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ABSTRACTS

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MODELLING PECTORAL FIN ADDUCTION OF GOBIES. *D. Adriaens, D. Decleyre* and *W. Verraes.* University of Ghent, Lab. Morfologie en Systematiek der Dieren, Ledeganckstraat 35, B-9000 Gent.

In general *Pomatoschistus lozanoi* cannot be regarded as a good swimmer. Swimming occurs by means of short swimming-darts where the propulsion is generated through tail-fin motion and pectoral fin adduction. The latter was analysed by means of a physical model, constructed on the basis of the morphological structure of the pectoral girdle and fin of *P. lozanoi.* Two springs were attached to the medial side of the shoulder- and fin-plate, simulating the contraction forces of the fin adductor muscles. The model-fin was operated in water. Two types of displacement were video-recorded : one where both the fin-plate and the shoulder-plate could rotate freely (type I) and another where the shoulder-plate was restricted in a forward rotation by Baudelot's ligament between the shoulder-plate and the neurocranium (type II). Using the coordinates of the model-parts, the pressure drag on the shoulder- and fin-plate was calculated. In both motions the shoulder-plate exerted a forward rotation, producing a negative pressure drag and so resulting in a braking effect during forward propulsion. In order to calculate the pressure drag on the fin-plate, an equation point had to be taken into account, *i.e.* the point on the fin-plate where the backward rotation speed of the fin-plate equals the forward rotation speed of the shoulder-plate-end. In type I displacement, the positive pressure drag produced by the backward rotation of the fin-plate is neutralised through a negative pressure drag generated by the forward rotation of the shoulder-plate. In type II displacement, the shoulder-plate is stopped in its forward rotation, so only a positive pressure drag is generated.

ECOTOXICOLOGICAL EVALUATION OF AN INDUSTRIAL EFFLUENT. *M. Baillieul, L. Bervoets* and *R. Blust.* University of Antwerp (RUCA), Dept. of Biology, Groenenborgerlaan 171, 2020 Antwerp.

An ecotoxicological evaluation of an industrial effluent was conducted to assess the current impact of the discharge on a freshwater river and to derive discharge levels that would allow for a partial recovery of the ecosystem. This was done by a combination of toxicity tests, field surveys and chemical analysis. Toxicity tests were performed on the effluent and water from different locations along the river course. Solutions of the main effluent component ($CaCl_2$) were tested to identify its contribution to the overall toxicity. Dilution water was collected from an uncontaminated location of the same river. Acute and chronic toxicity was tested using *Daphnia magna* ; acute toxicity was also tested on carp (*Cyprinus carpio*) and three resident species (*Gobio gobio, Asellus aquaticus, Hydropsyche angustipennis*). The field survey included the inventarisation of the aquatic communities and analyses of a set of physi-

cal and chemical variables. Using this strategy it was possible to : 1) detect stress that cannot be attributed to the chemical composition of the water (e.g. oxygen stress) ; 2) identify the most important chemicals responsible for the acute or chronic toxicity of the effluent ; 3) assess the evolution of the toxicity of the river along its course. The results indicate the importance of incorporating toxicity tests, field surveys and chemical analysis in environmental impact assessments of effluents.

THE EFFECT OF CADMIUM ON CULTURED INSECT CELLS : DEVELOPMENTAL AND ULTRASTRUCTURAL ASPECTS. *B. Braeckman, H. Raes* and *P.H. De Rycke*. University of Ghent, Laboratory of Zoophysiology, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.

We used the AEAL-cell culture (derived from the dipterous *Aedes albopictus*) to study the effect of $CdCl_2$ on invertebrate cells at sublethal Cd-concentrations. In order to define the sublethal region we first determined the lethal concentration by using a neutral red viability assay. The lethal Cd-concentrations after 24 hrs of treatment was found to be 4 ppm. The sublethal concentration range was tested in long term experiments : growth curves showed that between 1 and 4 ppm of $CdCl_2$, cell multiplication was almost completely halted. Between 0.25 and 1 ppm the cells were able to multiply but their multiplication was inhibited dose dependently. Based on these results cells were treated with 0, 0.3, 1 and 2 ppm $CdCl_2$ for 8, 16, 24 and 48 hrs respectively. The morphological effects were studied both at S.E.M. and at T.E.M. mode. Morphologically the cells are of the fibroblast type ; they resemble insect granulocytes (blood cells). Cd-treatment induced several alterations in relation to the control ultrastructure. At the S.E.M. mode cell blebbing and rounding were the most characteristic effects, they are probably indicative for the disturbance of the cytoskeleton. Fine sections showed blebbing, swelling of the mitochondria and condensation of the chromatin. We also found dilatation of the R.E.R.-cisternae and an increase in size and number of secondary lysosomes. The former effects are clearly pathological, the latter can probably be interpreted in terms of a defence system.

EFFECTS OF VARIOUS EXTERNAL CALCIUM LEVELS ON THE UPTAKE OF RADIOACTIVE COBALT BY A FRESHWATER FISH, *CYPRINUS CARPIO*. *S. Comhaire*. University of Antwerp (RUCA), Onderzoeksgroep voor ecofysiologie en biochemie, Groenenborgerlaan 171, 2020 Antwerpen.

The effects of various external calcium levels on the uptake of radioactive cobalt by the carp were examined. Fish were acclimated to different external calcium levels during a 14 day period previous to the experiments. The uptake of cobalt and calcium was measured after a 3 hour incubation period of the fish in water with different calcium concentrations and activities of 1480 Bq ^{45}Ca/l and 740 Bq ^{57}Co/l. Apparently the concentrations of calcium in the water during the experiment was of greater importance on the uptake of both elements than the concentrations of calcium of the water during the acclimation period. Both cobalt and calcium uptake were high at low external calcium concentrations, and a significant correlation between the uptake of the elements was observed. By adding calcium channel antagonists (i.e. lanthanum and cadmium) to the water we measured a decrease of both ^{45}Ca and ^{57}Co uptake with concentrations of cadmium exceeding 10^{-6} M. The size of decrease however was not the same for both elements. Adding lanthanum to the water had no effect on the uptake of cobalt nor calcium. These results suggest that although calcium and cobalt

uptake are correlated, the uptake of both elements does not appear to involve the same uptake mechanism.

FUNCTIONAL MORPHOLOGICAL STUDY OF THE FEEDING APPARATUS IN *CROCODILUS NILOTICUS* (REPTILIA). *K. D'Août, J. Cleuren* and *F. De Vree*. Dept. Biology, University of Antwerp (U.I.A.), Universiteitsplein 1, B-2610 Antwerpen.

The morphology and function of the craniocervical complex and its associated musculature were studied in *Crocodilus niloticus* by anatomical and experimental techniques. Movements of jaws and neck were recorded by a magnetoresistive gape-transducer and a tri-axial accelerometer. The kinematic output was correlated with the activity pattern of all jaw muscles and one cervical muscle. Feeding in *Crocodilus niloticus* can be divided into six sequential stages : acquisition, holding, killing, reduction, transport and swallowing. The intraoral transport of the prey is an inertial process and is accomplished by rapid movements of the jaws and neck in which a backward acceleration is imparted to the prey. Analysis of the kinematic gape profile and the muscle activities indicates three inertial bite types (repositioning, killing/crushing, transport) and a non-inertial swallowing cycle. During jaw opening the lower jaw is depressed by contraction of the m. depressor mandibulae and the upper jaw is elevated by dorsal cervical muscles. Fast jaw closure is achieved by simultaneous contraction of all jaw adductors in all inertial bites. Pulsatile activity of all jaw adductors generates the crushing force at the end of the killing/crushing bites. Toward the end of the feeding sequence closing of the jaws is achieved by the activity of less muscles, in swallowing cycles only two jaw adductors remain active. Supported by FKFO grant nr. 2.9005.90.

STRESS INDICATORS IN THE ENERGY METABOLISM OF THE COMMON CARP. *G. De Boeck*°, *R. Blust*° and *A.M. Van der Linden**. ° University of Antwerp (RUCA), Dept. of Biology, Groenenborgerlaan 171, 2020 Antwerp, Belgium ; * University of Antwerp (UIA), Dept. of Medicine, Universiteitsplein 1, 2610 Wilrijk, Belgium.

The aim of our study is to measure the energy status of the common carp as a subclinical and sensitive indicator of environmental stress. Establishing a direct quantitative relationship between short-term physiological responses of the organism and its long-term performance (survival, growth, reproduction) under the mixed stress of a changing environment, would allow to detect stress and predict its results before irreversible changes have occurred. Different aspects of the changes in the energy metabolism of *Cyprinus carpio* are investigated. First of all the energy status of the carp is examined by the AEC or adenylate energy charge (ATP + 1/2ADP)/(ATP + ADP + AMP) which is monitored using NMR (Nuclear Magnetic Resonance) scanning. Secondly, the oxygen consumption/nitrogen production ratio is measured in order to know the relative use of protein versus lipid and carbohydrate consumption. For this purpose a respiration chamber has been developed in which pH, O_2 and NH_4^+ can be measured directly. As it is possible to change the water during the experiment, short-term effects as well as long-term effects can be monitored. Finally glycogen, lipid and protein content is determined in order to evaluate the general condition of the fish, and RNA/DNA ratio to assess for protein synthesis activity.

FUNCTIONAL MORPHOLOGICAL STUDY OF THE CERVICAL SYSTEM IN *TRIONYX* (REPTILIA : CHELONIA : CRYPTODIRA). *S. Geerts, J. Van Damme, P. Aerts* and *F. De Vree.* Dept. of Biology, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerpen.

The relationship between the form and movement in the neck of softshell turtles (*Trionyx spiniferus* and *T. ferox*) was studied by descriptive anatomical and biomechanical approaches. The change in orientation between the neck vertebrae during head and neck retraction was recorded in anaesthetized animals using radiography and computerized tomography. Predictions concerning the potential range of movements are based on anatomical analysis and manipulation of bone-ligament preparations. Rotations in the sagittal plane are possible in all joints, but dorsiflexions are larger than ventroflexions, except in joint D1-V8. Lateral flexions and small axial rotations are limited to the distal region of the neck, anterior to V6. The bicipital intercentral hinge joints between the proximal vertebrae and the greatly enlarged zygapophyseal joints in D1-V8 restrict motion to rotations in the sagittal plane. Judging from the CT scans, most of the head and neck retraction results from sagittal rotations at the five proximal most cervical joints (D1-V8 through V5-4). The kinematic pattern during head retraction is characterized by ventroflexion in joint D1-V8 followed by a sequence of successive dorsi- and ventroflexions in joints V8-7 through V5-4 progressing in proximo-distal direction. The range of angular changes is approximately equal in all joints. An hypothesis concerning muscular activities during head retraction and extension is proposed. Supported by FKFO grant nr. 2.9005.90.

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE DOPAMINERGIC AND SEROTONINERGIC SYSTEM IN THE BRAIN OF THE CHICKEN (*GALLUS DOMESTICUS*). *E. Ghijsels, L. Moons* and *F. Vandesande.* Zoologisch Instituut, Naamsestraat 59, 3000 Leuven.

The present work is situated in a long term project trying to elucidate the role of the biogenic amines in the antithesis between growth and reproduction in the chicken (*Gallus domesticus*). From several observations it has become clear that the monoamines play an important role in the modulation of appetite and food intake and at the same time exert effects on the male and female reproductive behavior as well as on the secretion of the gonadotrophic hormones. In a first approach, we analysed the distribution of these monoaminergic neurotransmitters in the brain of two physiologically different groups of a broiler chicken breed, i.e. *ad libitum* fed broiler chickens and food restricted animals, designated for reproduction. A light microscopic immunocytochemical study revealed the dopaminergic and the serotoninergic system in the brains of food restricted and *ad libitum* fed broiler chickens of 4, 10 and 16 weeks old. We were able to describe the localization of tyrosine hydroxylase (TH), L-DOPA, dopamine (DA) and serotonin (5-HT) throughout the whole central nervous system of the chicken. From this semiquantitative study we could, however, not find any clear differences in the distribution pattern nor concentration between the two types of food regime.

FEEDING IN *AGAMA STELLIO* (REPTILIA) : KINEMATICS AND MUSCLE ACTIVITY. *A. Herrel, J. Cleuren* and *F. De Vree*. Dept. Biology, University of Antwerp (U.I.A.), Universiteitsplein 1, B-2610 Antwerpen.

The feeding in *Agama stellio* (a generalised insectivorous lizard) was examined. For that purpose cineradiographic and electromyographic experiments were undertaken. Some aspects of the physiology of the jaw muscles were studied by stimulation experiments. In the EMG experiments the activity of the jaw, tongue and hyoid muscles was recorded simultaneously, in combination with cineradiography or high speed film (200 fps). The feeding cycle is divided into different bite types : strike, reduction/transport (which could not unambiguously be separated) and swallowing. Bites are further divided into subsequent kinematic phases : slow open (SO), fast open (FO), fast close (FC) and slow close/power stroke (SC/PS). Reduction and transport cycles are characterized by a clear FO and a pronounced SC/PS. Swallowing differs from reduction and transport by a short FO and the absence of SC/PS. The depression of the lower jaw during FO is achieved by activity in the jaw opener ; however no activity in this muscle is present during SO. Our experiments have shown this phase to be the result of the protrusion of the tongue and the fitting of the tongue to the prey. Jaw closure is achieved by simultaneous contraction of all jaw adductors. Stimulation experiments show that during feeding the neuronal stimulation frequency is 80Hz, which is twice the fused tetanus frequency. Supported by FKFO grant nr. 2.9005.90.

SELECTION OF STABLY TRANSFORMED *DROSOPHILA* S2 CELLS AFTER COTRANSFECTION WITH pBmNPV IEG lacZ AND pUChsHyg BY HYGROMYCIN B RESISTANCE. *I. Janssen, A. De Loof* and *R. Huybrechts*. K.U.L., Laboratorium voor Ontwikkelingsfysiologie, Naamsestraat 59, 3000 Leuven.

Recombinant baculovirus mediated expression of foreign proteins in insect cells is by definition transient, because of the lytic properties of the virus. By using the promoter of an immediate early gene from a silkmoth specific baculovirus, *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV), we developed a continuous expression system with *Drosophila* S2 cells. Up till now the gene coding for β-galactosidase under BmNPV IEG promoter control (pBmNPV IEG lacZ) is used as reporter gene. After cotransfection with this reporter gene and an antibiotic resistance gene, the selection of stably transformed cells can be carried out in the presence of the antibiotic. Methotrexate and hygromycin B have both been tested but only hygromycin B is lethal to S2 cells. The hygromycin resistance construct (pUChsHyg) contains the resistance gene under control of a *Drosophila* heat-shock promoter. The cotransfection was done both with linear and supercoiled DNA using different ratio's of both constructs. Three weeks after transfection, antibiotic resistant colonies appeared. The majority of the transformed cells expressed the β-galactosidase IEG fusion protein, even after subculturing these cells for up to 6 months. This expression remained present in non-selective conditions. Western blotting showed that the expression of β-galactosidase is limited to the transformed cells. Southern analysis of these cells proved that the foreign DNA was integrated in the hosts genome.

ASSESSMENT OF MORPHOLOGICAL DEFORMITIES IN *CHIRONOMUS RIPARIUS* LARVAE (DIPTERA, NEMATOCERA) IN A CHRONIC EXPOSURE TEST WITH CADMIUM. *L. Janssens de Bisthoven*[1], *J. Postma*[2], *A. Vermeulen*[1] and *F. Ollevier*[1]. [1]Catholic University of Louvain, Lab. Ecologie en Aquacultuur, Naamsestraat 59, B-3000 Leuven, Belgium ; [2]University of Amsterdam, Department of Aquatic Ecology, Kruislaan 320, 1098 SM Amsterdam, The Netherlands.

Chronic exposure tests with cadmium (0, 3, 9, 27 ppm ; static with replacement ; cellulose substrate) were performed on *Chironomus riparius* larvae (Diptera, Nematocera) during three successive generations. Head capsule deformities were assessed in 4th instar larvae of each generation. Heavy mechanical wearing of the buccal structures and a high percentage of antennae lacking distal segments was observed in each experimental condition. Moreover, there was a general trend towards maximum percentages of larvae with structures showing mechanical wear and loss of antennal segments in the 9 and 27 ppm conditions of the second generation and in the 27 ppm condition of the third generation. This indicates a gradual weakening of the chitineous structures in subsequent generations of larvae which were subjected to a constant cadmium stress. This experiment could not induce so-called « Kohn-gaps » (mentum gaps), as usually encountered in aquatic systems polluted with heavy metals. However, splitting of the medial teeth of the menta increased abruptly when the larvae were exposed to cadmium stress.

THE SIGNIFICANCE OF MORPHOLOGICAL DEFORMITIES IN CHIRONOMID LARVAE (DIPTERA, NEMATOCERA) FOR SEDIMENT QUALITY ASSESSMENT. *L. Janssens de Bisthoven, C. Huysmans, P. Parren* and *F. Ollevier*. Catholic University of Louvain, Lab. Ecologie en Aquacultuur, Naamsestraat 59, B-3000 Leuven.

There is increasing field and experimental evidence that morphological deformities in riparian chironomid larvae do reflect contaminant stress (heavy metals and pesticides) of fluvial sediments (Hamilton/Saether Hypothesis). Domestic pollution of inland waters in Flanders (Belgium) is commonly assessed with the Belgian Biotic Index (BBI), which relies on the presence of oxygen sensitive macro-invertebrates in the macrobenthos community. In order to investigate whether there is any relationship between water quality (organic pollution) and sediment quality (micropollutants), deformities in *Chironomus riparius* larvae were matched against BBI for several watercourses in Flanders. Preliminary results, relying on 14 sites, show an inverse relationship between BBI and deformities of the median teeth of the mentum and a positive relationship between BBI and deformities of the pecten epipharyngis and the inner teeth of the mandibles. Deformities in other zonations of mentum and mandibles and in the antennae showed no relationship with BBI. The deformity response is clearly not unequivocally associated with domestic water pollution (BBI), but is rather reflecting the presence of other stress factors, which occur in the aquatic system independently from organic pollution. Therefore, deformities in chironomid larvae give complementary information to the BBI about the pollutional state of a water body.

MORPHOLOGICAL DEFORMITIES IN *CHIRONOMUS RIPARIUS* LARVAE (DIPTERA, NEMATOCERA) FROM THE DYLE WATERSHED : IN SITU EVIDENCE OF A RELATIONSHIP WITH MICROPOLLUTANTS. *L. Janssens de Bisthoven* and *F. Ollevier*. Catholic University of Louvain, Lab. Ecologie en Aquacultuur, Naamsestraat 59, B-3000 Leuven.

Morphological deformities in mentum, mandibles, antennae, premandibles and pecten epipharyngis of the head capsules of 4th instar *Chironomus riparius* (Diptera, Nematocera) larvae from the Dyle watershed were quantified. These data were matched against total concentrations of 7 sediment bound heavy metals and 20 sediment bound pesticides, using canonical correlation analysis and multiple regression analysis. The 7 sites were ranked in the same order for the deformity scores, as for the overall pollution. HCB and a number of PCB's appeared to be associated to antennal deformities, while the other pollutants showed a more disparate association with the other structures. Deformed larvae contained significantly more lead and copper than normal larvae. These field data strongly suggest a positive relationship between the occurrence of morphological deformities in midge larvae and the presence of heavy metals and pesticides in the fluvial sediments. Adequate interpretation of these deformities will conduct towards the elaboration of a practical biomonitoring tool for the screening of industrial and agro-chemical pollution in the lowland rivers of Flanders, Belgium.

BIOMECHANICAL ANALYSIS OF THE VERTICAL JUMP OF *GALAGO SENEGALENSIS*. *H. Plompen, P. Aerts, D. De Clercq* and *F. De Vree*. Universiteit Antwerpen (UIA), Departement Biologie, Universiteitsplein 1, B-2610 Wilrijk.

Galago senegalensis (a small prosimian), is known to jump as high as 2.25 m. A simple model was used to calculate the power required for jumping, assuming a strict synchronization between joint rotations and muscular contraction. Judging the power demands for a 2.25 m jump, at least 125 % (!) of the body mass of *Galago* must be jumping muscle. To explain this obvious discrepancy, three hypotheses can be formulated : (a) the used model overestimates the required power, (b) the muscles can produce much more power than generally assumed and (c) a power amplifying mechanism is in play (*i.e.* a catapult system, implying pre-straining of elastic structures). To test these hypotheses, the push-off phase of 19 jumps of three animals were recorded by means of a force plate (measuring ground reaction forces) and a NAC 1000 video (500 frames/s ; registration of the body kinematics). The obtained data allow to calculate the required instantaneous external power output very accurately. On the basis of these results the first hypothesis must be rejected. The second hypothesis is considered to be doubtful as then muscles should perform more than four times better than the estimated optimum for vertebrate muscles. Thus the results point at the presence of a power amplifying mechanism. However, preliminary morphometry excludes the most obvious system : the achilles tendon. Further research is required.

LEAF QUALITY AND HERBIVORY PATTERNS IN *AGROMYZA PHRAGMITIDIS* (DIPTERA, AGROMYZIDAE). *J. Scheirs* and *L. De Bruyn*. Department of Biology, Evol. Biol. Grp, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerpen, Belgium.

Agromyza phragmitidis Hendel, 1922 is a monophagous leafminer of common reed, *Phragmites australis* (Cav.) Trin. ex Steud. We examined the distribution of *A. phragmitidis*

within its macrohabitat, the reed bed, and its microhabitat, the reed stems. Reed stems were collected in different reed beds. In the laboratory the following leaf characteristics were measured : leaf height on the shoot, total leaf and mine area, water, protein and sugar content. We observed the highest concentrations of leafmines of *A. phragmitidis* in the shaded areas of the reed bed. In its microhabitat, the mines of *A. phragmitidis* are concentrated on the leaves in the mid-region of the reed stem. We provided evidence that the leaves in this mid-region have a higher water content and a larger leaf area than the other leaves on the reed stem. Further analyses showed a strong positive relation between leaf area and number of larvae in the mine of that leaf. When the leaf characteristics of the leaves with and without mines of the mid-region were compared, we observed a higher water content, a lower protein content, and a larger total leaf area in the mined leaves. L.D.B. is a senior research assistant of the N.F.S.R.

CERVICAL MOVEMENTS DURING PREY CAPTURE IN THE AUSTRALIAN SNAKE-NECKED TURTLE *CHELODINA LONGICOLLIS* (REPTILIA ; CHELONIA ; PLEURODIRA). *J. Van Damme*, *P. Aerts* and *F. De Vree*. Dept. of Biology, University of Antwerp (U.I.A.), Universiteitsplein 1, B-2610 Antwerpen.

Kinematics of the neck movements in *Chelodina longicollis* during frontal strike movements were studied by means of X-ray cinematography (50 frames/s). Radio-opaque markers were inserted in the neck vertebrae of two specimens of *Chelodina longicollis*. Digitalisation of these markers allowed to calculate the degree of head retraction and joint angles between the successive vertebrae during neck movements. In *Chelodina* lateral rotations between cervical vertebrae are most important (i.e. Pleurodira). A frontal strike cycle consists of a fast extension of the head and neck towards the prey item, followed by a much slower retraction phase. Individual differences in joint rotations are noticeable during the strike : the main centres of motion are observed in V5-4 (i.e. joint between vertebrae 4 and 5), V6-5 (V5 = biconvex vertebra) and, depending on the individual, V8-7 (turtle 2) or V9-8 (turtle 1). These joints also show the largest starting angle at the beginning of the strike. Angular changes in V7-6 are less conspicuous. During the head retraction phase, angular changes in the more proximal joints are an inversion of those during protraction. Supported by IWONL grant nr. 910091 and FKFO grant nr. 2.9005.90.

A MORPHOLOGICAL STUDY OF THE *PHYLLOTRETA TETRASTIGMA* COMPLEX (COLEOPTERA, CHRYSOMELIDAE, ALTICINAE). *P. Verdyck, L. De Bruyn, J. Scheirs,* and *J. Verwaerde*. University of Antwerp (RUCA), Department of Biology, Evolutionary Biology Group, Groenenborgerlaan 171, 2020 Antwerpen, Belgium.

The *Phyllotreta tetrastigma*-complex comprises three sibling species : *P. tetrastigma, P. flexuosa* and *P. dilatata*. At present the separation of these species is based on colour characteristics. However intraspecific colour variation is often very large and interspecific overlap exists. To find out whether these sibling species differ morphologically, we started a biometrical study of their habitus. For each species a population was analyzed. Fifteen measurements were taken from the habitus of both males and females. We found that differences between males and females of the same species were smaller than differences between animals of the same sex but from different species. To see which characters attributed the most to the separation of the species a discriminant analysis was performed. Different charac-

ters attributed to a greater or lesser extent to the separation of the three species. P.V. is an I.W.O.N.L.-bursar, L.D.B. is a senior research assistant of the N.F.S.R.

SEQUENCE DETERMINATION OF THE SMALL RIBOSOMAL SUBUNIT RNA OF *PHASCOLOSOMA GRANULATUM* LEUCKART 1828 (SIPUNCULA). *B. Winnepenninckx*[1], *T. Backeljau*[2] and *R. De Wachter*[1]. [1]Departement Biochemie, Universitaire Instelling Antwerpen, Universiteitsplein 1, B-2610 Antwerpen; [2]Koninklijk Belgisch Instituut voor Natuurwetenschappen, Afdeling Malacologie, Vautierstraat 29, B-1040 Brussel.

The phylogenetic relationships of the Sipuncula, a phylum of unsegmented coelomate worms, are difficult to evaluate on the basis of morphological, anatomical, embryological or paleontological data. We determined the first complete 18S rRNA sequence of a representative of the phylum, viz. *P. granulatum*. The new sequence was aligned by hand with those of other Metazoa, including Chordata, Arthropoda, Mollusca, Pentastomida, Platyhelminthes and Cnidaria. From the tree we obtained using the neighbour-joining distance method to this data set, it is concluded that the Sipuncula are a sister group to the Mollusca. The latter phylum, represented by Bivalvia, Gastropoda and Polyplacophora, appeared to be a monophyletic group. However this is a very preliminary hypothesis, which has to be tested further by addition of complete 18S rRNA sequences of some crucial metazoan groups (e.g. Annelida) to our data set.

IN SITU HYBRIDISATION FOR GAP-43 IN THE BRAIN OF THE ADULT CAT (*FELIS CATUS*). *G. Wouters, L. Arckens, F. Vandesande* and *G.A. Orban*. University of Leuven, Department of Biology, Lab. Neuro-endocrinologie en Immunologische Biotechnologie, Naamsestraat 59, B-3000 Leuven.

After development of the cat has been completed, the GAP-43 (Growth Associated Protein-43) concentration substantially decreases in most of the adult neurons that are thought to be involved in memoryformation (1). We examined in which of the brain structures the level of GAP-43 remains high. We approached the problem at genetic level, searching for the localisation of GAP-43-mRNA by radioactive (^{35}S) in situ hybridisation. Summarizing, we can say that the hippocampus and structures that are involved in smell-processes show the highest levels of GAP-43-mRNA. In the tractus opticus, the corpus callosum and the commissura anterior there is no RNA detectable. Worth mentioning are the structures which express more GAP-43 anterior in the brain compared to posterior : the caudate-putamen, parts of the amygdala and nuclei in the thalamic domain, including the colliculus superior and the corpus geniculatum mediale.

(1) M.B. KENNEDY (1988) — Nature *335* : 770-772.

SOME HISTOPATHOLOGICAL EFFECTS OF PCBs (AROCLOR 1260) ON THE LIVER AND GONADS OF THE BARBEL (*BARBUS BARBUS*). *C. Adam, G. Goffinet, J.L. Hugla* et *J.P. Thome*. Université de Liège, Institut de Zoologie, Laboratoire de Morphologie, Systématique et Ecologie animale, Unité d'Ecotoxicologie des Micropolluants Organiques, Quai Van Beneden 22, B-4020 Liège.

PCBs are widely distributed in the environment and have been reported to reach high concentrations in upper trophic levels of aquatic food chains. Liver as the main organ of detoxication is likely to accumulate high quantities of such xenobiotics which can alter its morphology. Common barbels were contaminated with food containing 12.5 µg/g of Aroclor 1260 for 75 days. Samples of livers, testes and ovaries were taken from contaminated and control fish and observed under light and electron microscopy. The light microscopic study showed that there were no observable differences in the general architecture of the liver and the testes of PCB-treated and untreated fish. Electron microscopic study indicated some alteration of the liver ultrastructure of contaminated fish. The most frequently encountered changes were increase of rough endoplasmic reticulum, drastic reduction of glycogen, dissolution of mitochondria contents and appearance of myelin figures. Some damage appeared in the spermatozoa originating from contaminated fish, particularly in the head region. Light and electron microscopic studies of ovaries showed that this organ is less developed in contaminated fish than in control fish. PCBs induce changes in the ultrastructure of liver and spermatozoa, and also affect the development of the ovary ; hence, we can suppose that they could affect the reproduction processes. J.L.H. acknowledges an IRSIA grant.

EFFECTS OF TUMOR NECROSIS FACTOR-α (TNF-α) ON THE PROLIFERATION AND DIFFERENTIATION OF PORCINE PREADIPOCYTES. *C. Boone, N. De Broux, C. Genart, N. Hauser* and *C. Remacle*. Laboratoire de Biologie cellulaire, Université Catholique de Louvain, Place Croix du Sud, 5, 1348 Louvain-la-Neuve, Belgium.

TNF-α is a cytokine having multiple effects notably on the immune system, the general metabolism and the development of adipose tissue. The effects of TNF-α (5, 10, 25, 100 and 200 U/ml) were analysed on the proliferation and differentiation of pig preadipocytes. The cells obtained from inguinal adipose tissue of pietrain pig (± 7 days), were inoculed in a serum-free DMEM/F12 (1:1) medium containing 5,5 nM fibronectin and in a serum-additioned medium (10 % FBS). After 24 hours, the defined medium was replaced by another containing 10mg/ml transferrin, 0,2 nM T3, 8,7 nM insulin, 100 nM cortisol, 50 µM β-mercaptoethanol and 100 µM ascorbic acid and the FBS medium was replaced by another containing 2,5 % PS, 14,5 nM insulin and 100 nM cortisol. The different doses of TNF-α were also added at this time. In both media, the proliferation was estimated by tritiated thymidine uptake on the third day of culture and differentiation was recorded by determining LPL activity (early marker) and GPDH activity (late marker). The total cell number and the ratio of differentiated cells were also counted at the end of the culture. In the serum-added medium, the stimulation of proliferation reached 150 % (tritiated thymidine uptake) when 10U/ml TNF-α was added and the cells were numerically 2,5 times more when 200 U/ml of TNF-α was added. In contrast, the LPL activity was less than 1 % of the control values and the GPDH activity less than 20 %. The number of differentiated cells was also dramatically too low. In the chemically-defined medium, the stimulation of proliferation reached 500 % with 200 U/ml TNF-α. The inhibition of the differentiation was less pronounced in comparison with the serum-added medium. FBS : Fetal Bovine Serum, T3 : Tri-iodothyronin,

PS : Porcine Serum, GPDH : Glycerol-3-Phosphate Dehydrogenase, LPL : Lipoprotein Lipase.

SPATIAL DISTRIBUTION OF THE WESTERN FLOWER THRIPS *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) ON SWEET PEPPER PLANT. *I. Charlier.* Unité d'Ecologie et de Biogéographie, Université Catholique de Louvain, Place Croix du Sud, 5, 1348 Louvain-la-Neuve.

With a long term aim to develop a viable biological thrips management programme on sweet pepper, the spatial distribution of the population of different stages of *Frankliniella occidentalis* (*i.e.* egg, larvae 1, larvae 2, nymph 1, nymph 2 and adult) was determined on the plant. Out of the total number of eggs, 99,8 % were found to be on the leaves, and the rest on the flowers sepals, whereas the mobile individuals were found to be on the leaves, within the flowers, and on the fruits. In this last case, many individuals were found between the ovary and the sepals. Among the immature individuals, larvae 1 showed a higher preference for fruits (48,2 %) than flowers (27,7 %) and leaves (24,1 %), and larvae 2 prefered principally flowers (46,5 %) and fruits (40,1 %) rather than leaves (13,4 %). On the other hand, nymphs (1 and 2) were exceptionally found on the plants as nymphosis takes place predominantly in soil. The few nymphs inhabiting the plant were distributed equally within the flowers and under the sepals of the fruits. Adults were principally met within the flowers (68 %), then on fruits (26,5 %) and on leaves (5,5 %).

MORPHOLOGICAL AND PHYSIOLOGICAL APPROACH OF BIOLUMINESCENCE IN THE OPHIUROID *AMPHIPHOLIS SQUAMATA* (DELLE CHIAJE, 1828) (ECHINODERMATA). *D. Deheyn*[1], *J. Mallefet*[2] and *M. Jangoux*[1]. [1]Laboratoire de Biologie marine, C.P. 160, Université Libre de Bruxelles, Avenue F.D. Roosevelt, 50, 1050 Bruxelles. [2]Unité de Biologie animale, Place Croix du Sud, 5, 1348 Louvain-la-Neuve.

Amphipholis squamata is a small bioluminescent polychromatic ophiuroid (max. disc diameter *ca.* 2.5 mm) in which only the arms are bioluminiscent. Ultrastructural studies done before and after light emission allowed to determine that the light-producing cells occur within the spinal ganglia of the arms (these ganglia occur at the spine base along the arms). It was demonstrated that black-coloured adult individuals produce a much more intense light than those having a beige colouration. This could be related to a difference in the number of photocytes and/or in the amount of photoproteins within these cells. It was also demonstrated that, in each investigated group of ophiuroids (i.e., black-coloured *versus* beige-coloured), small and large individuals behave differently as far as the cinetic parameters of the produced light are considered. It is suggested that these differences could be related to either the maturation of the nervous system or the acquiring of sexual maturity.

PRELIMINARY ESTIMATIONS OF FORAGING AND POPULATION DENSITIES OF GREAT APOIDS (HYMENOPTERA, APOIDEA : *BOMBUS, HABROPODA)* IN SOUTHERN FRANCE. *G. Duhayon.* Service de Zoologie, Université de Mons-Hainaut, Avenue Maistriau, 19, 7000 Mons.

During the year 1992, the author has estimated the densities of the populations of great apoids in the « Massif des Maures » (SE-France, Var) by testing marking-recapture observa-

tions. The foraging densities were : 1-10 spec./m^2 for *Bombus terrestris,* 1-7 spec./m^2 for *Megabombus ruderatus,* 1-7 spec./m^2 for *Megabombus pascuorum,* < 1 spec./m^2 for *Habropoda tarsata.* For *Bombus terrestris* the population density was estimated only once and was < 1 spec./m^2 . Good relations between the estimations of marking-recapture observations and eliminatory trapping were obtained.

INVESTIGATIONS ON THE METAMORPHIC PROCESSES IN THE ECHINOID, *PARACENTROTUS LIVIDUS* (ECHINODERMATA). *P. Gosselin* and *M. Jangoux,* Laboratoire de Biologie marine, Université de Mons-Hainaut, Avenue Maistriau, 19, 7000 Mons.

Three successive stages occur during the metamorphic period of the echinoid *Paracentrotus lividus* where individuals are able to metamorphose (competent larvae), transform (metamorphic larvae), and carry out their imaginal organogenesis (postlarvae) respectively. The postlarval life ends when the mouth and the anus are open, which leads to the appearance of exotrophic juveniles. Competent larvae possess appendages (viz. primary podia) allowing them to test the substrate and to fix on it (the occurrence of metamorphosis depends on the inductive capabilities of the substrate). Metamorphosis is a short event (1 h) in which eversion of the adult rudiment and the lyse of most larval tissues takes place. Imaginal organogenesis starts before larvae become competent (differentiation of the rudiment) but takes place mostly in postlarvae which are endotrophic. During postlarval life, the echinoid skeleton starts its organogenesis and the ambulacral system and imaginal gut start differentiation. Postlarvae bear protective and adhesive appendages (pedicellariae and multifid spines ; primary podia) that will regress in early juveniles.

EFFECT OF PCB ON THE REPRODUCTION OF BARBEL (*BARBUS BARBUS*) : FIRST EXPERIMENTAL RESULTS. *J.L. Hugla, C. Adam, J.C. Philippart* and *J.P. Thome.* Institut de Zoologie, Laboratoire de Morphologie, Systématique et Écologie animale, Unité d'Écotoxicologie des Micropolluants Organiques, Université de Liège, Quai Van Beneden 22, B-4020 Liège.

PCBs are ubiquitous micropollutants, which are known to be inductors of hepatic microsomal oxydases in fishes. These enzymes are involved in the catabolism of steroid hormones. As a consequence, chronic exposure of fishes to PCBs may cause adverse effects on reproduction and hatchability of eggs. 4-years old common barbels from a captivity-reared brood-stock were held in aquaria and intoxicated by food containing supra-environmental concentrations of 2.5 and 12.5 µg PCB/g, respectively during 50 and 75 days. All male fish became sexually mature at the same time. The PCBs content of their milt increased in relation to the contamination level. However, neither the number of spermatozoa per mm^3, nor the duration of their stages of mobility was affected. Control females and those contaminated with 2.5 µg PCBs/g in the food became gravid at the same moment. The number of eggs artificially stripped from the treated fish was reduced by 50 % compared to the control fish. The mean weight of the ovules was not affected by the intoxication. Female fish contaminated at a higher value never reached sexual maturity, as a possible consequence of the PCB intoxication. Control and contaminated eggs were artificially fertilized with sperm of control fish. A significant correlation was observed between total egg mortality and PCB burden in the gonad. High PCB concentrations in the eggs affected hatching success, and increased hatching duration and malformation rate of larvae. As a conclusion, supra-environmental

PCB concentrations can affect the reproduction biology processes of the common barbel. Further research is needed to verify experimentally the impact of intermediate and environmental concentrations. J.L.H. acknowledges an IRSIA grant.

INFLUENCE OF GRAZING RATE ON THE PCBs TRANSFER DYNAMICS IN APLANKTONIC ROTIFER SPECIES, *BRACHIONUS CALYCIFLORUS* PALLAS. *C. Joaquim-Justo*[1], *Y. Marneffe*[1], *J.P. Descy*[2] and *J.P. Thome*[1]. [1]Institut de Zoologie, Laboratoire de Morphologie, Systématique et Écologie animale, Unité d'Écotoxicologie des Micropolluants Organiques, Université de Liège, Quai Van Beneden 22, B-4020 Liège. [2]Facultés Universitaires Notre-Dame de la Paix, Rue de Bruxelles, 61, B-5000 Namur.

The presence of PCBs in terrestrial and aquatic ecosystems has been reported by many authors since the sixties. These widespread micropollutants are highly resistant to any biodegradation process and possess acute lipophilic characteristics. As a consequence, these xenobiotics are accumulated in living organisms. Such a contamination can occur via two pathways : the direct route through exchange surfaces ; the indirect way by contaminated food ingestion. This study deals with the establishment of the relative importance of both these pathways in the contamination of plankton, which has not yet been actually defined. In a medium contaminated by means of 5 µg PCBs/l, rotifers (*Brachionus calyciflorus*) accumulated up to 40 µg PCBs/g D.W. within a few hours. When fed with PCB contaminated algae (*Dictyosphaerium ehrenbergianum* containing 20 µg PCBs/g D.W.), rotifers reached a concentration of 4 µg PCBs/g D.W. after 48 hours. Finally, if the animals were contaminated by both pathways at the same time, a concentration of 30 µg PCBs/g D.W. was measured after 24 hours. Obviously, in these experiments where a high PCB concentration in water was considered, *Brachionus calyciflorus* was mainly contaminated by the direct pathway. Nevertheless, when using the ingestion rate determined in laboratory experiments and the concentration of PCBs reported in natural phytoplankton of the river Meuse, the calculated PCB contamination level of rotifers coincides with the PCB concentration measured in situ. This would mean that in natural ecosystems, the PCB contamination of zooplankton can entirely occur by the indirect pathway i.e. by absorption of contaminated food.

INTRASPECIFIC GROUP EFFECT IN THE FALSE SPIDER MITE (*BREVIPALPUS PHOENICIS* (GEIJSKES)) POPULATION. *F.J.S. Kennedy*. Unité d'Écologie et de Biogéographie, Université Catholique de Louvain, Place Croix du Sud 5, 1348 Louvain-la-Neuve.

« Group effect » is defined as the effect of population density on the physiological processes of the individuals in a population which is *sensu stricto* different from the « density-dependant » effects. To study this effect in the population of false spider mite (*Brevipalpus phoenicis* (Geijskes)), a pest on citrus, tea, papaya, passion fruit, palms and many other ornamental plants, two levels of densities were considered *viz.*, 3 and 50 mites per leaf discs of 9.5 cm^2 size respectively. The results of the experiment are presented below :

1	Density levels	3 eggs/disc	50 eggs/disc
2	Number of eggs kept	120	500
3	Fertility of the eggs	118 (98.33 %)	470 (94.00 %)
4	Egg hatching period	10.25 days	9.53 days
5	Developmental time	26.77 days	19.55 days
6	Immature mortality	6 (5.00 %)	52 (10.40 %)

7	Total life cycle	52.01 days	41.01 days
8	Eggs laid per female	40.68	39.71

It seems from this study that grouping of the individuals shortens the developmental time and total life span without significantly reducing the oviposition of eggs. The mechanism for such reduction can be explained by two hypotheses. The first one postulates that the presence of cumulatively more hormones present in the higher density level could have stimulated the faster development. However it would be necessary to demonstrate the secretion of such hormones. The second hypothesis rests upon the tactile stimulation among the immature mites as observed in many mite populations (1). The « group effect » discussed here will probably influence the demography of the false spider mite.

(1) G. VAN IMPE (1984) — In : D.A. GRIFFITHS and C.E. BOWMAN (Eds.) Acarology *VI* : 1 : 617-621.

INU I : THE INULINASE GENE OF *KLUYVEROMYCES MARXIANUS* VAR. *MARXIANUS*. *O. Laloux, J.-P. Cassart, J. Delcour* and *J. Vandenhaute*. Unité de Génétique Moléculaire, Facultés Universitaires Notre-Dame de la Paix, rue de Bruxelles 61, B-5000 Namur, Belgium.

Inulinase (E.C.3.2.1.7.) hydrolyses inulin, a plant polymer which consists of long linear chains of D-fructoses (5 to 40 moieties) ended at one extremity by a glucose moiety, used as carbohydrate storage essentially in the family Compositae. Among the microorganisms producing inulinase the yeast *Kluyveromyces marxianus* (ATCC12424) is commonly used for its ability to ferment inulin to ethanol directly or as an enzymatic source for pure fructose production (1). The cell wall inulinase was purified from *K. marxianus* by anion exchange chromatography and its N-terminal 33-amino acid sequence was established. On the basis of this sequence, two sets of oligonucleotide primers were derived and used in a PCR reaction on *K.m.* cDNA. A completely homologous probe deduced from the sequence of the PCR fragment was used successfully in a screen of a *K.m.* genomic library. The inulinase gene (*INU 1*, accession number X57202) encodes a 555-amino acid precursor protein with a typical N-terminal signal peptide flanked by 3 consecutive putative cleavages sites (2). In addition, it was shown that *Saccharomyces cerevisiae* transformants produce and secrete inulinase at high level (70 % of the inulinase activity was in the supernatant). Comparison of the inulinase sequence with known invertases suggests that they belong to the same family. A structural and functional analysis of the promoter sequence is currently in progress.

(1) E. J. VANDAMME and D. G. DERYCKE (1983) — Adv. appl. microbiol., *29* : 139-176.
(2) O. LALOUX, J.-P. CASSART, J. DELCOUR, J. VAN BEEUMEN and J. VANDENHAUTE (1991) — FEBS Lett., *289* : 64-68.

ECOTOXICOLOGY OF THE POSIDONIA MEADOW IN THE MEDITERRANEAN : DISTRIBUTION OF THE HEAVY METALS AND DYNAMICS OF CADMIUM ACCUMULATION IN *POSIDONIA OCEANICA* (L.) DELILE. *G. Ledent, M. Warnau, A. Temara* and *Ph. Dubois.* Laboratoire de Biologie marine, C.P. 160/15, Université Libre de Bruxelles, Avenue F.D. Roosevelt, 50, 1050 Bruxelles.

The seagrass, *Posidonia oceanica,* is widely distributed in the infralittoral zone of the Mediterranean region where it forms dense communities called « meadows ». Due to their situation close to the coast, these meadows are directly subjected to anthropogenic pollution. The present work focuses on the contamination of this ecosystem by heavy metals. The concentration of 7 metals (Zn, Pb, Cd, Fe, Cr, Cu, Ti) was measured in 4 anatomic compartments of the seagrass (*viz.* photosynthetically active leaf, dead leaf, rhizome, and root) during the annual cycle in 3 stations of the western Mediterranean region (Calvi, Corsica ; Ischia, Italy ; Marseille, France). The results show that (1) seagrasses sampled in Ischia were the most contaminated, (2) there is a seasonal determinism in the contamination of the photosynthetically active leaves, (3) such a determinism is not observed in the rhizomes sampled in Marseille and Calvi. Experimental contaminations *in situ* of the *P. oceanica* by Cd in solution showed that (1) the accumulation of Cd by the leaves is an active process resulting in a concentration of it ranging from 652 to 1088 after 5 days, (2) the Cd-loaded leaves quickly decontaminate when returned to natural conditions (a contamination level corresponding to 10 % of the initial burden is reached after 6 days), and (3) Cd concentration in rhizomes is not affected by short-term contaminations (5 days). These experiments suggest that *P. oceanica* is a bio-indicator of metal pollution at two temporal scales : short-term for the leaves, long-term for the rhizomes.

STIMULATION AND REGULATION OF EGG-LAYING OF THE COCCIDIPHAGOUS LADYBIRD *CRYPTOLAEMUS MONTROUZIERI.* *O. Lemaitre.* Laboratoire de Biologie Animale et Cellulaire, C.P. 160/12, Université Libre de Bruxelles, Avenue F.D. Roosevelt, 50, 1050 Bruxelles.

C. montrouzieri is a coccidiphagous ladybird, often used in biological control against the populations of mealybugs. We have studied the stimulation and the regulation of the ladybird's egg-laying. These two features are almost unknown for the predaceous insects.The stimulation initiating the oviposition behaviour is due to the presence of a substance in the cuticular waxes of every stage of *Planacoccus citri* and only in the ovisacs of *Eupulvinaria hydrangeae*. Afterwards, a second stimulation occurs, which is due to the perception of enclosed places where the eggs have to be laid. We pointed out the existence of an epideictic pheromone which inhibits the female's laying proportionally to the number of conspecific larvae encountered. This pheromone is present in the larvae's cuticular waxes, which are lost when the larvae forage. In addition, when *C. montrouzieri* meets this pheromone it moves to an acceptable laying place and scatters its eggs.

ELIMINATION OF BRYOZOAN COLONIES DEVELOPING IN THE COOLING SYSTEMS OF THE NUCLEAR POWER PLANT OF TIHANGE. *Y. Marneffe* and *J.P. Thome*. Institut de Zoologie, Laboratoire de Morphologie, Systématique et Écologie animale, Unité d'Écotoxicologie des Micropolluants Organiques, Université de Liège, Quai Van Beneden 22, B-4020 Liège.

For many years, the development of bryozoan colonies has induced strong disturbance in the functioning of cooling systems of thermoelectric and thermonuclear power plants. In the nuclear power plant of Tihange (Belgium), significant biomasses of these organisms (essentially *Plumatella emarginata*) have developed in the basins beneath the cooling towers. The chlorine injection (as NaClO) is the usual way to clean the pipe of the cooling system. The free available chlorine (FAC) is relatively inefficient towards bryozoans. However the bryozoan colonies appear to be more sensitive to chlorine when ammonia present in water induces monochloramines formation. We had confirmed this hypothesis by ecotoxicological experiments. The aim of this work was to study 1) the stability of monochloramines when humic acids are present in the water, 2) the biocide efficiency of monochloramines towards bryozoans and 3) the NH_4^+ concentrations necessary to form, *in situ,* sufficient concentrations of monochloramines. These experiments have shown that : monochloramines are more toxic than free chlorine for bryozoans ; monochloramines are more stable than free chlorine in the presence of humic acids ; young colonies (1 to 10 polypids) are more sensible than the old ones ; the maximal effect of chlorination in the cooling system is obtained when the ammonia concentrations reach 1 to 1,5 mg NH_4^+/l and when the monochloramine concentrations reach 6 mg/l for 1 to 2 hours.

ISOLATION AND PURIFICATION OF A FROG-DERIVED PROTEINACEOUS CHEMOATTRACTANT ELICITING PREY ATTACK BY GARTER SNAKES. *C. Remy, R. Wattiez, P. Falmagne* and *G. Toubeau*. Service d'Histologie, Service de Chimie biologique, Université de Mons-Hainaut, Avenue Maistriau, 21, 7000 Mons.

A potent proteinaceous chemoattractant, eliciting prey attack by checkered garter snakes (*Thamnophis marcianus*), was isolated from aqueous washes of the frog *Rana temporaria* by means of preparative electrophoresis. The biological activity of frog extract was not modified by lyophilization but was lost after proteolytic digestion. A purified glycoprotein appeared highly attractive to garter snakes, as demonstrated by a snake bioassay (lures covered with test sample or control solution). This protein showed an apparent molecular weight of 21 kDa estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (PAGE-SDS). If a reducing agent (β-mercaptoethanol) was omitted during isolation, the chemoattractant tended to form high-molecular-weight aggregates suggesting the presence of free thiols in freshly prepared frog extract. However, in contrast to other studies, the biological activity seemed to be retained in the extracts even in the absence of reducing agents. These results suggest that a water-soluble glycoprotein molecule, produced by frogs, may be a vomeronasal stimulus used by garter snakes for prey trailing.

SEXUAL AND SOCIAL BEHAVIOUR OF *OREOCHROMIS AUREUS* (PISCES : CICHLIDAE) : ENDOCRINE PROFILES. [1] *G. Skoufas,* [1] *P. Poncin,* [2] *E. R. Kühn,* [2] *N. Byamungu,* [2] *B. Cuisset,* [1] *Ch. Mélard* and [1] *J. Cl. Ruwet.* [1] Service d'Éthologie et de Psychologie animale, Université de Liège, Institut de Zoologie, Quai Van Beneden, 22, B-4020 Liège and [2] KUL, Laboratorium voor Vergelijkende Endocrinologie, Zoölogisch Instituut, Naamsestraat 61, B-3000 Leuven.

The relationships between relative social dominance and T4, T3, Testosterone (T), Estradiol 17β (E2) and 11-ketotestosterone (11-keto) plasma levels were determined for 14 pairs of males and 7 pairs of females Tilapia *Oreochromis aureus* in aquaria. Sexually mature males show a ritual aggressive behaviour. Significant lower levels of circulating E2 and 11-keto have been observed in the subordinate males, but circulating T4, T3 and T levels were not significantly affected. Dominant females exhibit higher levels of circulating T3, T and 11-keto than the subordinates. A significant linear correlation has been observed in the females between circulating T levels and five aggressive patterns (« mouth fighting », « jagen », « lateral display », « tail beating », « biting »). Isolated males exhibit significantly higher levels of circulating T3, E2 and 11-keto than the isolated females, but T level was not significantly affected. The reproductive behaviour exhibits the mating patterns of « quivering » or « body quiver » which are unusual in the other species of the genus *Oreochromis.* After spawning and fertilization of the eggs, the females develop a mouthbreeding behaviour for the protection of eggs and young.

MORPHOLOGICAL VARIATIONS BETWEEN POPULATIONS OF *CARABUS PROBLEMATICUS* (CARABIDAE : COLEOPTERA), AT THE INTRAREGIONAL, INTERREGIONAL AND INTRACONTINENTAL LEVELS. *D. Stilmant* and *M. Dufrêne.* Unité d'Écologie et de Biogéographie, Université Catholique de Louvain, Place Croix du Sud, 5, 1348 Louvain-la-Neuve.

What is the influence of *Carabus problematicus* population isolation on their morphology, their isolation being natural or due to human activities? To answer this question, 19 measurements of 756 specimens, from 18 populations, were taken and analysed using multivariate techniques (PCAs, CDAs, FDAs). There are morphological differences at all the levels of the analysis, and a reallocation discriminant analysis shows that at least 60 % of the females and 54 % of the males are correctly assigned to their original populations. At the intraregional level, the differences shown are mainly shape differences. Surprisingly, two populations of *C. problematicus* separated by 8 kilometers show significant morphological differences. At the interregional level, the differences of size become more important but the shape variation stays the most significant. The first axis of the intracontinental DCAs, analysed on populations grouped by region, separates the 3 regions (Scotland, Ardennes, Provence). All the areas of a same country stay together. This discrimination is based, for the greatest part, on size differences, but there is an overlapping zone between Scotland and Belgium. Only French specimens are well isolated. For the Provence sample, all the males are reallocated to their group. This study confirms the classic systematics of *C. problematicus* (1).

(1) R. JEANNEL (1941) — Faune de France, *39,* Paris ; Ed. LECHEVALIER, 571 pp.

BIOGEOGRAPHY OF SMALL CARPENTER BEES OF NORTH AFRICA AND OCCIDENTAL EUROPE (HYMENOPTERA, ANTHOPHORIDAE, CERATININI).
M. Terzo[1], *P. Rasmont*[2] and *M. Dufrêne*[2]. [1]Service de Zoologie, Université de Mons-Hainaut, Avenue Maistriau, 19, 7000 Mons. [2]Unité d'Écologie et de Biogéographie, Université Catholique de Louvain, Place Croix du Sud, 5, 1348 Louvain-la-Neuve.

The authors have studied the small carpenter bee fauna of Occidental Europe, focusing on France and Belgium. 2600 specimens were studied from museum origins and from personal collections on flowers and in nests. 13 species are found in Occidental Europe. Of them, 2 are new for Europe and 1 is new for France. Their systematics has been revisid. A numerical taxonomic analysis emphazises the most discriminant characters between similar species (1). The present results complete the check-list of North African species of DALY (2). The presence of endemic species in Maghreb and South Spain and the reduction of the number of species in north of Mediterranean Sea emphazises the presence of a secondary dispersion center in this region for small carpenter bees.

(1) M. TERZO (1992) — Mémoire de licence, Université de Mons-Hainaut, 98 + XI pp. Mons, Belgique.
(2) H. V. DALY (1983) — Systematic Entomology, *8* : 29-62.

PREY CAPTURE IN SCLEROGLOSSAN LIZARDS.
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Many studies have shown that scleroglossan lizards capture preys with the jaws only (1). The prey capture kinematics of four species (*Lacerta viridis, Gerrhosaurus major, Zonosaurus laticaudatus,* and *Tiliqua scincoides*) of three families (Lacertidae, Cordylidae, and Scincidae) was quantitatively studied. Two prey items (mealworm and cricket) were used. The lacertid *L. viridis* always captured both prey types with the jaws only. The cordylid *G. major* captured the mealworm with the tongue and the cricket with the jaws. The cordylid *Z. laticaudatus* and the scincid *T. scincoides* captured the mealworm with the tongue, and the cricket with the jaws. In *G. major* and *T. scincoides,* the tongue was used for prey bringing into the buccal cavity as in iguanians, whereas, in *Z. laticaudatus,* the tongue was used for maintaining the prey on the substratum and facilitate the capture by the jaws. The mode of prey capture is highly variable in the studied scleroglossans. The gape cycle in *G. major* and *T. scincoides* is divided in three stages ; slow opening, fast opening, and fast closing as in iguanians. The division in slow and fast stages of the gape angle during prey capture with the jaws only or with the tongue by *Z. laticaudatus* was not present.

(1) K. SCHWENK and G.S. THROCKMORTON (1988) — J. Zool. (Lond.), *219* : 153-175.

QUANTITATIVE ANALYSIS OF FEEDING BEHAVIOUR IN THE SCLEROGLOSSAN *ZONOSAURUS LATICAUDATUS* (REPTILIA : SQUAMATA : CORDYLIDAE). *J.-M. Urbani* and *V.L. Bels*. Laboratory of Functional Morphology, University of Liège, Quai Van Beneden 22, B-4020 Liège, Belgium.

The four phases of the feeding behaviour (capture, reduction, transport into the buccal cavity, and deglutition or cleaning) were studied quantitatively. 7-13 cycles were digitized per phase for four individuals. The displacements of the head, the tongue, the jaws, and the prey were measured for each cycle. Two types of prey were used (mealworm and cricket). The mealworms were captured with the tongue or the jaws, and the cricket with the jaws only. The tongue in mealworm capture was used for maintaining the prey on the substratum and facilitate the capture by the jaws. A multivariate analysis of variance (MANOVA) was performed on 18 kinematic variables. This analysis was a mixed-model design that crossed the fixed phase effect with the fixed prey effect and the fixed use of the tongue. In order to explore the kinematic relationships between the four feeding phases, a principal component analysis was performed using 10 variables depicting the jaw displacements. Three main conclusions were obtained from the data : (a) the tongue was used for prey reduction, transport and deglutition as in *Lacerta viridis* (1), and the iguanians (2), (b) the capture phase was highly variable, and (c) the evolutionary transformation of the tongue to chemoreception does not prevent its use during all the feeding phases.

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THE HABITAT OF THE *OLIVA* (MOLLUSCA, GASTROPODA) OF HANSA BAY (PAPUA-NEW GUINEA). *C. Van Osselaer* and *B. Turch*. Laboratoire de Bio-Écologie, Université Libre de Bruxelles, Avenue F.D. Roosevelt, 50, 1050 Bruxelles.

Oliva are burrowing, mainly nocturnal carnivores, feeding on live or dead prey. They are a typical (and often dominant) component of the molluscan assemblage of tropical soft benthos. Their ecology is practically unknown. 73 stations in Hansa Bay have been sampled by SCUBA diving or dredging. For each station, accurate depth was recorded, the water motion estimated and the sediment analyzed for carbonates, organic carbon, granulometric distribution and color. 27 species of *Oliva* were collected, each having a limited spatial distribution. Most *Oliva* species were found to be cryptic : the colour of the mantle and the shell closely match the sediment. No decisive substrate choice was exhibited in aquarium experiments on several species. The distribution is explained neither by depth alone nor by the nature of substrate alone. The observed distribution of the *Oliva* seems largely controlled by predator selection. The hitherto unknown predator(s) must have a good vision and be diurnal (as evident by the generalized crypsis in *Oliva* and the higher mortality of non-cryptic specimens). Predation must be very important (as evident by the large percentage of scar-bearing individuals). We suggest that the nocturnal activity of *Oliva* might be a counter-adaptation to diurnal predation.