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GENOTYPE-DEPENDENT DAYTIME VERTICAL DISTRIBUTION OF *DAPHNIA MAGNA* IN A SHALLOW POND

by

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SUMMARY

The daytime vertical distribution of *Daphnia magna* STRAUS genotypes, characterized by differences at two enzyme loci (*Got* and *Pgi*), was analysed in a shallow pond. Though all genotypes tended to occur predominantly in the lower half of the water column, we observed significant differences in the vertical distribution of genotypes marked by differences at the *Got* locus. Our results indicate that, in shallow water bodies too, the vertical distribution of zooplankton is not merely random, and that vertical distribution and migration patterns may be genotype-dependent in such small-scale systems.

Keywords : Daphnia, vertical distribution, genotype, habitat selection.

INTRODUCTION

A substantial amount of the information on diurnal vertical migration of zooplankton has been derived from laboratory experiments on *Daphnia magna* (CLARKE, 1931 ; HARRIS and WOLFE, 1955 ; RINGELBERG, 1964 ; CALABAN and MAKAREWICZ, 1982 ; DUMONT *et al.*, 1985 ; DE MEESTER, 1991a). *D. magna* is indeed easy to culture and, due to its size, easy to manipulate. Moreover, there is an abundance of background information available on the physiology, ecology and genetics of this species (PETERS and DE BERNARDI, 1987), including a great deal of data on phototactic behaviour under laboratory conditions (reviewed by RINGELBERG, 1987). However, *D. magna* being a pond species, there is little information available on its vertical migration behaviour in the field. RINGELBERG (1964) reports on a nocturnal vertical migration (evening ascent) of a *D. magna* population in a shallow canal. RANTA and NUUTINEN (1985) also observed nocturnal migration of *D. magna* in shallow rock-pools.

In previous work, we have shown that there is a significant genetic component to the variability in phototactic behaviour in *D. magna* (DE MEESTER, 1989, 1991a, 1993b ; DE MEESTER and DUMONT, 1989 ; VAN UYTVANCK and DE MEESTER, 1990). The vertical distribution of different genotypes in outdoor containers is in concordance with expectations drawn from laboratory observations on phototactic behaviour (DE MEESTER, 1993a). These results suggest that the vertical daytime distribution of *D. magna* in natural populations may be genotype-dependent. Studies using electrophoretic markers have shown such genotype-dependent vertical distributions for typical lake *Daphnia* species (MÜLLER and SEITZ, 1993) as well as for *D. pulex* in a large deep pond (6.5 m depth ; WEIDER, 1984). In the present study, we analyse the vertical distribution of *D. magna* genotypes in a shallow pond. Experiments on *D. magna* clones isolated from this pond have provided evidence for intrapopulational variability in phototactic behaviour (DE MEESTER, 1991b).

MATERIAL AND METHODS

The study site is Driehoekvijver (Heusden, Eastern Flanders, Belgium), a small (1/2 ha surface area) and shallow (< 1 m depth) eutrophic pond. *D. magna* and *D. pulex* are the most abundant cladocerans, but their densities are subject to rapid and extensive changes due to periodical flushing, and partial drying out of the pond in summer. The pond contains fish (*Rutilus rutilus* LINNAEUS and *Gasterosteus aculeatus* LINNAEUS), but they are never abundant, probably due to the unstable nature of the pond system.

Samples were taken on 5 February 1992, 14.00 h, by towing a series of rectangular plankton nets simultaneously through the water over a distance of 1.2 m, after which all nets were closed simultaneously. Each net was 10 cm high, with a sampling surface of 50 cm^2 (total volume sampled per net : 6 liter). The nets were mounted on a wooden bar in such a way that the complete water column was sampled with a depth interval of 10 cm. The sampling site was 60 cm deep, with an artificial (concrete) bottom, facilitating efficient sampling of the near-bottom water layer. From each depth sample, 30 adult *D. magna* females were randomly picked out for electrophoretic analysis and preserved in liquid nitrogen. Only 15 adult females were analysed from the upper waterlayer (0-10 cm), because of the low number of animals caught. The remainder of the sample was fixed with formaldehyde (4 %) for subsequent counting.

Cellulose acetate electrophoretic analysis was done according to the methods described in HEBERT and BEATON (1989). Small animals (< 2.5 mm) were homogenized in 50 µl Tris-glycine buffer solution (pH = 8.5), larger animals in 70 µl. Two enzymes were screened : glutamate-oxaloacetate transferase (*Got*, EC 2.6.1.1.) and phosphoglucose isomerase (*Pgi*, EC 5.3.1.9.).

We tested the null hypothesis that there is no genotype-dependent vertical distribution by means of G-tests of Independence (SOKAL and ROHLF, 1981) grouping individuals according to their genotype at the two loci (*Got* and *Pgi*). Two-locus as well as pooled one-locus genotype frequencies were tested. Though analyzing the

loci separately (pooled data) results in a potential loss of information, the statistical reliability is increased considerably due to higher cell frequencies.

RESULTS

The number of adult *Daphnia magna* caught in the different nets is given in Table 1. The animals are not uniformly distributed, but show a tendency to reside close to the bottom. Almost 50 % of the adults were found in the lower 10 cm, whereas less than 3 % occurred in the upper 10 cm.

TABLE 1

Depth distribution of adult *Daphnia magna* in Driehoekvijver, 5 February 1992, 14.00 h. N : number of adults caught ; $N\,l^{-1}$: density per liter ; % : percentage of total population caught at given depth ; avg.: average.

Depth	N	$N\,l^{-1}$	%
0-10 cm	17	2.8	2.4
10-20 cm	32	5.3	4.6
20-30 cm	42	7.0	6.0
30-40 cm	130	21.7	18.5
40-50 cm	150	25.0	21.4
50-60 cm	330	55.0	47.1
avg.	116.8	19.5	

All possible two-locus genotypes were represented in the population (Table 2). An overall RxC G-Test of Independence indicates a genotype-dependent vertical distribution when the data are pooled according to genotype at *Got* (G-statistic = 25.98, df = 10, $p < 0.005$). When the data are pooled for genotype at *Pgi*, the null hypothesis is not rejected (G-statistic = 8.11, df = 10, $p > 0.5$). Pooling depth strata two by two (0-20 cm, 20-40 cm, 40-60 cm) yields similar results (*Got* : G-statistic = 9.77, df = 4, $p < 0.05$; *Pgi* : G-statistic = 1.90, df = 4, $p > 0.5$). The vertical distribution of genotypes heterozygous for *Got* is found to be significantly different from that of both homozygous genotypes by G-tests ($p < 0.001$). No significant differences were observed in the vertical distribution of *Pgi* genotypes (G-tests, all $p > 0.1$).

Analysing the frequency data of all two-locus genotypes that represent > 5 % of the population yields some additional differences (see Table 2), but caution should be taken in interpreting these results, as sample sizes are small. Figure 1 shows that, though the genotypes marked by differences at the *Got* locus were all

TABLE 2

Genotype of 165 adult *Daphnia magna* sampled at different depths in the Driehoekvijver. Number of animals sampled at depth 0-10 cm is 15, and 30 at all other depths. n° : number designated to genotypes ; % : relative abundance of genotype in pooled sample (corrected for reduced sample size at depth 0-10 cm) ; G : pairwise comparisons of distribution over depth of genotypes (in case of two-locus genotypes, only those representing > 5 % of the sampled individuals analysed) by G-tests, numbers refer to genotypes for which the vertical distribution is significantly different at p = 0.05 ; ns : no significant differences were observed for any of the comparisons ; / : data not analysed due to low sample size (< 5 % of the population).

A : Two-locus (*Pgi* — *Got*) genotypes.

Depth	SS,SS	SF,SS	FF,SS	SS,SF	SF,SF	FF,SF	SS,FF	SF,FF	FF,FF
n°	1	2	3	4	5	6	7	8	9
0-10 cm	1	2	3	1	5	3	0	0	0
10-20 cm	3	7	7	1	3	4	0	1	4
20-30 cm	4	5	6	1	4	6	1	2	1
30-40 cm	0	6	5	1	7	9	0	1	1
40-50 cm	2	9	6	1	2	4	1	3	2
50-60 cm	5	9	5	1	3	2	1	1	3
Total	15	38	32	6	24	28	3	8	11
%	8.9	22.2	19.4	3.9	16.1	17.2	1.7	4.4	6.1
G	2, 3, 5, 6	1,5,6	1	/	1, 2, 9	1, 2, 9	/	/	5, 6

B : One-locus genotypes.

Depth	*Pgi*			*Got*		
n°	SS 1	SF 2	FF 3	SS 1	SF 2	FF 3
0-10 cm	2	7	6	6	9	0
10-20 cm	4	11	15	17	8	5
20-30 cm	6	11	13	15	10	5
30-40 cm	1	14	15	11	17	2
40-50 cm	4	14	12	17	7	6
50-60 cm	7	13	10	19	6	5
Total	24	70	71	85	57	23
%	14.5	42.5	43.0	51.5	34.6	13,9
G	ns	ns	ns	2	1, 3	2

more abundant in the lower 30 cm of the water column than in the upper 30 cm, the extent to which the animals aggregate in the near-bottom 10 cm was genotype-dependent.

DISCUSSION

Our results clearly indicate a genotype-dependent daytime vertical distribution of *Daphnia magna* in a shallow pond. Genetic differentiation in vertical distribution and migration patterns in *Daphnia* have been reported before (WEIDER, 1984; STIRLING *et al.*, 1990; MÜLLER and SEITZ, 1993). Our observations show that such habitat partitioning may occur in a very shallow system, on a scale of less than one meter. Previous work on clones isolated from the same pond had already indicated significant interclonal differences in phototactic behaviour (DE MEESTER, 1991a, 1991b). In concordance with these previous results, the present observations indicate that genotypes with a preference for a shallow day-depth are absent or rare in this population. Indeed, all genotypes are predominantly found in relatively deep water (see Table 2, Figure 1). This may be explained by the fact that this population co-occurs with fish (mainly *Rutilus rutilus* and *Gasterosteus aculeatus*). So far, we have not been able to hatch positively phototactic clones out of ephippia collected from the pond (DE MEESTER, pers. obs.). However, positively phototactic clones have been obtained in the laboratory through selfing clones isolated from the Driehoekvijver (DE MEESTER, 1991a), indicating that this population has not lost

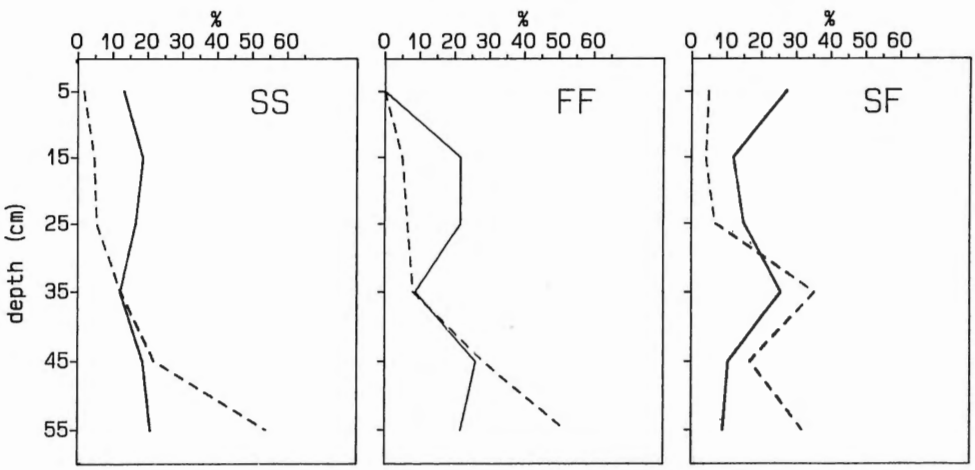

Fig. 1. — The vertical daytime distribution of *Daphnia magna Got* genotypes in Driehoekvijver, 5 February 1992 : Solid line : % adults of given genotype observed at different depths, when the same number of animals is analyzed at all depths (relative distribution). Broken line: estimates adjusted for differences in density of the *Daphnia* population at different depths (estimate of absolute distribution).

the capacity for an evolutionary change of its daytime distribution, *e.g.* by becoming partly positively phototactic, if the environmental conditions would allow so.

Our results are based on one vertical profile and a resolution of only two loci. The differences in vertical distribution that were observed for genotypes marked by their alleles at the *Got* locus most probably are the result of a linkage disequilibrium effect, though a direct effect of *Got* on phototactic behaviour or a physical linkage of the *Got* locus with loci influencing phototactic behaviour, cannot be excluded. Resolution of more loci might reveal more clear-cut vertical distribution patterns, as the multi-locus genotypes thus obtained would more closely correspond to true single genotypes.

Irrespective of these limitations, our results indicate that the vertical distribution of zooplankton in shallow ponds is not merely random, and that vertical distribution and migration patterns may be genotype-dependent even in such small-scale systems. This adds to the confidence that differences in phototactic behaviour between *D. magna* clones isolated from this population, as observed in the laboratory (*e.g.* DE MEESTER, 1991a, 1991b), are not merely laboratory artefacts. In order to evaluate the full extent of genetic differences in vertical migration patterns of *Daphnia* and other zooplankton in shallow ponds, a more comprehensive study is needed, analysing the vertical distribution of genotypes over several day-night cycles.

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THE INFLUENCE OF SPERM PRECEDENCE PATTERNS AND MATING COSTS ON COPULATION DURATION IN ODONATES : PREDICTIONS AND SUPPORTING DATA.

by

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SUMMARY

NUYTS and MICHIELS (1993) developed a model that predicts how insect copulation duration should vary under varying conditions of intra-male competition, mating systems and copulatory mechanisms in order to optimize the male's reproductive success. In this paper, we compare the predictions of this model with data of odonates. In general, the data seem to support the predictions, especially the prediction that territoriality, a lower take-over probability, a higher female encounter rate or a shorter time between copulation and first oviposition bout shorten copulation. We conclude that the model is well-suited for odonates, and point out how it can have some practical use.

Key-words : optimality model, copulation duration, Odonata, sperm competition, reproductive success, territoriality, take-over, encounter rate, oviposition.

INTRODUCTION

Copulation duration varies widely in odonates, both within one species (UÉDA, 1979 ; MILLER, 1983 ; NOMAKUCHI *et al.*, 1984 ; SIVA-JOTHY, 1987 ; ALCOCK, 1988 ; SIVA-JOTHY and TSUBAKI, 1989a ; WOLF *et al.*, 1989 ; MICHIELS, 1992) and between species (WAAGE, 1984a, 1986). The authors listed discuss many different explanations for these differences. Only few of them show that some of these hypotheses are evolutionarily stable. In this paper, we present the (relevant) predictions of a theoretical model (NUYTS and MICHIELS, 1993 ; NUYTS and METZ, unpublished) and use these to explain the observed differences in copulation duration. The strength of our model is threefold :

(i) The model assumes that males optimize copulation duration to maximize their reproductive success. As a results, the predictions are both causal and evolu-

tionarily stable. By adding stability we reinforce the importance of existing hypotheses.

(ii) All but one of the results presented in the literature can be explained by this model. Hence, it seems to provide a unifying theory on the influence of parameters on the copulation duration of odonates. It is, however, sufficiently flexible to be applied to a wide range of different taxa.

(iii) All this suggests that the model can be used to indicate new research avenues in dragonfly copulatory behaviour.

TABLE 1

Abbreviations used in the text

t :	copulation duration.
h :	time between copulation and first oviposition.
ovi + :	copulation duration increases with increasing time between copulation and oviposition.
take + :	copulation duration increases with increasing probability of take-over between copulation and oviposition.
enc + :	copulation duration increases with increasing time to encounter the next receptive female.
cost − :	copulation duration decreases with increasing territorial or energetic costs of a copulation.

THE MODEL OF NUYTS AND MICHIELS (1993)

The model assumes that male reproductive success is a monotone increasing, concave or sigmoid function of copulation duration (Fig. 1). It also assumes that copulation duration is not limited because of sperm depletion and that it has no effect on the probability of a take-over, nor on the time between encounters with receptive females. This implies, amongst other things, that rival males never interrupt a copulating pair. In several odonates, a short copulation can yield a high immediate second male's precedence (P_2), since the last male's sperm initially has a positional advantage. But, due to long term sperm mixing, this advantage may decrease with time. Hence, P_2 decreases in time even without rematings. In such a species longer copulations are necessary to maintain a high P_2 over time, since longer copulations increase the volume of the male's sperm and/or decrease the volume of the rival's sperm within the female sperm stores.

If every male has only one optimal copulation duration (only inter-individual differences between conspecific males), the following predictions can be made.

(1) Males that face higher risks will copulate longer. It is as if they make the best of a bad job, by exploiting their opportunities more extensively. More precisely :

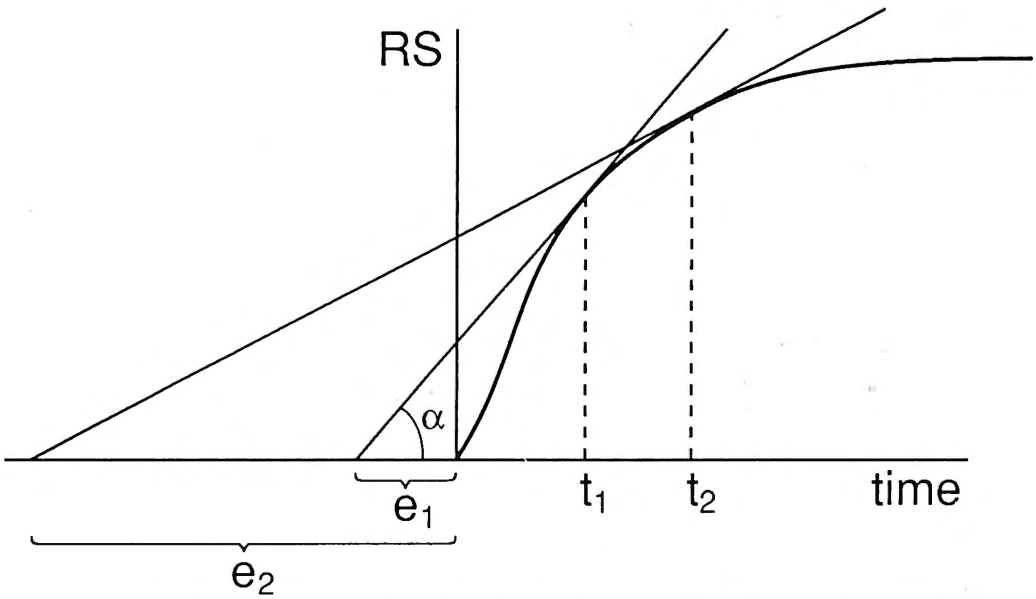

Fig. 1. — A simple representation of the maximization principle. The only costs taken into account are the encounter time to the next receptive female (e) and the copulation duration (t) (cfr PARKER and STUART, 1976). Copulation duration is optimised if

$$\frac{\text{reproductive succes}}{\text{costs}} = \frac{RS}{e + t} = \text{tg } (\alpha) \text{ is maximized.}$$

A larger encounter time increases optimal copulation duration. The optimal duration, associated with e_1 (e_2) is noted as t_1 (t_2).

(1a) If P_2 decreases in time due to long term sperm mixing, a male that can expect a longer time between copulation and the first oviposition, will copulate longer. (Prediction *ovi*+).

(1b) A higher take-over probability before or during the first oviposition bout will result in an increase of the optimal copulation duration for individual males, if long term sperm mixing occurs. (Prediction *take*+).

(1c) The optimal copulation duration will also increase with the female encounter time for the male. (Prediction *enc*+). Courting is included as part of the encounter time. If guarding duration is independent of the copulation duration, it has two contradictory effects. As guarding needs time, it has the same influence on t as the encounter time, and so it prolongs copulation. But since guarding reduces the probability of a take-over, it should also shorten copulation duration.

(2) Males that face higher energetic and territorial costs copulate shorter. As an essential difference between costs and risks, risks are independent of the copulation duration, whereas the costs depend on the copulation duration. If guarding duration is positively correlated with copulation duration, in the same way as are the energetic costs, it has only a negative effect on t. (Prediction *cost*−).

If every species has only one optimal copulation duration the predictions (ovi +), (enc +) and (cost-) stand firm. For mathematical reasons with biological relevance, the model does not allow a prediction on (take +) in this situation (NUYTS unpublished).

DISCUSSION OF ODONATE EXAMPLES

In the following discussion, abbreviations are used according to Table 1.

Within species

MILLER (1983) found significant differences in copulation duration between territorial (190.6 ± 330s) and nonterritorial (1699s ± 1398s) *Orthetrum chrysostigma* (BORM) males. Since territorial males encountered females nine times more often than nonterritorial males, this difference is in agreement with prediction (enc+) of the general model. Also, post-copula take-overs were less common in territorial than in nonterritorial males, since the former guard ovipositing females (MILLER, 1983). If sperm mixing occurs in *O. chrysostigma*, this lower number of take-overs for territorial males will also shorten t (take+). Finally, territorial costs must be taken into account (cost-), since twice a territorial male was seen to terminate copulation abruptly in order to chase an intruder (MILLER, 1983). This suggests that a male shortens t in response to the increased risk of a successful take-over of his territory. Continued copulation would make it increasingly difficult to chase away the intruder.

In *Orthetrum cancellatum* (L.), territorial males at the water have short copulations (21.0 ± 13.5 s) resulting in 10-15 % removal of rival sperm (SIVA-JOTHY, 1987). Wandering males mated for 894 ± 142 s, resulting in almost 100 % removal. SIVA-JOTHY (1987) attributed this difference to the very short encounter time for territorial males relative to the opportunistic males (16 times longer) (enc+), and, secondly, the short time between copulation and a mate's first oviposition for territory holders ($h \approx 0$) (ovi+). A third possible reason might be a difference in the number of take-overs. According to SIVA-JOTHY (1987) females deposited on average half their clutch before being grasped by a territorial male. Hence, if there are no take-overs by opportunistic males away from the water, both territorial and opportunistic males had a take-over probability of aprox. 0.5 per female. If there were unnoticed take-overs by opportunistic males, the number of take-overs could lead to increased copulation duration in opportunistic *O. cancellatum* (take +).

Mnais pruinosa pruinosa SELYS males show three alternative mating tactics, each characterized by a specific copulation duration (SIVA-JOTHY and TSUBAKI, 1989a, b). Territorial males defend pieces of wood, have copulations of intermediate length and guard their ovipositing females. Nonterritorial, wandering males have long matings at feeding sites. Sneaky males secure females that are ovipositing in another male's territory. Their copulations are short. Probably to avoid discovery by the territory owner, as this invariably results in remating of the female with the

territory owner (SIVA-JOTHY and TSUBAKI, 1989a,b). In our notation this means that the number of take-overs depends on t, which we assumed not to be so. Hence, the model is not valid for sneaky *M. p. pruinosa* males. Under natural conditions, the time between copulation and the first oviposition is longer for nonterritorial males than for territory owners (SIVA-JOTHY and TSUBAKI, 1989a). Since long term sperm mixing has been demonstrated (SIVA-JOTHY and TSUBAKI, 1989a), a smaller h is predicted to result in a shorter copulation duration (ovi+). Territory owners experience fewer take-overs than nonterritorial ones (SIVA-JOTHY and TSUBAKI, 1989a). Since mixing has been demonstrated and more than one oviposition bout can be taken into account (NOMAKUCHI, 1988 ; SIVA-JOTHY and TSUBAKI, 1989a), fewer take-overs will shorten t (take+). By experimentally introducing males and females, SIVA-JOTHY and TSUBAKI (1989b) found that t did not depend on the number of territorial intrusions by other males, nor on the female encounter rate. Only the place where a female was captured and copulated with explained variation in t. From this, we have the feeling that insects don't measure encountertime, but the presence or absence of an oviposition site and that they use this as an on/off trigger to adjust their copulation duration. Both in *Mnais pruinosa* (SIVA-JOTHY and TSUBAKI, 1989b) and in yellow dungflies *Scatophaga stercoraria* (L.) (WARD and SIMMONS, 1991) males do not adapt their copulation duration when the encountertime is reduced in an experimental way. But under natural conditions the encountertime is smaller in presence of an oviposition site (*S. stercoraria* : PARKER, 1971 ; *M. pruinosa* : SIVA-JOTHY and TSUBAKI, 1989b). As a result males adapt their copulation duration if females are presented near versus away from the ovipositon site (*M. pruinosa* : SIVA-JOTHY and TSUBAKI, 1989b ; *S. stercoraria* : WARD and SIMMONS, 1991). The influence of the encountertime seems only to exist in copulations at versus away from the oviposition site. Independence of the rate of territory intrusions seems to disagree with the model (cost−).

In *Sympetrum danae* (SULZER) a large variation in copulation duration exists (MICHIELS, 1992). Copulations shorten with increasing temperature, with time of the day, and for each additional mating of the male. The correlation with temperature probably has a physiological origin and has been mentioned earlier for insects (PARKER, 1971 ; UÉDA, 1979 ; LARSSON, 1989 ; PETERSSON, 1990). At high temperatures, males mate more rapidly with the same results than at low temperature. The time effect is most probably due to an increasing mating probability during the first half of the day (MICHIELS, 1992), which is equivalent to a decreasing encounter time (enc +). *S. danae* pairs mate before 13h00 (mean solar time), and hardly afterwards. Apparently, males become sperm depleted when mating several times a day (MICHIELS, 1992). PARKER (1992) modelled a comparable situation, and showed that it is adaptive to decrease copulation duration with each additional mating.

In all the former examples, males « make the best of a bad job », by adapting their copulation duration to a worse situation. In *Leucorrhinia intacta* (Hagen), the situation is different. WOLF *et al.* (1989) found a significant difference in t between territorial and nonterritorial males. The short copulations (t_1) of territory owners resulted in less than 100 % immediate precedence, whereas the longer copulations

of nonterritorial males (t_2) ensured 100 % immediate precedence. But territorial males that left their territory while in copula copulated as long as nonterritorial ones. Apparently, the territorial cost in *L. intacta* is high, and this cost shortens copulation. According to WALTZ and WOLF (1984) the overall gain per cost was equal for both tactics :

$$\frac{G(t_1)}{TC(t_1) + C(t_1)} = \frac{G(t_2)}{0 + C(t_2)},$$

with G(t) denoting the reproductive success when copulating during time t, TC(t) the costs due to copulating while territory owner, and C(t) all the other costs of copulating. If we assume that the probability of intrusions is greater in a territory than in the nearby grass, copulating in a territory will require more energy ($TC(t) > 0$). Occasionally, territory holders in copula chased away intruders (WOLF *et al.*, 1989), again increasing TC(t). When the territorial cost exceeds the benefits (the higher probability of meeting another female the same day), males will abandon their territory and become nonterritorial, resulting in diminished costs. We conclude that the two tactics are part of a mixed Evolutionary Stable Strategy (ESS), whereas two different ESS's, one with a lower pay-off, were concerned in the other species. Also in this species, the predictions (cost-) and (enc+) are fulfilled.

In *Calopteryx maculata* (de Beauvois) the conditions for an intraspecific difference in copulation duration exist. Territorial males have territorial costs (cost-) that result from mating due to a temporary absence from the territory (WAAGE, 1973). They encounter as much or more receptive females than wandering males (WAAGE, 1973) (enc +) and have a smaller take-over probability (WAAGE, 1979) (take+). But an essential assumption of the model is not fulfilled by this species. There is a biological limit for the copulation duration : after 1.0-1.5 minutes, 88-100 % of previous inseminated sperm is removed, and own sperm is inseminated (WAAGE, 1979). This means that the P_2-function is not concave everywhere, but it becomes horizontal after some copulation duration t_0 (for $t > t_0 : P_2(t) = 1$). Hence it is simply useless to copulate longer than this duration.

Between different species

WAAGE (1984a, 1986) compared different species, and suggested that short copulations are related to high mating rates, territoriality and non-contact guarding, intermediate copulation durations are related to low mating rates, nonterritoriality or opportunism and oviposition in tandem, and long copulations with infrequent mating and no postcopulatory guarding. This is in agreement with the predictions (enc+) and (cost−) of the model useable for interspecific comparison.

Males that have on average energetically more costly copulations should copulate shorter than males that copulate energy-efficiently (cost−). Costs can be higher when copulation takes place in flight or at a less optimal temperature. Male dragonflies that copulate in flight do so for a few seconds only (SAKAGAMI

et al., 1974), while copulations on a perch last a few minutes to several hours (CORBET, 1980).

CONCLUSION AND FURTHER SUGGESTIONS

From the examples above, we conclude that our model is appropriate to predict copulation behaviour for odonates. Only one (experimental) result was in contradiction with the model (*M. pruinosa* (cost−)). Therefore the model can be used to generate or contradict hypotheses and to point out experiments to be done, such as :

(a) UÉDA (1979) interprets the long copulation duration of wandering relative to territorial males in *Sympetrum parvulum* BARTENEFF as a kind of postcopulatory guarding. His major argument is that at low densities the copulation duration of both types of males is the same. This hypothesis might be correct, but a part of the longer duration at high densities can also be caused by *e.g.* higher encounter time (enc+) and increased take-over risk (take+) for wandering males (UÉDA, 1979), and probably a higher territorial cost (cost−) for the territory owners.

(b) In *Erythemis simplicicollis* (HAGEN) territorial males copulate more frequently (enc+), they have a higher probability of immediate oviposition (ovi+), and they guard their mates more effectively than nonterritorial satellites (take+) (MCVEY, 1981). Long term sperm mixing has been proven (MCVEY and SMITTLE, 1984). Hence, we strongly expect a difference between territorial and nonterritorial males. Data to support this prediction are not yet available.

(c) WAAGE (1984b) found the same restriction in *Calopteryx dimidiata* BURMEISTER as in *C. maculata*. Therefore we predict no different copulation durations.

(d) In order to see if an on/off trigger is a good explanation for the independence between copulation duration and artificially decreased encountertime, it might be interesting to decrease experimentally the encountertime for males at and away from the oviposition site in other species.

(e) If a species doesn't fulfill several predictions, it might be worthwhile to look for an overriding factor, such as a very large difference between males, rather than between groups of males, or a strong female influence.

(f) If the encounter time is very large, relative to lifetime, the optimisation principle as such may not be fulfilled. Then the right currency to optimize is lifetime reproductive success, rather than gain per unit of time. Time loss is not important anymore. Then we expect the copulation duration to be limited by other factors, such as sperm depletion, the maximal P_2 being reached after some time, an external physical constraint or female unwillingness to continue. Large encounter times are reported for *Hetaerina vulnerata* Selys (e = 3.5 days (ALCOCK, 1982)). Analogously, no variation in copulation duration will be caused by the parameters in the model if the mating system limits the males to one mating a day, as is the case in *e.g.* *Argia apicalis* (Say) (BICK and BICK, 1965), *Ischnura graellsii* RAMBOR (CORDERO, 1990), and *Ischnura elegans* (van der Linden) (MILLER, 1987). We expect the parameters of the model not to influence the copulation duration.

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PROALES CHRISTINAE (ROTIFERA, PROALIDAE) : A NEW SPECIES FROM THE LITTORAL OF THE NORTH SEA

by

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SUMMARY

A new rotifer species, *Proales christinae* sp. nov. (Rotifera, Monogononta : Proalidae), collected from hydroids washed ashore on the beach of the North Sea, Belgium, is described.

Keywords : Proales christinae, Rotifera, North Sea, hydroids.

INTRODUCTION

The marine rotifer fauna of Belgium is virtually undocumented (see DE RIDDER, 1961, 1989, 1992). For this reason, I recently started sampling different marine littoral habitats along the Belgian coast. The new species described here, was obtained from a collection of miscellaneous hydroids.

DESCRIPTION

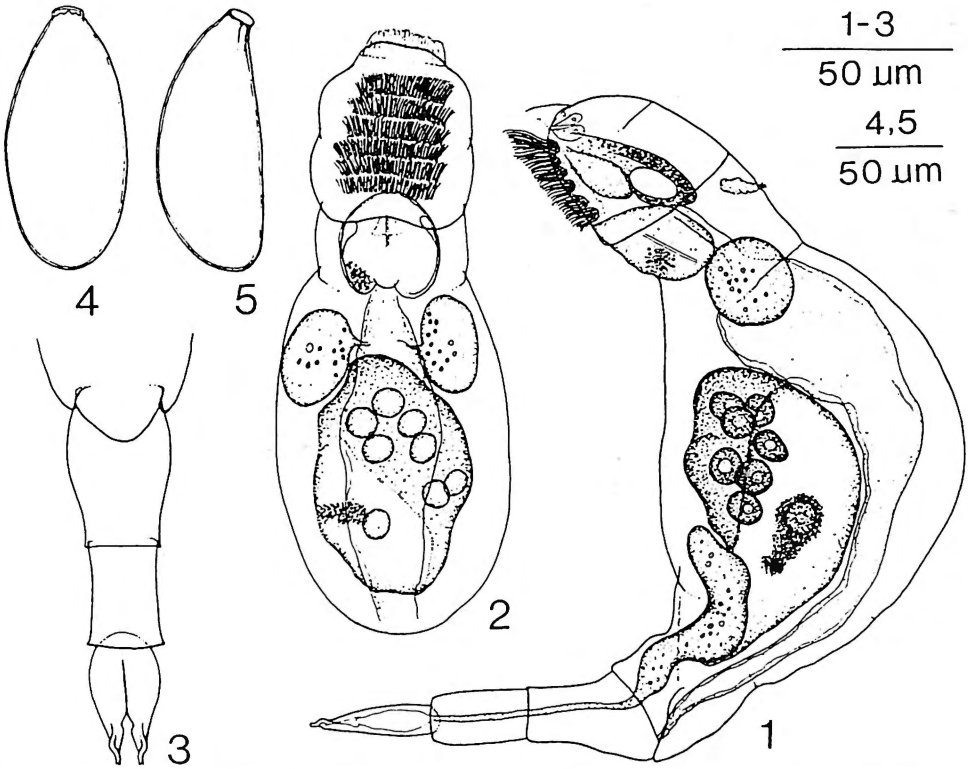
Proales christinae sp. nov.
(Figs 1-9)

Type locality. Westende, Belgium, beach of the North Sea. Coordinates : 51°09′35″N, 2°46′10″E.

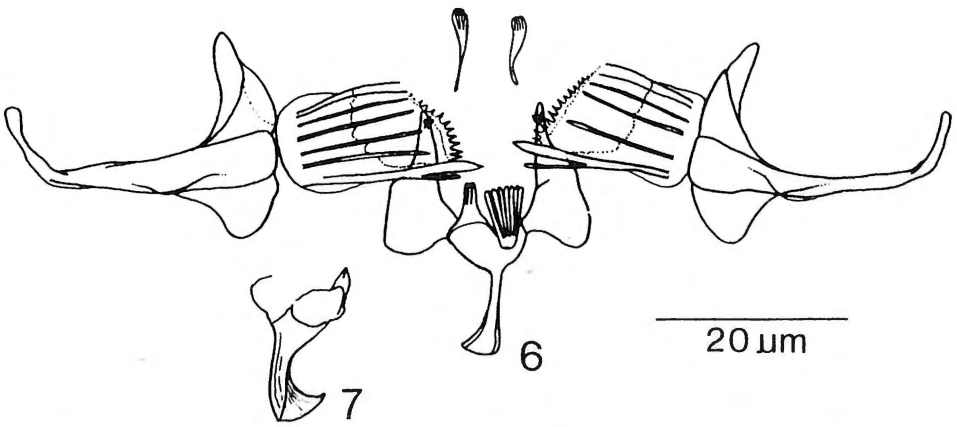
Type material. All specimens obtained from unidentified hydroids washed ashore on the beach ; April 1993.

Holotype : a female mounted in glycerine, deposited in the Koninklijk Belgisch Instituut voor Natuurwetenschappen, Brussels, Belgium, N° AI. 28.040.

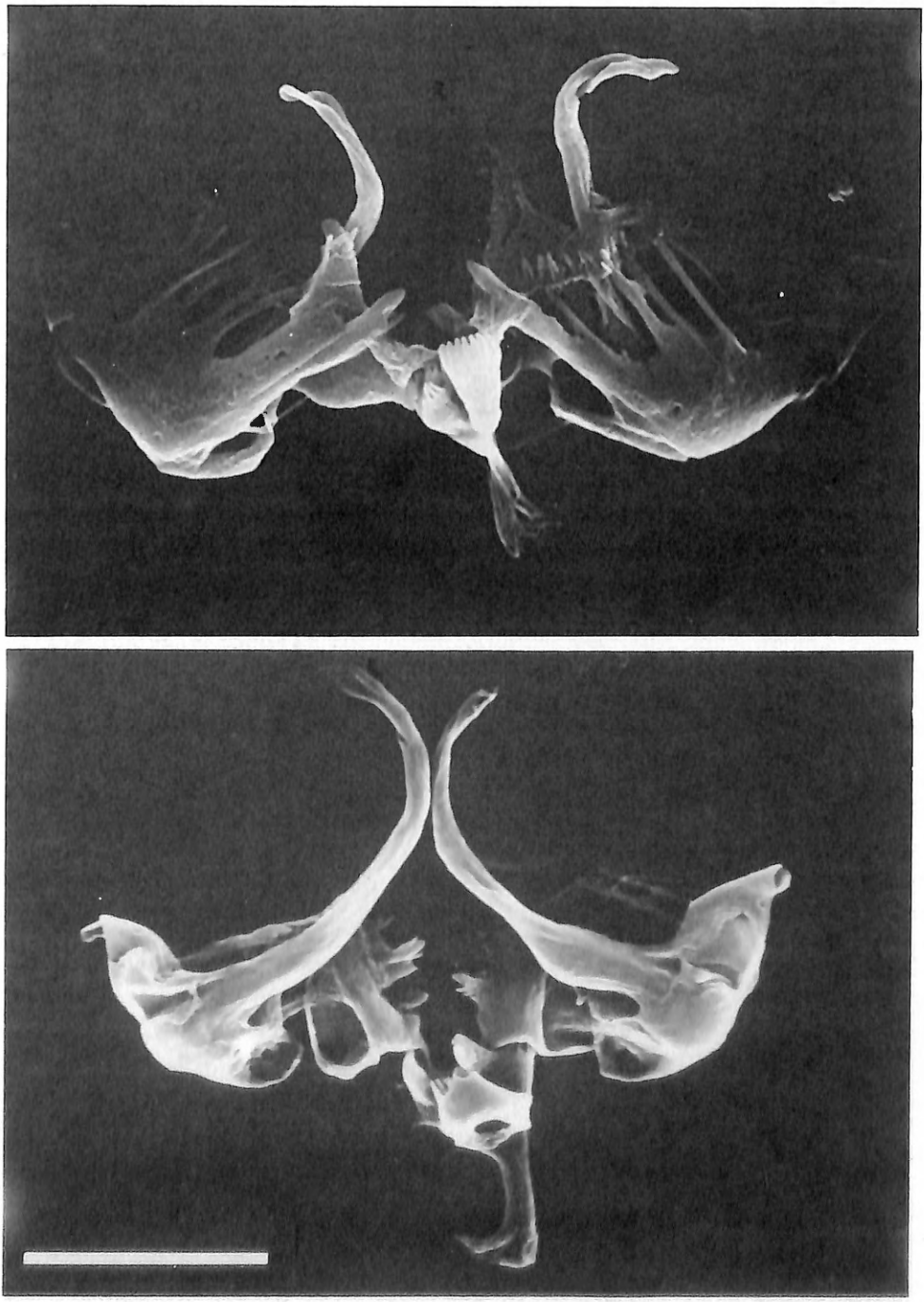
Paratypes : 5 females, one trophi preparation and one subitaneous egg mounted in glycerine in the K.B.I.N. ; 25 females mounted in glycerine, 5 trophi preparations in glycerine and 3 trophi mounted for SEM with the author in the Department of Biology, R.U.C.A.



Figs 1-5. — *Proales christinae* n. sp. — 1. Lateral view, ♀ holotype. — 2. Ventral view, ♀ paratype (foot omitted). — 3. Foot with toes, holotype. — 4. Subitaneous egg, dorsal view. — 5. Ibidem, lateral view.



Figs 6-7. — *Proales christinae* n. sp. — 6. Trophi. — 7. Fulcrum, lateral view.



Figs 8-9. — *Proales christinae* n. sp., SE micrographs of trophi, different views. Scale bar 10 µm.

Body elongate, stout, fusiform in dorsal view, ventrally bent in lateral view ; broadly oval in cross-section, higher than wide, greatest diameter near mid-length, fairly hyaline. Head and neck region narrower than trunk ; head offset by dorsal transverse fold, occasionally with dorsal transverse fold anteriorly ; neck offset from trunk by shallow, dorsal transverse fold. Trunk arched dorsally, more or less abruptly narrowing posteriorly ; tail distinct, rounded posteriorly. Foot moderately short, c. 1/5 total length, two pseudosegments of equal length. Toes straight, more or less lanceolate in lateral view, abruptly ending in tubular points, in dorsal view with straight inner margins and curved outer margins ; inner margins with short indentation prior to tubules, tubules laterally outcurved prior to their free end. Corona ventral, an anterior row of close-set cilia (reduced circumapical band ?) and at some distance (reduced apical field ?) from the latter six transverse, pre-oral bands of close-set cilia ; lateral ciliary tufts absent. Dorsal antenna unpaired, short. Mouth near posterior edge of corona. Retrocerebral organ with sac and a pair of subcerebral glands. Brain egg-shaped, surrounded by large retrocerebral sac, two ducts, sac and ducts granular. Eyespots absent ? No distinct constriction between stomach and intestine. Gastric glands of medium size, globular, slightly compressed laterally, short-stalked. Pedal glands large, elongated, extending into trunk, with reservoir in toes. Vitellarium rounded, 8 nuclei.

Trophi modified malleate. Rami triangular, inner margins, smooth, three to four short projections prior to tip at underside of rami ; basal apophyses asymmetric : right apophyse small, 3-toothed, left large, triangular, 7-toothed. Fulcrum short, in ventral view rod-shaped ; slightly expanded posteriorly ; in lateral view with expanded, ventrally recurved, hook-shaped posterior end. Left and right uncus with one principal and 5-6 subsidiary teeth ; principal tooth with single accessory toothlet ; principal teeth slightly clubbed, others linear ; a supplementary comb of sharp, delicate teeth behind tips of uncinal teeth. Manubria long, slender, strongly incurved posteriorly, head with broad inner and shorter outher lamella. Two small, club-shaped epipharyngeal elements.

Subitaneous egg elongate oval in dorsal view, ventrally flattened in lateral view ; short-stalked ; smooth.

Measurements

Body length 270-320 µm, heigth 74-88 µm, width 65-70 µm ; toe 32-36 µm.

Trophi : ramus 12-14 µm, fulcrum 6-7 µm, uncus 12-15 µm, manubrium 16-20 µm.

Subitaneous egg (L × W × H) : 114-122 × 45-55 × 45-51 µm.

Derivation of name. The species is named after doctoranda Christine Friedrich (Institut für polarökologie, Kiel), in reminiscence of the time spent with the attractive rotifers from arctic sea ice.

Differential diagnosis. *Proales christinae* n. sp. is close to *P. gonothyraeae* described by REMANE (1929). It differs from the latter in the following characters : trunk much more arched ; a posteriorly rounded tail, vs. tail absent ; longer (about

twice) and more lanceolate toes ; two subcerebral glands, vs. subcerebral glands absent ; rami with denticulate basal apophyses, vs. basal apophyses absent ; unci 6-7-toothed, vs. thin uncinal plates with 4-5 ribs ; lamellae on head of manubria larger ; dome-shaped epipharynx absent, vs. present.

Proales christinae and *P. gonothyraeae* are easily distinguished from all other *Proales* species displaying a long foot, by the ventrally placed corona. The trophi of *P. christinae* show some resemblances with those of *P. reinhardti* (EHRENBERG, 1834), but the latter has smaller manubrial lamellae, less (4-5) uncinal teeth and smooth basal apophyses.

Biology

So far, *P. christinae* has been found only in collections of hydroid polyps, which suggests a close association with a hydroid host. It remains to be established if it is associated with a single species, or whether it occurs on a wide variety of hosts. At the moment we can only guess about the type of relationship involved : is it parasitic, symbiotic, commensal or epizoic ? The related *P. gonothyraeae* lives on the epidermis of the hydroid polyp *Laomedea loveni* (ALLMANN). It is considered (REMANE, 1929) to be an ectoparasite that pierces the skin with its acute rami, and feeds on the epidermal cells. Instead of the cardate trophi type as found in *P. gonothyraeae, P. christinae* shows a modified malleate type, which suggests another mode of feeding and maybe another host relationship.

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ROTIFERA AND TARDIGRADA FROM SOME CRYOCONITE HOLES ON A SPITSBERGEN (SVALBARD) GLACIER

by

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SUMMARY

Seven species of rotifer (2 bdelloids and 5 monogononts), and two species of tardigrade were identified in 8 cryoconite holes from a Spitsbergen glacier.

Keywords : Rotifera, Tardigrada, Arctic, cryoconite holes, glacier, Spitsbergen.

INTRODUCTION

Cryoconite holes are water-filled depressions in the surface of glaciers. They are up to 60 cm deep and from a few millimetres to a metre or more in diameter. The holes are usually found in the ablation zone, and often occupy a vast area. They have been reported from the Arctic, and to a lesser extent from the Antarctic, and also from some glaciers in temperate regions (WHARTON *et al.*, 1985). They are formed by the absorption of solar radiation by the dark, wind-borne inorganic and organic sediment (cryoconite) that accumulates on the surface (*e.g.* PHILIPP, 1912 in STEINBÖCK, 1936 ; GRIBBON, 1979). Subsequent melting of the underlying ice produces cylindrical holes. At the bottom of the holes there is a more or less gelatinous and sandy, dark brown to blue-black sediment, containing micro-organisms. Besides physical factors (absorption of direct and diffuse solar radiation, downward convection of warm water, etc.), biological activities such as photosynthesis of algae, metabolic processes due to growth and reproduction of micro-organisms, etc., may also contribute to their formation and growth (STEINBÖCK, 1936 ; GERDEL and DROUET, 1960 ; MCINTYRE, 1984).

Cryoconite holes have been found to contain bacteria and fungi (GERDEL and DROUET, 1960), algae (*e.g.* WITTROCK, 1885 ; VON DRYGALSKI, 1897 ; GERDEL and DROUET, 1960 ; WHARTON and VINYARD, 1983 ; WHARTON *et al.*, 1981), ciliates (STEINBÖCK, 1938, 1957) and rotifers and tardigrades. Information on the last two groups is rather scanty with many doubtful identifications. Reports of rotifers are

restricted to *Brachionus* sp. by VON DRYGALSKI (1897), *Philodinavus paradoxus* (MURRAY, 1905) (sub *Microdina paradoxa* MURRAY, 1905) by GERDEL and DROUET (1960) (1), both from the Greenland Ice Cap, and « probably » *Philodina aculeata* EHRENBERG, 1832 and *P. roseola* EHRENBERG, 1832 by STEINBÖCK (1957) from cryoconite holes on a glacier in the Stubaier Alps.

References to tardigrades are almost as rare as for rotifers. Thus VON DRYGALSKI (1897) mentioned « probably » *Macrobiotus hufelandi* SCHULTZE, 1833 from the Greenland Ice Cap, STEINBÖCK (1957) reported *Macrobiotus* sp. from the Stubaier Alps, RAMAZZOTTI (1968) found *Hypsibius janetscheki* RAMAZZOTTI, 1968 and *H. convergens* (URBANOWICZ, 1925) from the Nare glacier (Himalaya); DASTYCH (1985) listed *Diphascon recamieri* RICHTERS, 1911, *Hypsibius dujardini* (DOYÈRE, 1840) and *H. arcticus* MURRAY, 1907 from Spitsbergen.

The present paper reports on the rotifers and tardigrades from a small number of cryoconite holes from a Spitsbergen glacier, sampled during a biological expedition of Antwerp University.

MATERIAL AND METHODS

Eight cryoconite holes in the ablation zone of the Hyrnebeen glacier (Hornsund, Spitsbergen, Svalbard) were sampled on 15.08.1991. Geographic coordinates are 77°49'33"N, 16°14'43"E. The holes were 20-30 cm deep and 8-12 cm broad. Water temperature was about 0.2° C. Physical and chemical characteristics of the water (measured at one hole only) were as follows pH 4.62, conductivity 38 μScm^{-1}, turbidity and true colour 100 % transmission, chlorides 5.5 mgl^{-1}, sulphates 7.0 mgl^{-1}, orthophosphates $< 0.1\ mgl^{-1}$, ammonium nitrogen $< 0.1\ mgl^{-1}$, nitrites and nitrates 0.0 mgl^{-1}, Na 4.4 mgl^{-1}, K 0.8 mgl^{-1}, Ca 1.2 mgl^{-1}, Mg 0.51 mgl^{-1}, Si 0.08 mgl^{-1}.

Since there was no suitable method of separately sampling the cryoconite and the supernatant, the mixture was stirred up until it was homogeneous and a sample taken of it. Seven of the samples were immediately preserved with formalin; the other one was kept cool and used for culture experiments began three days later at the laboratory in Antwerp.

All the tardigrades present were counted; differential counts of rotifers were based on 500 individuals per sample. Size classes between ecdyses of tardigrades were separated by plotting data for body length, grouped in 5 µm size classes, as cumulative percentages on probability paper (LEWIS and TAYLOR, 1967).

(1) The « diatom » mentioned on p. 264 and pictured in fig. 20 by these authors are jaws of a bdelloid rotifer.

RESULTS AND DISCUSSION

The cryoconite was dark black, slightly gritty with much plant debris and some coarser mineral particles, remains of feathers and a solitary bird vertebra. Microscopically the organic and inorganic material was loosely bound by hyphae of fungi. All samples contained small numbers of red unicellular algae and larger quantities of the desmids *Ancylonema nordenskioeldi* BERGGREN, 1871 and *Cylindrocystis* sp. Two samples showed important numbers of a filamentous member of the Cyanobacteria. Ciliates (at least four species) were present at low numbers in all samples. Rotifers and tardigrades were abundant in all samples.

Rotifera

All the cryoconite holes contained high densities of bdelloids (2 species) and small numbers of monogononts (5 species). Two (*Keratella cochlearis* (GOSSE, 1851) and *K. quadrata* (O.F. MÜLLER, 1786)) are invariably planktonic and live in the water on top of the sediment. The other species are benthic.

Macrotrachela insolita DE KONING, 1947

The species preponderated (> 99 % of total number of rotifers) in all samples. The body is carmine red and up to 300 µm long. The teeth on the trophi were variable and had a dental formula of 2/2, $2/2_{+1}$, $2_{+1}/2_{+1}$ and rarely $2_{+2}/2_{+1}$. Many eggs were seen, measuring 50-53 µm × 75-80 µm. They were oval, with one or two poles slightly indicated. This is a highly variable cosmopolitan species, usually found among terrestrial and submerged mosses, algae and litter. The closely related (synonymous ?) *M. habita* (BRYCE, 1894) was found in terrestrial and submerged mosses on Spitsbergen by BRYCE (1897 sub *Callidina habita* BRYCE, 1894 ; 1922) and SUMMERHAYES and ELTON (1923).

Philodina acuticornis odiosa MILNE, 1916

Two individuals were observed in each of two samples. This species is ubiquitous and probably cosmopolitan. The *Philodina erythrophthalma* EHRENBERG, 1832 and *P. acuticornis* MURRAY, 1902, mentioned by BRYCE (1897, 1922) from terrestrial mosses collected in Spitsbergen, probably refer to this species.

Dicranophorus permollis permollis (GOSSE, 1886)

Found in two holes, one and four individuals. This species has a holarctic distribution. At Svalbard it has been recorded from terrestrial mosses (BRYCE, 1897 sub *Diglena permollis* GOSSE, 1886) and submerged mosses in a pond (DE SMET, 1990 sub *Encentrum* sp.).

Encentrum mucronatum WULFERT, 1936

Two specimens in one sample only. A cosmopolitan and ubiquitous species that has been reported from Svalbard in submerged mosses, puddles and shallow pools (DE SMET, 1990).

Keratella cochlearis (GOSSE, 1851)

One specimen in each of two holes. These individuals belonged to the form *macracantha* (Lauterborn). This cosmopolitan and eurythermic species is rare at Svalbard, where it has been reported from ponds and lakes (THOMASSON, 1958, 1961 ; AMRÉN, 1964c).

Keratella quadrata (O.F. MÜLLER, 1786)

Observed only once. The species is cosmopolitan and eurythermic ; found in fresh waters of all types, also saline waters. It was present in 16 of the 72 ponds and puddles studied by AMRÉN (1964a, b, c) on Spitsbergen. According to the latter it is not found in lakes and extreme arctic ponds. No other Svalbard records.

Lecane closterocerca (SCHMARDA, 1859)

Only a single specimen was found. This ubiquitous and cosmopolitan species has been reported from Svalbard by DE SMET (1988) ; OLOFSSON (1918), BRYCE (1922) and SUMMERHAYES and ELTON (1923) identified their species as *Monostyla cornuta* (O.F. MÜLLER, 1786). Never very common, it is found amongst vegetation and in the littoral zone of ponds.

Tardigrada

Eutardigrada (two species) were abundant in each of the cryoconite holes examined.

Diphascon recamieri RICHTERS, 1911

The species was found in seven of the eight samples and the 735 specimens collected amounted to 10 % of the total tardigrade population. The percentage in individual holes ranged from 1 to 18 %. This is an arctic-alpine species with a holarctic distribution ; hydrophilous and cold-stenotherm. It has been reported from Svalbard by RICHTERS (1911), WEGLARSKA (1965, sub *Hypsibius recamieri* RICHTERS, 1911)), DASTYCH (1985) and VAN ROMPU and DE SMET (1994) ; among terrestrial and submerged mosses. It was the dominant species in 7 cryoconite holes studied by DASTYCH (1985).

The total body length of the 264 specimens measured, varied from 154 to 394 µm ; the buccal tube length ranged from 40 to 97 µm. Total body length plotted against frequency is shown in Fig. 1. The multimodal distribution provides evidence

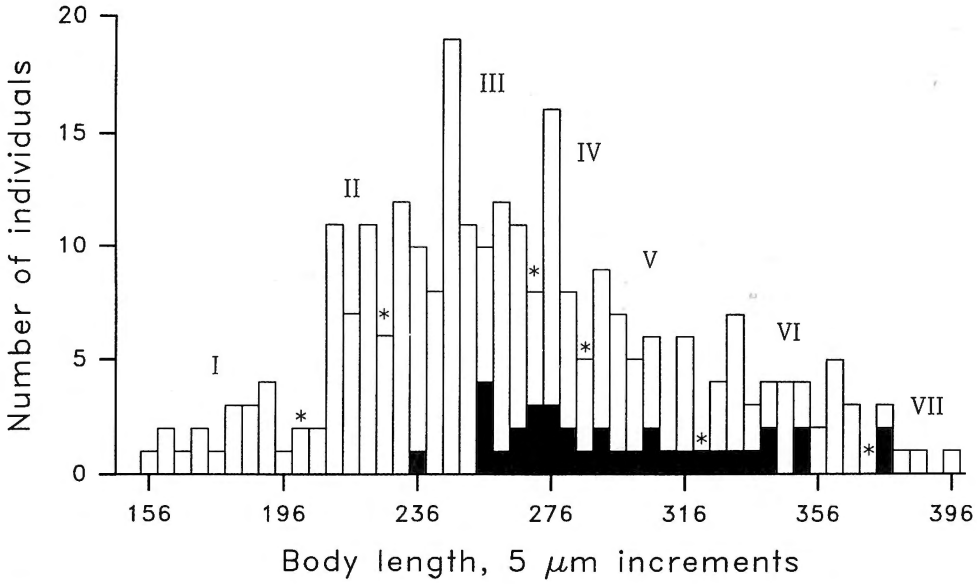

Fig. 1. — *Diphascon recamieri.* — Frequency distribution of body length (5 μm size increments). Points of probable ecdyses indicated by asterisks; black = animals with eggs; instars indicated by Roman numerals.

for the presence of different age classes. Determination of the size classes suggested that there were 7 size classes and 6 lengths, as indicated by asterisks in Fig. 1, where ecdyses probably occurred. The average egg dimensions were 61 × 65 μm. According to HALLAS (1972) the length of the larvae should not deviate considerably from 3 × the egg diameter. Expected length of the largest larvae of *D. recamieri* would therefore be about 195 μm, which agrees with the upper size limit of 204 μm noted for the first size class. Thirteen percent of the population carried eggs. The number of eggs per individual ranged from 1-3 (average 2). Developing eggs were present in animals from 255-376 μm long; exuvia with eggs were found in animals from 236-333 μm. Animals longer than 204 μm and less than 236 μm may therefore be classed as juveniles. From the evidence of Fig. 1 it is suggested that there is only one juvenile instar. Reproduction occurs in periods between several ecdyses (instars 3-7); the last ecdysis occurs at about 371 μm. From the size-frequency histogram it further follows that adults preponderate, making up 88 % of the total population, with the third instar showing the highest numbers of individuals (38 %). The dominance of middle-sized animals, including individuals capable of egg production, has been explained by HIGGINS (1959), POLLACK (1970) and MORGAN (1977) as a rapid development of juveniles, leading to early recruitment into these middle-size classes, which are slower growing and persist longer. Lower numbers of animals in the larger size classes may reflect a higher mortality, possibly stemming from the effects of egg production (MORGAN, 1977).

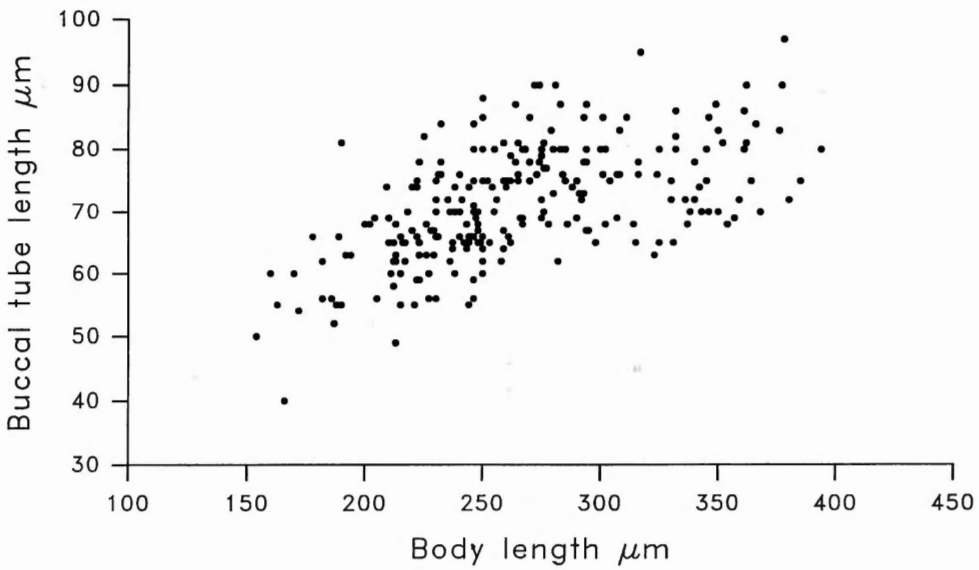

Fig. 2. — *Diphascon recamieri.* — Relation of buccal tube length to body length.

The relationship between buccal tube length and body length is shown in Fig. 2. Both dimensions are positively correlated and increase fast up to about 250 µm. When body length exceeds 250 µm, buccal tube length increases only slightly with increasing body length. A similar relationship, whereby the buccal tube reaches its maximum length early in life and retains it without appreciable change thereafter has been reported for *Echiniscoides sigismundi groenlandicus* KRISTENSEN and HALLAS, 1980 by KRISTENSEN and HALLAS (1980) and *Pseudobiotus augusti* (MURRAY, 1907) by KATHMAN and NELSON (1987). The latter authors suggest that the appropriate size for the food supply is reached early, and is independent of the final size of the mature animal.

Isohypsibius granulifer THULIN, 1928

This species was present in all samples. A total of 6.369 individuals, or 90 % of the total tardigrade number, was collected. The share of *I. granulifer* varied from 82-100 % in the different samples. It is a hydrophilous species with a cosmopolitan distribution, and is usually the most dominant taxon in submerged mosses from arctic freshwater habitats (VAN ROMPU and DE SMET, 1991). It has been reported from Svalbard by DE SMET *et al.* (1988), VAN ROMPU and DE SMET (1988, 1991, 1994) and DASTYCH (1985) who mentions *Isohypsibius ? granulifer* from mosses on stones near stream.

Of the 590 individuals measured, the shortest was 118 µm and the longest 425 µm ; buccal tube length ranged from 19 to 49 µm. Size-frequency and cohort analysis suggest 5 possible ecdysis points and 6 size classes (Fig. 3). Animals in the

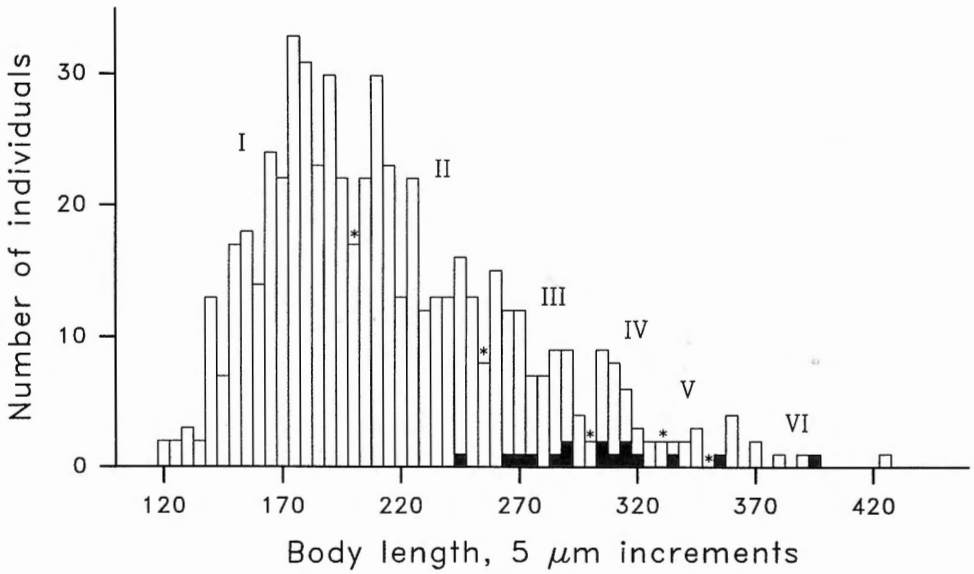

Fig. 3. — *Isohypsibius granulifer*. — Frequency distribution of body length (5 μm size increments). Points of probable ecdyses indicated by asterisks ; black = animals with eggs ; instars indicated by Roman numerals.

first size class were 118-202 μm long. Average egg-size was 58 × 70 μm. According to the three times rule of HALLAS (1972), the larval stage should therefore include animals to about 210 μm long, which agrees fairly well with the observed upper limit (202 μm) of the first size class. Only 2.7 % of the population was carrying eggs. Egg number per individual varied from 2 to 6 (average 4). Developing eggs were found in animals from 247 to 393 μm long ; exuvia with eggs were not seen. Animals longer than 202 μm and less than 247 μm are assumed to be juveniles. The ecdysis points suggest that there is only one juvenile instar. Egg production occurs during instars 3 to 6. The last ecdysis point is indicated at 350 μm length. In contrast with *Diphascon recamieri*, where 88 % of the population were adults, the majority (75 %) of the individuals of *Isohypsibius granulifer* were larvae (48 %) and juveniles (37 %). This preponderance of smaller-sized classes, together with the smaller number of instars (6 versus 7 in *D. recamieri*), suggests a slower development for *I. granulifer*.

Buccal tube length and body length are positively correlated. The relationship is slightly curvilinear (Fig. 4), there being an indication of a proportionately faster increase of body length for animals from 225-425 μm. A similar relationship has been found in *Macrobiotus islandicus* RICHTERS, 1904 by HIGGINS (1959), *M. grandipes* SCHUSTER, TOFTNER and GRIGARICK, 1977 by SCHUSTER *et al.* (1977), *Isohypsibius saltursus* SCHUSTER, TOFTNER and GRIGARICK, 1977 by WAINBERG and HUMMON (1981), and *Milnesium tardigradum* DOYÈRE, 1840 by SCHUETZ (1987).

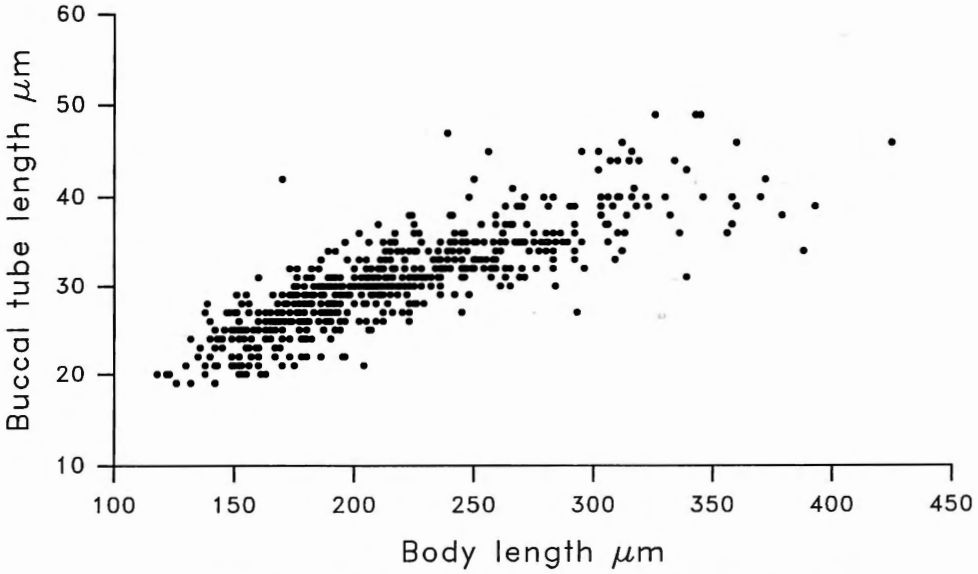

Fig. 4. — *Isohypsibius granulifer*. — Relation of buccal tube length to body length.

Life spans of active tardigrades have been estimated to range from 3 to 30 months, with 3 to 11 ecdyses occurring during this period (*e.g.* HIGGINS, 1959 ; FRANCESCHI CRIPPA and LATTES, 1967 ; POLLOCK, 1970 ; SCHUSTER *et al.*, 1977 ; WAINBERG and HUMMON, 1981 ; RAMAZZOTTI and MAUCCI, 1983 ; KATHMAN and NELSON, 1987). Our results for *Diphascon recamieri* and *Isohypsibius granulifer* from cryonite holes suggest 7 and 6 instars respectively. Since cryoconite holes are only ice-free during 2-3 months, the animals will be in a latent state of cryobiosis during the rest of the year. Because of these cryptobiotic periods, the total life span of these tardigrades may therefore extend to several years.

CONCLUDING REMARKS

It is clear that cryoconite holes can be regarded as individual ecosystems with distinct boundaries, energy flow and nutrient cycling (WHARTON *et al.*, 1985). They are inhabited by a species-poor microflora and microfauna, with specific representatives living in or on the cryoconite sediment, and in the water on top of it. Only few species show mass development. Rotifers (mainly the bdelloid *Macrotrachela insolita*) appeared to be the largest component by number of individuals, in the cryoconite holes studied. Tardigrades, represented by *Diphascon recamieri* and *Isohypsibius granulifer*, also occurred in substantial numbers. Ciliate populations were insignificant. Most of the species found feed on algae, bacteria and/or detritus. As such, tardigrades and most of the rotifers probably utilise the same niche and may be in competition for food. The rotifer *Dicranophorus permollis* is

a voracious carnivore, feeding *inter alia* on small rotifers. This means that the ecological pyramid is formed by three trophic levels.

Species other than those found by us have previously been reported (for references see Introduction), and still more will presumably be found in future research. The species composition in the holes probably depends on latitude and length of ice-free period, hole dimensions (cryoconite holes can be regarded as islands), age of hole, nutrients available, etc.

Most of the rotifers and tardigrades found are widespread or cosmopolitan species. Their reproductive strategies are strongly orientated towards achieving maximum dispersal : they reproduce parthenogenetically and form resting eggs and/or resting stages, which can be passively transported by wind and birds. Passive dissemination on the glacier, and to some extent active dispersal, will often take place when continuous melting during summer causes the water in cryoconite holes to overflow.

Cryoconite holes probably persisted during the Pleistocene, and played an important role in the biological colonization of the newly exposed aquatic and terrestrial habitats following deglaciation (STEINBÖCK, 1936 ; WHARTON *et al.*, 1985 ; DE SMET, 1993).

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TWO MORE NEW SPECIES OF *LECANE* (ROTIFERA, MONOGONONTA) FROM THAILAND

by

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SUMMARY

Two new species of *Lecane*, *L. shieli* n. sp. and *L. thailandensis* n. sp. are described from two localities in Thailand. *L. shieli* n. sp. is known from the type locality only, and occupies an isolated position in the genus, whereas *L. thailandensis* n. sp. is also known from China, and is close to *L. latissima*.

Key words : Rotifera, taxonomy, *Lecane*, new species, Thailand.

INTRODUCTION

The genus *Lecane* NITZSCH, 1827 is, with over 160 valid species, one of the most species-rich of all Rotifera. It reaches its highest diversity in the tropics and subtropics, but representatives are also found in the Arctic and Antarctic. Although many species are cosmopolitan, a considerable number have restricted distributions, are endemic or have been found on single occasions only (SEGERS, 1994a). Considering that the rotifer fauna of South East Asia has received relatively little attention so far, it came as no surprise to find representatives of three hitherto unknown species in samples from Thailand. Of one of these species, a single specimen only was found. The taxon is figured and commented upon, but not named.

MATERIAL AND METHODS

Samples were collected using a 30 μm mesh-size plankton net, and preserved in 4 % formaldehyde. Specimens were picked under a Wild M5 dissection microscope, and examined using a Olympus CH2 microscope. Trophi were isolated by dissolv-

ing tissues with NaOCl. Scanning Electron Microscopy (S.E.M.) was performed using a JEOL JSM-840 microscope on critical-point dried specimens.

All measurements are in µm.

RESULTS

Representatives of hitherto unknown *Lecane* were present in two samples from Sakon Nakhon province (North-East Thailand). One species occurred as a single specimen (Fig. 1). It may have been confused with *L. aculeata* (JAKUBSKI, 1912) (Fig. 2) in the past, as it bears a strong but superficial resemblance to that species. However, it is much larger, and its antero-lateral spines are prolongations of the ventral plate, rather than emerging from between the ventral and dorsal plate as in *L. aculeata.*

Two species appeared in numbers, sufficient to warrant description. They are as follows :

Lecane shieli n. sp.

Figs 3a-b

Material examined

Holotype (RIR 35) and five paratypes (RIR 34) deposited in the royal Belgian Institute for Natural Sciences, Brussels (K.B.I.N. : IG 28055)). Five paratypes in the collection of the Institute of Animal Ecology (RUG) and in the second author's collection each. All from Nam Pung reservoir, Sakon Nakhon province, Thailand, 4 June 1993 (type locality). Many more specimens, all parthenogenetic females, present in the sample.

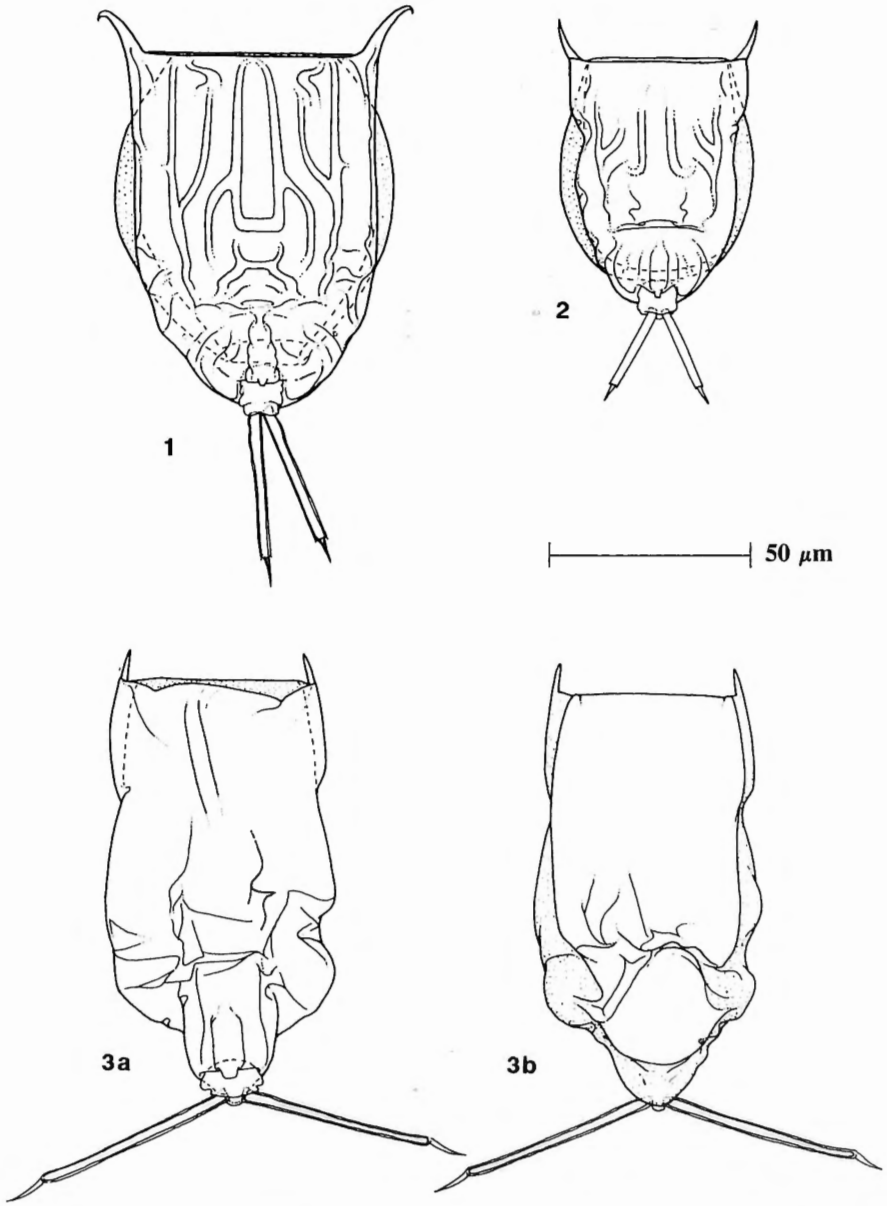
Differential diagnosis

L. shieli n. sp. can hardly be confused with any congener, by its characteristic soft and elongate lorica, long toes and peculiar claws. The species appears close to *L. eutarsa* HARRING and MYERS, 1926 and relatives, but cannot be mistaken for any of these by the above-mentioned characters.

The species keys out to *L. eswari* DHANAPATHI, 1976 using the identification key by SEGERS (1994a), but can, however, not be confused considering the stiffer, less elongate lorica, shorter antero-lateral spines and toes, and straight claws of *L. eswari.*

Description

Parthenogenetic female (male unknown) : lorica relatively soft, irregularly folded through conservation. Dorsal plate consistently narrower than ventral plate, elongate. Head aperture margins nearly coincident, straight or with irregular



Figs 1-3. — 1. *Lecane sp.*, ventral view. — 2. *L. aculeata* (JAKUBSKI), ventral view. — 3. *L. shieli* n. sp. a : ventral view, b : dorsal view.

notches. Antero-lateral corners armed with long, sharp and straight spines. Ventral plate longer than wide, generally parallel-sided, irregularly folded. No lateral sulci. Foot plate especially separated, coxal plates indistinct. Prepedal fold narrow,

elongate, distally with median projection. Foot pseudosegment short, not or scarcely projecting. Toes long and slender, nearly parallel-sided, slightly outcurved distally. Claws weakly curved, inserted eccentrically.

Measurements : dorsal plate length 94-101, width 41-46, ventral plate length 99-115, width 51-59, toe length 48-51, claw length 7-11.

Etymology

The species is named after Dr. R. J. Shiel (Albury, Australia), in recognition of his work on Rotifera.

Distribution and ecology

Only known from the type locality. At the time of sampling, temperature was at 26° C, conductivity 85 µS and pH 7.8. A list of the accompanying rotifer fauna is presented in table 1.

TABLE 1

Rotifer record of a sample from Nam Pung reservoir, Sakon Nakhon province, Thailand.

Ascomorpha ovalis (BERGENDAL, 1892)
Brachionus angularis GOSSE, 1851
B. dichotomus SHEPHARD, 1911
f. *reductus* KOSTE & SHIEL, 1980
B. falcatus ZACHARIAS, 1898
Colurella uncinata (O.F. MÜLLER, 1773)
f. *bicuspidata* (EHRENBERG, 1832)
Euchlanis dilatata EHRENBERG, 1832
Keratella cochlearis (GOSSE, 1851)
K. lenzi HAUER, 1953
K. tropica (APSTEIN, 1907)
Lecane batillifer (MURRAY, 1913)
L. bulla (GOSSE, 1851)
L. closterocerca (SCHMARDA, 1859)
L. crepida HARRING, 1914
L. curvicornis (MURRAY, 1913)
L. furcata (MURRAY, 1913)
L. hastata (MURRAY, 1913)
L. hornemanni (EHRENBERG, 1834)
L. leontina (TURNER, 1892)
L. ludwigii (ECKSTEIN, 1883)
L. luna (O.F. MULLER, 1776)
L. lunaris (EHRENBERG, 1832)
L. papuana (MURRAY, 1913)
L. shieli n. sp.
L. rhenana HAUER, 1929
L. thailandensis n. sp.
Lepadella acuminata (EHRENBERG, 1834)
L. biloba HAUER, 1958
L. discoidea SEGERS, 1993
L. ehrenbergi (PERTY, 1850)
L. rhomboides (GOSSE, 1886)
Macrochaetus collinsi (GOSSE, 1867)
Mytilina ventralis (EHRENBERG, 1832)
Ploesoma hudsoni (IMHOF, 1891)
Scaridium longicaudum (O.F. MÜLLER, 1786)
Testudinella patina (HERMANN, 1783)
Trichocerca braziliensis (MURRAY, 1913)
T. chattoni (DE BEAUCHAMP, 1907)
T. similis (WIERZEJSKI, 1893)
T. tropis HAUER, 1938

Lecane thailandensis n. sp.

Figs 4a-i

L. hornemanni in WANG, 1961.

Material examined

Holotype (RIR 32) and one paratype (RIR 33) deposited in the royal Belgian Institute for Natural Sciences, Brussels (K.B.I.N. : IG 28055). One paratype in the collection of the Institute of animal Ecology, RUG and in the second author's collection each. Two specimens on S.E.M. preparation. All from Nam Pung reservoir, Sakon Nakhon province, Thailand, 4 June 1993 (type locality). A single specimen from Nong Takai swamp, Sakon Nakhon province, Thailand, 5 June 1993.

In total, 10 specimens, all parthenogenetic females, were seen.

Differential diagnosis

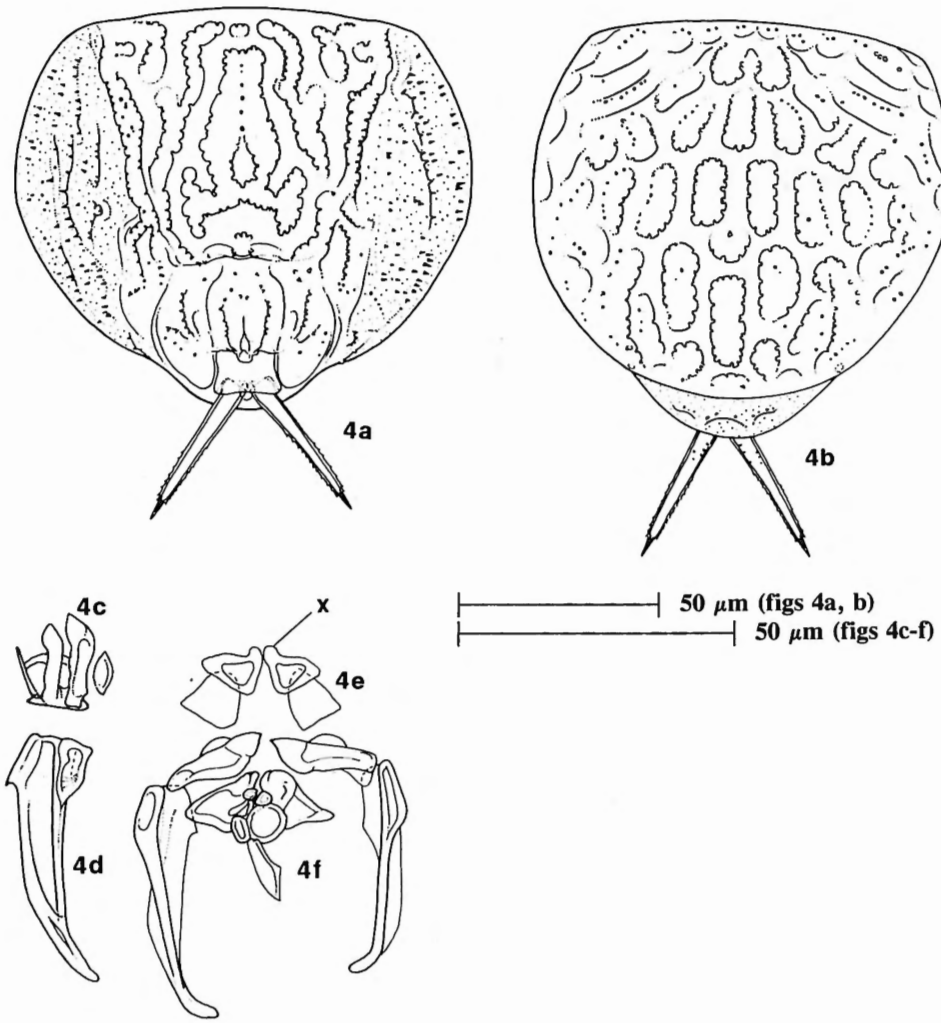
L. thailandensis n. sp. keys out to *L. latissima* YAMAMOTO, 1955. The two species differ by the lorica surface, which is strongly pustulated in *L. thailandensis* n. sp., and smooth in *L. latissima*. Differences in ecology and distribution of the two species add to the diagnosis : *L. latissima* is a well-known cold-stenotherm, *L. thailandensis* n. sp. is probably an Oriental, warm water species.

Both *L. thailandensis* n. sp. and *L. latissima* are distinguished from the related *L. hornemanni* (EHRENBERG, 1834) by their toes bearing claws, from *L. ruttneri* HAUER, 1938 by their larger size and dorsal plate being wider than long, and from *L. abanica* SEGERS, 1994 by their larger size and rounded lorica.

Description

Parthenogenetic female (male unknown) : Lorica relatively stiff. Dorsal plate wider than ventral plate, armed with rows of spines and with ornamental folds. Head aperture margins nearly coincident, straight or slightly convex, with rounded antero-lateral corners. Ventral plate slightly longer than wide, with incomplete transverse and longitudinal folds, ornamented with rows of spines. No lateral sulci. Foot plate broad, coxal plates rounded triangular. Prepedal fold relatively broad, elongate, distally with median projection. Foot pseudosegment constricted medially, not projecting. Toes long, slightly tapering to distally, with small spicules laterally and dorsally. Claws incompletely separated, bent dorsad.

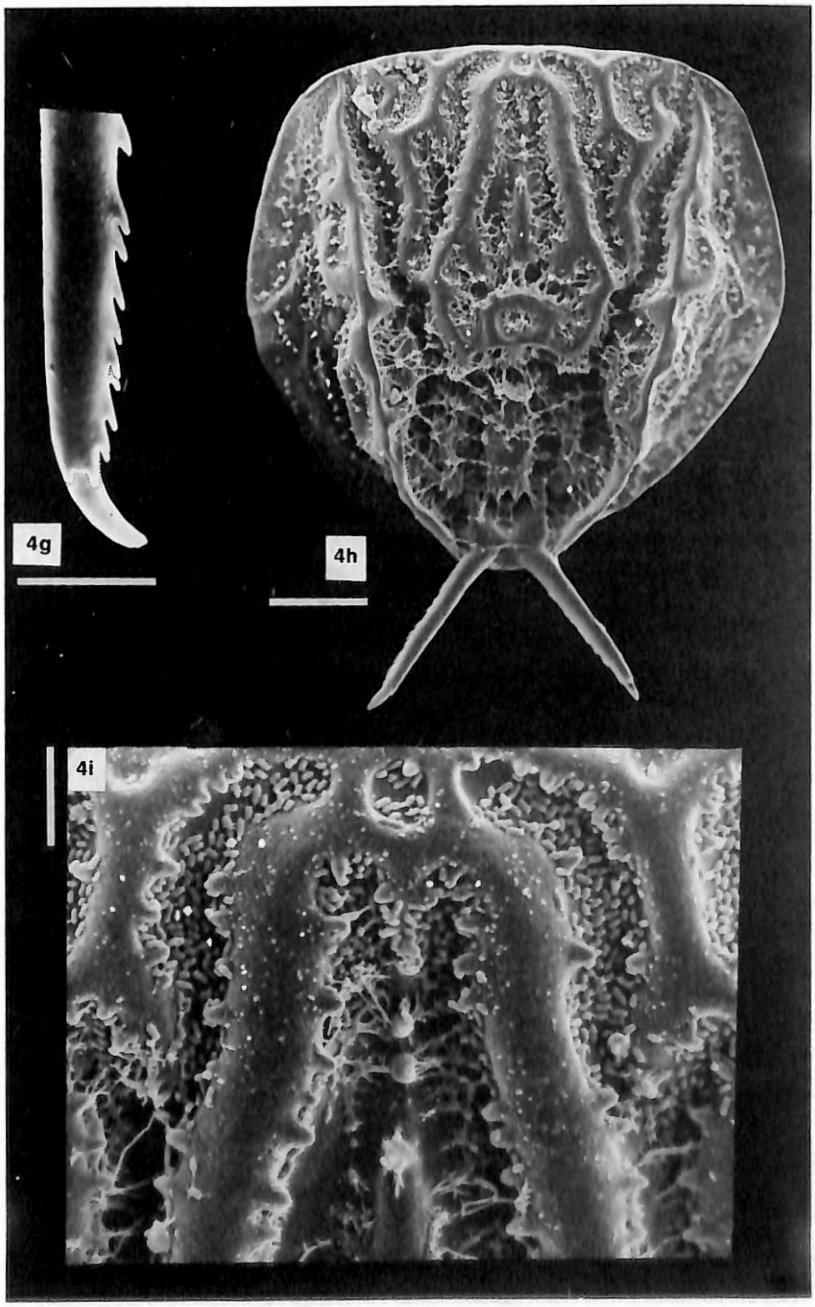
Measurements : dorsal plate length 85-95, width 95-110, ventral plate length 98-105, width 61-85, toe length 33-38, claw length 5-6.



Figs 4a-f. — *Lecane thailandensis*. — a. ventral view. — b. dorsal view. — c. right uncus, anterior view. — d. manubrium, lateral view. — e. preuncinal plates, ventral view ('x' : antero-median projection). — f. trophi, ventral view.

Etymology

The species name refers to the country from which the species was first recognised, Thailand.



Figs 4g-i. — *Lecane thailandensis*, S.E.M. photographs. — g. toe and claw, lateral view. — h. ventral view. — i. detail of ornamentation (scale bars : g, i : 5 µm, h : 20 µm).

Distribution and Ecology

L. thailandensis n. sp. was found in two samples from Sakon Nakhon province of Thailand, the Chinese record is from Donqian Lake, Zhejiang Province. The species may be Oriental.

Ranges for some environmental characteristics of localities in which this species was found are : temperature 26-33° C, conductivity 85-110 µS, pH 6.5-7.8. The rotifer fauna of the type sample is as in table 1.

Comments

By their similar lorica morphology, *L. thailandensis* n. sp. is closely related to *L. latissima*. Differences between the two are in the presence of an ornamented lorica in the former, whereas the lorica is smooth in the latter, and in the different ecology and distribution of the two species. Apart of these, differences in trophi morphology are apparent : compare figs 4c-f with the relevant drawings of *L. latissima* in SEGERS (1994b). A more pronounced asymmetry of the rami and rounded shape of the bulla rami in *L. thailandensis* n. sp., and differences in shape of the unci, manubria and, especially, preuncinal plates are apparent. On the other hand, the presence of a single, clearly discernable antero-median pair of rounded projections on the preuncinal plates ('x' in Fig. 4e), a character not seen in any other species examined, may point to their kinship. Obviously, these observations should be interpreted with care, as the observation angle appears not to be exactly the same in both cases, and only single specimens are compared.

The drawing of *L. hornemanni* by WANG (1961) undoubtedly represents a misidentified *L. thailandensis* n. sp. WANG (*op. cit.*) did not report armed toes for his material. This is probably a consequence of the minute size of the toe spines, rather than being factual.

ACKNOWLEDGEMENTS

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ECOPHYSIOLOGY OF SALT ACCLIMATISATION IN CRUSTACEANS : A MINI REVIEW (*)

by

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SUMMARY

With their marine, fresh water and euryhaline representatives, crustaceans exhibit almost any of the known possible patterns of osmo-ionoregulation. That group therefore appears as a choice material to tackle the question of ecophysiology of salt acclimatisation from a comparative point of view. In crustaceans, osmo-ionoregulation can be effected in two different ways the significance of which is always to avoid water movements at the cellular level. The first one, of general occurrence and considered as a prerequisite for adaptation to salinity changes, is to maintain the intracellular fluid isosmotic to the extracellular fluid, either the body fluids, or that of the environment. The second one is to control the concentration of the extracellular fluids at a more or less constant level regardless of the external salinity. This review will focus on the second way where mechanisms are active essentially in boundary epithelia. The gills will be shown to be the prominent structure responsible for the blood NaCl balance and regulation in marine, marine euryhaline and brackish water species. The review will therefore deal mostly with recent physiological and ultrastructural data on gill tissue and provide information leading to a characterization of the particular mechanisms and driving forces at work at that level. It will refer largely to experiments using perfused preparations of gills isolated from the chinese crab *Eriocheir sinensis,* taken as a model. The applicability of the chinese crab model to other crustaceans will be considered. It will be shown also that the cuticle lining the epithelium is largely involved in ionic regulation in crustaceans. It does contribute indeed to reduce ionic leaks in regulators and yet allows for the entry of ions across specific « channels » at the sites where active uptake takes place. An attempt is made to understand how both the cuticle and the epithelium fit in a working epithelio-cuticular complex.

Keywords : Crustacea, cuticle, ecophysiology, epithelium, gill, ionoregulation, Na^+, Cl^-, osmoregulation, potential difference, ultrastructure.

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INTRODUCTION

A review centered on the Ecophysiology of salt acclimatisation in crustaceans should consider all the processes at work in the control of the thermodynamic activity of water in the different biological fluids of the species considered. In crustaceans, the inorganic ions Na^+ and Cl^- are the major osmotic effectors found in these fluids, either extracellularly or intracellularly, while in addition cellular fluid contains significant amounts of amino acids and peptides of actual osmotic importance. Such a review should therefore consider any kind of metabolic and transport process involved in the adjustment of the level of both these inorganic and organic constituents. Viewed in this way, the subject is very complex and much too voluminous to be dealt with in such a brief review. As a matter of fact, for many years, the composition of the haemolymph has been studied in the various groups of crustaceans as a function of the salinity of the environment. All together, these investigations represent a huge amount of information which corroborates the

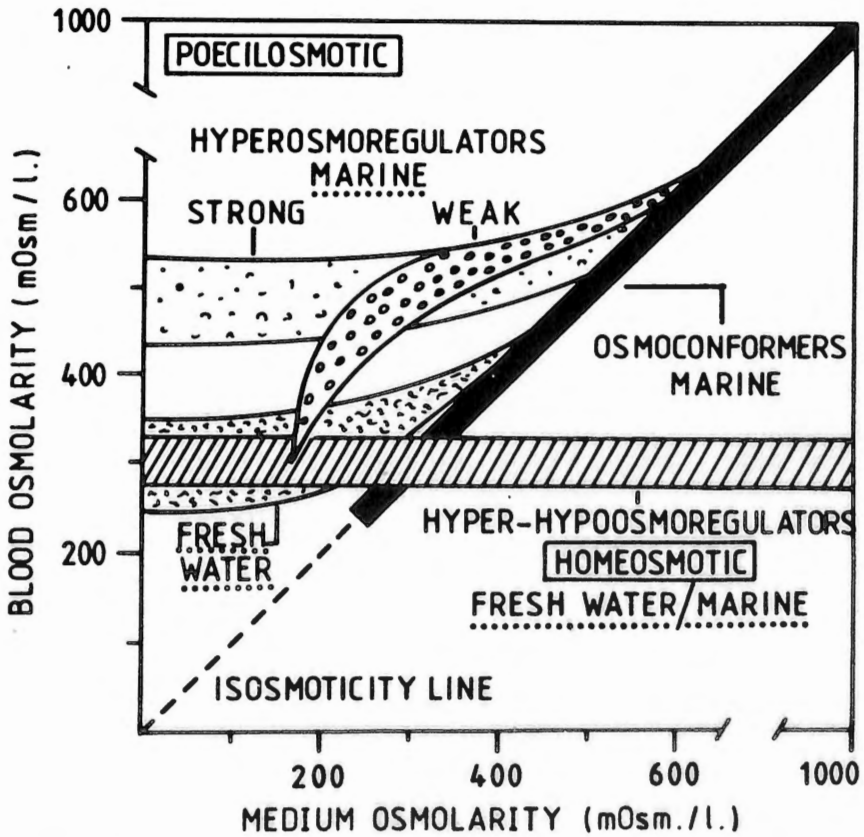

Fig. 1. — Patterns of haemolymph osmoregulation in aquatic crustaceans.

assertion that crustaceans exhibit almost all of the possible patterns of haemolymph composition. From that abundant material, it has been possible to draw several generalizations for the most representative patterns encountered in animals living in a range of media from concentrated sea water (SW) to freshwater (FW) and terrestrial environments (Fig. 1).

A first and basic way to effect osmotic regulation is to maintain the intracellular fluid isosmotic to the extracellular fluid, either of the external medium or of the body fluid. In media of fluctuating salinities, the demand of the medium may then be difficult to meet. All the mechanisms involved in this kind of regulation or adjustment have been gathered under the general heading « Isosmotic regulation of cellular fluids ».

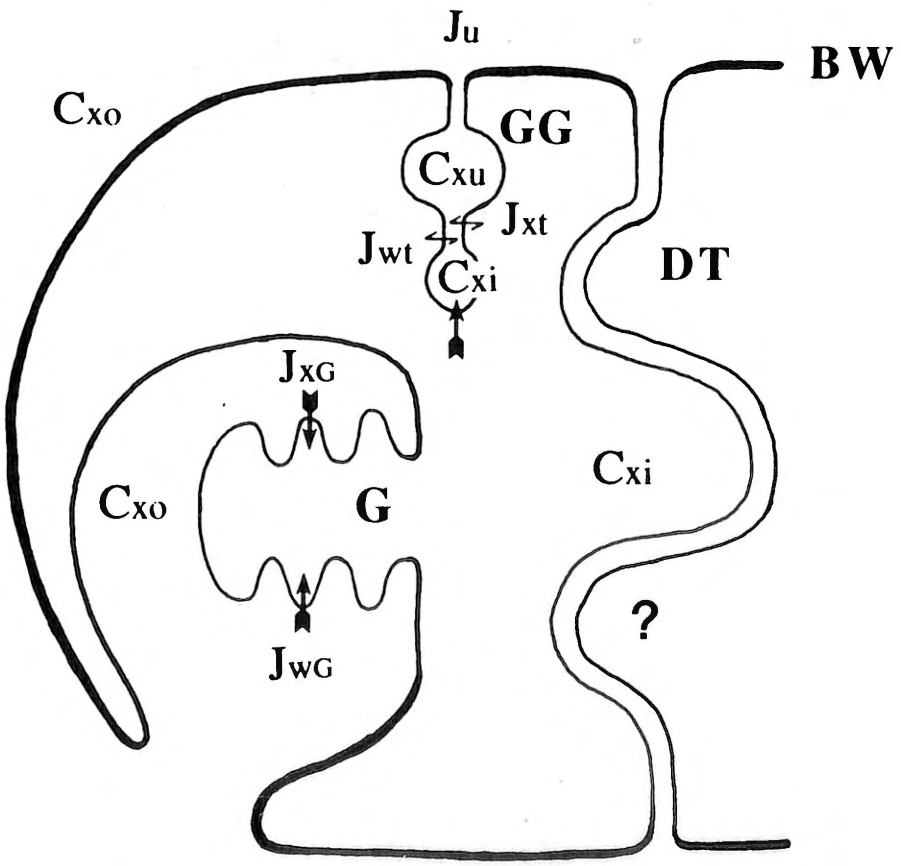

Fig. 2. — Schematic representation of a theoretical crustacean.

BW : body wall ; C : concentration ; DT : digestive tract ; f : filtrate ; G : gill ; GG : green gland ; i : internal medium (haemolymph) ; J : flux ; o : outside (external medium) ; t : tubule ; u : urine ; w : water ; x : inorganic compound x.

Another process to achieve osmoregulation has been evolved by many species, a process working always aside from the mechanisms of isosmotic regulation of intracellular fluid. That second way is to maintain the osmotic concentration of the extracellular (body) fluids more or less constant regardless of the salinity of the surrounding medium. These processes ensure an « Anisosmotic regulation of the body fluids ». They preserve internal tissues from drastic and sudden salinity changes that could impair life mechanisms of the cell, or, at least, induce costly adjustments. Both processes work together to control water movements at the cellular level.

This review intends to concentrate on this latter category of crustaceans, *i.e.* on species that hyperregulate in dilute media and that are osmoconforming in salt concentrated ones. Such animals do achieve anisosmotic regulation of their blood in dilute media.

Two types of basic mechanisms relevant to the « pumps and leaks » system are implicated in the control of the blood NaCl level : (1) « limiting processes » acting on the permeability properties of epithelial structures in order to minimize the diffusive movements of ions ; (2) « compensatory processes » driving active movements of NaCl to counterbalance the diffusive fluxes. These mechanisms appear to be at work essentially in boundary epithelia, such as the body walls, the gut, the excretory organs, and the gills (Fig. 2). Their study has been reviewed regularly and the reader interested in an overall panorama of this literature since the early 1960's is referred to the following review articles and volumes : POTTS and PARRY, 1964 ; SCHOFFENIELS and GILLES, 1970 ; GILLES, 1975 ; LOCKWOOD, 1977 ; GREENAWAY, 1979 ; SPAARGAREN, 1979, KIRSCHNER, 1979 ; GILLES, 1979 ; MANTEL and FARMER, 1983 ; GILLES and PÉQUEUX, 1983, 1985 ; PÉQUEUX and GILLES, 1988. Compensatory influx of NaCl in the gut appears to be related mainly to water absorption in hypo-osmoregulators ; the role of this organ in the overall blood salt balance and osmoregulation is, however, far from being clear. Compensatory NaCl reabsorption has also been described in the excretory organs of some freshwater species. Such a mechanism has never been found in brackish water species nor in marine ones. Clearly, the gill appears up to now as the prominent structure implicated in blood NaCl balance and osmoregulation.

STRUCTURE AND FUNCTION OF THE CRUSTACEAN GILL EPITHELIUM AS RELATED TO ENVIRONMENTAL SALINITY

Since the early works of KROGH (1938, 1939) active uptake of NaCl at the gill level has been considered to play an essential part in blood osmoregulation in many hyper-regulating aquatic species. Since then, indirect evidence that supports the model has been accumulating while most of these experiments were performed on whole animals. This failed to provide conclusive information leading to a clear characterization of the particular mechanisms and driving forces at work at the gill level. Part of this problem has been overcome during the past years with the use of perfused preparations of isolated gills. It is clear that much of our understanding

of the physiology of that organ has been gained in investigations on such preparations. The biochemical approach which has been developed concurrently will not be considered here. It deals essentially with the two enzymes Na^+, K^+ ATPase, and carbonic anhydrase. An account of the function of carbonic anhydrase in crustacean gills has been given by HENRY (1984) and BURNETT (1984). The reader interested in Na^+, K^+ ATPase is referred to different papers on the topic : TOWLE *et al.*, 1976 ; PÉQUEUX and GILLES, 1977 ; SPENCER *et al.*, 1979 ; NEUFELD *et al.*, 1980 ; PÉQUEUX and CHAPELLE, 1982 ; SIEBERS *et al.*, 1982 ; PÉQUEUX *et al.*, 1983, 1984 ; PÉQUEUX and GILLES, 1984, 1988 ; TOWLE, 1981, 1984a,b.

The results obtained following this line of approach, *i.e.* experimentation on isolated perfused gills, clearly establish that a distinction, or even in some species like the chinese crab *Eriocheir sinensis* (H. Milne-Edwards) a clear-cut distinction, must be made between the three most posteriorly located pairs of gills and the anterior ones. Several sets of evidence suggest indeed that the various pairs of gills of decapods are not functionally equivalent. This has been indicated by experiments on histology and ultra structure (COPELAND and FITZJARRELL, 1968 ; BARRA *et al.*, 1983), as well as on characterization of ions fluxes (KOCH *et al.*, 1954 ; PÉQUEUX and GILLES, 1981).

As a matter of fact, if all gills of crabs are macroscopically similar, microscopic and ultrastructural observations indicate they are lined by quite different epithelia. The epithelium of the anterior gills is much thinner (2-4 µm) than in the posterior ones (10 µm and more). There is little or no folding of the plasma membrane and the extracellular space under the cuticle is extremely reduced. The amount of intracellular organelles seems also to be very limited (BARRA *et al.*, 1983). Clearly, these gills appear to be lined only by an epithelium of the « respiratory » type. Conversely, the large epithelial cells of the posterior gills are characterized by a complex and well-developed network of apical folds which delimits a large extracellular compartment under the cuticle when animals are acclimatised to dilute media. Mitochondria are the most abundant organelles in these cells ; they may fill almost completely the cytoplasmic space. Basolaterally, another important infolding system coming into tight contact with mitochondria penetrates deeply into the cells (BARRA *et al.*, 1983). This kind of structure appears to be quite characteristic of « salt-transporting » epithelia, it is found indeed in most tissues implicated in ion transport and osmoregulation (BERRIDGE and OSCHMAN, 1972 ; TAYLOR and TAYLOR, 1992).

As stated above, these differences in structural organization of gills of euryhaline crabs when in dilute media have functional correlates. Early experiments of KOCH (1934) studying the staining of gills with silver salts already suggested about 50 years ago, that gills pairs showing fixation of silver, *i.e.* the three posterior pairs, are the only ones involved in active Na^+ uptake. This has been largely confirmed later on by experiments using perfused preparations of gills and radioisotopes. From the data reported in Table 1, it can be concluded indeed that the epithelium of the anterior gills is permeable to Na^+ ions but quite impermeable to Cl^-. It has been established that Na^+ movements are essentially passive and fit in with the

TABLE 1

NaCl flux in perfused Gills isolated from the crab *Eriocheir sinensis*.

Acclimation medium	Na^+ or Cl^- in saline (mM) OUT		IN	ANTERIOR GILLS Influx (μequiv./gww/h)	Efflux	POSTERIOR GILLS Influx (μequiv./gww/h)	Efflux
Na^+ flux							
FW	240	Na^+	240	285 ± 87	277 ± 84	260 ± 61	N.S.
	1		240	6 ± 2	112 ± 74	21 ± 7	N.S.
	10		240	85 ± 14	247 ± 13	142 ± 60	N.S.
SW	500	Na^+	480	3448 ± 960	2079 ± 632	N.S.	N.S.
Cl^- flux							
FW	280	Cl^-	280	N.S.	N.S.	2273 ± 539	875 ± 168
	1		280	N.S.	N.S.	16 ± 3	138 ± 39
	10		280	N.S.	N.S.	568 ± 66	469 ± 2
SW	560	Cl^-	560	N.S.	N.S.	2353 ± 826	2514 ± 724

Mean results of at least 5 experiments ± S.D.
N.S.. = not significant; FW = fresh water; SW = sea water.

Ussing's equation of the fluxes ratio. Conversely, there is no Na^+ efflux in the posterior gills, while there is an influx that must therefore be linked to the activity of active processes. Cl^- ions cross the epithelium in both ways, but influx generally overcomes the efflux. It is clear that posterior gills only are able to transport NaCl transepithelially against a concentration gradient. Further studies conducted on these perfused preparations have uncovered a number of transport processes located in the apical and the basolateral membranes and that can yield a tentative cellular model accounting for the NaCl absorptive process observed when animals are acclimatised to dilute media (for review, see for instance PÉQUEUX and GILLES, 1988).

A MODEL OF NaCl TRANSPORT IN *E. SINENSIS* POSTERIOR GILL — APPLICABILITY TO OTHER CRUSTACEANS

A schematic drawing taking into consideration the different experimental results available at present is given in Fig. 3 . While this model has to be considered as basically tentative, it agrees with the data obtained when both sides of the preparation are bathed with the same saline i.e., in the absence of a transepithelial concentration gradient. It is worth noting that in the freshwater chinese crab, the

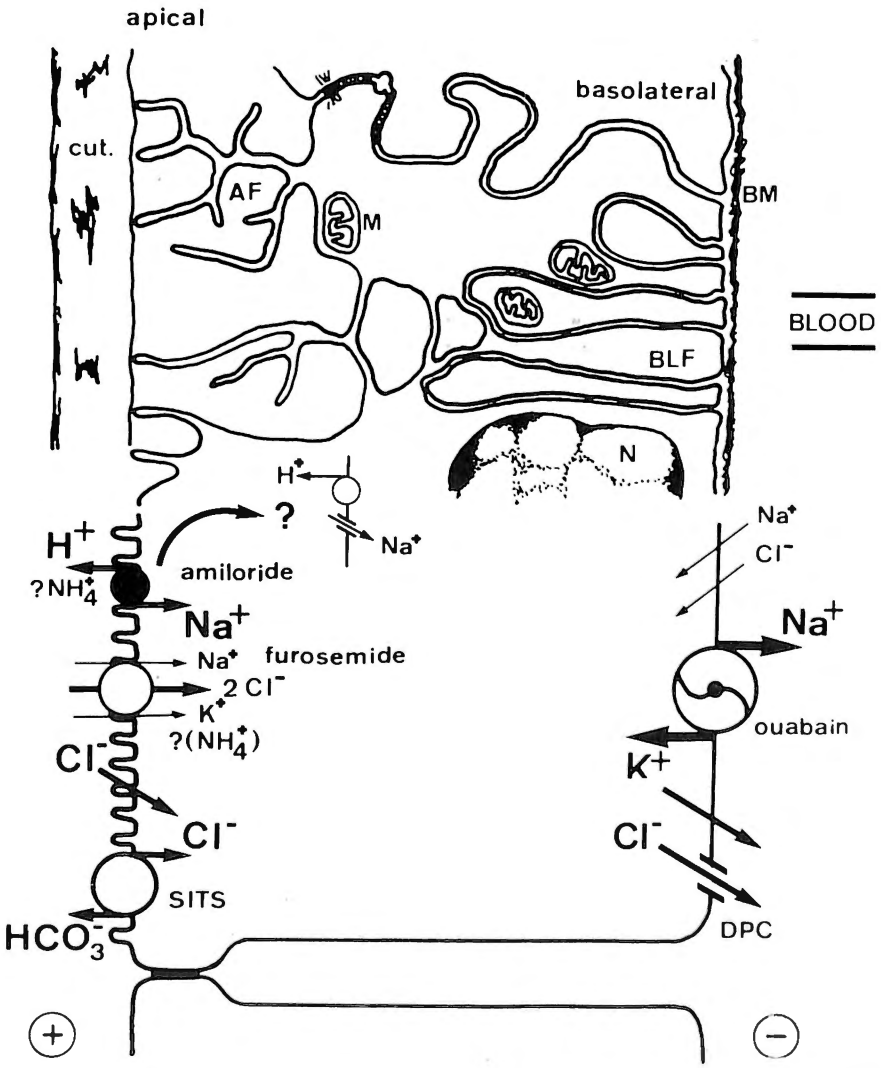

Fig. 3. — Functional model of POSTERIOR gills of FW chinese crabs, *E. sinensis*. Both sides of the epithelium are bathed with the same « FW bloodlike » saline. Explanations in the text.

intracellular level of Na^+ and Cl^- in the gill epithelium is higher than in the external medium and lower than in the serosal/haemolymph one. Therefore, the movements of these ions from the external medium to the intracellular and haemolymph compartments cannot occur by diffusion only. Active transport processes at both sides have to be taken into consideration. To be pointed out are the few following distinctive features : (1) Na^+ and Cl^- are transported independently ;

(2) the permeability of the epithelium to Na^+ is extremely low ; (3) the epithelium is quite permeable to Cl^- ; (4) the basic driving mechanism is a Na^+/K^+ pump whose activity is linked to a $(Na^+ + K^+)$ATPase system and which has been established to reside in the basolateral membranes ; (5) a NaK2Cl mechanism is thought to be at work at the apical side of the epithelium and involved in the entry of Na^+ and Cl^- into the cell ; (6) Na^+ inward movement at the apical side also seems to be largely related to an amiloride-sensitive Na^+/H^+ exchange process ; (7) a SITS-sensitive Cl^-/HCO_3^- exchange system is involved in the transport of Cl^- at the apical side ; (8) Cl^- ions leave the cell at the basolateral side using a Cl^- conductive pathway which is very likely driven by the K^+ concentration gradient known to generate a potential difference oriented haemolymph side positive.

The question which arises now is that of whether the *E. sinensis* model of gill transport can be applied to other crabs , as for example the shore crab *Carcinus maenas* (L.), a well-known weak regulator. This model is by no means exclusive of others. However, it could be quite satisfactorily applied to other euryhaline crabs by adding pathways for Na^+ outward movements or by considering that the transport characteristics of their gills are a mixture of the characteristics described separately for the anterior and posterior gills of the chinese crab.

The relative importance of these processes and/or of these specific characteristics could vary greatly from one species to another, thus determining their ability to hyperosmoregulate in dilute environments. In *C. maenas*, the anterior gills are mostly lined with a respiratory-like epithelium, while both a respiratory and a salt-transporting epithelium are found in the posterior gills. However, the salt-transporting tissue never exceeds 30 % of the whole lamellar surface (COMPÈRE *et al.*, 1985 ; 1989). The large unidirectional Na^+ fluxes that occur in *C. maenas* gills (WANSON and PÉQUEUX, 1981 and unpublished results), at variance with the situation found in *E. sinensis*, could thus be satisfactorily related to the fact that in *E. sinensis*, the posterior gills are essentially lined with an epithelium of the transporting type (with a low Na^+ permeability), while in *C. maenas,* both the respiratory (with a high Na^+ permeability) and the transporting (with low Na^+ permeability) epithelia are present. A similar situation would also occur in the blue crab *Callinectes sapidus* (Rathbun) (COPELAND and FITZJARREL, 1968).

These results lead to the interesting conclusion that the structural organization of the gills can be directly related to their functional properties, hence to the osmoregulatory capabilities of the considered species. They substantiate the idea that the model of gill structural and functional organization described for the chinese crab *E. sinensis* can be applied quite satisfactorily to other euryhaline crabs. They moreover establish that this species is an almost unique model with respect to the study of not only ion transport processes, but also the structure-function relation in a NaCl-transporting epithelium. It is however not actually complete, and a precise view of the mechanisms at work in the gill tissue of crustacea, and also of the mechanisms involved in the overall osmoionoregulation function in crustacea entails the study of the physiological properties of a structure which always has been ignored by the physiologists : the cuticle.

PERMEABILITY PROPERTIES OF THE CRUSTACEAN CUTICLE

Up to now, most of the physiologists have taken for granted that the thick cuticle covering the body is impermeable while that of the gills is highly permeable, which implies that the permeability of crustacea only depends upon the gill epithelium.

Recent work however demonstrated that this is far from being the case and that the various cuticles may exhibit various degrees of permeability that can be related to the osmoregulatory performances of the species considered (for review, see LIGNON and PÉQUEUX, 1990 ; PÉQUEUX and LIGNON, 1991).

These studies have been conducted by measuring, in a comparative way, diffusional transcuticular potentials and electrical conductance in several species of decapod crustaceans. It is established that the cuticle permeability depends upon the species considered and its ionoregulation capability, upon the localization of the gill in the gill chamber or even upon the topographic region in a single gill and upon the nature of the ionic species.

In each case, the cuticle must always be considered as a diffusion barrier for the main osmotic effectors, Na^+ and Cl^-. However, the efficiency of this barrier is low in the case of the stenohaline osmoconformers. In hyperregulators, the efficiency of the cuticular barrier is much higher. What emerges from the studies of Lignon and coworkers is that a clear-cut correlation can be established between cuticular ionic permeabilities, either branchial either extrabranchial, and the environmental salinity that can withstand the three following groups : (1) marine conformers (*Homarus gammarus* L., *Maia squinado* Risso and *Nephrops norvegicus* L.), (2) moderate regulators (*Carcinus maenas*) and euryhaline conformers (*Cancer pagurus* L.) and (3) powerful regulators (*Astacus leptodactylus* Escholtz and *Eriocheir sinensis*). For instance, soft or calcified cuticles of the body and of the gut are 500 to 5,000 times less permeable in regulators as compared with conformers (LIGNON and PÉQUEUX, 1990). For each species, the gill cuticle is the most permeable while the carapace and the soft body cuticle are the least permeable. Anyway, the gill cuticle of strong regulators is less permeable than the carapace of osmoconformers.

To be pointed out also is the fact that, in hyperregulators, the cuticle exhibits important differences in its electrical characteristics between the various pairs of gills or even between different topographic regions of the same gill. As a matter of fact, the cuticle permeability of the crayfish gill lamina is low for all ionic species but Cl^- while, in gill filaments and in crab gills, permeability to cations is high and permeability to Cl^- is low. In addition, the cuticle shows a functional asymmetry which favours ionic influxes, this asymmetry being almost inexistant in osmoconforming species.

It is thus clear that the cuticle behaves as a structure complementary to the epithelium with respect to ionoregulation. There is no doubt the reduced cuticular permeability of regulators when in dilute media results in the reduction of diffusional ionic leaks. It also forces cnsideration of the anatomical subcuticular space in the gill as a genuine physiological compartment where recycling of ions by the

cellular uptake systems before they cross the cuticle could take place in an efficient way. Such a process could well account for the low « apparent » permeability of the epithelium reported above, for instance, in the posterior gills of the chinese crab in the case of Na^+ ions.

It is also remarkable that the body cuticle permeability is generally low enough foo most of the ionic exchanges take place at the gill level. However, this permeability is conversely large enough to account for a NaCl loss as large as the urine loss in the crayfish. Fron both these lines of evidence, the conventional view of an impermeable body cuticle and of a highly permeable gill cuticle in crustaceans appears actually as a very crude approximation.

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ACID-BASE HOMEOSTASIS IN AQUATIC ANIMALS EXPOSED TO NATURAL AND PERTURBED ENVIRONMENTS

by

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SUMMARY

Keeping an appropriate acid-base state in the various body compartments of animals is of prime importance for many basic living processes. What is preserved is not a constant pH value but rather a constant relationship between pH and body temperature, which tends to stabilize the protein electrical charge and, more generally, conformation and function of macromolecules. Acid-base homeostasis requires a balance between metabolic production and controlled excretion of two classes of acids or bases : the volatile carbonic acid whose elimination depends on respiratory regulations ; and fixed acids and bases, usually excreted in association with ion exchanges. In aquatic animals, these functions are heavily challenged by large natural changes of respiratory gases, oxygen and carbon dioxide, as well as of total salinity or of particular ions in the environment. The effects of each of these factors in isolation have been well studied in laboratory conditions, but integrated responses to the changes of many factors as it occurs in the natural setting are less well known. Variations of ambient or internal CO_2 are not a strong stimulus to breathing in aquatic crustaceans and fishes, and respiratory compensations are thus of little importance in acid-base homeostasis. On the contrary, aquatic organisms are usually able to quickly get rid of large fixed acid or alkaline loads by coupling their excretion with gill ionic exchanges. Such excretory processes also serve to compensate acid-base disturbances induced by changes of the respiratory qualities of the water. The well-known impact of various pollutants (heavy metals, ammonia, acid waters...) on gill structure and ionoregulatory mechanisms can also considerably disturb acid-base balance in aquatic animals. Such disturbances may serve as very sensitive tests of sublethal toxicity.

Keywords : acid-base regulation, aquatic animals, gill ion exchange, intertidal rockpools, pollutants.

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INTRODUCTION

The maintenance of an appropriate hydrogen ion activity is probably one of the most basic requirements of living systems. Much recent work has revealed that a large range of animal cells are able to efficiently compensate cytosolic acid-base deviations arising from endogenous metabolic byproducts or from extracellular disturbances. The well-known constancy of blood pH in man and homeothermic vertebrates probably represents a first line of defense that facilitates cellular acid-base homeostasis. More recently, it has become clear that such a precise extracellular acid-base regulation is also working in lower vertebrates and invertebrates. Rather than extensively reviewing the abundant literature on this topic (see TRUCHOT, 1987), the present short account will focus on aquatic animals and try to answer a few simple questions. Do water-breathing animals possess mechanisms to maintain acid-base homeostasis ? Are these mechanisms similar to or different from those at work in air breathers ? How are these mechanisms used in natural aquatic environments ?

But before examining these particular questions in some detail, it is important first to raise a more general problem which has recently been much clarified thanks to studies on lower animals.

WHAT IS THE REGULATED ACID-BASE VARIABLE AND WHY ?

Acid-base homeostasis is commonly thought to mean maintenance of a constant blood pH. But this concept is valid only as long as body temperature remains steady. In poikilotherms, many observations have shown that blood pH in fact decreases as body temperature increases (RAHN *et al.*, 1975 ; REEVES, 1977). This pH change depends both on the thermal properties of body fluid buffers and on physiological adjustments that maintain a new steady value after a temperature change (*e.g.* TRUCHOT, 1978). The slope of the relationship between blood pH and temperature has raised some debate, but it is generally agreed that it is similar *in vivo* and in an *in vitro closed* system, its value amounting to 0.015 to 0.020 pH units/°C (reviewed by TRUCHOT, 1987). This is just the value required to keep a constant difference between the physiological pH and the neutral pH of pure water, which also decreases at increasing temperature. This means that blood pH is regulated in such a way that the *relative alkalinity* (or the ratio $[OH^-]/[H^+]$, see RAHN and HOWELL, 1978) is kept constant (Fig. 1). Another striking consequence of the observed pH/temperature slope is that the degree of dissociation of the most important protein buffer group at physiological pH, the imidazole group of histidine, is also kept nearly constant (REEVES, 1972). This so-called *alpha-imidazole regulation* results in the maintenance of a relatively unchanged electrical charge on proteins, which presumably stabilizes their structure and functional properties in the face of temperature changes (WHITE and SOMERO, 1982 ; SOMERO, 1986). Beyond the maintenance of a constant blood pH, or of a constant blood pH/temperature relationship, acid-base regulation may thus have the meaning of an *homeostasis of*

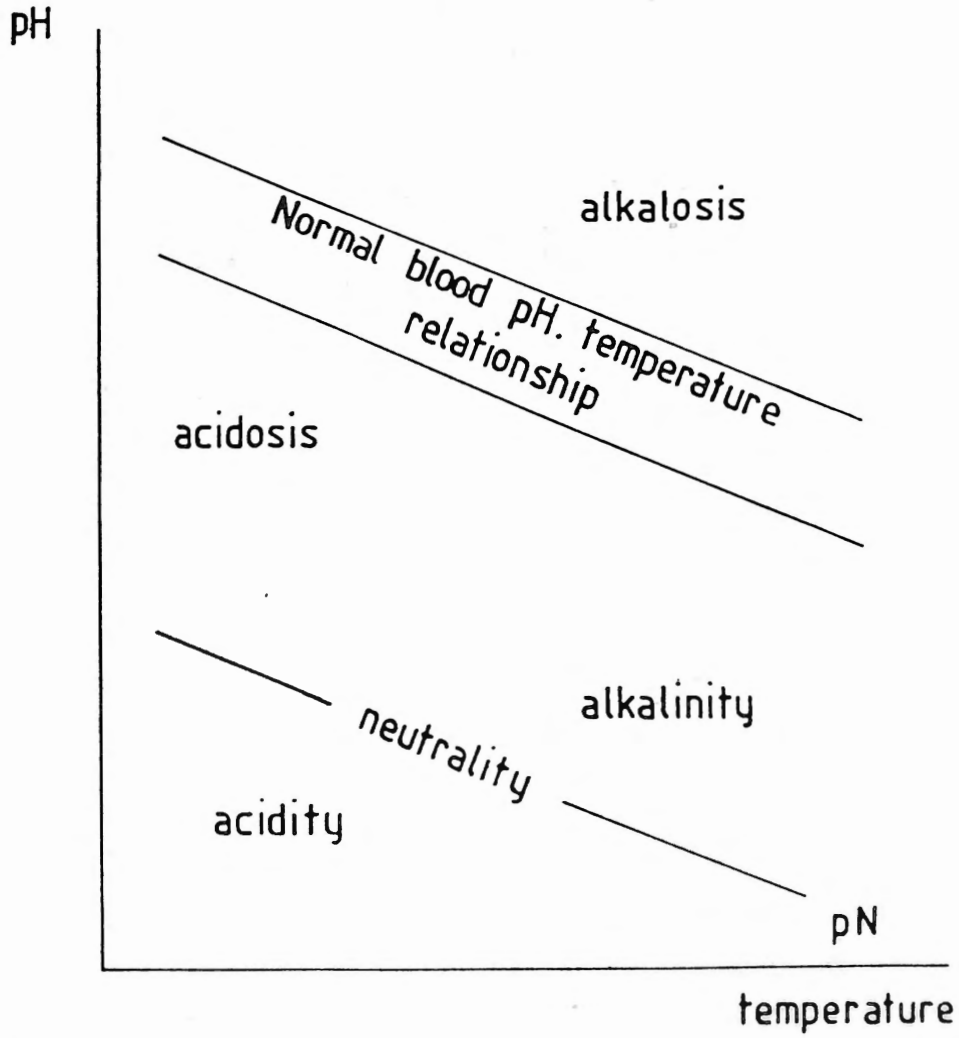

Fig. 1. — The normal blood pH *vs* body temperature relationship observed in poikilothermic animals. The regulated blood pH value decreases as temperature increases in such a way that the difference between biological pH and the neutral pH of pure water (pN) remains constant through the whole temperature range. In the same way as acidity and alkalinity of aqueous solutions are defined by deviations from the pN/temperature line, acidosis and alkalosis designate departures not from an unique pH value but from the normal blood pH/temperature relationship.

protein function. A similar relationship between intracellular pH and temperature has been found (*e.g.* RODEAU, 1984), showing that the concept also applies to intracellular fluids.

SOME ASPECTS OF ACID-BASE HOMEOSTASIS IN AQUATIC ANIMALS

Acid-base regulatory mechanisms

Body fluid acid-base balance is permanently challenged by acidic and alkaline substances which are produced by cell metabolism and must thus be eliminated. There are in fact two broad categories of such products : first, the volatile acid CO_2 whose excretion is controlled by respiratory gas exchanges, and second, non volatile or so-called fixed acidic or alkaline equivalents, whose elimination must comply with electroneutrality constraints and is by consequence necessarily coupled with ion exchanges. The primary purpose of gas and ion exchanges being not acid-base homeostasis, it should be pointed out that these processes can be involved as *disturbing factors* as well as *regulatory mechanisms* of acid-base balance. For example, reduced CO_2 elimination relative to its production leads to an increase of body fluid Pco_2 (hypercapnia) and by consequence to a pH decrease of respiratory origin (respiratory acidosis). Conversely, a restricted excretion of metabolically-produced acidic equivalents or an excessive elimination of alkaline equivalents (both taking place mainly via the urinary route in higher terrestrial vertebrates) could lead to a decrease of body fluid pH of metabolic origin (metabolic acidosis). In mammals, respiratory acid-base disturbances are known to be compensated at least partly by modulation of renal excretion of acidic or alkaline equivalents (metabolic compensation). In addition, ventilatory adjustments of blood Pco_2 in response to pH changes can contribute to the regulation of metabolic acid-base disturbances (respiratory compensation).

In many fishes and crustaceans, acid-base disturbances of respiratory origin are efficiently compensated with a progressive recovery of blood pH resulting from an increased bicarbonate concentration. This response has been much studied by exposing the animals either to ambient hyperoxia, which entails a reduced ventilatory activity and an endogenously-generated elevation of internal Pco_2, or to ambient hypercapnia which leads to CO_2 loading from the external medium. As shown in Fig. 2, the increase in plasma [HCO_3^-] compensating for the hyperoxia-induced respiratory acidosis is obtained in trout by a sustained net outflux of fixed acid to the ambient water via the gill route. At return to normoxia, a transient alkalosis disappears rapidly thanks to a branchial base efflux (or acid influx). These responses affecting fixed acid or base excretion are very similar to those observed in terrestrial vertebrates, but the contribution of urinary excretion is here always minor (Fig. 2). As for ionic regulation, the role of the gills in acid-base control is prominent. Similar acid-base compensations have been observed in crustaceans (*e.g.* TRUCHOT, 1979).

Branchial excretion of acid-base equivalents is in fact coupled to ion exchanges. It has long been postulated that freshwater animals take up Na^+ and Cl^- ions from the ambient water in exchange for excretion of H^+ (or NH_4^+) and HCO_3^-, respectively, in order to maintain ion homeostasis. Coupled Na^+ and H^+ movements probably do not take place by a direct Na^+/H^+ antiport but rather by

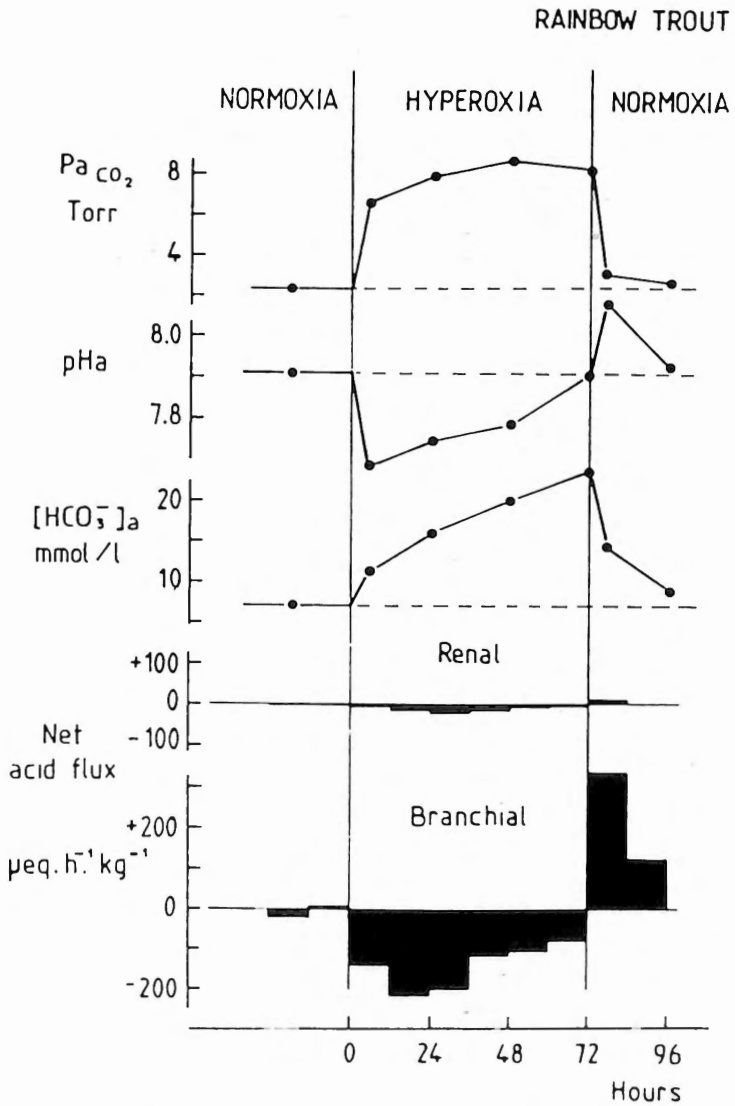

Fig. 2. — Simultaneous changes of arterial blood acid-base status (pH, Pco_2 and plasma HCO_3^-) and of net transfer of acidic equivalents via renal and branchial routes in the rainbow trout *Oncorhynchus mykiss* during exposure to hyperoxic water followed by recovery in normoxic water. Net acid flux is considered negative when fixed acid is lost, as measured by titration of ambient water. During hyperoxia, a transient respiratory acidosis brought about by an increase of blood Pco_2 is progressively compensated thanks to an increase of HCO_3^- concentration. This increase results from a mainly branchial acid loss, which is readily reversed to an influx of acid (or base efflux) upon return to normoxic water whereas [HCO_3^-] rapidly decreases. Drawn from values published by HOBE *et al.* (1984), WHEATLY *et al.* (1984) and WOOD *et al.* (1984).

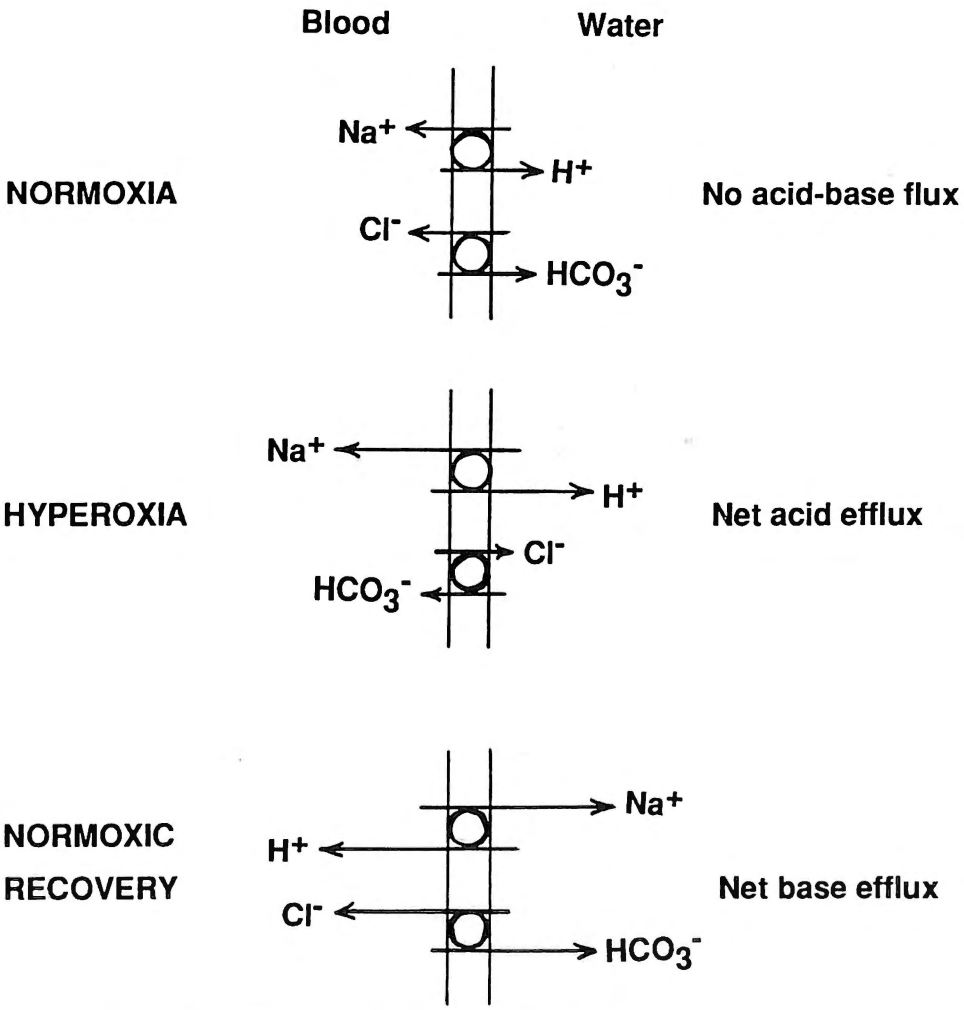

Fig. 3. — General principles of adjustments of the transfers of acid-base equivalents coupled to gill Na^+ and Cl^- exchanges in the rainbow trout *Oncorhynchus mykiss*, in response to respiratory acid-base disturbances (Derived from flux values published by WOOD *et al.*, 1984). Sodium and chloride are stoichiometrically exchanged against acid and base equivalents labelled H^+ and HCO_3^-, respectively. In normoxic steady acid-base state, NaCl uptake against equal effluxes of H^+ and HCO_3^- result in no measurable net acid-base flux. Hyperoxia induces a respiratory acidosis (see Fig. 2), accompanied by an increased Na^+ net influx and a reversed Cl^- net flux. Both increased H^+ excretion and bicarbonate uptake result in a net acid efflux increasing plasma bicarbonate and compensating the respiratory acidosis. During normoxic recovery, a transient alkalosis (see Fig. 2) is also compensated by a net base efflux resulting from H^+ uptake and HCO_3^- excretion, respectively coupled to increased Cl^- influx and Na^+ outflux.

a proton pump electrically coupled to an apical Na^+ channel (AVELLA and BORNANCIN, 1989). But, whatever the exact mechanisms, it has been shown that gill ion exchanges can be modulated to ensure acid-base compensations (WOOD, 1991). Indeed, measurable directional changes of Na^+ and Cl^- fluxes consistently explain associated acid-base movements in trout (Fig. 3), and a 1/1 linear relationship between the net acid-base flux and the difference of Na^+ and Cl^- net fluxes indicate that the Na^+/H^+ and Cl^-/HCO_3^- exchanges are the only mechanisms involved (WOOD *et al.*, 1984).

Whether respiratory modulation of blood Pco_2 can compensate for metabolic disturbances in aquatic animals remains little documented and controversial. Regulation of ventilatory activity in water-breathers is mainly oriented to meet oxygen demand, presumably because oxygen is poorly available in water due mainly to its low solubility (DEJOURS, 1981). The poor responsiveness of ventilatory control to increased Pco_2 and/or decreased pH is clearly illustrated by the sustained depression of gill water flow rate under hyperoxia, despite a prevailing and often marked respiratory acidosis. Some data nevertheless suggest that ventilation may be responding to acid-base disturbances in certain circumstances, and particularly when ion exchange limitations presumably impede metabolic compensations. For example, crayfish exposed to very poorly mineralized water typically exhibited a metabolic acidosis which was, however, moderated by a marked decrease of Pco_2 apparently resulting from a higher than normal ventilatory activity (BURTIN *et al.*, 1986).

Acid-base balance in naturally variable aquatic environments

Many aquatic environments may undergo wide spatial and temporal changes of many physical and chemical factors among which ion and gas composition are potentially disturbing for acid-base balance. We will focus here mainly on respiratory gases, O_2 and CO_2, and pH, the variations of which are mainly caused on a diel basis by biological processes, respiration and photosynthesis. These variations are particularly marked in small water bodies such as rockpools left at low tide on the seashore (TRUCHOT and DUHAMEL-JOUVE, 1980). Typically, these biota become hypoxic at night while water Pco_2 increases moderately and pH decreases. Conversely, during the day, photosynthesis is active and water Po_2 usually reaches high levels, accompanied by a large reduction of Pco_2 and a huge increase of pH. Additionally, in temperate regions, diel changes of temperature are well marked and may also strongly affect acid-base balance.

Obviously, situations encountered in nature are often very different from those explored in single-factor laboratory experiments, in that some variables can act synergistically and some others antagonistically on acid-base balance. For example, during the day, ambient hyperoxia will induce gill hypoventilation and respiratory acidosis, while concomitant reduction of water Pco_2 and increase of pH could be expected to favor internal alkalosis. Organismal acid-base disturbances resulting from such antagonistic influences can hardly be predicted and must be directly studied either *in situ* or in simulated environments, which has been rarely done. As

an exemple, we will comment some data we have obtained on shore crabs *Carcinus maenas* put in an outdoor tank populated with algae, a device which has proved to qualitatively and quantitatively reproduce quite well natural variations of many factors as observed in rockpools (TRUCHOT, 1986). The most interesting outcome of these data was that blood pH variations were much more moderate that could be predicted from changes of ambient water oxygenation alone, essentially because they were damped by the influence of concomitant Pco_2 variations. In addition, changes of blood bicarbonate concentration were so moderate that they could not be taken as evidence that metabolic compensations of respiratory acid-base disturbances took place in these conditions. Rather interestingly, when measured pH values were plotted as a function of water temperature, they gave a slope much alike that observed at variable temperature but constant gas conditions in laboratory experiments. A rather surprising conclusion can be drawn from these data. Even if the shore crab possesses elaborate mechanisms to maintain acid-base homeostasis in the face of environmental changes, these mechanisms are apparently not used when the animal is exposed to natural rockpool conditions. In fact, the concerted changes of ambient factors in this environment seems appropriate enough to lead passively to the optimal acid-balance.

Effects of pollutants on acid-base balance in aquatic organisms

Acid-base homeostasis in aquatic animals is strongly dependent on a proper functioning of the gill which is the main site of respiratory and ionic exchanges, two processes involved in acid-base compensations. Being actively irrigated by the respiratory water flow, the large surface and the thin epithelial lining of the gill is also the most prominent interface between the aquatic organism and the environment. As a consequence, it has been shown to be very sensitive to toxicant action (MALLATT, 1985). In fact, the gill epithelium of many fishes and crustaceans is apparently the first target of many pollutants as demonstrated by important and relatively unspecific cytological damages, even upon exposure to sublethal levels. Among accompanying physiological perturbations, restriction of gas exchange is caused mainly by thickening of the water-blood barrier or by mucus accumulation. Although less studied, acid-base disturbances are probably always present and may even constitute the first and most sensitive symptom of sublethal contamination. As shown by our observations on the shore crab, *Carcinus maenas,* exposed to sublethal copper levels, there is first a progressively developing metabolic acidosis (Fig. 4), which does not result from anaerobic lactic acid generation but is probably ascribable to some yet undefined perturbation of gill ion exchanges (BOITEL and TRUCHOT, 1989). Indeed, even if no changes of hemolymph ion concentrations are apparent in full strength sea water where the crab is isosmotic and almost isoionic, copper exposure causes a typical loss of hemolymph ions in dilute sea water (BOITEL and TRUCHOT, 1990). Concomitant changes of hemolymph Pco_2 may or not ensure partial compensation of the primary metabolic acidosis. Interestingly, these acid-base disturbances appear reversible after weeks of sublethal exposure to copper in the shore crab (Fig. 4). Disappearance of the metabolic acidosis coincides with

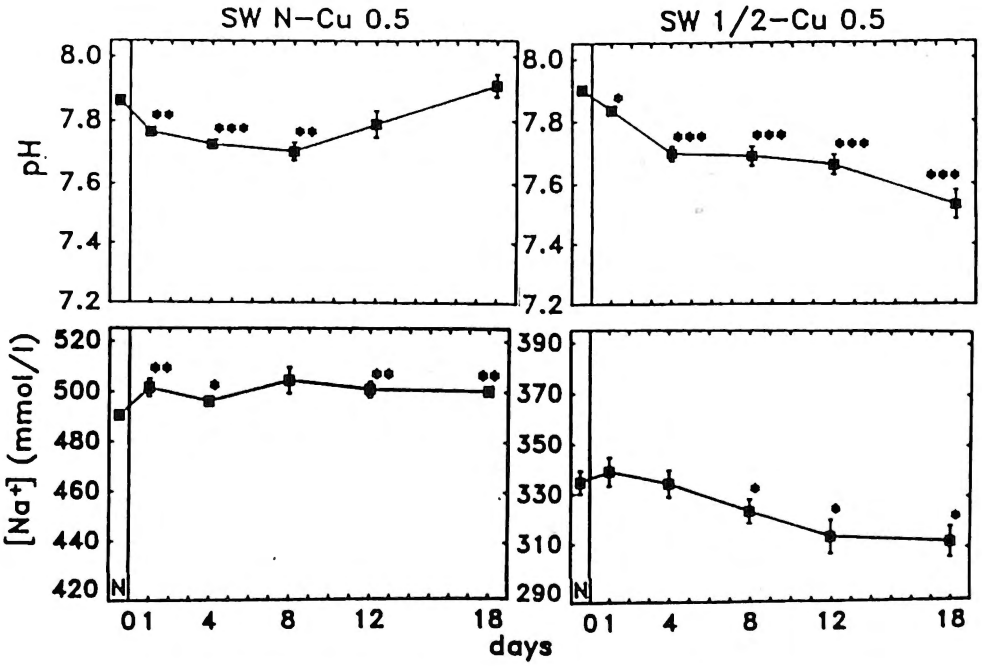

Fig. 4. — Changes of hemolymph pH and Na^+ concentration induced by sublethal exposure to waterborne copper (0.5 mg/l) in the shore crab *Carcinus maenas* acclimated to full strength (SW N) and dilute (SW 1/2) seawater. A well marked acidosis progressively recovers after 18 days in full strength but not in dilute sea water. Copper exposure induces no important ionic disturbance in full strength sea water but the animals progressively lose sodium in a dilute medium when exposed to the toxicant. N : values measured the day before copper exposure. (Redrawn in a modified form from BOITEL and TRUCHOT, 1990).

anatomical repair of the gill epithelium and recovery of normal oxygen levels in hemolymph (NONNOTTE *et al.*, 1993). These and other data indicate that acid-base disturbances could provide very sensitive tests of toxicant sublethal exposure. Inasmuch as the toxicity of many pollutants depends on water chemistry, studies of acid-base effects could prove appropriate to understand such variations.

CONCLUSION

Like higher terrestrial vertebrates, aquatic animals are endowed with efficient mechanisms to achieve extracellular acid-base homeostasis. Among these mechanisms, transfer of acid-base equivalents in coupling with ion exchanges at the gill level appears of prime importance while respiratory compensations are likely minor or absent. Being related to both respiratory and ion exchange functions, acid-base parameters are particularly useful to evaluate physiological, pathological

and adaptational responses of aquatic animals to any natural or anthropogenic changes in their environment.

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CELLULAR MECHANISMS OF SALT SECRETION BY THE MALPIGHIAN TUBULES OF INSECTS (*)

by

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SUMMARY

Malpighian tubules secrete primary urine and the hindgut and rectum perform the fine regulation of its composition. The system plays an important role in the regulation of the salt and water content in insects : after eclosion and before flight some insects must lose a lot of water in order to reduce their weight ; or after a meal the blood sucking bug *Rhodnius*, for instance, or the female mosquito need to eliminate a high load of NaCl and water ; the mealworm and the desert beetle on the other hand use a cryptonephric system, built by the Malpighian tubules and the rectum, to reabsorb practically 100 % of the water present in the excreta.

Formica, the species described in this paper, is an omnivorous, continuously feeding species. K^+ secretion is essential in the formation of primary urine by the Malpighian tubules of *Formica*. In the present paper a model for this K^+ secretion is discussed in detail and a brief comparison is made with other species living in different conditions and/or exposed to different salt and water loads. The prime mover for salt secretion in the tubule is a V-type H^+ATPase in the luminal membrane in parallel with a Na^+/H^+ or K^+/H^+ antiporter. Uptake mechanisms for K^+ and/or Na^+ at the haemolymphal side may differ according to the species : in tubules of *Formica* uptake of K^+ through high conductance channels occurs in the presence of a high K^+ concentration. At lower K^+ concentrations a K/Cl and a Na/K/2Cl cotransporter also become functional in these tubules. In some species an appreciable Na^+ conductance is present (e.g. *Aedes aegypti*) or a Na^+/K^+-ATPase may play a role (e.g. *Rhodnius*). The pathway followed by the accompanying anion (mostly Cl^-) is still controversial.

Keywords : Malpighian tubule, electrophysiology, KCl secretion, intracellular measurements, cable analysis, *Formica*.

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INTRODUCTION

Insects can regulate the composition of their blood within narrow limits, although some species live in extreme and variable conditions and have widely varying diets (EDNEY, 1977; STOBBART and SHAW, 1974). A necessary link in this homeostatic regulation is the formation of primary urine by the blind ending Malpighian tubules. As blood pressure in insects is low, the urine is formed not through pressure filtration, but by secretion of salt osmotically followed by water. Metabolites and toxic components may diffuse passively or be secreted actively into the lumen (PHILLIPS, 1981). The primary urine is then voided into the intestine, just below the midgut and fine regulation is achieved by the rectum according to the animal's needs (PHILLIPS, 1977).

MADDRELL (1977; 1980) and PHILLIPS (1981) summarized facts and hypotheses on ion and water transport in the Malpighian tubules as they were known and imagined at that point in time. The facts and insights concerning the ionic basis of the fluid secretion were updated in 1993 by NICOLSON.

Briefly, fluid secretion by most insect tubules is driven by active transport of K^+ ions, while anions (mostly Cl^-) and water follow passively (see PHILLIPS, 1981). Exceptions are, on the one hand some so-called primitive species in the evolutionary trends in insects (*e.g., Libellula,* NICHOLLS, 1985) that rather use Na^+, an ion that is present in abundance in their haemolymph, and, on the other hand, blood-sucking species, that can switch from a mixed Na^+/K^+ in basal conditions to a preferentially Na^+ driven fluid secretion (*e.g. Rhodnius,* MADDRELL, 1980; *Aedes,* BEYENBACH and PETZEL, 1987) or that always preferentially transport Na^+ (*e.g. Glossina,* GEE, 1976). The reason for this shift from NaCl to KCl secretion in modern insects may be that most ancestral insects (like locusts, cockroaches and houseflies today) had a $NaCl^-$rich, low K^+ haemolymph (SUTCLIFFE, 1963), but fed on succulent plant material with the opposite ionic ratios. Fresh lettuce for example contains (in mmol per kg tissue water) 110 K^+, 14 Na^+ and 35 Cl^- (PHILLIPS, 1977). So the need to retain Na^+ and eliminate excess K^+ may have been solved in insects by using K^+ secretion in the primary urine production process. Blood-sucking species have then switched back to a predominantly Na^+ driven secretion at least in stimulated conditions, *i.e.* just after a blood meal. In the latter species the weight may increase several times after feeding, making the mosquito for instance vulnerable to predators. A rapid loss of weight is realized by increasing the rate of primary urine formation by the Malpighian tubules of blood feeders (NIJHOUT and CARROW, 1978; PETZEL *et al.*, 1987, MADDRELL, 1963). A similar stimulation of salt and water secretion by the tubules of butterflies causes loss of fluid (and of weight) after eclosion and before flight as was shown by RYERSE (1978) and NICOLSON (1976; 1980).

Other insects may need to retain as much water as possible. An extraordinary structure present in many Coleoptera and some Lepidoptera is the cryptonephric rectal complex. The structure consists of the blind end of the Malpighian tubules being closely apposed to the rectum, the whole being enveloped by a perinephral membrane. The extremely high concentrations of KCl (over 3 M, MACHIN and

O'DONNELL, 1991 ; O'DONNELL and MACHIN, 1991) realized in the lumen of the Malpighian tubules in some species like the mealworm (*Tenebrio molitor*) and the desert beetle (*Onymacris*) allow the system to reabsorb almost 100 % of the water from the excreta present in the rectum and to take up water vapour directly from the atmosphere (reviewed by O'DONNELL and MACHIN, 1988).

In the present paper we want to concentrate on a model for KCl transport across the Malpighian tubule cell in unstimulated conditions, based on recent information obtained with electrophysiological techniques on Malpighian tubules of the forest ant *Formica polyctena*. The forest ant is an omnivorous, continuously feeding species. So a diet containing both K^+ and Na^+ will be taken in, but the animal will not normally be exposed to extreme conditions of salt and water load as is the case in blood feeders. Adults in contrast with the larval stage (FLORKIN and JEUNIAUX, 1974) have a haemolymph with a high Na^+ over K^+ content (see VAN KERKHOVE *et al.*, 1989). The preference of the Malpighian tubule was found to be for KCl secretion however (VAN KERKHOVE *et al.*, 1989). This is in agreement with the findings for most modern insects (PHILLIPS, 1981). It was shown that the transepithelial K^+ transport always occurred against a large electrochemical gradient. K^+ transport must thus be transcellular. Cl^- transport is mostly passive. The Cl^- transport pathway could be either across the cells or paracellularly, across the shunt. Data will be described in detail allowing the construction of a model for the different steps of the K^+ transport.

From the model derived for this tissue, differences with other species living in different conditions and/or exposed to different salt and/water loads will be briefly commented on.

MATERIAL AND METHODS

The methods used in this study have been described in detail elsewhere, but will be briefly summarized.

Dissection and experimental set-up (see also VAN KERKHOVE *et al.*, 1989)

After decapitation the ventral abdominal sternites were removed and the midgut with the Malpighian tubules attached to it was removed. One Malpighian tubule was cut off, as close to the midgut as possible and transferred to a bathing droplet of about 50 to 100 µl covered with paraffin oil. *Formica* Malpighian tubules are short (2 to 3 mm) so the Ramsay method (RAMSAY, 1953) for measurement of fluid secretion had to be adapted. The cut end of the tubule was sucked into a holding pipette and partly pulled out of the bathing droplet. The part in the oil was nicked and secretory fluid leaving the tubule was collected every 10 min with a collecting pipette. Fluid secretion rates in control Ringer, containing 51 mM K^+ and 143 mM Cl^-, were typically between 100 and 200 pl/min. The bathing droplet could be continuously refreshed with the help of a perfusion and a suction pipette and the com-

position of the bathing solution could be easily changed in order to study the effect of ion concentrations or of different drugs.

Intracellular and intraluminal measurements with double barrelled ion-sensitive or conventional microelectrodes

In order to be able to puncture the cell (or the lumen) with a microelectrode both ends of the tubule were fixed in a holding pipette. The preparation of the double-barrelled ion-sensitive (K^+, H^+ or Cl^- sensitive) or of the conventional electrodes has been described extensively by ZHANG *et al.* (1994), LEYSSENS *et al.* (1992, 1993a and b) and DIJKSTRA (1993).

Cable analysis

The electrical properties of the epithelium and of its basal (i.e. bath side) and luminal cell membrane can be studied by perfusing the lumen of an isolated tubule with Ringer solution of a known composition and by sending a small electrical current across the cell layer. At the same time the transepithelial and transmembrane voltage deflections can be measured as caused by the passage of this current. This allows the calculation of the transepithelial and the transmembranal resistances. Also, if the luminal and bath perfusion solutions are symmetrical, the equivalent active short circuit current can be calculated. The theoretical considerations and the cable equations in the study of *Formica* Malpighian tubules have been described in WELTENS *et al.* (1992) and DIJKSTRA *et al.* (1994a).

MODEL AND DISCUSSION

The effects of different drugs and changes in the ion composition of the bathing fluid were studied on fluid secretion, on the cellular and luminal ion concentrations and on the electrophysiological parameters of the tubule. From the data a model emerged unraveling the different mechanisms that may be involved in the transfer of K^+, H^+ and Cl^- across the epithelium. Fig. 1 gives an overview of the mechanisms present for which evidence was found (see Table 1 and Fig. 2). The consequences and interpretation of these characteristics for KCl transport will be discussed.

The effect of bath K^+ (and Na^+) concentration on fluid secretion

Malpighian tubules of *Formica polyctena* show a strong dependence on the presence of K^+ in the bathing fluid (Table 1 and 2). In a K^+ free, high Na^+ medium no fluid is secreted (VAN KERKHOVE *et al.*, 1989). If no Na^+ is present but the K^+ concentration is high, fluid secretion reaches a high rate. So fluid secretion is very sensitive to the K^+ concentration in the bathing medium. No Na^+ needs to be present to sustain the fluid secretion, at least in high K^+ conditions.

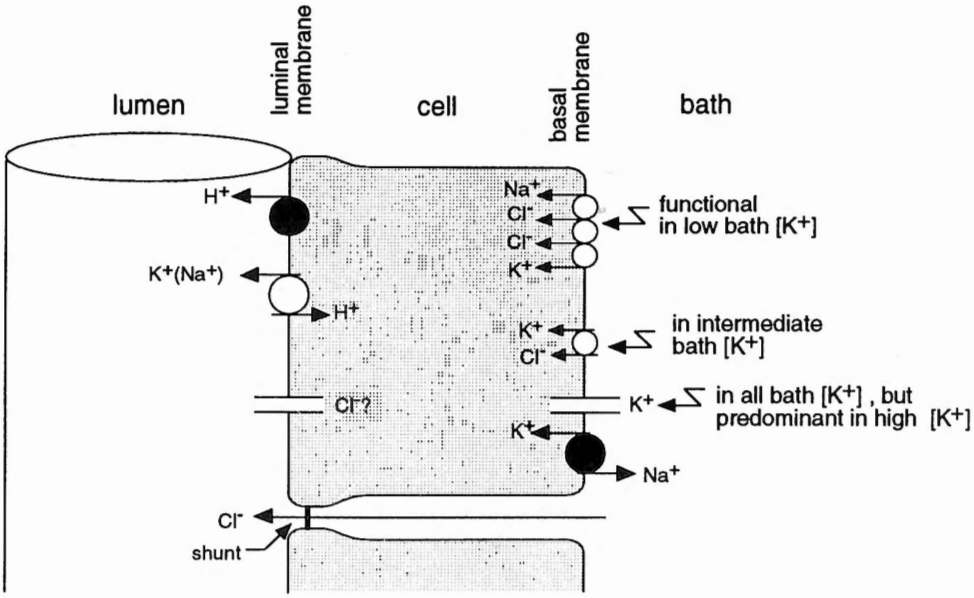

Fig. 1. — Overview of the transport mechanisms that may play a role in the salt secretion by the Malpighian tubules of *Formica* in unstimulated conditions. The relative importance of the Na/K/2Cl and the K/Cl cotransporter for basal K^+ uptake decreases with increasing bath K^+ concentration. In the presence of a high K^+ concentration uptake takes place primarily through the conductive K^+ channel. A Na/K pump was found with immunocytochemical techniques, but does not necessarily play a role in KCl secretion. A V-type H^+ATPase creates a proton concentration gradient across the luminal membrane. This gradient is sufficiently high to drive K^+ extrusion via an electroneutral H^+/K^+ antiporter. The pathway for Cl^- is still controversial.

Electrochemical gradients for K^+ and Cl^- across the epithelium and across the basal and luminal cell membrane

Data from LEYSSENS *et al.* (1993a) and DIJKSTRA (1993) allow us to show the profile of the electrochemical gradients for the two ions that are preferentially transported by the Malpighian tubule of *Formica* (see Fig. 2). The data show that both K^+ and Cl^- are concentrated in the lumen. K^+ however is secreted not only against a concentration but also against an electrical gradient, the lumen being positive with respect to the bath. So K^+ transport is active and must be transcellular. Cl^- on the other hand is transported against a concentration gradient representing an opposing force of about 12 mV. But the luminal positive potential of 39 mV is large enough to attract Cl^- into the lumen against its concentration gradient (total force being 39 mV − 12 mV = 27 mV).

When estimating the electrochemical gradients across the basal membrane (bath side) K^+ seems to be passively distributed, the concentration and the electrical potential force exactly opposing each other. The large active step for K^+ resides

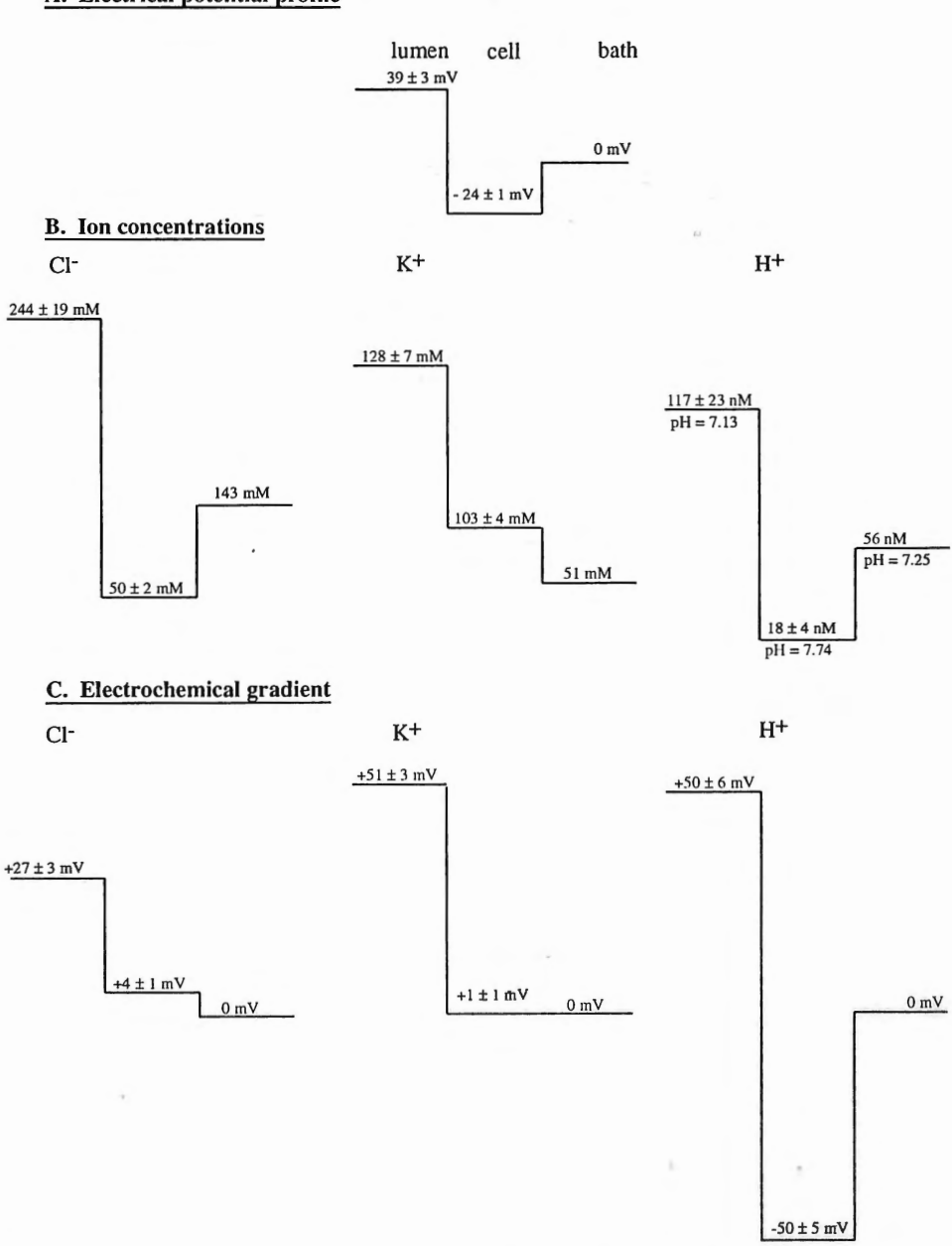

Fig. 2. — Profiles across the Malpighian tubule of *Formica* in the presence of 51 mM K^+, 63 mM Na^+, 143 mM Cl^- and pH 7.2. Summary of data from LEYSSENS *et al.* (1993a and b) and DIJKSTRA (1993). The data for K^+, Cl^- and H^+ were obtained from different series of tubules. Mean values ± SE (n = 6 to 63).

almost completely in the luminal membrane. The mechanism for this K^+ extrusion will be discussed below. The thermodynamic electrochemical gradient for Cl^- movement is in the direction of secretion both across the basal (4 mV) and the luminal membrane (23 mV).

K^+ uptake mechanisms across the basal membrane

K^+ channels

The basal membrane (bath side) has a high K^+ selectivity and a very low resistance ($4\Omega.cm^2$, see WELTENS *et al.*, 1992; LEYSSENS *et al.*, 1992; DIJKSTRA *et al.*, 1994a) and, as shown above, the electrochemical gradient for K^+ is very small. If K^+ was indeed distributed passively across the basal membrane, no net passive movement of K^+ would occur across this membrane. It is possible however that the conductance of this membrane is so high that an inward electrochemical driving force of 1 mV is large enough to drive sufficient K^+ into the cell to sustain the observed net K^+ transport (LEYSSENS *et al.*, 1993a). Net K^+ secretion in control conditions is calculated to be $0.2\ mA/cm^2$. If all the K^+ uptake across the basal membrane (bl) was via the conductive K^+ channels with conductance, g_{K+}, we can write :

$$\text{Net } K^+ \text{ secretion} = I^{bl}_{K+} = g^{bl}_{K+}.\ (\text{electrochemical } K^+ \text{ gradient in mV})$$
$$= 1/R^{bl}_{K+}.\ (\text{electrochemical } K^+ \text{ gradient in mV})$$
$$= 0.2\ mA/cm^2 = (1/5\Omega.cm^2).1mV$$

The value for the basal membrane resistance derived from the data was $4\Omega.cm^2$. When the resistance is that low an electrochemical gradient of 1 mV would indeed be large enough to explain the total K^+ uptake necessary to sustain the transepithelial K^+ secretion.

(A) Electrical potential differences across the epithelium (Vte), *i.e.* lumen with respect to the bath, and across the basal membrane (V_{bl}), *i.e.* cell with respect to bath. The electrical potential difference across the luminal membrane can be derived as follows : $V_{lu} = -[V_{te} - V_{bl}] = -[39\ mV - (-24\ mV)] = -63$ mV (cell negative with respect to the lumen).
(B) Ion concentrations : Cl^- and K^+ in mM and H^+ in nM. The ratio of the luminal over intracellular H^+ concentration is large enough (6.5) to drive K^+ extrusion across the luminal membrane against its concentration gradient (ratio for $K^+ = 1.2$).
(C) Electrochemical gradient across the epithelium (V^{te}) and across the basal membrane (μ_{bl}) for Cl^-, K^+ and H^+ in mV. The electrochemical gradient (μ) for an ion (i) is calculated as [the electrical potential difference (see A) across the barrier minus the equilibrium potential for the ion across the same barrier]. The equilibrium potential is calculated from the ion concentrations (see B). The electrochemical gradient (μ) for ion (i) transfer from cell to lumen across the luminal membrane is $[\mu_{te}i - \mu_{bl}i]$. It can be seen that the large active step for K^+ secretion (about 50 mV) is situated entirely across the luminal membrane. The electrochemical gradient across the basal membrane is not statistically different from zero. Driving forces for Cl^- movement are in the direction of secretion across all barriers. Protons could passively enter the cells from the bath side, if a H^+ channel or carrier was present. They are actively pumped out of the cell into the lumen against both a concentration and an electrical gradient.

TABLE 1

Effect of drugs or ion composition of the bathing solution on fluid secretion rate in isolated Malpighian tubules of *Formica**

Effect of	Bath K^+ and Na^+ concentration			mechanism
	5 or 10 mM K^+ 108 or 113 mM Na^+	51 mM K^+ 62 mM Na^+	113 mM K^+ Na^+ free	
Fluid secretion in control solution	slow	intermediate	fast	
$Ba2^+$ (6 mM)	↓↓	↓↓	↓↓	K^+ channels
Omission of Na^+	↓	no effect	no effect[a]	K/Cl or Na/K/2Cl cotransporter
Cl^- substituted by Br^-	↓	↑		
Bumetanide 10^{-5} M	↓	no effect		
10^{-4}.M	↓↓	↓	no effect	
Quabain 10^{-3} M	no effect			Na/K pump
Schering 28080 10^{-4} M	no effect	no effect		K/H pump
Vanadate 10^{-3} M		↓↓	↓↓[b]	Na/K pump or H^+ pump
Bafilomycin A1 5.10^{-6} M		↓↓		H^+ pump
NEM** 5.10^{-4} M		stops		H^+ pump

* *Summary of results from* WELTENS *et al.* (1992), DIJKSTRA (1993), LEYSSENS (1993) and LEYSSENS *et al.* (submitted). Symbols : moderate (↓), severe (↓↓) or complete (stops) inhibition of fluid secretion ; moderate (↑) stimulation or no change (no effect) in secretion.
** NEM : N-ethyl-maleimide.
[a] Omission of 3.5 mM Na^+ (*i.e.* by titrating pH of solution with KOH instead of NaOH) did not result in a change in fluid secretion rate.
[b] Note that vanadate inhibits fluid secretion in the absence of Na^+.

Such a gradient is very difficult to detect as it is within the technical limitations of the experimental methods (see Fig. 2 : the 'measured' gradient was of the order

of 1 mV and not significantly different from zero). The fact however that fluid secretion was drastically slowed down (see Table 1) and the basal membrane hyperpolarized in the presence of Ba^{2+}, a well known K^+ channel blocker, led us to believe that the blocked K^+ movement was an inward K^+ current, providing at least part of the K^+ ions needed to sustain the K^+ extrusion across the luminal membrane (LEYSSENS *et al.*, 1993a, WELTENS *et al.*, 1992).

Electroneutral K/Cl and K/Na/2Cl cotransport

K^+ channels seem to be the main pathway for K^+ uptake in a high K^+ concentration in the bath. Bumetanide, a blocker of the K/Cl or Na/K/Cl cotransporters, had no effect on fluid secretion in a high bath K^+ concentration (see Table 1). When K^+ is lowered (and Na^+ raised) however cation/anion cotransporters seem to gain in relative importance. In these media the fluid secretion is much slower, but part of the K^+ uptake is now realized by the mentioned cotransporters. In low bath K^+ the cell inward electrochemical gradient for K^+ decreases (LEYSSENS *et al.*,1993a) and clearly, less K^+ is available for uptake through channels. The necessary concentration gradients are present on the other hand for the cotransporters to be functional : experiments measuring the intracellular Cl^- and K^+ concentration showed that in an intermediate bath K^+ concentration (51 mM) the cell inward gradient for Cl^- is large enough to drive K^+ uptake via a K/Cl cotransporter, in 5 or 10 mM K^+ the Na^+ inward gradient is needed to drive a K/Na/2Cl cotransporter (LEYSSENS *et al.*, 1993a ; DIJKSTRA, 1993). Experiments using specific blockers (bumetanide) or ion substitutions (omission of Na^+ or substition of Cl^- by Br-) corroborate the above hypothesis (see Table 1 and DIJKSTRA, 1993 ; LEYSSENS *et al.*, 1994) : omission of Na^+ from the bathing solution (replaced by N-methyl-D-glucamine) caused a partial inhibition of fluid secretion, but only in a low K^+ concentration (10 mM), not in 51 mM. In other tissues a concentration of 10^{-5} M bumetanide is enough to block the Na/K/2Cl cotransporter, a higher dose is necessary before the K/Cl cotransporter is affected (see ELLORY and HALL, 1988 ; PALFREY and O'DONNELL, 1992). A concentration of 10^{-5} M partially blocked fluid secretion of the Malpighian tubule in 5 mM bath K^+, but not in 51 mM. In the latter K^+ concentration a dose of 10^{-4} M bumetanide was needed before a partial inhibition was observed (see Table 1). Also Br^-, that is known to inhibit the Na/K/2Cl but to stimulate the K/Cl cotransporter (ELLORY and HALL, 1988), had opposite effects on fluid secretion in 10 mM K^+ (inhibition) and in 51 mM K^+ (stimulation) (Table 1). The evidence strongly suggests that part of the K^+ uptake occurs via a K/Cl cotransporter in 51 mM K^+ or via a Na/K/2Cl cotransporter in a lower K^+ concentration (5 to 10 mM) in the bath.

Active pumps

No conclusive physiological evidence was found for a role of a Na/K ATPase (no effect of ouabain) or a K/H ATPase (no effect of Schering compound 28080) in sustaining the fluid secretion of the tubules of *Formica* (Table 1 ; LEYSSENS *et al.*, 1994). Immunocytochemical techniques however revealed that a Na/K pump may

be present in the basal membrane of *Formica* Malpighian tubules (GARAYOA, personal communication).

Also a high dose of vanadate, a blocker of P-type ATPases like the Na/K pump, inhibited fluid secretion (see Table 1). An effect on a Na/K pump can not be excluded (DIJKSTRA *et al.*, 1994b). But vanadate also inhibited fluid secretion in the absence of Na (see Table 1) and it depolarized the luminal membrane. It is possible therefore that the luminal H^+ pump is affected by vanadate (LEYSSENS *et al.*, submitted) as was shown in yeast cells by BELTRÁN and NELSON (1992).

K^+ extrusion across the luminal membrane, the role of luminal H^+ turnover

For a long time the K^+ extrusion was thought to be the result of an electrogenic K^+ ATPase, hypothesized by HARVEY and NEDERGAARD in 1964 in the midgut of a silkworm. In 1986 however WIECZOREK and coworkers found that the luminal membrane of the goblet cells of the *Manduca sexta* midgut contained a vacuolar type H^+ ATPase. The same group (WIECZOREK *et al.*, 1989 and 1991) presented evidence that the K^+ extrusion is the result of an exchange of cellular K^+ for luminal H^+. Depending on the function of the epithelium and the stoichiometry of the antiporter the H^+ ATPase needs to build up either a large electrical potential difference to extrude K^+ (if one K^+ is exchanged for two H^+ as in *Manduca sexta* for instance, AZUMA and WIECZOREK, 1993), or a large H^+ concentration gradient (if the antiporter is electroneutral).

Up to now data obtained for the *Formica* Malpighian tubule all indicate that a vacuolar type H^+ ATPase may be present. Evidence was presented by WELTENS *et al.* (1992) and DIJKSTRA *et al.* (1994b) : bafylomycin A1 and NEM (N-ethylmaleimide) both known to block the V-type H^+ ATPase inhibited fluid secretion and depolarized the luminal membrane of the tubule cell. ZHANG *et al.* (1994) found that an appreciable proton concentration gradient is built up across the luminal membrane (see also Fig. 2). Also DIJKSTRA *et al.* (1994b) showed that acidifying the lumen of luminally perfused tubules or applying bafilomycin A1 blocked the active equivalent short circuit current.

The H^+/K^+ exchanger in parallel with the proton pump is probably an electroneutral one for one exchanger in *Formica* tubules : the luminal proton concentration gradient is always cell inward and larger than the K^+ gradient in conditions where the tubule secretes fluid (Fig. 2 ; ZHANG *et al.*, 1994) ; when fluid secretion is slowed down (in low bath K^+ concentration for instance or in the presence of Ba^{2+}) or when it is completely blocked by DNP the ratio of the proton over the K^+ concentration gradient reaches a value close to one (ZHANG *et al.*, 1994 ; Leyssens *et al.*, 1993b).

Intrinsic regulation of K^+ secretion by K^+. Cross talk between the basal and luminal membranes

In an epithelium in steady state the quantity of ions or other substances transferred across the basal and luminal membranes must always be closely matched. Otherwise the cells would accumulate or lose material and swell or shrink.

In Malpighian tubules of *Formica* the rate of transport increases with the bath K^+ concentration : more K^+ is taken up across the basal membrane per unit time, more K^+ must therefore leave the cell across the luminal membrane via the H^+/K^+ antiporter. Factors determining the rate of turnover of the H^+/K^+ exchanger are : (1) the rate at which the protons can be recycled by the active electrogenic proton pump (see Fig. 1), (2) the affinities of H^+ and K^+ for the exchanger and (3) the relative ratios of luminal over intracellular $[H^+]$ and $[K^{++}]$: $\{[H^+]_{lu}/[H^+]_{cell}\}/\{[K^+]_{lu}/[K^+]_{cell}\}$. The latter condition holds if the exchanger is electroneutral as is probably the case in *Formica* Malpighian tubules.

The rate of recycling of H^+ by the electrogenic proton pump will depend on the luminal electrical potential difference (V_{lu}) against which the pump must transfer the protons into the lumen. The electrophysiological properties of the *Formica* Malpighian tubules provide an intrinsic regulation of V_{lu} : the luminal membrane has a much higher resistance than the basal membrane (about 50 times, WELTENS *et al.*, 1992 ; DIJKSTRA *et al.*, 1994a). This means that the electromotive forces that determine the basal membrane potential (V_{bl}), *i.e.* mainly the K^+ diffusion potential in the case of the *Formica* Malpighian tubule (LEYSSENS *et al.*, 1992), will be 'imposed' on the luminal membrane (for theoretical considerations, see WELTENS *et al.*, 1992). As a consequence, when bath K^+ is raised, V_{bl} depolarizes, reducing at the same time V_{lu}. So the electrical gradient against which the H^+ pump must secrete the protons decreases and proton cycling can occur at a much faster rate.

A second phenomenon is that, the intracellular K^+ concentration of the cells increases and reaches a new steady state value when the bath K^+ is raised (LEYSSENS *et al.*, 1993a). Total cation concentration in the cell will remain constant, but K^+ is presumably replacing Na^+. Clearly, if $[K^+]_{cell}$ increases the turnover of the H^+/K^+ exchanger will be facilitated.

The pathway for passive Cl^- secretion

This pathway is still controversial. It may be transcellular (*i.e.* across both cell membranes) and/or paracellular (*i.e.* across the shunt). In *Formica* Malpighian tubule only one cell type is present (GARAYOA *et al.*, 1992). So a cell type specialized in Cl^- transport as in frog skin for instance does not seem likely in these tubules.

A rough estimate can be made of the relative importance of the transcellular or shunt pathways. If chloride travels across the epithelium via the cellular pathway, a basal carrier transport for chloride has to be present since no evidence was found for an appreciable Cl^- conductance across this membrane (LEYSSENS *et al.*, 1992). Experimental results showed that K/Cl and Na/K/Cl cotransporters may be present (see Fig. 1 and Table 1), but from the data it is clear that these cotransporters

sustain but part of the fluid secretion and that they function only in the presence of an intermediate or low bath K^+ concentration. At the luminal side a Cl^- channel could be present as is the case in many NaCl and KCl transporting epithelia. As explained above the movement (I) of ion (i) across a conductive membrane depends (1) on the electrical potential difference (V) across the barrier, (2) on the concentration gradient, expressed as the equilibrium or Nernst potential, E_i, and (3) on the specific conductance of the barrier for the ion, g_i. $I_i = g_i . (V - E_i)$.

We expect the Cl^- conductance (g_{Cl}) to be low since the total conductance of the luminal membrane is low (50 times lower than the basal membrane conductance, WELTENS *et al.*, 1992 ; DIJKSTRA *et al.*, 1994a). Therefore the driving force ($V - E_{Cl}$) should be rather large to obtain an appreciable Cl^- current across this membrane.

In low bath K^+ fluid secretion is slow (see Table 1), but V_{lu} is relatively high (LEYSSENS *et al.*, 1992). As stated above in low bath K^+ the relative importance of Cl^- uptake into the cell via the cation/anion cotransporters increases too. So part of the Cl^- secretion may pass through the cell. V_{te}, *i.e.* the driving force for Cl^- across the shunt on the other hand is small, this means that the contribution of the shunt in Cl^- transfer may decrease.

In high bath K^+ the opposite is true. V_{lu} decreases and V_{te} increases : Cl^- movement across the shunt seems to be favoured.

A detailed investigation of the specific Cl^- conductances and driving forces across all barriers is needed, before a final conclusion can be drawn, but the data seem to suggest that in high K^+, when secretion is fast, most of the Cl^- movement will be across the shunt. K^+ uptake across the basal membrane will be via the K^+ channel, independent of Cl^- and Cl^- uptake by the cells may be small. When bath K^+ is lowered and secretion slows down, some Cl^- (together with part of the K^+) may be taken up by the cell via the cation/anion cotransporters. This amount of Cl^- may leave the cell through a Cl^- channel in the luminal membrane be it at a relatively slow rate (due to the low Cl^-conductance), but taking advantage of the large electrical driving force (V_{lu}) present in low K^+ conditions. To complete the picture the E_{Cl} in all K^+ concentrations should be known as well.

Cl^- is far from equilibrium both across the shunt and across the luminal membrane (see Fig. 2 in the presence of 51 mM K^-). Therefore Cl^- may be a rate limiting step in the salt transport and a site for regulation. When fluid secretion was increased by the second messenger cAMP, it was found that the basolateral Na/K/2Cl cotransporter is stimulated (DE DECKER, 1993), accelerating Cl^- entry into the cell. In these circumstances the luminal (and transepithelial) electrical potential difference approaches the Cl^- equilibrium potential (DIJKSTRA, 1993), suggesting that the conductance for Cl^- (luminal and shunt ?) may have increased. More data are required however before it can be decided how much of this Cl^- is passing either through the shunt or through the cell.

BRIEF COMPARISON WITH OTHER SPECIES

The high K^+ dependence of fluid secretion of *Formica* Malpighian tubules is in sharp contrast with for instance tubules of the tsetse fly, *Glossina morsitans*, where fluid secretion is primarily dependent on $Na+$, although a minimal amount of K^+ needs to be present in a high Na^+ medium in order to keep the fluid secretion going at a high rate (GEE, 1976). In the ant only in low K^+ high Na^+ medium Na^+ becomes of importance in sustaining part of the fluid secretion (LEYSSENS *et al.*, unpublished). In *Musca* for instance a minimal amount of Na^+ is needed to maintain the fast fluid secretion rate in high K^+. Table 2 gives an overview of possible patterns of dependence on K^+ and Na^+ in a few species.

TABLE 2

Fluid secretion rate by isolated Malpighian tubules as a function of the Na^+ and K^+ concentration in the bath.

Species	Composition of bathing solution				References
	K^+ free high Na^+	low K^+ high Na^+	high K^+ low Na^+	high K^+ Na^+ free	
Formica	0	+	+++	+++	VAN KERKHOVE *et al.* (1989)
Carausius	0	+	+++	+++	PILCHER (1970)
Teleogryllus	§	+	+++	+++	MARSHALL *et al.* (1993)
Pieres	+	+	+++	+++	NICOLSON (1976)
Musca	(+)	++	+++	(+)	DALTON and WINDMILL (1980)
Glossina	0	+++	+	0	GEE (1976)
Libellula	+++	+++	+	0	NICHOLLS (1985)

Symbols : +++ (= high secretion rate), ++ or + (= intermediate), (+) (=low) and 0 (= secretion stops).
§ Data not available.

One explanation for the observed differences is that the importance of K^+ versus Na^+ channels may vary. Na^+ channels for instance are more important in the mosquito especially when the tubule is stimulated (see SAWYER and BEYENBACH, 1985). This makes sense in an animal that has to be able to get rid of a high NaCl load after the female mosquito has taken a blood meal. The animal is primarily interested in ingesting the blood proteins in order to prepare its eggs. The NaCl and water that are taken in at the same time have to be excreted as fast as possible to allow the animal to lose the extra weight that prevents it from flying away and make it an easy prey for predators.

The relative role played by the cation/anion cotransporters may also be different. Inhibitors of the anion/cation cotransporters (furosemide, bumetanide) were

reported to reduce fluid secretion in *Rhodnius*, (O' DONNELL and MADDRELL, 1984), in *Aedes*, at least in stimulated conditions (HEGARTY *et al.*, 1991), in *Locusta* (BALDRICK *et al.*, 1988) in *Glossina* (ISAACSON and NICOLSON, unpublished, cited in NICOLSON, 1993) and in *Drosophila* (WESSING *et al.*, 1986). It would be interesting to make a comparative study of the kinetics and ion affinities of these systems in the different species.

A role for a Na/K pump is mostly studied using cardiac glycosides, well known inhibitors of this pump in vertebrate tissue. Insects may have developed resistance against this plant alkaloid (Malpighian tubules of some species can even secrete it, MEREDITH *et al.*, 1984) and the effect of ouabain or similar compounds on fluid secretion of Malpighian tubules is variable (ANSTEE and BOWLER, 1979). In *Rhodnius* ouabain even stimulated fluid secretion, possibly because the cells become rich in Na^+ (MADDRELL and OVERTON, 1988).

Evidence for the presence of a proton pump in the Malpighian tubule of insects was first obtained by BERTRAM *et al.* (1991), when they showed that bafilomycin A1 stopped fluid secretion in tubules of *Drosophila*. ISAACSON and NICOLSON (unpublished, cited in NICOLSON, 1993) for *Onymacris* and *Glossina* and PANNABECKER and BEYENBACH (1993) for *Aedes* observed the same phenomenon.

Preferential secretion of either a K^+ or a Na^+ rich fluid may be due to differences in the affinity of the luminal proton/cation exchanger for either K^+ or Na^+. MADDRELL and O'DONNELL (1993) controlled the ion composition of the intracellular milieu using gramicidin that inserts into the basal membrane as non-selective cation permeable channel. They showed that in the blood sucking insect, *Rhodnius*, the tubules secrete a Na^+ rich fluid even when the cellular milieu contains equal concentrations of K^+ and Na^+. The system [proton pump plus proton/cation antiporter] clearly shows a preference for Na^+ over K^+.

Inhibition of fluid secretion by amiloride (reviewed by NICOLSON, 1993), even in the absence of Na^+ (LEYSSENS and ZHANG, unpublished results) is in favour of the presence of a luminal proton/cation antiporter.

In conclusion Malpighian tubules of different species seem to possess similar tools to transport KCl and/or NaCl, i.e. ion channels, cation/anion cotransporters, a Na/K pump in the basal membrane on the one hand, a proton pump and proton/cation antiporters (and Cl^- channels ?) in the luminal membrane on the other. Differences in the kinetics and in the relative importance (*e.g.* silent or active in control versus stimulated conditions) of these systems may allow the tubule to perform its function according to the specific needs of each species.

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