

## ABSTRACTS

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

by

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

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

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

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**ENTOMOFAUNE COMPARÉE  
DES TERRILS D'HENSIES ET ST-ANTOINE**

par

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

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

by

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

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

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

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

by

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

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

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

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

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

by

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

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

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

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

by

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

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

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

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

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

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

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

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

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

by

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

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

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

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

by

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

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

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

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

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

by

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

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

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

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

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

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

**MINERALIZATION OF ADULT MOUSE BONE MARROW IN VITRO**

by

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

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

The mineralization process has a shorter latency period in the presence of serum containing TCM as compared to a serum free TCM. The serum probably contains growth factors for the primary cultures. Serum components in the TCM influence the physiology of the bone formation in vitro. Stromal cells start mineralization in serum containing TCM, they contain the cells responsible for mineralization. The immortalized cells provoked mineralization immediately and with the same course in serum containing TCM and serum free TCM. The immortalized cells are probably differentiated cells with a self-regulating capacity for the mineralization. Effects of the bone growth regulating hormones, parathyroid hormone and vitamin D<sub>3</sub> on the mineralization are not measurable by the <sup>85</sup>Sr uptake. This can be caused by the insensibility of the <sup>85</sup>Sr technique or by the separation of the <sup>85</sup>Sr metabolism and the cellular process in which parathyroid hormone and vitamin D<sub>3</sub> act. Parathyroid hormone and vitamin D<sub>3</sub> modified the alkaline phosphatase activity of the immortalized cells. This provides evidence for the presence of receptors for the hormones in the immortalized cells.

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

by

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

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



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

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

by

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

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

(1) GUPTA *et al.*, 1985; *J. Cell. Sci.*, **74**, 137-152.

(2) SACCHI *et al.*, 1986; *FEBS*, **204**(2), 213-218.

(3) KNOWLES *et al.*, 1987; *Bioch. et Bioph. Acta*, **924**, 509-518.

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

by

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

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

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

by

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

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

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

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

by

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

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

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

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

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

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

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

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

by

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

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

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

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

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## RED SPIDER MITE DAMAGE ASSESSMENT FOR GREENHOUSE TOMATOES

by

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

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

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

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

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

by

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

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

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

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

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

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

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

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

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

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

by

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

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

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

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

\* Supported by an « IWONL » grant.

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

by

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

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

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

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

by

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

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



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

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

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

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

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

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

par

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

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

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

- Marquage de 7 antigènes membranaires révélé par immunofluorescence indirecte (F4/80, 2.4G2, MAC-1, THY1.2, 21.1.1, L3T4a) et directe (IgM).
- Evaluation du pouvoir phagocytaire vis-à-vis de billes de latex fluorescentes et de bactéries fluorescéinées (*Micrococcus lysodeikticus*).
- Comparaison de la présentation de l'antigène de classe II (Ie) du complexe majeur d'histocompatibilité en association avec différentes concentrations du peptide à processor.

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

L'analyse des résultats est résumée comme suit :

- 1) Les cellules des densités 1.050 à 1.060 g/ml appartiennent à la lignée des lymphocytes (elles sont L3T4a +, THY1.2 + et IgM +). De plus, le test de phagocytose est faible.
- 2) Les cellules des densités 1.064 à 1.096 g/ml appartiennent à la lignée des monocytes-macrophages (elles sont 2.4G2 +, F4/80 +, les tests de phagocytoses et la présentation de *Ie* sont très positifs). De plus, il existerait parmi ces macrophages de la même lignée cellulaire des stades différents de maturation. En effet, les cellules des densités 1.064 à 1.082 g/ml seraient plus immatures que celles des densités 1.087 à 1.096 g/ml (hétérogénéité de positivité lors des différents tests effectués).
- 3) Les cellules des densités 1.104 et 1.117 g/ml appartiennent à la lignée des polymorphonucléaires et des mastocytes.

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

## BIODEGRADATION AND PRESERVATION OF FISH VERTEBRAE IN MARINE AND ESTUARINE CONDITIONS

by

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

Anoxic conditions were created by use of closed jars, containing sediment sampled *in situ* (organoclastic sand, CaCO<sub>3</sub>-poor sand or estuarine « slikke ») in the Calvi Bay (Corsica) or Le Guillec estuary (Bretagne). When the jars were opened, enzymatic hydrolytic activity assessments were performed by use of Apizym kits. Samples were subsequently processed for S.E.M. observations.

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

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

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

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

#### SEROLOGICAL IDENTIFICATION OF *AEROMONAS* SPECIES ISOLATED FROM FISH

by

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

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

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

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

by

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

In order to obtain a reasonable amount of material, a number of the identified peptides were synthesized (Texas and Leuven ; Merrifield's method, (2)). After synthesis, decoupling and deprotection, they all were purified on Seppak-columns (C<sub>18</sub>) and by RP-HPLC ( $\mu$ -Bondapak phenyl).

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

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

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

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

\* Supported by IWONL grant.

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PRELIMINARY DATA ON LIPID REQUIREMENTS  
OF *CLARIAS GARIEPINUS* LARVAE

by

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

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

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

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

by

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

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

by

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

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

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

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

by

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

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

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

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

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

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

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

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