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Ostracoda in the Earth and Life Sciences

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Earth sciences Statistics	
Infraspecific variability in shape in Neobuntonia airella: An exposition of geometric morphometry R.A. Reyment & F.L. Bookstein	291
Cobb Mountain spike of the Kuroshio Current detected by Ostracoda in the lower Omma Formation (Early Pleistocene), Kanazawa City, central Japan: Analysis of depositional environments K. Ishizaki, T. Irizuki & O. Sasaki	315
Analysis of morphological changes through ontogeny: Genera Baffinicythere and Elofsonella (Hemicytherinae) Toshiaki Irizuki & Osamu Sasaki	335
Life sciences Genetics	
Clonal diversity in parthenogenetic ostracodes J.E.Havel & P.D.N.Hebert	353
Genetic variability in parthenogenetic populations of <i>Heterocypris incongruens</i> (Crustacea, Ostracoda) Valeria Rossi, P.Giordano & P.Menozzi	369
Life sciences Microchemistry	
Salinity tolerance, morphology and physiology of the osmoregulatory organ in Ostracoda with special reference to Ostracoda from the Aral Sea N.V.Aladin	387
Chemical composition of Leptocythere psammophila carapaces (Ostracoda, Crustacea) Dietmar Keyser, Patricia Behrens, Manfred Niecke & Anne Marie Bodergat	405
Life sciences Speleology	
Podocopid Ostracoda from freshwater caves of Australia and New Zealand R.F.Maddocks & T.M.Iliffe	421
Anchialine podocopid Ostracoda of New Caledonia R.F.Maddocks, T.M.Iliffe & S.Sarbu	439
Life sciences Limnology/palaeolimnology	
Ostracoda (Crustacea) from eutrophic and oligotrophic maar lakes of the Eifel (Germany) in the Late and Post Glacial Burkhard W.Scharf	453
Benthic ostracods in the pre-alpine deep lake Mondsee: Notes on their origin and distribution Dan L. Danielopol, Mathias Handl & Yu Yin	465

Salinity tolerance, morphology and physiology of the osmoregulatory organ in Ostracoda with special reference to Ostracoda from the Aral Sea

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ABSTRACT: All Ostracoda that inhabit continental inland waters are capable of osmore-gulation. Hyperosmotic regulation is present in freshwater Ostracoda, hypoosmotic regulation in hyperhaline Ostracoda, and the combination of hyperosmotic (at salinities below 8 ppt) and hypoosmotic (at salinities over 8 ppt) in brackish water Ostracoda. The hyperosmotic regulation in Ostracoda is determined mainly by the amount of salts consumed with the food. Hypoosmotic regulation in Ostracoda is determined mainly by excretion of salts in special cells located in the non-calcified zone of the inner shell layer.

The Aral Sea, formerly the world's fourth largest lake in area, is disappearing. Over the last 30 years its level has dropped 15 m and its salinity has increased from 8-10 ppt to 30 ppt, because more than 60% of the Aral Sea by volume has been desiccated. Regression has resulted from reduced inflow caused primarily by withdrawals of water from two large rivers due to irrigation. At present only one species of Ostracoda inhabits the Aral Sea instead of the 11 species that were previously found. The change is consistent with osmoregulation capacities of the Ostracoda concerned.

KEYWORDS: Recent Ostracoda, salinity tolerance, osmoregulation organs, Aral Sea.

1 INTRODUCTION

Very little is known about salinity tolerance and osmoregulation organs in Ostracoda. The majority of ostracod species inhabit the oceans and continental fresh and brackish water lakes; some other species live in highly mineralised continental water bodies. The present study focuses on salinity tolerance, morphology and physiology of the osmoregulation organs in Ostracoda, with special reference to Ostracoda from the Aral Sea. It forms part of a more extensive study covering various aspects of the osmoregulatory biology of the Ostracoda and summarises some previously published papers (Aladin 1983a,b, 1984a,b, 1985, 1986a-c, 1987a-c, 1988a-c, 1989a-c; Tones 1983; Aladin & Schornikov 1986a,b).

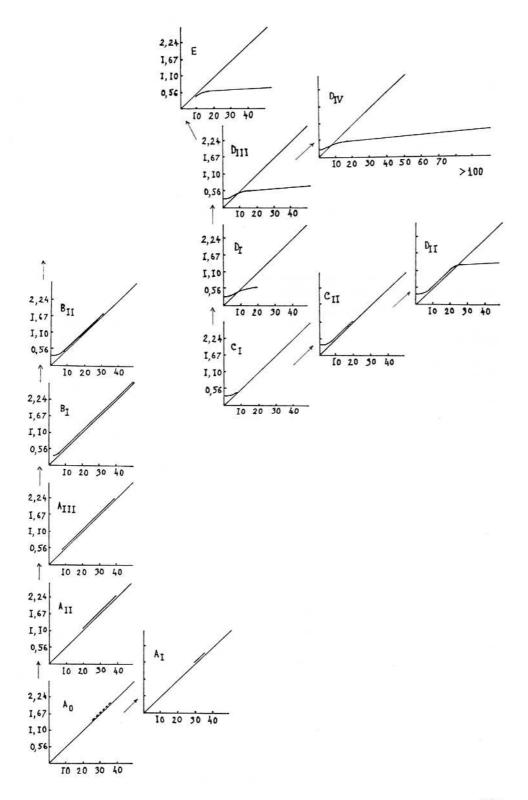
2 MATERIAL AND METHODS

The animals and water used for the experiments were collected from the following seas: Barents, White, Baltic, Black, Azov and Japanese; from big salt lakes: Caspian and Aral;

and from freshwater lakes near St Petersburg and Moscow, and the Lithuanian, Ukrainian, Azerbaijanian, Kazakh and Uzbek Republics. Some Ostracoda were raised in the laboratory from resting eggs. Dried muds containing these eggs were collected in Canada, USA, Peru, Bolivia, Argentina, Italy, India, Seychelles and Australia. The freezing point of the Ostracoda haemolymph was measured by microcryoscopical methods. For morphological studies of osmoregulation organs, both scanning and transmission electron micrographs were used. Full details on techniques of microcryoscopy and microscopy were given in an earlier paper (Aladin 1983a).

Figure I. Osmoconformity and osmoregulation in Ostracoda. Vertical axis = freezing point (depression) of haemolymph in °C; horizontal axis = water salinity in ppt; bisector line = isoosmotic line; curved line = freezing point (depression) of haemolymph. A0 = hypothetical ancestor of osmoconformers. Different types of osmoconformity and osmoregulation in Ostracoda are presented to give a plausible indication of the evolution of osmoconformity and osmoregulation functions. Arrows show the possible evolutionary connection between different types of osmoconformity and osmoregulation in Ostracoda.

Osmoconformers (AI to AIII): AI, Osmoconformers-I, stenohaline marine Ostracoda: Vargula norvegica, Euphilomedes nipponica, Boroecia borealis, Neonesidea mutsuensis, Cythere uranipponica, Cytherois sp. A, Paradoxostoma ussuricium, Paradoxostoma brunneum, Paradoxostoma sp. A, Paradoxostoma sp. B, Paracytheridea paulii, Semicytherura sp. A, Semicytherura sp. B, Hemicytherura sp. A, Loxoconcha tarda, Loxoconcha sp. A, Hirschmannia viridis, Xestoleberis sp. A, Ponthocythere japonica, Callistocythere hayamenensis, Coquimba sp. A. AII, Osmoconformers-II, marine Ostracoda: Philomedes brenda, Discoconchoecia elegans, Sclerochilus (Praesclerochilus) verecundus, Paradoxostoma sp.C, Cytheroma karadaginis, Leptocythere fabaeformis, Howeina camptocytheroidea, Hemicytherura kajiyamai, Loxoconcha uranouchiensis, Loxoconcha harimensis, Loxoconcha sp. B, Cytheromorpha acupunctata, Cytheromorpha japonica, Spinileberis quadriaculeata, Hemicythere emarginata, Urocythereis margaritifera, Robustaurila assimilis, Pontocythere subjaponica, Xestoleberis hanaii, Coquimba sp. B, Doratocythere tomokoae, Aspidoconcha sp. A. AIII, Osmoconformers-III, euryhaline marine Ostracoda: Jonesia simplex, Paradoxostoma intermedium, Paradoxostoma sp. D, Acetobulastoma hyperboreum hyperboreum, Cythere lutea, Cytherura similis, Semicytherura undata, Hemicytherura bulgarica, Microcytherura nigrescens, Loxoconcha fragilis, Loxoconcha pontica, Loxoconcha bulgarica, Loxoconcha sp. C, Bicornucythere bisanensis, Hiltermannicythere rubra, Hemicythere villosa, Cytheridea papillosa, Pontocythere bacescoi, Xestoleberis depressa, Xestoleberis decipiens, Callistocythere sp. A. Osmoregulators (BI and BII, CI and CII, DI to DIV, E). BI, Confohyperosmotics-I, widely euryhaline marine Ostracoda: Cytherois cepa, Leptocythere pellucida, Leptocythere lacertosa, Leptocythere histriana, Tanella supralittoralis, Semicytherura nigrescens, Loxoconcha impressa, Loxoconcha aestuarii, Loxoconcha elliptica, Hirschmannia viridis, Spinileberis pulchra, Xestoleberis aurantia. BII, Confohyperosmotics-II, brackish water Ostracoda of marine origin: Cyprideis torosa torosa, Tanella sp. A. CI, Hyperosmotics-I, fresh water Ostracoda: Candona marchica, Candona schweyeri, Dolerocypris fasciata, Cyclocypris ovum. CII, Hyperosmotics-II, (or secondary confohyperosmotics-I), brackish water Ostracoda of fresh water origin: Limnocythere inopinata, Limnocythere stationis, Darwinula stevensoni, Cypris decaryi, Cyprinotus edwardi, Cyprinotus salina, Heterocypris incongruens, Alboa worooa, Cyclocypris laevis, Sarscypridopsis aculeata, Cypridopsis vidua, Plesiocypridopsis newtoni. DI, Amphiosmotics-I, some Caspian and Aral brackish water Ostracoda of freshwater origin: Leptocythere bacuana, Amnicythere cymbula, Galolimnocythere aralensis, Loxoconcha lepida, Cytheromorpha fuscata, Tyrrhenocythere amnicola donetziensis. DII, Amphiosmotics-II, some Australian euryhaline Ostracoda of freshwater origin: Diacypris spinosa, Mytilocypris praenuncia. DIII, Amphiosmotics-III, terrestrial and aquatic euryhaline Ostracoda of fresh water origin: Terrestricythere ivanovae, Terrestricythere pratensis, Potamocypris steueri. DIV, Amphiosmotics-IV, widely euryhaline Ostracoda of freshwater origin: Cyprideis torosa amphiosmotica, Eucypris inflata. E, Hypoosmotics, secondary marine Ostracoda of freshwater origin: Gerdocypris complanata, Propontocypris maculata.



3 RESULTS AND DISCUSSION

109 species of Ostracoda from 20 families were studied by means of microcryoscopical methods. The results are given in Figure 1.

The Ostracoda are capable of both osmoconformity and osmoregulation. It is possible to distinguish 3 levels among the osmoconformers (Figure 1.AI, AII, AIII).

- 1. Osmoconformers-I (Figure 1.AI) are stenohaline, tolerating a salinity range approximately from 30 ppt up to 36 ppt. In this study, 21 species of osmoconformers-I were examined: Vargula norvegica, Euphilomedes nipponica, Boroecia borealis, Neonesidea mutsuensis, Cythere uranipponica, Cytherois sp. A, Paradoxostoma ussuricum, Paradoxostoma brunneum, Paradoxostoma sp. A, Paradoxostoma sp. B, Paracytheridea paulii, Semicytherura sp. A, Semicytherura sp. B, Hemicytherura sp. A, Loxoconcha tarda, Loxoconcha sp. A, Hirschmannia viridis, Xestoleberis sp. A, Pontocythere japonica, Callistocythere hayamenensis, Coquimba sp. A.
- 2. Osmoconformers-II (Figure 1.AII) for the most part are typical marine Ostracoda living in a broader salinity range, approximately from 20 ppt up to 40 ppt. In the present study 22 species of osmoconformers-II were examined: *Philomedes brenda*, *Discoconchoecia elegans*, *Sclerochilus* (*Praesclerochilus*) verecundus, *Leptocythere fabaeformis*, *Howeina camptocytheroidea*, *Hemicytherura kajiyamai*, *Loxoconcha uranouchiensis*, *Loxoconcha harimensis*, *Loxoconcha* sp. *B*, *Cytheromorpha acupunctata*, *Cytheromorpha japonica*, *Spinileberis quadriaculeata*, *Hemicythere subjaponica*, *Xestoleberis hanaii*, *Coquimba* sp. *B*, *Doratocythere tomokoae*, *Aspidoconcha* sp. A.
- 3. Osmoconformers-III (Figure 1.AIII) are euryhaline marine Ostracoda living in a yet broader range of salinities approximately from 8 ppt up to 40 ppt. For this study 21 species of osmoconformers-III were considered: Jonesia simplex, Paradoxostoma intermedium, Paradoxostoma sp. D, Acetobulastoma hyperboreum hyperboreum, Cythere lutea, Cytherura similis, Semicytherura undata, Hemicytherura bulgarica, Microcytherura nigrescens, Loxoconcha fragilis, Loxoconcha pontica, Loxoconcha bulgarica, Loxoconcha sp. C, Bicornucythere bisanensis, Hiltermannicythere rubra, Hemicythere villosa, Cytheridea papillosa, Pontocythere bacescoi, Xestoleberis depressa, Xestoleberis decipiens, Callistocythere sp. A. Broadening of salinity tolerance to cope with such a large range of salinities must be connected with developing cell resistance to damage effected by freshening as well as by hypersalinisation and also with improving the cells' capability to regulate their volume.

Among the osmoregulators it is possible to distinguish: confohyperosmotics (Figure 1.BI, BII), hyperosmotics (Figure 1.CI, CII), amphiosmotics (Figure 1.DI, DII, DIII, DIV) and hypoosmotics (Figure 1.E). Confohyperosmotics must be regarded as primitive osmoregulators because they are capable of combining osmoconformity (at high salinities) with hyperosmotic regulation (at low salinities).

1. It is possible to distinguish 2 levels among the confohyperosmotics (Figure 1.BI, BII). Confohyperosmotics-I (Figure 1.BI) are widely euryhaline marine Ostracoda living at salinity ranges from 2 ppt to 50 ppt approximately. For this study, 12 species of confohyperosmotics-I were examined: Cytherois cepa, Leptocythere pellucida, Leptocythere lacertosa, Leptocythere histriana, Tanella supralittoralis, Semicytherura nigrescens, Loxoconcha impressa, Loxoconcha aestuarii, Loxoconcha elliptica, Hirschmannia viridis, Spinileberis pulchra, Xestoleberis aurantia. Confohyperosmotics-II (Figure 1.BII) are brackish water Ostracoda of marine origin, in which hyperosmotic regulation is imposed

together with a simultaneous decrease in the isoosmoticity range limit. These Ostracoda are capable of existing normally even in fresh water. Confohyperosmotics-II demonstrate isoosmoticity at salinities from 30 ppt down to 8 ppt, more seldom to 14 ppt, whereas at lower salinities they demonstrate hyperosmoticy. In the present study, two species of confohyperosmotics-II were examined: *Cyprideis torosa torosa*, *Tanella* sp. A.

- 2. Among the hyperosmotics, it is possible to distinguish two levels (Figure 1.CI, CII). Hyperosmotics-I (Figure 1.CI) are typically freshwater Ostracoda in which the internal medium is hyperosmotic respective to the external one in the whole salinity tolerance range from freshwater up to a salinity of 8 ppt. In the present study, 4 species of hyperosmotics-I were examined: *Candona marchica*, *Candona schweyeri*, *Dolerocypris fasciata*, *Cyclocypris ovum*. Hyperosmotics-II (Figure 1.CII), or, as they might more properly be named, secondary confohyperosmotics-I, are brackish water Ostracoda of freshwater origin. They demonstrate isoosmoticity at salinities from 8 ppt up to 14 ppt, more seldom to 20 ppt; and at lower salinities hyperosmoticity of the internal medium (haemolymph) respective to the external one is obtained. In the present study, 12 species of hyperosmotics-II (or secondary confohyperosmotics-I) were examined: *Limnocythere inopinata*, *Limnocythere stationis*, *Darwinula stevensoni*, *Cypris decaryi*, *Cyprinotus edwardi*, *Cyprinotus salina*, *Heterocypris incongruens*, *Alboa worooa*, *Cyclocypris laevis*, *Cypridopsis aculeata*, *Cypridopsis vidua*, *Plesiocypridopsis newtoni*.
- 3. Among the amphiosmotics (the term is applied to Ostracoda combining hyperosmotic regulation at low salinities with hypoosmotic regulation at high ones), it is possible to distinguish four levels (Figure 1.DI, DII, DIII, DIV). Amphiosmotics-I (Figure 1.DI) are some Caspian and Aral Sea brackish water Ostracoda of freshwater origin. Their haemolymph is hyperosmotic in freshwater and at salinities up to 8 ppt but is hypoosmotic at salinities from 8 ppt up to 14-16 ppt, more seldom to 20 ppt. In the present study, 6 species of amphiosmotics-I were examined: Leptocythere bacuana, Amnicythere cymbula, Galolimnocythere aralensis, Loxoconcha lepida, Cytheromorpha fuscata, Tyrrhenocythere amnicola donetziensis. Amphiosmotics-II (Figure 1.DII) include some Australian euryhaline Ostracoda of freshwater origin. Their haemolymph is hyperosmotic in the interval from freshwater up to a salinity of 8 ppt, isoosmotic from 8 ppt up to 20-24 ppt, and hypoosmotic from 20-24 ppt up to 50 ppt approximately. In the present study, two species of amphiosmotics-II were examined: Diacypris spinosa, Mytilocypris praenuncia. Amphiosmotics-III (Figure 1.DIII) are terrestrial and aquatic euryhaline Ostracoda of freshwater origin with a haemolymph that is hyperosmotic from freshwater up to salinities of 8 ppt and hypoosmotic from 8 ppt up to 50 ppt. In the present study, three species of amphiosmotics-III were examined: Terrestricythere ivanovae, Terrestricythere pratensis, Potamocypris steueri. Amphiosmotics-IV (Figure 1. DIV) are widely euryhaline Ostracoda of freshwater origin. At this level, the capacity for hypoosmotic regulation is most pronounced. Because of this ability, most amphiosmotics-IV Ostracoda can endure salinisation up to 100 ppt hypoosmoticity, and some of them can tolerate 200 ppt and more. In the present study, two species of amphiosmotics-IV were examined: Cyprideis torosa amphiosmotica, Eucypris inflata. The amphiosmotics-IV level is the most perfect because it determines very wide euryhalinity together with the simultaneous maintenance of relative osmotic homeostasis in the ostracode metabolism.
- 4. Among the hypoosmotics, it is possible to distinguish only one level (Figure 1.E). Hypoosmotics are euryhaline secondarily marine Ostracoda of freshwater origin, in which the internal medium is hypoosmotic respective to the external one over the whole salinity

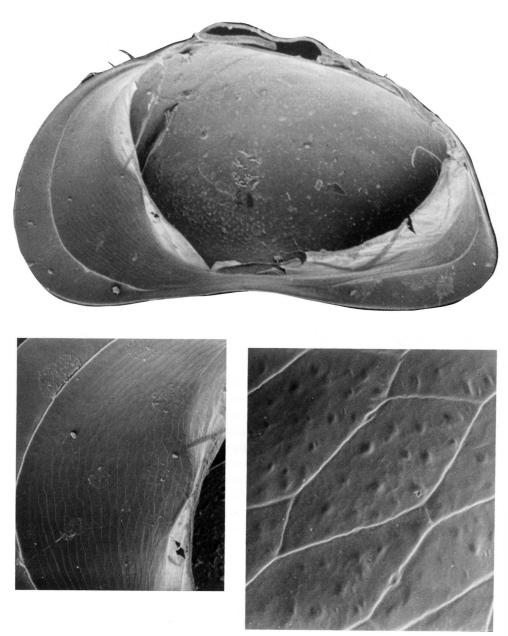


Figure 2. A-C: RV of *Mytilocypris praenuncia*, raised under laboratory conditions within the salinity interval from fresh water to slightly brackish water (<4 ppt). The LV and the body of the organism are removed in order to show the non-calcified zone of the inner shell layer. A, general internal view. B, anterior part of the non-calcified zone of the inner shell layer. C, high magnification of the anterior part of the non-calcified zone of the inner shell layer. Cells have clear borders and there are numerous holes or depressions in the cuticle.

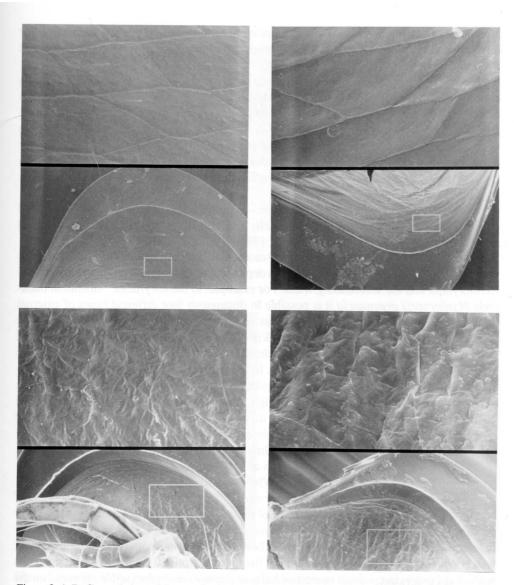


Figure 3. A-D: General view of the anterior and posterior parts of the non-calcified zone of the inner shell layer of *Mytilocypris praenuncia*, raised under laboratory conditions. Anterior view on left, posterior view on right. A-B, within the salinity interval from 4 ppt to 8 ppt, more seldom to 12 ppt. Cells have clear borders but the numerous holes or depressions in the cuticle are lacking. C-D, within the salinity interval from 8-12 ppt to 20-24 ppt. It is impossible to distinguish either holes or depressions in the cuticle or even cell borders.

tolerance range from brackish water at 8 ppt up to salinities of 50 ppt approximately. For this paper, two species of hypoosmotics were examined: *Gerdocypris complanata* and *Propontocypris maculata*.

Hyperosmotic regulation in adult Ostracoda is determined mainly by the amount of salts consumed with the food (Belyaev 1950; Aladin 1984b) and probably also by reabsorption of salts via the antennal gland. But ostracode embryos cannot feed under egg envelopes, and thus their hyperosmotic regulation is determined mainly by absorption of salts in special cells located in the non-calcified zone of the inner shell layer (Aladin 1988a). After leaving the egg envelopes the young organism can feed, and following the first postembryonal moult, the special osmoregulation cells tend to disappear, sometimes completely (Aladin 1988a).

On the other hand, hypoosmotic regulation – in both adults and embryos – is determined mainly by excretion of salts from special cells which also are located in the non-calcified zone of the inner shell layer (Aladin 1983a, 1984a, 1987a,b, 1988a-c, 1989c).

The external morphology of Ostracoda osmoregulation organ special cells, located in the non-calcified zone of the inner shell layer, is dependent on the osmotic gradient between the haemolymph and the surrounding water. For example, in the euryhaline Australian ostracode Mytilocypris praenuncia it is possible to distinguish four different types of external morphology in these cells. When organisms were raised in laboratory conditions, within the interval from freshwater to slightly brackish water (<4 ppt), cells with clear borders and numerous holes or depressions in the cuticle appeared on the surface of the non-calcified zone of the inner shell layer (Figure 2A-C). In this salinity interval, Mytilocypris praenuncia is capable of strong hyperosmotic regulation and the osmotic gradient between the haemolymph and surrounding water is high. When organisms were raised within the interval from 4 ppt to 8 ppt, more seldom to 12 ppt, cells with clear borders but without numerous holes or depressions in the cuticle appeared on the surface of the non-calcified zone of the inner shell layer (Figure 3A,B). In this salinity interval Mytilocypris praenuncia is capable of weak hyperosmotic regulation and the osmotic gradient between the haemolymph and the surrounding water is low. When organisms were raised within the interval from 8-12 ppt to 20-24 ppt neither holes or depressions in the cuticle nor even borders of cells could be distinguished on the surface of the non-calcified zone of the inner shell layer (Figure 3C,D). In this salinity interval Mytilocypris praenuncia is incapable of osmotic regulation and there is no osmotic gradient between the haemolymph and the surrounding water. When organisms were raised within the interval from 20-24 ppt to 44-48 ppt, cells with clear borders but without numerous holes or depressions in the cuticle appeared on the surface of the non-calcified zone of the inner shell layer. But new structures appeared in this salinity interval on the surface of the non-calcified zone of the inner shell layer. These structures are called caplike structures and are considered to be a type of salt gland (Figure 4A-C). In the salinity interval from 20-24 ppt to 44-48 ppt Mytilocypris praenuncia is capable of strong hypoosmotic regulation and the osmotic gradient between the haemolymph and the surrounding water is high.

The special cells located in the non-calcified zone of the inner shell layer are very much alike in their ultrastructure. All of them have a dense cytoplasm with numerous mitochondria distributed throughout a lacunar system. The cuticular site of the non-calcified zone of the inner shell layer is characterised by a high permeability to ions, in contrast to the other parts of the organism (Aladin 1983a, 1984a, 1987a, 1988a, 1989c). Recently, the ultrastructure of the special cells located in the non-calcified zone of the inner shell layer of some

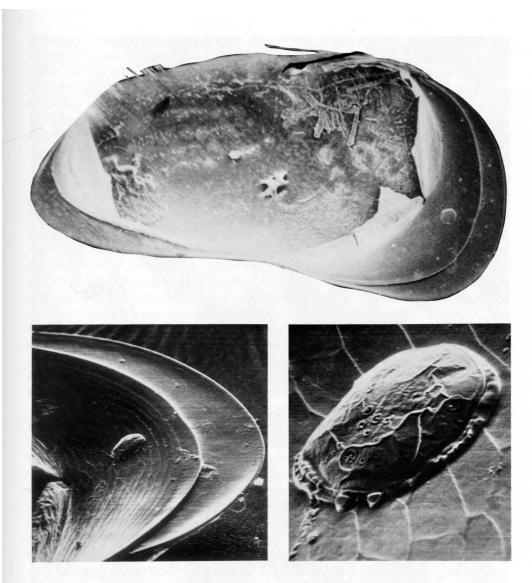


Figure 4. A-C: LV of *Mytilocypris praenuncia*, raised in laboratory conditions within the salinity interval from 20-24 ppt to 44-48 ppt. The RV and the body of the animal have been removed in order to show the non-calcified zone of the inner shell layer with caplike structures on the anterior. A, general internal view. B, anterior part of the non-calcified zone of the inner shell layer carrying a caplike structure. C, high magnification of a caplike structure on the same animal. Individual cells have well demarcated borders and show numerous holes or depressions in the cuticle; around the caplike structure is a cuticular ring.

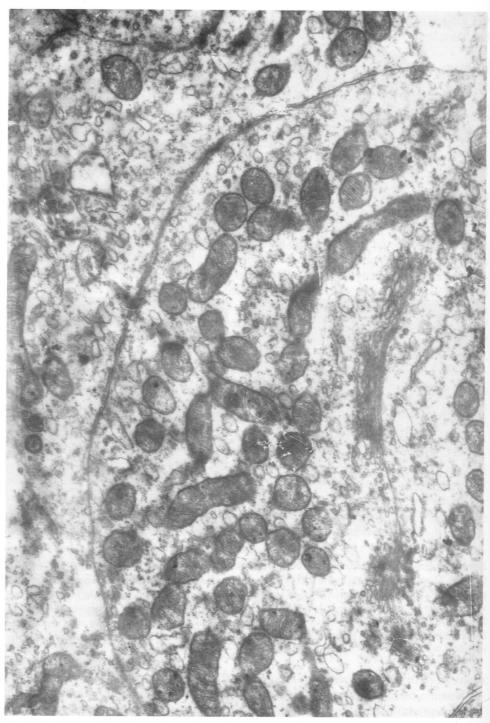


Figure 5. The basal zone of ion-transporting cells of the caplike structure of *Mytilocypris praenuncia*. Numerous mitochondria are distributed throughout a lacunar system (right-centre of field), forming part of a complex Golgi.

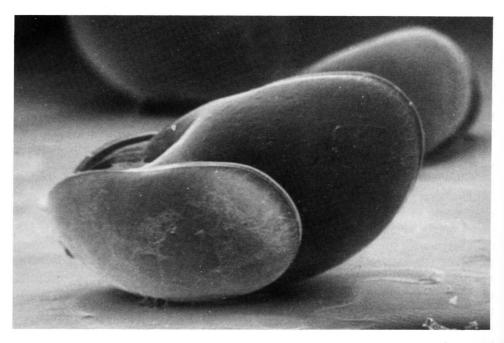
podocopid Ostracoda was studied very carefully by Keyser (1990). These cells exhibit structural features that are relevant to both ion-transporting and osmoregulation functions.

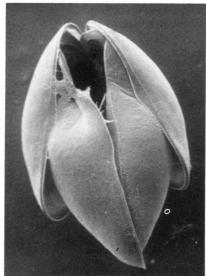
It is possible to compare the ultrastructure of the special cells that form caplike structures in Ostracoda (Figure 5) with the ultrastructure of the nuchal (neck) organ in marine Cladocera and nauplii of *Artemia salina* (Hootman & Conte 1975; Khlebovich & Aladin 1976; Potts & Durning 1980; Aladin 1982, 1991; Meurice & Goffenet 1982, 1983, 1990; Halcrow 1982). From such comparison, it appears that the caplike structures like the nuchal (neck) organs are a type of salt gland that functions to excrete salts from the haemolymph.

It is important to underline the fact that all changes in the external morphology of the special cells located in the non-calcified zone of the inner shell layer including the appearance or disappearance of the caplike structures take place only after moulting (Aladin 1987c, 1988a, 1989c). In the case of Ostracoda, for example, all physiological changes from one to another different levels of osmoconformity or types of osmoregulation (hyperosmotic, amphiosmotic, hypoosmotic) and any resulting changes of osmotic gradient between the haemolymph and the ambiental water can be morphologically realised and fixed on the external surface of the non-calcified zone of the inner shell layer only after a moult. Thus, when *Mytilocypris praenuncia* or other species of Ostracoda that are capable of osmoregulation were transferred from one salinity to another in the laboratory, the osmotic concentration of their haemolymph in accordance with the osmoregulation capacities of the organisms reached new stable levels and new osmotic gradients with the surrounding water over several hours, but all associated morphological changes on the external surface of the non-calcified zone of the inner shell layer were expressed only after moulting.

It is also important to emphasise that during moulting in Ostracoda the osmotic concentration of the haemolymph usually decreases or increases. In the case of hyperosmotic regulation in freshwater or slightly brackish water conditions, the osmotic concentration of the haemolymph decreases strongly in direct proportion to the increase in organism volume increase (Figures 6A-C, 7A). In the case of hypoosmotic regulation in brackish water or seawater conditions, the osmotic concentration of the haemolymph increases strongly in directly proportional quantity to the increase in organism volume (Figure 7B). In the case of osmoconformity in brackish water or seawater conditions, the osmotic concentration of haemolymph always decreases, but only very slightly, and it is impossible to register any corresponding increase in organism volume. Of course, after moulting, the osmotic concentration of the haemolymph quickly reaches the same stable level that was achieved before moulting. The duration of this stabilisation is in agreement with the osmotic difference between the haemolymphs before and after moulting. The shortest time taken for this osmotic stabilisation in the laboratory was less than 2 hours and the longest was 26 hours.

In the present study, a very complicated manner of osmotic regulation was registered in the ectoparasitic ostracode *Acetabulastoma hyperboreum hyperboreum*. It was shown that this species is an osmoconformer-III (Figure 1.AIII), but only when not in contact with its host amphipod *Gammarus oceanicus* (Aladin 1986a). When *Acetobulastoma hyperboreum hyperboreum* was in contact with the host the osmotic concentration of the haemolymph was changed as if the ectoparasite was confohyperosmotic (Figure 1.B). Thus, the salinity tolerance range of this ostracode was wider when it was in contact with *Gammarus oceanicus* from less than 4 ppt to more than 32 ppt, but when not in contact with the host it ranged only from 16 ppt to 32 ppt. After a study of the anatomy of *Acetobulastoma*





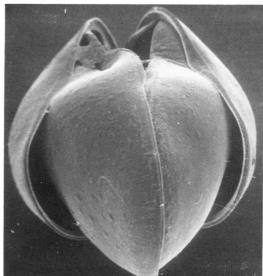
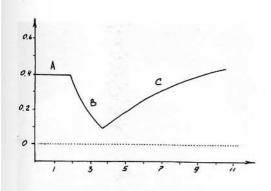


Figure 6. A-C: Moulting of *Heterocypris incongruens* that was raised under laboratory conditions within the salinity interval from freshwater to slightly brackish water (<4 ppt). A, lateral view. B, dorsal view. C, posterior view.



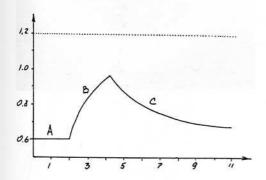


Figure 7. A-B: Changes of freezing point (depression) of haemolymph before, during and after moulting. Vertical axis = freezing point (depression) of haemolymph in °C; horizontal axis = time in hours; dotted line = freezing point (depression) of surrounding water; curved line = freezing point (depression) of haemolymph. A, freshwater: before (A), during (B) and after (C) moulting. B, brackish water with a salinity of 22 ppt: before (A), during (B) and after (C) moulting.

hyperboreum hyperboreum, it seems that the three trough-like holes on the attachment disk of the haemocoelic cavity of the ostracode are in contact with the haemocoelic cavity of the host amphipod (Figure 8A, B). Gammarus oceanicus is a true confohyperosmotic-I (Mantel & Farmer 1983), thus it is possible to hypothesise that the haemolymphs of the ectoparasite and of the host are mixing, and because of this, when Acetobulastoma hyperboreum hyperboreum is in contact with the amphipod at low salinities (<8 ppt) the internal medium of the ectoparasite becomes hyperosmotic in relation to the surrounding slightly brackish water.

4 THE ARAL SEA

The Aral Sea, formerly the world's fourth largest lake in area, is disappearing. Between 1960 and 1990, its level dropped nearly 15 m, its area decreased more than 40%, and its volume decreased more than 60% (Figure 9). Regression of the water level has resulted from a steadily reduced inflow caused primarily by withdrawals of water for irrigation (Micklin 1988, 1991; Micklin & Bond 1988). Because of this desiccation of the Aral Sea,

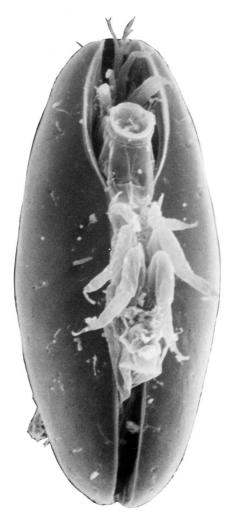




Figure 8. A-B: The ectoparasitic ostracode *Acetobulastoma hyperboreum hyperboreum*. A, general ventral view. B, attachment disc with three trough-like holes. Through these holes the haemocoelic cavity of the ectoparasite makes contact with the haemocoelic cavity of its host amphipod.

its salinity has increased over the last 30 years from 8-10 ppt to 28-30 ptt and this has led to changes in flora and fauna (Aladin 1991; Aladin & Potts 1991; Williams & Aladin 1991).

Particularly serious changes have take place in the ostracode fauna. In the early 1960s, 11 species inhabited the Aral Sea: Darwinula stevensoni, Candona marchica, Cyclocypris laevis, Plesiocypridopsis newtoni, Cyprideis torosa amphiosmotica, Amnicythere cymbula, Tyrrhenocythere amnicola donetziensis, Limnocythere (Limnocythere) dubiosa, Limnocythere (Limnocythere) inopinata, Limnocythere (Galolimnocythere) aralensis, Loxoconchissa (Loxocaspia) immodulata.

From 1974 to 1976, the average salinity of the Aral Sea increased to more than 14 ppt. Then, the Ostracoda of freshwater origin became extinct: *Darwinula stevensoni*, *Candona*

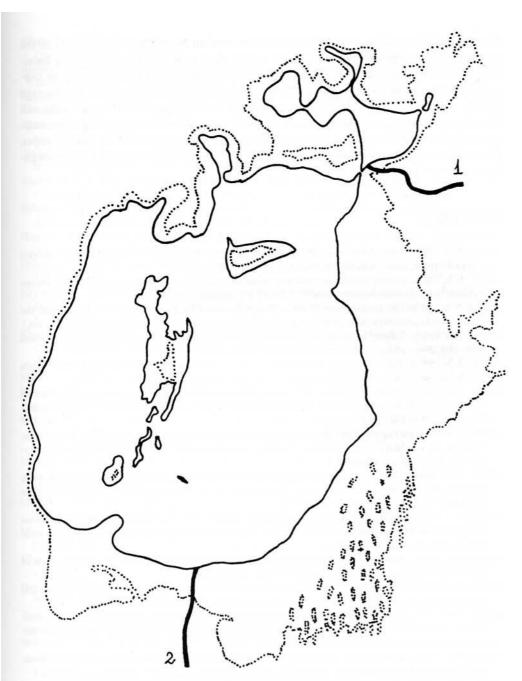


Figure 9. Schematic drawing of the Aral Sea. Dotted line = shore line in 1960; unbroken line = the shore line in 1991; 1 = Syrdarya River, 2 = Amudarya River.

marchica, Cyclocypris laevis, Plesiocypridopsis newtoni, Limnocythere (Limnocythere) dubiosa, Limnocythere (Limnocythere) inopinata, Loxoconchissa (Loxocaspia) immodulata. All these species were only capable of hyperosmotic regulation. From 1985 to 1987,

the average salinity of the Aral Sea increased to more than 24 ppt. As a result, nearly all the brackish water ostracodes became extinct: Amnicythere cymbula, Tyrrhenocythere amnicola donetziensis, Limnocythere (Galolimnocythere) aralensis. These species were capable of a very weak hypoosmotic regulation in brackish water. At present, the average salinity of the Aral Sea is 28-30 ppt, or greater. Only one ostracode now inhabits this salt lake: Cyprideis torosa amphiosmotica. This species is capable of a very strong hypoosmotic regulation in high salinities. As confirmed by the experimental data cited in this paper, the documented changes in the Aral Sea ostracode fauna are consistent with the osmoregulation capacities of these crustaceans.

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