0.001$). The difference at this stage is probably due to down regulation of the receptor by the pulsatile secretion of GH, which is more pronounced in the FC line than in the GR line (2). The adult GR chickens also showed a higher specific binding, but less pronounced than in the growing stage ($P < 0.05$). This difference was due to a higher amount of GH receptors, while the receptor affinity was unchanged. No differences were measured in triiodothyronine, thyroxine or GH plasma concentrations. Therefore, since GH secretion is probably not different between the lines in the adult stage, the difference in amount of GH receptors between the two lines at that age are likely due to another mechanism.

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- 39 EXTRA-PAIR PATERNITY IN THE BLUE TIT (*PARUS CAERULEUS*) : EVIDENCE FROM DNA FINGERPRINTING.** B. Kempenaers and A.A. Dhondt - University of Antwerp (UIA).

Behavioural observations of a Blue Tit population indicated that males guarded their fertile female to ensure their paternity. It could be shown that the mate guarding intensity was highest for monogamous males and lowest for polygynous males guarding their secondary female. If mate guarding intensity reflects the certainty of paternity, one expects polygynous males to suffer more from loss of paternity. To check this DNA fingerprints were produced for 304 nestlings from 36 nests. We detected

33 cases of extra-pair paternity (11 % of the nestlings) in 11 nests (31 % of the broods). In only 1 out of 9 nests of polygynous males (5 nests from primary females, 4 from secondary ones) extra-pair paternity was detected. In 10 out of 27 nests of monogamous males extra-pair paternity was found. This study shows that mate guarding in the Blue Tit can not prevent that females engage in copulations with other males. Although polygynous males guarded less intense, they only rarely suffered from lost paternity. These results are discussed in the light of what is known about the benefits from extra-pair paternity for the females.

40 LACK OF GEOGRAPHIC VARIATION IN TANZANIAN *MASTOMYS* : ZOOGEOGRAPHIC CONSEQUENCES. *H. Leirs, C. Heyrman, R. Verhagen and W. Verheyen* - University of Antwerp (RUCA).

Species of the genus *Mastomys* are the most common indigenous rodents in subsaharan Africa. Morphologically they are hard to distinguish, but in some cases there are clear karyological differences. Currently, two species with $2N=32$ chromosomes are recognized : one from West Africa extending eastward, the other one from South Africa extending northward. The border zone between both species is supposed to be located somewhere in East Africa. In order to test this hypothesis, we collected more than 6000 specimens of *Mastomys* from different localities in Tanzania (1985-89). All of 34 investigated karyotypes showed a diploid chromosome number of $2N=32$. Local seasonal variation in morphometric characters was larger than the variation between localities. An electrophoretic analysis of 9 enzyme loci in 167 individuals from 9 localities revealed neither geographic patterns, nor genetic subdivisions among the Tanzanian species. We therefore conclude that all these Tanzanian *Mastomys* belong to a single species which, according to chromosome banding data, should very probably be referred to as the South African *Mastomys natalensis* (SMITH 1834). It is also suggested that the border zone with the West African *M. huberti* will thus probably be situated more to the north.

41 LIFE HISTORY VARIATIONS IN SUBSEQUENT GENERATIONS OF *MASTOMYS* RATS. *H. Leirs, R. Verhagen and W. Verheyen* - University of Antwerp (RUCA).

Mastomys rats are the most common indigenous rodents insubsaharan Africa. Their breeding activity is strongly related to rainfall conditions. Off-season heavy rains induce off-season breeding. In Tanzania three different types of generations exist in *Mastomys*. The most common generation (A) is born in the main breeding season and breeds in the next main breeding season. A second type (B) are animals that are born in the main breeding season but that will reproduce already after off-season abundant rainfall. The

third generation type (C) is born in the off-season breeding period and will breed in the next main breeding season. Life histories of these three generation types are different with regard to growth, reproduction and survival. Generation A has a long growth stop, has several consecutive litters and reaches an old age ; generation B has a short growth stop, breeds only once or twice and then dies off ; generation C grows very fast, reproduces at young age and has a moderate life-span. Litter sizes are largest for generation A and slightly but significantly lower in the others (Student-Newman-Keuls $p < 0.05$). The occurrence of these generation types depends on climatic conditions. They have an important effect on population dynamics.

42 SEASONAL BREEDING IN *MASTOMYS* RATS : INDUCTION OR INHIBITION BY ENVIRONMENTAL FACTORS ? H. Leirs, R. Verhagen and W. Verheyen - University of Antwerp (RUCA).

Mastomys rats, common African rodents with great economical and public health importance, display well-defined seasonal breeding patterns. These patterns have been explained as an opportunistic response to changing environmental conditions. However, seasonality can also be an evolutionary adaptation to temporal instability : environmental triggers (proximate causes) induce reproduction at such a moment that the young will enter the population under optimal conditions (ultimate cause). Proximate causes are not necessarily related to reproductive requirements, but they let the animal anticipate favorable periods. Such mechanism can only have evolved in predictably unstable environments. In contrast, the so called opportunistic species live in unpredictable habitats. They are thought to reproduce continuously, regardless of the conditions under which the young will enter the population. Only when available resources drop below a certain level, reproduction is inhibited. Although *Mastomys* has many characteristics of a typical opportunist, we present field data, theoretical arguments and literature information to support the existence of environmental triggers for reproduction. The start of the breeding season is well related with milieu conditions but the end is not. Habitat predictability must be considered at time-scales that are relevant within the animals life-span. Recent literature and own laboratory experiments indicate possibly stimulating effects of growing grass on reproduction. We conclude that even in an opportunist like *Mastomys*, short-term predictive triggers for seasonal reproduction are advantageous.

- 43 LUMINESCENCE AND FLUORESCENCE OF DISSOCIATED PHOTOCYTES FROM *AMPHIPHOLIS SQUAMATA* (ECHINODERMATA).** *J. Mallefet, G. Germain** and *F. Baguet* - Université Catholique de Louvain (UCL), Louvain-la-Neuve and *Université de Montréal, Canada.

Although the luminescence of the ophiuroid *Amphipholis squamata* was first described by VIVIANI in 1805, it remained poorly understood. In the first attempt to characterize the basic units of that luminescence, it was showed (1) that luminous cells, termed photocytes, are located deep into the calcareous plates of the exogenous skeleton of *A. squamata*. Recent physiological data suggest that the luminescence may be controlled by a cholinergic nervous mechanism (2). It is still unclear whether acetylcholine is associated with neurons innervating photocytes or elicits luminescence by acting directly on photocytes. This work describes the method we used to dissociate the photocytes embedded into the calcareous skeleton and the evidences that make us believe that these cells are still in good physiological condition. The incubation of tiny chopped brittle star arms in a succession of baths consisting in pronase cocktail (0.5 % at 30°C) and divalent cations free sea water provided us the dissociated cells. The samples of cell suspensions (200 µl) were stimulated by potassium chloride (KCl 200 mM); the so elicited luminous responses were monitored by an IP 21 photomultiplier connected to a chart recorder. Kinetics of those responses were similar to these obtained from isolated arms, but with lower magnitudes. Responses were observed from dissociated cells maintained for up to 5 days in simple culture medium, which consist of ASW added with glucose (0.1 %) and gentamycin (50 mg/l). The comparison of fluorescence spectra (peak at 550 nm) of the dissociated cells and the luminous sites of the entire arms showed no differences. The development of this new method provides us a useful tool for further physiological investigations of the luminous control in echinoderms.

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- 44 REVISION OF THE ARCHIMONOCOLIDIDAE (PLATYHELMINTHES) MEIXNER, 1938.** *P.M. Martens, E.R. Schockaert* and *M.C. Curini-Galletti** - Limburgs Universitair Centrum, Diepenbeek and *Università di Pisa, Pisa, Italy.

The family Archimonocelididae Meixner, 1938 has been revised. The family contains two subfamilies, each comprising two genera : the Archimonocelidinae with *Archimonocelis* Meixner, 1938 and *Meidiama* Marcus, 1946 and the Calvirinae subfam. nov. with *Asilomaria* Karling, 1966 and *Calviria* gen. nov. with respectively

20, 2, 1 and 3 species, 13 of which are new for science. The type species of the family is redescribed. Material of all the Archimonocelididae genera has been studied and supplementary remarks are made on some known species. The genus *Archimonocelis* has a cosmopolitan distribution while the genus *Meidiama* is restricted to the South American coast, *Calviria* to the Mediterranean and *Asilomaria* to the Pacific North American coast.

45 SIMILAR FEEDBACK MECHANISMS ARE INVOLVED IN AFFECTING THYROTROPIC ACTIVITIES OF TRH AND CRF IN CHICKEN EMBRYOS. R. Meeuwis, E. Decuyper and E.R. Kühn - Catholic University of Leuven (KUL)

The aim of our experiment was to show that negative feedback mechanisms already exist in chicken embryos, 18 days of age. Embryos were injected with saline or T₃. Half an hour later a second injection of either saline, TRH or CRF was given. Blood was taken 15 min, 1 and 2 hours after the second injection. Using different RIA protocols T₄, T₃ and corticosterone plasma levels were determined. As expected T₃-preinjection resulted in augmented plasma T₃ levels. Results for T₄ (ng/ml plasma) are shown in the table below.

		Sal.	TRH	CRF
Sal.	15 min.	3.906 ±.628	3.578 ±.687	4.506 ±.853
	1 hour	6.477 ±.908	18.693 ±2.907***	12.827 ±2.372*
	2 hours	2.206 ±.510	8.499 ±1.256***	7.399 ±1.763**
T ₃	15 min.	6.922 ±1.007	8.567 ±.951	9.718 ±.891*
	1 hour	3.820 ±.480	11.086 ±1.571**	6.481 ±.876
	2 hours	1.503 ±.359	3.776 ±.950	3.834 ±1.181

(*: P<.05, **: P<.01, ***: P<.001 vs. saline (ANOVA)).

As in previous experiments (2) CRF as well as TRH is capable of stimulating thyroidal T₄ release, probably through activation of TSH secretion (1). After T₃ preinjection this thyroidal response is clearly less pronounced and vanishes completely after 2 hours. Meanwhile the increase in corticosterone plasma levels following CRF injection does not seem to be affected since the responses in saline and T₃ preinjected animals are similar. These results also indicate that in 18 day old chicken embryos a negative feedback mechanism for thyroid hormones, affecting thyrotrophic cells in the pituitary, is already present.

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46 THE TAXONOMIC STRUCTURE OF THE "OLIVA OLIVA COMPLEX" (MOLLUSCA, GASTROPODA, OLIVIDAE). PRELIMINARY RESULTS. *O. Missa* - Université Libre de Bruxelles (ULB).

Oliva oliva (Linné, 1758) is the type-species of the vast tropical genera *Oliva*. This "species" is in fact a confused assemblage, the "*Oliva oliva* complex", whose taxonomic structure was unknown. A morphological approach is the sole approach applicable in practice to this problem. 22 measures have been carried out on 412 specimens (387 from the "*O. oliva* complex" and 25 from out-groups for comparison). This set of 9,064 measures constitutes the factual basis of our work. The "*O. oliva* complex" was compared to the nearest species. Its limits were restricted to a compact nucleus of specimens, difficult to separate on their total distribution. 17 local phena, some of which are sympatric and clearly distinct at local scale, were delimited and compared. Seven of these phena form clearly a morphological continuum L. The rest of the phena are all separable two by two. After removal of the continuum L, the rest of the sample splits clearly into three distinct groups X, G and B. Group X is the only one which is not separated from the group L. It follows that the taxonomic structure of the "*O. oliva* complex" consists of 3 separate morphological groups L+X, G and B. On sympatry considerations, the groups L+X, G and B must be considered as distinct species. The group X is constituted of two distinct sub-units, BB+SJ and AO of restricted distribution and considered as sub-species (geographic varieties) of L+X. No nomenclatural decisions have been taken at this stage, pending on examination of type-material. The three species L+X, G and B are sibling-species, hard to distinguish by simple visual examination on the whole sample. By contrast, they are easy to separate at local scale. This could be explained by the superposition (on a very broad distribution area) of non-correlated variation clines.

47 PRELIMINARY OBSERVATIONS OF BACTERIAL FEEDING BY PLUMATELLA FUNGOSA, PALLAS, 1768 (BRYOZOA, PHYLACTOLAEMATA). *Z. Moureau, E. Richelle** and *G. Van de Vyver** - Royal Belgian Institute of Natural Sciences and *Free University of Brussels (ULB).

According to several authors bacteria must be one of the food components of Bryozoans. However, until now there was no evidence of their ingestion and digestion by these invertebrates. The present work demonstrates the capacity of *Plumatella fungosa*, a freshwater bryozoan to feed on them. *P. fungosa* were cultivated in laboratory conditions from statoblasts collected in ponds. After hatching, young polypes were fed with formazan coloured *Escherichia coli* and *Aeromonas hydrophila*. Our results establish the ingestion and digestion of bacteria as shown by their transit in the digestive tract and the presence of damaged cells in fecal pellets. Experiments have been performed to quantify the ingestion rate of *E.coli*. This study points out the interest of filter-feeders as bryozoans in water purification.

- 48 DISPERSAL AND PHILOPATRY IN A SEABIRD : THE CASE OF THE KITTIWAKE (*RISSA TRIDACTYLA*).** G. Nève de Mévergnies and J.C. Coulson* - Université Catholique de Louvain (UCL), Louvain-la-Neuve and *University of Durham, United Kingdom

A classical view of seabird colonies was to consider them as distinct entities birth from the genetic and the demographic points of view (1, 2). The main aims of the present study are to assess the proportion of philopatric and emigrating individuals and, in the case of the latter, to obtain estimates of the distances moved between their natal colony and the colony in which they breed. Direct observation of colour-ringed birds in North-East England and the analysis of the British ringing data set of the *British Trust for Ornithology* were used in this investigation. A total of 126 birds originally colour-ringed in North Shields were located in colonies during the 1989 breeding season, and 286 ringing recoveries of Kittiwakes ringed as nestlings were recorded during the breeding season, of which 145 were most probably breeding adults (at least 4 years old). It is estimated that 36 % of the young are philopatric, and these are mainly males. A further 43 % breed in another colony within 100 km of the colony of birth. There are few young birds which recruit 100-400 km away. Birds which emigrate more than 100 km (21 %) show a marked tendency to be south, rather than north of their natal colony and British born Kittiwakes stay within the North Sea - Brittany complex. The previously held view that philopatry was virtually total in colonial seabirds is now untenable, arising out of biased observations. The fact that many young seabirds move to another colony raises a series of important questions. In particular, very little (3, 4) is known about the mechanism which attract young birds to establish colonies and this is an important field for new studies and has considerable importance in conservation and control of colonies.

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- 49 THE ENCYSTMENT OF THE CILIATED PROTOZOAN *PSEUDOMICROTHORAX AGILIS* MERMOD : DEVELOPMENT AND MORPHOLOGY.** S. Noël, N. Greco and J.C. Bussers - University of Liège

The vegetative form of *Pseudomicrothorax agilis* (Ciliophora Hypostomata Leptopharyngidae) is an oval and flattened cell about 50 µm long, with 12 furrows in which cilia are inserted. During encystment the cell progressively form a cyst wall. We propose 5 stages for this rapid phenomenon. Stage 1 : the cell comes to a standstill ; its

morphology is that of the vegetative form. The cell sticks to the substratum by ciliary thigmotactism. Stage 2 : the cell has lost the cilia and sticks to the substratum by mucous secretions. The first cystic layer appears. That's the ectocyst. The ectocyst is an electron-opaque layer and its texture is granular or/and microfibrillar. Stage 3 : in the middle of the furrows some ridges appear. The second cystic layer takes place. That's the chitinous mesocyst. Stage 4 : the ridges are separated by a narrow border. The third cystic layer, the endocyst, can be distinguished. Stage 5 : the cyst is mature, (stage reached about 10 to 16 hours after the beginning of encystment). Its morphology remembers the negative of the vegetative form. It's 30 μm long. The recent ultrastructural studies over 15 species who live in mosses reveal different processes and different structures. *Euplotes muscicola* (GRECO, 1986), for example, has only 2 cystic layers and the encystment is faster. The encysted cell can resist to diminution of water content of the xerophilic mosses where they live. This phenomenon takes place when the dew evaporates. The encystment of *P. agilis* is an outstanding adaptation to survive in the mosses.

50 FIGHTING FOR PERCHES IN THE BLACK-HEADED GULL (*LARUS RIDIBUNDUS*). E. Nuyts - Limburgs Universitair Centrum, Diepenbeek.

At first sight in fights for perches between flying and perching Black-headed Gulls (*Larus ridibundus* L.) the newcomer almost always seems to win. (This observation was made independently by British ethologists and myself.) The strategy that the owner loses the 'desired' object is rather unusual, and is known as the paradoxical strategy. Data were collected to support this hypothesis. Only in 5.5 % of the fights, physical contact was involved. 51 % of the fights were won by a flying gull, 44.5 % by a resting gull and 4.5 % ended in a draw. No significant difference in mean nor in frequency distribution was found for durations of fights won by a flying or a resting gull. Often, gulls did not retaliate if challenged. With these data, we tested six models that could represent the fighting strategy. We conclude that in our study site : 1) in general, fights are not settled by physical contact; 2) the gulls don't use a common or paradoxical Bourgeois strategy. In fact, the outcome of a fight is independent of ownership; 3) no hierarchical status influences the fights; 4) if gulls play a Hawk-Dove-Retaliator-Bully game, they all behave as Hawks. Hence a second key is necessary to analyse the fighting behaviour; 5a) fights can be wars of attrition with random rewards. Only under implausible conditions can 5b) fights be symmetric or asymmetric wars of attrition; 6) display duration transfer information in an assessor strategy.

- 51 IN VITRO AND IN VIVO DESATURATION AND ELONGATION OF LINOLEIC AND LINOLENIC ACID BY THE EUROPEAN EEL *ANGUILLA ANGUILLA* L : PRESENT STATE OF THE ART.** W. Pacolet, R. De Dyn, F. Fontaine, H. Moshage, R. De Schrijver, S. Yap and F. Ollevier - Catholic University of Leuven (KUL).

We investigated the essential fatty acid requirements of the European eel *Anguilla anguilla* L. using *in vivo* and *in vitro* techniques to determine the desaturation and the elongation of dietary and incubated linoleic 18:2(n-6) and linolenic 18:3(n-3) acid. The *in vivo* experiment was conducted during a 4 month feeding period using diets, containing 0.5 or 1 % of both linoleic and linolenic acid combined with 0, 1 or 2 percent, respectively of the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3). Growth rate, mortality, fat content and subsequently fatty acid composition of the muscle tissue are examined. For the *in vitro* methodology, we verified the bioconversion of linoleic and/or linolenic acid during 8 days incubation of eel hepatocytes in 'maintenance culture'. Analysis of the fatty acid composition after respectively 1, 4 and 8 days of these primary cultured hepatocytes compared with the data from the *in vivo* experiment will be discussed.

- 52 POPULATION DYNAMICS, SPATIAL DISTRIBUTION AND HOST-SPECIFICITY OF *LERNAEOCERA LUSCI* IN THE NORTH SEA AND THE DELTA AREA.** P. Parren, P. Van Damme and F. Ollevier - Catholic University of Leuven (KUL).

The aim of this study is to modelate and to describe the transmission of 3 parasite species (*Lernaecocera lusci*, *L. branchialis* and *L. minuta*). The underlying investigation is limited to the second part of the lifecycle of *L. lusci*, namely the infection of the final host. For the research, only bibs 0+ (*Trisopterus luscus*), the final host for this parasitic copepode, were used. The samples were taken with the aid of the Belgian researchship 'BELGICA'. The sampling period started in June 1988 and finished in September 1990. In the study-area, prevalences and intensities of infection were calculated in order to study temporal-and spatial infection patterns. Study of temporal patterns leads to the conclusion that the transmission of infective stages occurs in the summer (from June to September). The highest prevalence is found in November 1988. There are strong indications that the transmission of infective stages occurs in the coastal zone and the estuaries. Besides this, it is found that each individual can produce more than 2 egg strings. This ooservation leads to an hypothetical model of the infection on the final host. A biological model describing the development of *L. lusci* on its final host will be proposed when more information is available about the transmission dynamics of the intermediate host (*Solea solea*).

- 53 SELECTION OF AN ARTIFICIAL CONTROL DIET TO DETERMINE THE FATTY ACID REQUIREMENTS OF AFRICAN CATFISH, *CLARIAS GARIEPINUS*.** R. Pector, G. Caers, V. Blommaert, R. De Schrijver and F. Ollevier - Catholic University of Leuven (KUL).

Seven artificial reference diets were administered in duplo to African catfish larvae. The protein sources used in the respective diets were : 1) casein; 2) casein + yeast mixture a; 3) yeast mixture a; 4) casein + yeast mixture b; 5) yeast mixture b; 6) soya-isolate; 7) meat-isolate. All these protein sources contain only very few polyunsaturated fatty acids. Therefore, these diets could be used to determine later on the fatty acid requirements of *Clarias gariepinus* larvae. After yolk resorption, seven experimental groups of fish larvae (2.77 ± 0.38 mg) were fed one of these diets for 17 days. Partial or complete substitution of casein by the yeast mixtures had a positive effect on growth, survival and the condition factor of the larvae. The reference diets with pure casein or soya-isolate as protein sources resulted in significantly lower mean weights and higher mortalities. The lowest average weight, but the highest survival rate and a good condition factor were obtained with the meat-isolate diet. The results indicate that the basic nutritional requirements of the African catfish larvae were not fulfilled when casein, soya-isolate or meat-isolate were used as the only protein source in the diet. It was also concluded that the yeast mixtures are useful as food components in artificial control diets aiming at the determination of fatty acid requirements of *Clarias gariepinus* larvae.

- 54 AGE-RELATED CHANGES IN INHIBIN, LH- AND FSH-RESPONSE UPON LHRH IN EARLY POSTNATAL LIFE OF EWE LAMBS FROM DIFFERENT CROSSBREEDS.** R. Peeters, J. Van Isterdael, L. Rombauts, G. Verhoeven, D. Vanmontfort and E. Decuyper - Catholic University of Leuven (KUL).

During the early postnatal life (0-10 days and 9 weeks) the basal as well as the stimulated plasma concentrations after LHRH of LH, FSH, inhibin and P4 were investigated in 32 Cambridge crossbreeds (respectively CT and CF : Cambridge ram x Texel ewe or F2 crossbreeds ewe of Milkshopeep and Suffolk breed). After a two hour-period of intensive blood sampling (interval of 15 minutes) the ewe lambs received an intravenous injection of LHRH (50 μ g/0.5 ml NaCl 0.9 %) which was followed by a second intensive blood sampling period of two hours. The LH and FSH patterns after LHRH were highly significant ($P < 0.0001$) influenced by age, but not by crossbreed ($P > 0.05$). While FSH increased with 0.5 ng/ml in 30 minutes after injection at 0-10 days postpartum, the increase of this concentration was more pronounced at 9 weeks : 1.7 ± 0.8 to 5.8 ± 1.8 ng/ml. The difference between the LH responses of the two ages after LHRH was even so remarkable. At 0-10 days the increase of LH concentrations was six

fold after 1 hour (2.2 ± 1.3 to 14.3 ± 7.5 ng/ml) while the concentration increased from 2.7 ± 1.9 to 252 ± 156 ng/ml after 2 hours of LHRH in two month ewe lambs. The difference between the inhibin levels of the two ages was an interesting result. In both crossbreeds the levels decreased from 626 ± 395 U/L at 0-10 days to 156 ± 41 U/L at 2 months ($P < 0.001$). However the plasma inhibin concentrations did not vary during the 2 hour-period after LHRH. Contrary to the other hormones, P4 was significantly influenced by crossbreed. In the CF crossbreed P4 fluctuated around 2 ng/ml during the 4 hours at the two ages while in the CT crossbreed it decreased from 0.30 ± 0.12 ng at 0-10 days to 0.11 ± 0.07 ng/ml at 9 weeks. These results indicate that the sensitivity of the gonadal axis to LHRH stimulation changed remarkable during the early postnatal period of ewe lambs. The increased responses of FSH and LH to LHRH at the early postnatal age (9 weeks) can be linked to the decreased sensitivity of the pituitary for the negative feedback of gonadal steroids and probably not to a decreased inhibition by ovarian inhibin.

55 EXTERNAL STRUCTURES OF RACHIS, RAMI AND RACHIDIAL BARBULES OF FEATHERS BY MEANS OF SCANNING ELECTRON MICROSCOPY. *K. Perremans, A. De Bont and F. Ollevier*
- Catholic University of Leuven (KUL).

In this study external microstructures of rachis, rami and rachidial barbules of feathers from 196 bird species are investigated by means of scanning electron microscopy. The results of the intraspecific study, where feathers taken at fourteen different implantation sites were examined, show that within each species there are no intra-individual, inter-individual, sexual or age dependant differences in these microstructures. Only slight topological variations are detected. In the interspecific study, using the ninth primary, a whole lot of microstructures appearing in numerous combinations are discovered. These microstructures can be divided in 5 categories : 1) villi in different shapes, densities and configurations; 2) striations, from relatively smooth to roughly frayed in the presence of a few villi; 3) pits, with or without a core; 4) cell boundaries and 5) nature of the cell surface. Except for the group of the songbirds (Order Passeriformes), these microstructures can be used for the identification of bird feathers within each group of birds, because almost always clear cut differences are observed between the families studied.

56 RESPONSES OF MALE STARLINGS TO EXPERIMENTAL INTRASPECIFIC BROOD PARASITISM. *R. Pinxten, M. Eens and R.F. Verheyen* - University of Antwerp (UIA).

Intraspecific brood parasitism, in which parasitic females lay eggs in the nests of host females of the same species, has been reported in several populations of European starlings *Sturnus vulgaris*. Because parasitism usually depresses the reproductive success of starling hosts, they may be selected to recognize and remove parasitic eggs. The response of male and female starlings to single conspecific parasitic eggs added experimentally before or after clutch initiation was examined. The response of hosts to experimental parasitic eggs changed significantly after clutch initiation. Altogether 126 of the mimetic eggs introduced into 128 nests prior to onset of laying were removed, while none of the eggs added to 23 nests after clutch initiation was removed. Before clutch initiation, overall ejection rate of parasitic eggs by females was nearly 100 % and significantly higher than that of males (61 %). Ejection rates by males varied with time in relation to clutch initiation by their females. The results suggest that male starlings remove single parasitic eggs, but generally only till about 3-4 days before clutch initiation, when they usually start intensively mate guarding their females, whereas females remove such eggs up to the start of laying. This different response may be explained by the fact that males, unlike females, face the difficulty of having to decide whether a parasitic egg could be their female's first egg. This study provides the first experimental data demonstrating the ability of male passerines to remove intraspecific (parasitic) eggs added before clutch initiation.

57 MATE GUARDING AND MALE QUALITY IN THE GREAT TIT : A TEST OF THE "GOOD GENES"-HYPOTHESIS. *W. Plompen and A.A. Dhondt* - University of Antwerp (UIA).

During the breeding season 1991, we looked at a population of individually marked Great Tits (*Parus major*). The purpose was an investigation of mate guarding, from which the intensity was possibly related to male quality. Using song quality as a measure of male quality (1) we composed two quality groups consisting of six pairs each. We assumed that since song quality is related to lifetime reproductive success and is invariable during the lifetime of an individual, song quality provides a good measure of genetic quality of an individual. The "good genes"-hypothesis (2) predicts that extra-pair fertilizations are beneficial to females paired to a lower quality male, but not to females paired to a higher quality male. We therefore predicted that males in the low quality group would guard their mates more intensely than males from the high quality group. This difference was not found in our data. The data however did suggest that during the fertile period females paired to lower quality males flew away more frequently from their partner than the females paired to a higher quality male. From an

uncompleted paternity analysis on two nests, we can demonstrate that extra-pair fertilizations occur in Great Tits. Mate guarding and territorial behaviour seem to be the most important tactics performed by a male, to avoid loss of paternity. During the fertile period, long distance flights and intrusions in foreign territories are mainly initiated by females. We therefore conclude that in this period females take the initiative for extra-pair copulations.

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58 RECONSTRUCTION OF BELGIAN MEDIEVAL POPULATIONS DIET USING TRACE ELEMENTS ANALYSES OF BONES. C. Polet - Royal Belgian Institute of Natural Sciences.

Since the beginning of the years '80, new approaches of diet reconstruction for ancient populations has been developed (1). These studies are based on chemical analyses of bones (or teeth). Trace elements analyses have been used to discriminate herbivorous and carnivorous or terrestrial and marine alimentation, to evidence differences of social status, problems of intoxication, and so on. We have measured the concentrations of strontium, zinc and lead in ribs of two Belgian medieval populations : the skeletons from the cemetery of Torgny (prov. of Luxembourg, 6-7th centuries A.D.) and the skeletons from the Dunes Abbey of Koksijde (prov. West-Flanders, 12-15th centuries A.D.). Analyses were performed with X-ray fluorescence spectrometry. Both medieval populations are characterized by low lead levels and let suppose that they did not use this metal intensively (in contrast with the Romans). The high concentrations of strontium in the Koksijde population may indicate that an important part of their food came from the sea. The men and women from the cemetery of Torgny display significant differences in their strontium distribution. This can be explained by a diet difference (men eat more meat) or by the fact that many women were pregnant or in lactation. To elucidate the variability of those components and the effect of diagenesis, we will apply the same methods to a modern population and to soil samples from Torgny. Supported by an I.R.S.I.A. grant.

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59 THE CYTOSKELETON. *C. Remacle* - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

An intricate network of filamentous structures generates the maintenance of shape and motility of animal cells. *Mutatis mutandis*, this molecular "cytoskeleton" features functions exerted by organ systems, at a lower level of organization comprising cytobones, cytomuscles, and even cytonerves and cyto vessels. Only some fibers are true mixtures. Most structural elements of the versatile arrangement assemble and disassemble into building blocks. In terms of increasing stability of the polymer, are found tubulin microtubules, actin microfilaments, and the varied intermediate filaments. Each group consists of highly conserved molecules composing gene families probably derived from an original gene in a primordial eucaryotic cell, that was duplicated and modified during evolution. Due to the polarization of the monomer and the head-to-tail polymerization, actin microfilaments and microtubules are intrinsically polarized with plus and minus ends conferring directional information. This is not shared by intermediate filaments, whose polymerization implies tetramers formed by antiparallel, staggered side-by-side aggregation of two dimers. The specific properties of the cytoskeletal fiber according to species or cell type result from two different ways. In the case of intermediate filaments, several classes of cell-specific intermediate filament protein subunits exist. In the case of microfilaments and microtubules, the widely distributed actin or tubulin molecules can associate with binding proteins. These diverse binding proteins meet different requirements. In addition to GTP or ATP bound to the monomer and the monomer concentration, they participate to the regulation of polymerization-depolymerization rate. They ensure positioning of the cytoskeletal fiber to other homologous or heterologous fibers and to plasma or internal membranes. They exert ATP-driven motor action, like type I and II myosins, kinesin, and dynein. Several aspects of cytoskeleton functioning, such as the association with binding proteins, cross-linking, space-filling properties, polymerization, are headed up by local concentration in calcium ions, or protein phosphorylation.

60 INTRASPECIFIC NEST PARASITISM IN THE SPOTLESS STARLING (*STURNUS UNICOLOR* TEMM.). *A. Renard* and *R.F. Verheyen* - University of Antwerp (UIA).

We monitored a nestbox colony (N:42) of Spotless Starlings for the occurrence of intraspecific nest parasitism during one breeding season (Corbara, Corsica, 42°36'N, 8°55'E). Nests were checked daily during the prelaying, laying and incubation period. The eggs were numbered sequentially. The criteria used to determine intraspecific nest parasitism were: 1) the observation of more than one unmarked egg from one day to another in a clutch, 2) the appearance of eggs after completion of the host's clutch. The frequency of intraspecific nest parasitism was 33 % (N:15). One nest was parasitised twice; once during the laying period and once five days after the completion of the

clutch. It was also in that particular nest that the only case of egg removal in a parasitised nest was noted. From the eggs added after clutch completion, one egg, added after five days, did hatch four days after the host's pulli but the hatchling died two days later. The eggs added during the laying period (40 % of all parasitic eggs) all hatched. None of the parasitic pulli however survived longer than five days. It thus seems acceptable that the reproductive success of the parasitised females was, if ever, only slightly influenced; only one female suffered the loss of one egg. This study is merely a proof to the occurrence of intraspecific nest parasitism in the Spotless Starling. A long term study would allow us to deepen this strategy for this less dense colony breeder and compare it with intraspecific nest parasitism in the closely related European Starling (*Sturnus vulgaris* L.).

61 FRESHWATER SPONGES AS INDICATORS OF HEAVY METAL CONTAMINATION. *E. Richelle-Maurer, Y. Degoudenne, G. Van de Vyver and L. Dejonghe** - Free University of Brussels (ULB) and *Belgian Geological Survey

The objective of the present study is to investigate the ability of freshwater sponges to accumulate heavy metals in order to use this property for monitoring either geological or effluent discharge pollutions. Indeed sponges, being suspension feeders, filtering very large volumes of water due to their way of nutrition, are particularly exposed to pollution. Moreover, put in culture in laboratory conditions, they present the advantage of growing massively after transplantation in the field. Metal levels were measured in three freshwater sponge species common in Belgium, *Spongilla lacustris*, *Eunapius fragilis* and *Ephydatia mulleri*. Analyses were carried out using X-ray fluorescence (XRF) and inductively coupled plasma (ICP) spectrometry. The three species studied were found to accumulate the following metals according to different patterns. Fe and Mn are present in high concentration, reaching up to a few percent of dry weight; Zn and Ti accumulate to some extent up to one tenth of a percent, whereas the level of Br, Cu, Ni, Pb, Rb, Sr, V, Zr and Y is lower and greatly varies from one sample to another (in the order of ppm). Cr could be detected in very few samples only. Compared to the two other species, *E. fragilis* appears to be a worse accumulator. Works are on hand to determine species specificity of metal accumulation and to specify where within the sponge (skeleton, soft tissues, gemmules) the different metals are concentrated. The relationship between metal concentrations in sponges and those found in the ambient water and sediments will be established.

62 EFFECT OF BURSECTOMY ON THE ADRENAL CORTICOTROPHIC RESPONSE UPON ACTH *IN VIVO* AND *IN VITRO*.

G. Room, K. Delobelle, B.M. Goddeeris and E. Decuypere - Catholic University of Leuven (KUL).

From literature it is hypothesized that the adrenocortical response to ACTH is positively controlled by the bursa. The bursal factor, responsible for this, is called the 'bursal adrenal activator' (1). It is however not known by which part of the bursa this factor is produced. Our study was set up to gain information concerning this problem. Therefore the corticosterone response to ACTH was investigated in chickens, bursectomized in two different ways, namely hormonally (with testosterone) (3) or chemically bursectomized (with cyclophosphamide) (2). Chickens resulting from both types of bursectomy were subjected to an *in vivo* test. Synacthen, a synthetic adrenocorticotrophic hormone, was injected intravenously. Control chickens were given a saline injection. Bloodsamples were taken at 30, 90 or 150 minutes after the injection. With adrenals from the hormonally bursectomized chickens and their controls, a static incubation has been performed. After a preincubation of one and a half hour, the adrenals were incubated with synacthen during two hours. The results of the *in vitro* test indicate that the adrenals from the hormonally bursectomized chickens showed a lower corticosterone response to ACTH than the adrenals from the intact chickens. This is in agreement with the results of the *in vivo* experiment. Hormonally bursectomized chickens, characterized by a complete absence of the bursa, showed a lower plasma corticosterone response after stimulation with ACTH than the intact chickens. Chemically bursectomized chickens, characterized by a lack of B-cells but an intact bursal stroma, showed no reduced corticosterone response after ACTH stimulation compared to the response of the control group. From these experiments we can conclude that hormonal, but not chemical bursectomy reduced the adrenocortical response upon a synthetic ACTH. Therefore, a bursal factor responsible for this bursal endocrine function must be linked with the stromal cells of this organ.

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63 FINE STRUCTURE AND FUNCTION OF THE METAPLEURAL GLAND IN *ATTA* (HYMENOPTERA : FORMICIDAE). E. Schoeters and J. Billen - Catholic University of Leuven (KUL).

The metapleural gland in several members of the ant genus *Atta* is well developed and is composed of a large collecting chamber with numerous secretory units (each comprising one secretory cell and one duct cell) surrounding its thin cuticular wall and a more sclerotized reservoir. The collecting chamber and the reservoir are clearly

distinguishable, as can be seen on scanning electron micrographs of the internal sclerotized parts. Scanning microscopy also reveals the clustered organization of the secretory cells surrounding the collecting chamber. The secretion of the metapleural gland is released into intracellular collecting ducts surrounded by a microvillar sheath, each connected to the cuticular lining of the collecting chamber. Treatment of half thoraces in a 5 % potassium hydroxide solution made it possible to obtain a survey of the irregular shape of the collecting chamber. Observations on the outer wall of the metathorax and the pronounced bulla of the metapleural gland reservoir revealed the presence of a probably highly specialized cuticular structure beneath the narrow slit-like opening of the reservoir. This cuticular structure resembles a gutter, a conducting ridge which is thought to have its importance in transferring the white secretion from the reservoir opening to the hindleg coxae. Movements of the coxae then are probably responsible for further spreading of the secretion onto the ants body and in the fungus gardens within the nest. Preliminary experiments with pure metapleural gland secretion against the bacteria *Escherichia coli* and the entomogenous fungus *Metarhizium* confirmed an earlier hypoth esis, contributing antiseptic properties to the secretion.

64 AN ECOLOGICAL STUDY OF THE BENTHOS OF MANGROVES AND SURROUNDING BEACHES AT GAZI BAY, KENYA. J. Schrijvers and M. Vincx - University of Ghent .

Twelve sites in the mangroves around Gazi Bay, Kenya, are examined for density, diversity and biomass of meio- and macrobenthic infauna. The stations show divergent environmental factors that differ especially concerning human influence and vegetation (sandbank, *Sonneratia*, *Rhizophora*, *Avicennia*, *Cerriops* and *Bruguiera*). Only the macrobenthic Isopoda, Amphipoda, Polychaeta, Cumacea and Tanaidacea, and the meiobenthic Nematoda are determined up to the level of family (eventually even up to the level of genus or species). The total density hangs in between 1964 and 6101 ind./10cm² for the meiobenthos, in between 265 and 6025 ind./m² for the macrobenthos. The macrobenthic diversity (H') lies in between 1.65 and 3.35 and the total ashfree macrobenthic biomass lies in between 13 and 2079 mg/m². The nematode community has a diversity (H') of an average of 3.88 and a total average dryweight of 0.032 mg/10 cm². Mathematical processing of the data proves that patterns in the community ecology are only vaguely connected to the vegetation type. That connection only exists because the mangrove type forms the base of other, more defining factors, such as sediment, The difference between sandbank and mangrove sediment is actually very clear. Composition of the fauna may eventually be connected to the type of mangrove tree but there can not be decided on by means of this research. A comparison with data in literature shows that Gazi has larger densities and biomass than other 'similar' regions and that Gazi mangals in general also have larger densities and biomass than estuaries and lagoons (contrary to what ALONGI (1990) (1) claims).

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65 PARATENIC HOSTS OF THE SWIMBLADDER NEMATODE *ANGUILLICOLA CRASSUS* PARASITIC IN THE EUROPEAN EEL (*ANGUILLA ANGUILLA* L.). K. Thomas and F. Ollevier - Catholic University of Leuven (KUL).

During one year (March '90-'91) small freshwater fish species were sampled every month at the Kolenhaven, a deadending side-arm of the Albertcanal, and examined upon L3-larvae of *Anguillicola crassus*. Altogether 2088 fishes from 24 species were dissected. Except for 7 species of which we examined few numbers and one species which had been restocked in the Albertcanal, all other (16) species have been seen infected with L3-larvae at least once. In physoclistic fish species the larvae were found in the swimbladderwall : *Gymnocephalus cernua*, *Stizostedion lucioperca*, *Perca fluviatilis*, *Lepomis gibbosus*, *Gasterosteus aculeatus*, *Ictalurus nebulosus*, *Oreochromis niloticus* and *Gobio gobio*. In physostomic fishes the larvae remained in tissue surrounding the swimbladder : *Chondrostoma nasus*, *Alburnus alburnus*, *Scardinius erythrophthalmus*, *Rutilus rutilus*, *Leuciscus idus*, *L. leuciscus*, *L. cephalus* and *Tinca tinca*. Prevalence of infection differed from species to species but was generally higher in physoclistic fish species and highest in *Gymnocephalus cernua*. In this species the highest mean intensity (20.6 larvae/infected fish) was observed as well as the highest maximum number of larvae (146 larvae). Frequency distributions of the larvae in the different fish species show an overdispersed distribution. In physoclistic fish grown up L3-larvae were seen, larvae which will probably not be infective to an eel anymore. The percentage of grown up larvae on the total number of larvae found, differs among these fish species and was lowest in *Gymnocephalus cernua*.

66 KINEMATICS OF THE HEAD RETRACTION IN *CHELODINA LONGICOLLIS* (REPTILIA : PLEURODIRA). J. Van Damme and F. De Vree - University of Antwerp (UIA).

Morphology and kinematics of the cervical system of *Chelodina longicollis* are studied by means of X-ray cinematography. Therefore, radio-opaque markers were inserted in the neck vertebrae. Digitalisation of these markers allowed to calculate head retraction and joint angles between successive vertebrae, both in function of time. The head can be retracted either to the left or the right side, each following a fixed pattern of changing joint angles. This stereotype is most pronounced in the proximal joints. When retracted to the right side, the main bending point is located at the level of the articulation between the fifth and the sixth vertebra. In case of a left retraction, two major bending points are found : between vertebrae 4 and 5 and vertebrae 6 and 7. All these differences between left and right retraction movements might be related to the presence of initial joint angles, centrally in the completely protracted neck. Most likely, this initial cervical configuration, as well as the stereotyped movement patterns, are related to the escape function of the fast head retraction. An erroneous folding of the neck can thus be avoided. Based on this, a fixed neuro-motoric-pattern must be postulated for the neck retraction muscles.

67 ANALYSIS OF THE MITOGEN-INDUCED PROLIFERATION OF CHICKEN PERIPHERAL LYMPHOCYTES. *J. Van Den Block, K. Delobelle and B.M. Goddeeris* - Catholic University of Leuven (KUL).

The purpose of this work was to develop an *in vitro* proliferation test for chicken lymphocytes to represent the *in vivo* immunocapacity. The Nycodenz discontinuous gradient, with a density of 1.075 g/ml, was developed to isolate chicken peripheral lymphocytes. This was compared to other separation methods, namely centrifugation on a Ficoll gradient (density 1.077 g/ml) and slow speed centrifugation. Lymphocytes isolated on Nycodenz always proliferated much better than those isolated on Ficoll and they reached the concanavaline A-(ConA) induced proliferation level of lymphocytes that were isolated by slow speed centrifugation. The pokeweed mitogen-(PWM) induced proliferation of slow speed lymphocytes, however, was lower than that of Nycodenz-isolated lymphocytes. The proliferation of leukocyte populations, isolated of Ficoll, seemed to be inhibited by a high percentage of monocytes. This inhibition was stronger when ConA-induced proliferation was concerned than in the case of PWM-induced proliferation. Secondly the culture conditions for ConA- and PWM- stimulation were also optimised. Finally, we stated a difference in proliferative capacity between Hisex and Warren laying hens. The use of Nycodenz makes it possible to apply a discontinuous gradient to isolate chicken leukocytes that are to be used for mitogen stimulation.

68 THE EFFECT OF TRIIODOTHYRONINE T₃ SUPPLEMENTATION ON THE GROWTH HORMONE (GH) RECEPTOR CAPACITY IS INDEPENDENT FROM ITS EFFECT ON CIRCULATING LEVELS OF GH IN CHICKENS SELECTED FOR LOW OR HIGH ABDOMINAL FAT CONTENT. *A. Vanderpooten, W. Janssens, J. Buyse, E. Decuyper, B. Leclercq** and *E.R. Kühn* - Catholic University of Leuven (KUL) and *Centre de Recherches Avicoles, INRA, France.

In order to investigate the effect of selection for high or low abdominal fat content (1) and of T₃ supplementation on the hepatic GH receptor and on the circulating concentrations of GH and T₃, two lines of chickens, a fat line (FL) and a lean line (LL), were either fed a normal diet (control) or a T₃ supplemented (1 ppm) diet. After 10 days of treatment (at the age of 11 weeks) the animals were sacrificed and livers and blood samples were collected. Plasma concentrations were measured by means of radio-immunoassay and the GH receptor characteristics were determined in liver microsomal fractions as described earlier (2). The control LL chickens showed less specific GH binding ($P < 0.05$) than the control FL chickens due to a lower receptor capacity ($p < 0.001$). This difference was no longer present after T₃ supplementation, since T₃ reduced the number of measurable GH receptors ($P < 0.001$) to an equal level for both lines. The circulating GH and T₃ levels were lower ($P < 0.01$ and $P < 0.05$) for the FL

birds compared to the LL birds in the control condition, but not in the T₃ supplemented condition. Addition of T₃ to the diet resulted in a 20 fold increase of the plasma T₃ concentration, did not suppress the circulating GH concentrations, but abolished differences in circulating GH between both lines. Since the reduction of the number of GH receptors was not accompanied by a significant change in plasma GH concentration, the alteration of the receptor binding might be a direct effect of the high T₃ concentration. As the FL chickens were more sensitive for this T₃ effect than the LL chickens, the difference in GH receptor binding between the selection lines disappeared under the influence of this T₃ treatment.

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69 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISATION OF SOME HONEYBEE RACES (*APIS MELLIFERA* L.).
P. Van Erum and F. Jacobs - University of Ghent.

Two methods (morphometrics and allozyme analysis) which discriminate the 4 main European subspecies of *Apis mellifera* (*A. mellifera carnica*, *A. mellifera ligustica*, *A. mellifera mellifera* and *A. mellifera caucasica*) were compared in order to find out the best way to determine bee races. Samples of 10 to 50 honeybee workers were taken from colonies of known ancestry. A statistical treatment of 6 morphological measurements for each bee made it possible to identify all colonies and to recognize interracial hybrids. The allozyme analysis required hemolymph samples, which were exposed to iso-electrofocusing. Only malate dehydrogenase-allozymes were located. This polymorphic system could only differentiate between the race-group *A. mellifera carnica* - *A. mellifera ligustica* - *A. mellifera caucasica* and the race *A. mellifera mellifera*. Hybrids between the two were also recognized. The advantages of the two applied methods were discussed. The main conclusion is that both methods are adequate to discriminate among the 4 most important European subspecies of *Apis mellifera*, although the biochemical method requires more than one polymorphism system.

70 THE EFFECTS OF MATING ON THE MAIN BIOLOGICAL TRAITS OF *TETRANYCHUS URTICAE* KOCH (ACARI : TETRANYCHIDAE). *G. Van Impe - Université Catholique de Louvain (UCL), Louvain-la-Neuve.*

The aim of this study was to compare longevity and fecundity of mated and unmated females of the twospotted spider mite. To this end, 214 mated and 112 unmated females were observed, daily, during their complete adult lifetime. Experiments were performed on bean leaf discs, in controlled conditions (24°C). The mean adult

longevity was 14.9 days (s : 11.1) for mated females and 29.2 days (s : 16.3) for unmated ones, the mean difference being $14.3 (\pm 3.4)$ days ($\alpha = 0.05$). This proceeds partly from a longer oviposition period in virgin females (16.4 days) compared to mated individuals (8.7 days). In a same way, fecundity was higher for virgin females (104.8 eggs/fem; s : 46.9) than in mated mites (72.5 eggs/fem; s : 47.5), but daily fecundity appeared to be significantly lower in unmated females (3.5 eggs/fem.day; s : 4.0) than in mated ones (4.6 eggs/fem.day; s : 4.9). Thus, mating induces a higher daily rate of egg laying, but during a shorter oviposition period. In other terms, mated females tend to produce their (female) offspring more precociously, which contributes to induce the typical high rates of increase of *Tetranychus urticae* populations. On the other hand, a female remains unmated only if it is removed from its original population. This may occur, for instance, through phoretic dispersal of eggs or immature individuals. In this case, the virgin female appears to be, comparatively to mated females, well adapted to its colonizing role. Indeed, it will tend to live longer and to produce less eggs at the beginning of its adult lifetime but, as these eggs are exclusively male eggs (arrhenotokous parthenogenesis), the chance of mating with its own "sons" will be enhanced. Once fertilized, this solitary female will produce new females and thus set up a new population on the colonized plant.

71 MECHANISMS OF KCl TRANSPORT IN MALPIGHIAN TUBULES OF *FORMICA*. E. Van Kerkhove, R. Weltens, A. Leysens, N. De Decker, S.L. Zhang and P. Steels - Limburgs Universitair Centrum, Diepenbeek.

Malpighian tubules of *Formica* secrete K^+ , followed by Cl^- and water, against a transepithelial electrochemical gradient. The transport rate increases when the bath K^+ concentration, $[K^+]_{bl}$, is raised (1). Intracellular potential and K^+ activity measurements with conventional and double-barrelled K^+ -sensitive microelectrodes, respectively, revealed that across the basolateral membrane (bath side) a small, but cell-inward electrochemical gradient is present for K^+ . Across the apical membrane (luminal side), however, a large gradient is present, opposing K^+ extrusion. The active step of K^+ transport is located in the apical membrane (2). *Mechanisms of transport across the apical membrane* : it was proposed (3) that a V-type H^+ -ATPase in the apical membrane of another insect epithelium (midgut of *Manduca sexta*) is the prime mover in K^+ secretion. The H^+ pump builds up a proton gradient across the membrane. The latter gradient is then used by a K^+/H^+ antiporter to extrude K^+ from the cell into the lumen. In spontaneously secreting Malpighian tubules of *Formica* luminal and intracellular measurements with double-barrelled H^+ -sensitive microelectrodes showed that active transepithelial proton secretion occurs and that a large (103 mV) H^+ gradient is built up across the apical membrane. In control Ringer (51 mM K^+ , 57 mM Cl^- , pH = 7.25) the cell pH is alkaline (7.81 ± 0.04 S.E. n = 19) with respect to the lumen (7.45 ± 0.09 S.E. n = 12). Furthermore, when comparing the concentration gradient for K^+ ($[K^+]_{cell}/[K^+]_{lumen} =$

1.6) and H^+ ($[H^+]_{cell}/[H^+]_{lumen} = 2.3$) across the apical membrane, it is clear that the cell inward gradient for H^+ is large enough to drive K^+ extrusion with an electroneutral antiporter, exchanging 1 K^+ for 1 H^+ . *Mechanisms of intrinsic regulation of K^+ transport* : when bath $[K^+]_{bl}$ is increased 10 times, K^+ transport accelerates with a factor of about 5 (1). From cable analysis in luminally perfused tubules (same solutions used for luminal and bath perfusion) and from intracellular potential and K^+ measurements it could be concluded that $[K^+]_{bl}$ controls the transport rate in two ways : 1) the cellular K^+ concentration increases, when $[K^+]_{bl}$ is raised, so more K^+ is available in the cell for secretion across the apical membrane and 2) the apical over basolateral membrane resistance ratio is very high (44 ± 8 n = 6) in control conditions. Analysis of the electrical equivalent circuit of the epithelium shows that in that case the basolateral membrane will impose its electromotive force on the apical membrane. The basolateral membrane is very permeable for K^+ . So the electromotive force across this membrane is almost equal to the K^+ diffusion potential. The overall result is that an increase in $[K^+]_{bl}$ for instance will depolarize the basolateral, and at the same time, the apical membrane. The increase in $[K^+]_{cell}$ (see 1) and the decrease in electrical potential gradient to be overcome across the apical membrane (see 2) both contribute in lowering the total gradient against which K^+ secretion has to take place. This will facilitate K^+ , Cl^- and water transport.

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72 THE SOURCE OF IMMUNOREACTIVE INHIBIN IN THE CHICKEN OVARY. D. Vanmontfort, L. Rombauts, E. Decuypere and G. Verhoeven - Catholic University of Leuven (KUL).

The ovary of the domestic hen contains a hierarchy of yolk-filled follicles. The measurement of the steroids in the preovulatory follicles and serum have been reported. The objective of the present study was to determine whether immunoreactive inhibin occurs in the ovary of the hen and, if so, whether it is produced by the granulosa or thecal layer from the small and/or large follicles. In a first experiment, hens were subjected to an induced moulting program and blood samples were taken daily from the brachial vein from the time of food withdrawal during ten consecutive days. Induced egg laying stop did significantly decrease the plasma inhibin concentration. The observation that the inhibin plasma concentration decreased independently from the presented ay, *i.e.* in function of the day of the last layed egg or in function of the day of food restriction, may be linked with a preferential role for the small follicles to produce inhibin. In order to gain further insight in the steroidogenesis and source of inhibin within the ovary, the progesterone, oestradiol and inhibin concentrations of the theca and granulosa cells from

the four largest follicles of the ovarian hierarchy were measured in a second experiment and this at 4h intervals during the 24-26th ovulatory cycle. Our results are confirming the literature data about the oestradiol and progesterone concentration of the theca and granulosa cells during follicular maturation. Inhibin was mainly found within the granulosa tissue. The inhibin concentration was significantly ($P < 0.05$) higher in the F4 than in the F3 granulosa tissue and in the F3 compared to the F2 and F1 granulosa tissue ($P < 0.05$). There was no significant cyclic variation in inhibin concentration for individual follicles during the ovulatory cycle. The highest inhibin concentration coincide with the highest aromatase activity of the thecal layer and hence with the highest E2 concentration during the follicular maturation. However, the physiological significance of these changes in inhibin concentration during follicular maturation remained to be investigated.

73 PERITUBULAR SMOOTH-MUSCLE-LIKE CELLS IN THE QUAIL TESTIS. *L. Van Nassauw, F. Harrisson and M. Callebaut* - University of Antwerp (RUCA).

A considerable volume of work has been carried out on the arrangement and ultrastructure of the smooth-muscle-like cells in the connective tissue underlying the germinal epithelium of the vertebrate testis, particularly in mammals. Only one study has been devoted to the boundary tissue of seminiferous tubules in birds (1). In the present study, we have immunocytochemically localized desmin and -smooth-muscle actin, which are both mainly detected in smooth-muscle cells, in testes of adult quails. We demonstrated both proteins not only in the vascular smooth-muscle cells, but also in the spindle-shaped cells of the tunica albuginea testis and in spindle-shaped cells localized in the boundary tissue of seminiferous tubules. The latter were arranged in a single layer, forming a coat of smooth-muscle-like cells around the seminiferous tubules. In the wall of the testis, this coat was connected with the tunica albuginea testis. In the fowl, a multilayered boundary tissue was described (1) in which two distinct cell types are observed : an inner fibroblast-like cell and an outer myoid cell. It was observed (1) that with increasing age and activity, the boundary tissue of the seminiferous tubules becomes thinner. We conclude that, in quail testis, smooth-muscle-like cells are present and are similar to the contractile cells demonstrated in mammalian testes. We suggest that these cells may be important in sperm transport and in mechanical support.

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- 74 **THE FACTORS STRUCTURING THE MEIOBENTHOS COMMUNITIES IN THE SHELF BREAK AREA IN THE GULF OF BISCAY (N.E. ATLANTIC).** A. Vanreusel and M. Vincx - University of Ghent.

The meiobenthos of seven stations in the Gulf of Biscay ranging from 70 to 235 m depth are investigated in order to characterize the zone of transition from the continental shelves to the deep sea. The nematode communities, which are the dominant taxon, are relatively poor compared to those of shallow coastal areas. Small species are dominant and the total biomass is low. The species richness, however, is much higher than on the continental shelves, and even comparable to deep sea situations. The meiobenthos densities are correlated with densities of the microbial populations and with the oxygen supply. Chlorophyll a and sediment composition are also important structuring factors. The combined importance of food supply and oxygen is striking for the determination of the density of the meiofauna.

- 75 **BIOCHEMICAL CHARACTERIZATION OF FISHPATHOGENIC VIBRIO-SPECIES.** I. Van Roosbroeck, L. Grisez, R. Ceusters and F. Ollevier - Catholic University of Leuven (KUL)

A total of 262 strains of the Vibrionacea (of which about 14 were type-strains) were subjected to 152 biochemical and morphological tests. Most of these strains belong to the genus *Vibrio*, but also strains from related genera (*Aeromonas* and *Photobacterium*) and some *Pseudomonas*-strains have been included in this study. Besides 9 fishpathogenic *Vibrio*-species, some non-pathogenic strains were also investigated. For the testing, two teststrips (API 20E and API CH50, Biomérieux, Belgium) and 82 traditionally performed biochemical and morphological tests were used. The results of these tests were submitted to a cluster analysis (UPGMA from SAS). The dendrogram obtained from this cluster analysis shows the relation between the different fishpathogenic species and between pathogenic and the non-pathogenic vibrios. Special attention was given to the variance within the species *Vibrio anguillarum*. Based on the results, a determination key and a determination table for fishpathogenic *Vibrio*-species were made. The most important conclusion from this research is that there should be a partial review of the taxonomy within the genus *Vibrio*.

- 76 **GRIPPING MECHANISM IN DIDELPHIS INCLUDES PRE-HENSIVE PATTERNS.** K.J. van Zwieten, P.L. Lippens and M. Honinckx - Limburgs Universitair Centrum, Diepenbeek.

The hands of all primates, including man, possess convergent-divergent digits. The brush possum and the common opossum also have hands showing convergent positions (adduction of digits) and divergent positions (abduction of digits) (1). It is

evident that the muscular structure and grasping function of the opossum hand are adaptations for manipulating the food, and for an arboreal habitat. Within the limits of whole-hand control, the patterns used in locomotion and food grasping in the opossum correspond to different functional optima, which possibly cohere with the presence of two sets of long extensors of the fingers (2). Locomotion in the opossum is characterized by, among others, extension and abduction of the fingers at the end of the swing phase (3). In the present study, voluntary reaching in *Didelphis marsupialis* was analysed. Reach is initiated by progressive flexion of wrist and fingers, followed by extension of the wrist, and extension and abduction of the fingers. After the grasp, the hand returns to stance in the original position. These observations suggest that a comparable chain of prehensive patterns is included in both locomotion and gripping mechanisms.

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77 OXYGEN UPTAKE RATE AND HYPOXIA TOLERANCE IN CICHLID FISHES FROM DIFFERENT HABITATS. *E. Verheyen* and *W. Declair** - Royal Belgian Institute of Naturel Sciences, Brussels and *University of Antwerp (RUCA).

Cichlids are freshwater fishes that are widely distributed in the tropical areas of the world. Many morphological, ethological, ecological and genetical studies have bearing on the evolutionary processes that resulted in the Africal lacustrine cichlid fauna with its extreme ecological diversity and high degree of endemism. However, little is known about the physiological aspects of adaptive radiation observed in lacustrine cichlids. The present paper studies the oxygen consumption of five lacustrine species from oxygen rich water (*Tropheus moorei*, *Melanochromis auratus*, *Julidochromis marlieri*, *Eretmodus cyanostictus*, *Neolamprologus brichardi*), three other lacustrine species (*Astatotilapia burtoni*, *Nimbochromis venustus*, *Dimidiochromis compressiceps*) and two non-lacustrine cichlids (*Oreochromis niloticus*, *Steatocranus tinanti*) under declining dissolved oxygen levels. The species-specific critical oxygen tensions (P_c), defined as the PO_2 below which VO_2 ceases to be independent of ambient PO_2 , were determined for all the listed species. A surprising result is that there are important differences in the capacity for the regulation of oxygen uptake under hypoxia for species with essentially the same habitats where dissolved oxygen levels are concerned. It is suggested that differences in the capacity for the regulation of oxygen uptake rate under declining dissolved oxygen levels may be reflections of different ecological characteristics of the different ancestral lineages of the extant species.

- 78 TAXONOMIC AND PHYLOGENETIC STUDY OF SOME GASTROPODS FROM LAKE BAIKAL, SIBERIA (USSR).** *E. Verheyen, T. Sitnikova**, *D. Sherbakov** and *T. Backeljau* - Royal Belgian Institute of Natural Sciences, Brussels and *Limnological Institute, Siberian Division of the Academy of Science of the USSR, Irkutsk, USSR

A four year research program on the biochemical systematics of - mainly endemic - gastropods from Lake Baikal was started by the Biochemical Taxonomy Section at the RBINSc and the Limnological Institute at Irkutsk. During a two week visit in June 1991, a preliminary sampling program was carried out in the southern basin of the lake. Four localities at the eastern coast of the lake and at three sites along the Angara river in Irkutsk were sampled. Scuba divers collected eight gastropod species at 5-10 m depth in the lake, while in the Angara river the dense vegetation close to the shore was sampled for *Lymnaea* spp. The collected specimens were either frozen in liquid nitrogen for allozyme studies or fixated in 94 % ethanol for mtDNA sequencing. The initial study deals with taxonomical problems on some endemic taxa *i.e.* the Baicaliidae. Future studies will focus on the population genetics and phylogeny of this endemic family. Supported by FKFO-MI 30.35, "Systematics and Ecology of Selected animal groups in Lake Baikal".

- 79 INVENTARISATION OF THE FISH SPECIES OF THE ALBERT-CANAL AT GENK - LANGERLO.** *H. Verreycken, C. Belpaire* and *F. Ollevier* - Catholic University of Leuven (KUL).

In the framework of an investigation on the impact of the cooling water intake at the Electrabel power plant (Langerlo - Genk) on the fish populations of the Albertcanal, an inventarisation of the fish species of the Albertcanal was carried out. During a one year sampling period with fykes, gill-nets, trawl nets, electro-fishing and especially continuous sampling at the screens of the cooling water intake, 39 fish species were collected. Most of these 39 species such as eel, roach, bleak, pike perch and perch belong to the common species of the bream zone. Also some less common and even rare species as lampern, bitterling and wels were found. From the barbel and grayling zone chub, nose carp, dace, barbel, brown trout and salmon were determined; it is highly probable that these species have descended from the Meuse. All the salmons were marked and originated from a reintroduction experiment of the University of Liège. Sea trout, flounder and smelt are brackish water species but are also known to migrate in fresh water. Finally also some "exotic" species as rainbow trout, grass carp and tilapia were noticed, most of them probably coming from nearby fish culture stations.

- 80 HABITAT SELECTION IN BELGIAN LAPWINGS (*VANELLUS VANELLUS*) : TO BELONG TO A REGION OR TO A COMMUNITY ?** A. Versailles - Université Catholique de Louvain (UCL), Louvain-la-Neuve.

In Belgium, to belong to such or such region has the reputation to be "The" question. It seems to be the case for lapwings too. In my two study areas (one in the polders, near the coast and the other one in the Condroz) the surface areas, covered either by meadows or by crop fields, are comparable. But the lapwings do not choose the same nesting habitat at the same moment. In the polders, first breeders prefer meadows, later ones choose crop fields. In the Condroz, despite the lower hatching success in crop fields due to agricultural practices, are lapwings choosing nearly exclusively this nesting habitat. An inquiry by Belgian ringers and birdwatchers suggests that, before may 1st (sowing time), "flemish" lapwings prefer to place their nest in meadows and "walloon" ones in ploughing lands. After this date, all of them choose crop fields (maize, beet, garden peas). In Belgium, before the sixties, lapwings brood only in wet meadows, in the northern part of Belgium. Now, they become more or less common all over the country. Why are our "walloon" lapwings avoiding meadows ? We can imagine two explanations : 1) lapwings have progressively colonized the southern part of Belgium from the north. And they had to change their habitat choice because meadows, in this part of Belgium, are not suitable for them. In this case, the choice could be called regional. This hypothesis will be explored especially with remote sensing datas; 2) lapwings have colonized the southern part of Belgium from any other, southern or eastern, country where they brood in ploughing lands; and they continue, by tradition, to choose this habitat, despite it is no more the best choice. In this case, the choice would be a cultural one. This hypothesis will be explored with DNA analysis. Supported by an IRSIA grant and by a FDS project.

- 81 GENE TRANSFER AND EXPRESSION OF THE CMV- β GAL FUSION GENE IN EMBRYOS OF THE AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AND THE ZEBRAFISH (*BRACHYDANIO RERIO*).** F. Volckaert, B. Hellemans, A. Belayew* and F. Ollevier - Catholic University of Leuven (KUL) and *University of Liège.

Our research focusses on the *in vivo* testing of homologous inducible and tissue-specific fish promoters. In a first step, we wish to set up optimal conditions for foreign gene injection in early embryos of the African catfish (*Clarias gariepinus*). Zebrafish (*Brachydanio rerio*) were used in parallel as control. Embryos of both fish were injected with 0.5 to 50 pg of linearised CMV- β Gal during the one and two-cell stage. Survival till hatching (24 h) and yolk sac resorption (72 h) of African catfish did not differ among treatments. DNA integration was measured with dot blot and Southern blot hybridisation. Enhanced replication of the insert was observed till 8 h after fertilisation in African catfish (sommité stage) and zebrafish. A histochemical assay of β Gal

expression showed that 30 % of the catfish had a mosaic pattern at 24 h (no expression occurred before that time). Zebrafish showed 15 % mosaic expression at 48 h and 60 to 90 % transient expression at 3 and 4 days of age. It is concluded that 1) replication of a CMV-βGal linear construct occurs in African catfish and zebrafish, 2) expression of the CMV promoter occurs post-hatching, 3) CMV is suitable as a control promoter despite its human origin, and 4) African catfish is a suitable model for the assaying of foreign gene expression in embryos.

82 ELECTROPHYSIOLOGICAL INVESTIGATION OF THE MID-GUT OF THE COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA*) : EFFECT OF A NATURAL INSECTICIDE.
*R. Weltens, M. Peferoen**, *P. Steels* and *E. Van Kerkhove* - Limburgs Universitair Centrum, Diepenbeek and *Plant Genetic Systems, Gent.

The present work describes for the first time a method to study the electrophysiology of the isolated midgut of the small larval Coleoptera. The effect of the natural insecticide, δ-endotoxin, on the transepithelial potential (V_{te}) and the total transepithelial resistance (R_{tot}) was investigated. The crystal inclusions, typically produced during sporulation of *Bacillus thuringiensis*, contain proteins (δ-endotoxins) exhibiting a highly specific insecticidal activity after enzymatic breakdown (1). Intensive research over the past decade revealed a broad natural diversity of Bt strains and some insight in the mode of action of δ-endotoxins (2). Most studies of the histopathology and mode of action have been carried out on isolated larval lepidopteran midgut and on cultured insect cells of other tissues. The effect of the insecticide on the midgut of the smaller larval coleopteran species was restricted to morphological and histological effects (3) and to biochemical binding studies on microscopic slices (unpublished results). The results from these investigations should be confirmed by studies on isolated larval gut tissue for the following reasons: 1) the cells are alive, 2) the epithelium is free of peritrophic membrane and 3) no digestive enzymes attack the toxins. Therefore the direct admission of active toxin to living cells of the isolated epithelium can be investigated. Since it is known that the toxins form ionic channels (4) an electrophysiological approach is desirable. The composition of the Ringer solutions (1.5 mM NaCl, 15 mM K3-citrate, 7 mM KCl, 10 mM Ca-gluconate, 35 mM MgCl₂·6H₂O, 40 mM MgBr₂, 2.8 mM alanine and 12 mM Hepes; pH was adjusted to 7.2 and osmolality was adjusted to 370 mOsm by adding glucose) was based on the ion concentrations measured in the hemolymph (5). A larva was dissected under Ringer solution: the abdomen was opened and the complete gut was removed. The part proximal to the implantation of the Malpighian tubules was then isolated (about 2.5 mm) and transferred to the experimental set up. Both ends of this isolated midgut of larval *Leptinotarsa* were cannulated by double barreled glass pipettes and tied up: one barrel was used to perfuse the lumen ($\pm 11 \mu\text{l}/\text{min}$) and a 3 M KCl agar bridge (4 %) was brought into the second barrel to measure the transepithelial potential both at the perfusion (ΔV_{te}^0) and collection side (ΔV_{te}^L). Due to the surrounding muscle layer the intracellular potential

(ΔV_{bl}) could only be measured in a few cases by conventional 3 M KCl microelectrodes. Current was injected via the perfusion pipet (11 μ A during 1 second, every 10 seconds). From the according potential deflections it was possible to perform cable analysis. In symmetrically perfused guts (control Ringer) R_{tot} was $100 \Omega \cdot \text{cm}^2 \pm 9.5$ (n=19) and the voltage divider ratio (R_{ap}/R_{bl} with R= resistance, ap = apical membrane, bl = basolateral membrane) was 8 (preliminary result). The estimated short circuit current (V_{te}/R_{tot}) was $10 \mu\text{A}/\text{cm}^2$. The transepithelial potential is sensitive to changes in $[K]_{bath}$: when $[K]_{bath}$ is decreased from 75 to 7.5 mM K (substituted by Na) V_{te} alters from $+1.1 \text{ mV} \pm 0.9$ (n=53) to $-31 \text{ mV} \pm 1.5$ (n=48) in a freshly dissected epithelium. This 'K response' becomes smaller in time, because of a gradual depolarization in low $[K]_{bath}$. The reason for this decrease is unknown, but could be due to the absence of specific substrates *in vitro*. When toxin (*Bt tenebrionis*: CryIIIa) was added (17 $\mu\text{g}/\text{ml}$) no immediate response was seen on V_{te} , but the gradual depolarization of the potential in low $[K]_{bath}$ was much faster and more extreme than in normal conditions. R_{tot} decreased to very low (immeasurable) values within 20 to 30 minutes after admission (n=4). These effects were irreversible. The results can be explained by the membrane disruption, caused by binding of the toxin to the receptor (3, 6). From this we conclude that 1) our set up for the isolated perfused midgut of smaller insect species can be applied successfully for electrical circuit analysis and 2) that the technique allows to further explore the mode of action of δ -endotoxin on the pure epithelium. This work was supported by I.W.O.N.L. (Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw).

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83 CALCIFICATION RATE OF THE CARIBBEAN SCLEROSPONGE *CERATOPORELLA NICHOLSONI*. Ph. Willenz and W.D. Hartman* - Université Libre de Bruxelles (ULB) and *Yale University, Peabody Museum of Natural History, U.S.A.

It has long been believed that Porifera were not playing a significant role as framework builders on modern coral reefs. However, about 15 species with an unusual massive calcareous skeleton, bearing a striking resemblance to that of various corals, were found in submarine caves and cryptic habitats of coral reefs. They appear to be the survivors of reef-builder organisms of the Paleozoic and Mesozoic eras, such as stromatoporoids, chaetids and sphinctozoans, which were believed to be extinct. These

living fossils, the sclerosponges, are exceptionally abundant on the fore-reef escarpments of the North Jamaican reefs and are considered as important contributors to the calcareous framework at ranges greater than the lowest limit of distribution of scleractinians. Sclerosponges provide us with the unique opportunity to measure the growth rate of the calcareous skeleton of organisms which had an important geological role, as can be estimated by the abundance of ancient reefs they generated. Calcein, a fluorochrome stain taken up by calcifying tissues, has been employed to mark newly deposited aragonite in the skeleton of *Ceratoporelle nicholsoni* (Hickson), the most abundant sclerosponge of the Caribbean. Experiments were performed *in situ* in a reef tunnel at a depth of 28 m off Pear tree Bottom, Jamaica. Plastic bags containing a solution of the dye in sea water were tied around specimens and removed 24 hours later. Treatments were repeated on the same specimens after times ranging from one week to several months. Ground sections perpendicular to the surface of harvested samples were examined by fluorescence microscopy. A mean annual extension rate of less than 0.2 mm was calculated from the distance measured between successive fluorescent lines. These data are consistent with the hypothesis that the forebears of recent sclerosponges had been outcompeted as reef builders by the more rapidly calcifying scleractinian corals.

84 THE EVOLUTION OF BODY MUSCLE COMPOSITION OF *CLARIAS GARIEPINUS* (BURCHELL, 1822). H. Zamal, J. Maithya and F. Ollevier - Catholic University of Leuven (KUL).

The changes in gross body muscle composition of the African catfish *Clarias gariepinus* during growth was evaluated. Hundred *C. gariepinus* fingerlings were reared in 2 replicate culture tanks of 60 l in a flow-through system from 1 g to 383 g body weight (BW) and fed with *trouvit*. Protein levels in muscle were determined at different time intervals using Kjeldahl technique. Total fat content was analysed with the soxhlet method (petroleum ether extraction) after acid hydrolysis. Dry weight (DW) was determined by drying muscle samples at 100°C for a minimum of 16 h. Ash free dry weight (AFDW) by ashing the dried samples overnight at 550°C and subtracting these data from DW. The following data were obtained :

Weight and length relationship :	$W = 0.01 L^{2.84}$
Percentage of protein in the muscle :	$13.80 BW^{0.058}$
Percentage of ash in the muscle :	$2.95 BW^{-0.19}$

Fat content was considerably low (1.35 % - 2.66 %) throughout the whole experimental period and was not related with changes in BW. The following body muscle constituents were positively related to changes in live body weight of the fish : % protein (P<0.001), % DW (P<0.01) and % AFDW (P<0.001).

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