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Salinity tolerance and morphology of the osmoregulation organs in Cladocera with special reference to Cladocera from the Aral sea

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Abstract

The hyperosmotic regulation of adult Cladocera is determined mainly by the amount of salts consumed with the food and by reabsorption of salts in cells of the nuchal (neck) organ. The hypoosmotic regulation both in adults and embryos is determined mainly by excretion of salts in special epipodite cells or in cells of the nuchal (neck) organ. The salinity of the Aral sea for the last 30 years increased from 8–10‰ to 26–28‰, which led to changes in the Cladocera fauna. At present only 4 species of Cladocera inhabit the Aral sea instead of 14 species that were previously found. These changes are in agreement with osmoregulation capacities of Cladocera.

Note added in proof: Since this paper was accepted for publication, all Cladocera have disappeared from the Aral Sea. This happened when salinity reached 30–32‰. This disappearance was predicted by and agrees with earlier laboratory experiments with Aral Sea Cladocera (Aladin, 1982b).

Introduction

Very little is known about salinity tolerance and osmoregulation organs in Cladocera. Among the Cladocera more than 40 species live in brackish water. Eight species of Cladocera inhabit the oceans of the World and 5 species live in highly mineralised continental water-bodies. The present study focusses on salinity tolerance and morphology of the osmoregulation organs in Cladocera, with special reference to Cladocera from the Aral sea. It forms part of a more extensive study covering various aspects of the osmoregulatory biology of the Cladocera and summarizes the early published papers (Khlebovich & Aladin, 1976; Aladin, 1978, 1982a, 1982b, 1982c, 1983, 1984, 1987, 1988; Aladin & Andreev, 1984; Aladin & Plotnikov, 1985; Aladin & Valdivia Villar, 1987).

Materials and methods

Animals and water used for the experiments were collected from the seas: Barents, White, Baltic, Black, Azov and Japanise; from big salt lakes: Caspian and Aral; and from fresh water lakes near Leningrad and Moscow, and in the Karelian, Lithuanian, Ukrainian, Azerbaidjanian, Kazakh, and Uzbek Republics. Some Cladocera were raised in the laboratory from resting eggs. Dried mud containing these eggs were taken in Canada, USA, Peru, Argentina, Italy, Seychelles and Australia. The freezing point of the Cladocera haemolymph was measured by mean of micro-cryoscopical methods. For morphological studies of osmoregulation organs, both scanning and transmission electron micrographs were used. All the details of microcryoscopy and microscopy are given in an early paper (Aladin, 1982a).

Results and discussion

37 species of Cladocera from 10 families were studied by means of microcryoscopical methods. The results of this study are given in Fig. 1.

The Cladocera are able to osmoregulate. The hyperosmotic regulation of haemolymph (Fig. A, A_I, A_{II}) was found in freshwater and slightly brackishwater Cladocera: *Sida crystallina*, *Diaphanosoma brachyurum*, *Daphnia magna*, *Daphnia pulex*, *Daphnia longispina*, *Ceriodaphnia reticulata*, *Ceriodaphnia cornuta*, *Simocephalus vetulus*, *Bosmina longirostris*, *Macrothrix hirsuticornis*, *Chydorus sphaericus*, *Eurycercus glacialis*, *Echinisca capensis*, *Moina brachiata*, *Moina macrocopa*, *Polyphemus pediculus*, *Polyphemus exiguus*, *Cercopagis pengoi pengoi*, *Cercopagis pengoi aralensis*, *Apaqis cylindrata*, *Bythotrephes longimanus*
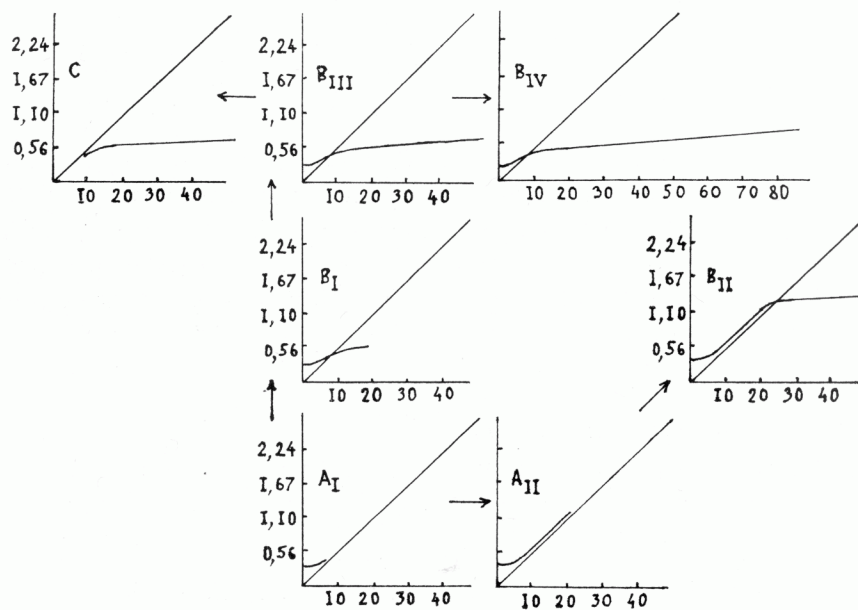

Fig. 1. Different types of osmoregulation in Cladocera.
vertical axis – freezing point (depression) of haemolymph, – °C
  horizontal axis – salinity of water, $\%_{oo}$.
  bisector line – isoosmotic line
  curve line – freezing point (depression) of haemolymph
  A – Hyperosmotic regulation of haemolymph
  A_I – Freshwater localities: *Sida crystallina, Diaphanosoma brachyurum, Daphnia magna, Daphnia pulex, Ceriodaphnia reticulata, Ceriodaphnia cornuta, Macrothrix hirsuticornis, Chydorus sphaericus, Eurycercus glacialis, Moina brachiata, Polyphemus pediculus, Bythotrephes longimanus arctica, Leptodora kindtii*.
  A_{II} – Slightly brackishwater localities: *Sida crystallina, Daphnia magna, Daphnia pulex, Daphnia longispina, Ceriodaphnia reticulata, Ceriodaphnia cornuta, Simocephalus vetulus, Bosmina longirostris, Macrothrix hirsuticornis, Chydorus sphaericus, Echinisca capensis, Moina brachiata, Moina macrocopa, Polyphemus exiguus, Cercopagis pengoi pengoi, Cercopagis pengoi aralensis, Apagis cylindrata*.
  B – Combination of hyperosmotic regulation of haemolymph (at low salinity) with hypoosmotic regulation (at high salinity)
  B_I – Brackishwater localities: *Podonevadne angusta, Cornigerius maeoticus hircus*
  B_{II} – Highly mineralised water localities in Australia: *Daphniopsis pusilla, Daphniopsis australis*
  B_{III} – Highly mineralised water localities: *Moina hutchinsoni, Evadne anonyx, Podonevadne trigona, Podonevadne camptonyx*.
  B_{IV} – Extremely high mineralised water localities: *Moina mongolica*
  C – Hypoosmotic regulation of haemolymph
  C_I – Marine localities: *Penilia avirostris, Pleopis schmackeri, Pleopis polyphemoides, Pseudevadne tergestina, Podon leuckarti, Evadne nordmanni*

arctica and *Leptodora kindtii*. The hypoosmotic regulation of haemolympha (Fig. 1, C_I) was found in the marine Cladocera: *Penilia avirostris*, *Pleopis schmackeri*, *Pleopis polyphemoides*, *Pseudevadne tergestrina*, *Podon leuckarti*, and *Evadne nordmanni*. The combination of the hyperosmotic regulation of haemolymph (at low salinity) with the hypoosmotic (at high salinity) (Fig. 1, B_I–B_{IV}) was found in Cladocera from brackish water and highly mineralised water: *Daphniopsis pusilla*, *Daphniopsis australis*, *Moina mongolica*, *Moina hutchinsoni*, *Evadne anonyx*, *Podonevadne trigona*, *Podonevadne camptonyx*, *Podonevadne angusta* and *Cornigerius maeoticus hircus*.

The hyperosmotic regulation in adults Cladocera is determined mainly by the amount of salts consumed with the food (Belyaev, 1950; Aladin, 1982c, 1984) and by reabsorption of salts in the maxillary gland (Aladin & Plotnikov, 1985). The hyperosmotic regulation in embryos of Cladocera is determined mainly by absorption of salts in cells of the nuchal (neck) organ (Aladin, 1982c,

Fig. 3. Head of older juvenile *Daphnia magna* without nuchal (neck) organ

Fig. 2. Nuchal (neck) organ on the head of the first instar of *Daphnia magna*

1 – Complicated surface of cells under nuchal (neck) organ cuticle

1988; Halcrow, 1982, 1985; Aladin & Valdivia Villar, 1987). In the brood pouch the embryos cannot feed and must have the special osmoregulation organ – nuchal (Neck) organ (Fig. 2). After leaving the brood pouch the young Cladocera can feed and after the first post-embryonal moulting, the nuchal (neck) organ has disappeared (Fig. 3).

The hypoosmotic regulation both in adults and embryos is determined mainly by excretion of salts in special epipodite cells (Fig. 4) (Aladin, 1978, 1982a, 1982c, 1983, 1988) or in cells of the nuchal (neck) organ (Fig. 5) (Khlebovich & Aladin, 1976; Potts & Durning, 1980; Aladin, 1982a, 1988; Meurice & Goffinet, 1982, 1983).

The ultrastructure of the nuchal (neck) organ cells and of the special epipodite cells are very much alike. All of them have a dense cytoplasm with numerous mitochondria distributed throughout a lacunar system (Fig. 6–7). The cuticular site of the nuchal (neck) organ cells and of special epipodite cells is characterized by a high permeability to ions, in contrast to the sur-
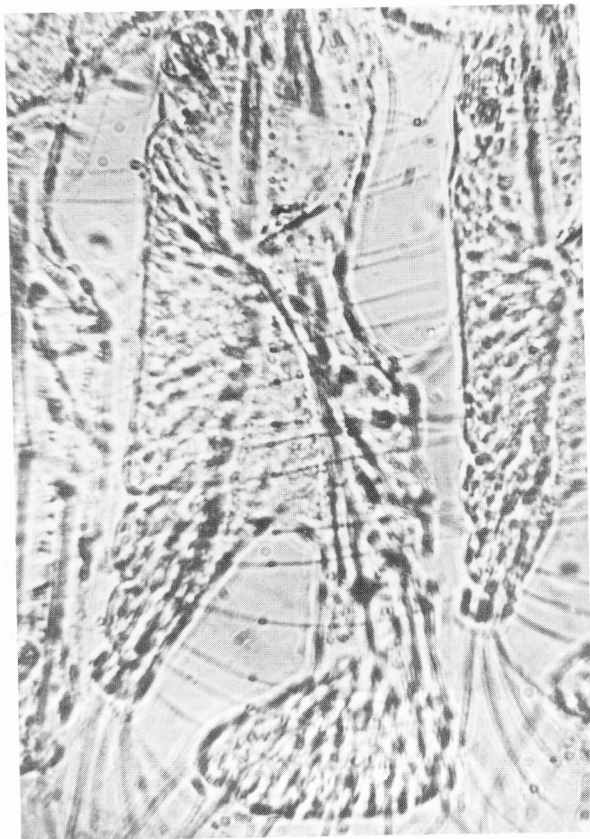
Fig. 4. Torocal appendage of *Penelia avirostris*
1 – special ion-transporting epipodite cells

rounding cuticle. These cells in Cladocera exhibits structural features that are relevant to an ion-transporting and an osmoregulation function (Khlebovicn & Aladin, 1976; Aladin, 1978, 1982a, 1982c, 1983, 1988; Potts & Durning, 1980; Meurice & Goffinet, 1982, 1983; Halcrow, 1982, 1985). It is possible to compare the morphology of osmoregulating organs in Cladocera with those in the salt-tolerant anostracan *Artemia salina*. Like *Artemia salina*, the Cladocerans *Penilia avirostris*, *Moina mongolica*, *Moina hutchinsoni*, *Daphniopsis pusilla* and *Daphniopsis australis* use the special epipodite cells for osmoregulation (Copeland, 1967). Like the nauplii of *Artemia salina*, the species *Pleopis schmackeri*, *Pleopis polyphemoides*, *Pseudevadne tergestina*, *Podon leuckarti*, *Evadne nordmanni*, *Evadne anonyx*, *Podonevadne trigona*, *Podonevadne camptonyx*,
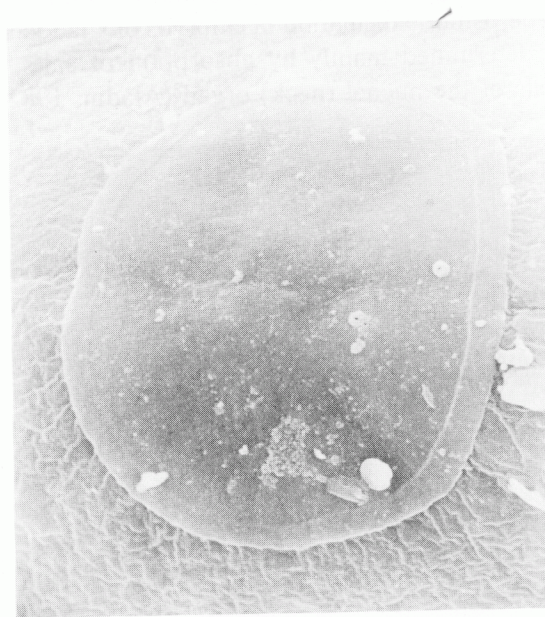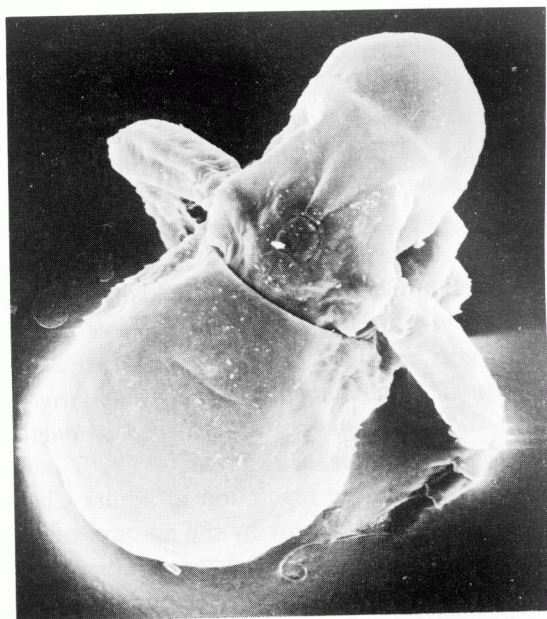

Fig. 5. Nuchal (neck) organ on the head of adult *Podon leuckarti* a – low magnification; b – high magnification
1 – nuchal (neck) organ

Podonevadne angusta, *Cornigerius macoticus hircus*, and embryos of freshwater and slightly brackishwater Cladocera use the nuchal (neck) cell organ for osmoregulation (Hootman and Conte, 1975).
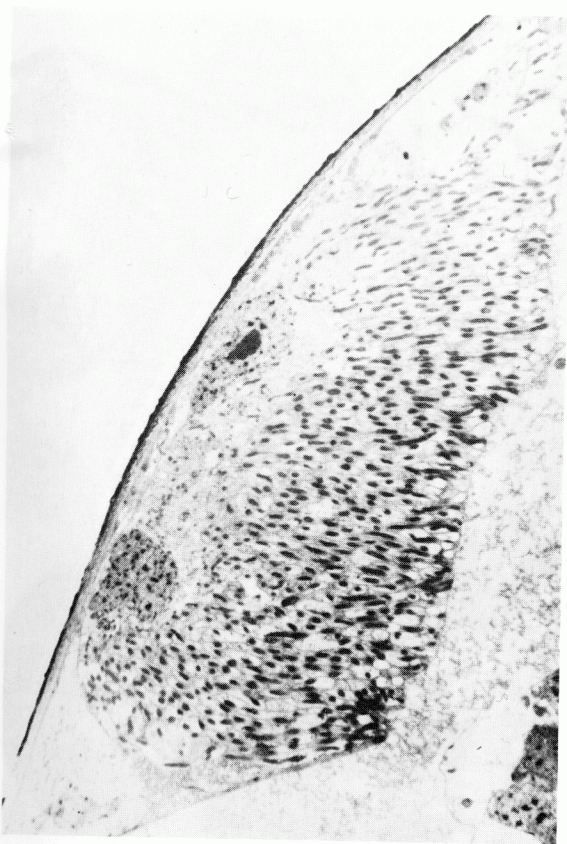
Fig. 6. Sagittal section of nuchal (neck) organ of adult
Evadne nordmanni
1 – nucleus
2 – lacunar ventral zone
3 – cuticle
4 – haemocoelic activity

Fig. 7. The basal zone of ion-transporting cell of nuchal
(neck) organ of adult *Evadne nordmanni*
1 – numerous mitochondria

The other structure that serves for osmoregu-
lation is the closed brood pouch of *Penilia
avirostris, Moina mongolica, Moina hutchinsoni,
Pleopis schmackeri, Pleopis polyphemoides,
Pseudevadne tergestina, Podon leuckartii, Evadne
nordmanni, Evadne anonyx, Podonevadne camp-
tonyx, Podonevadne angusta* and *Cornigerius
maeoticus hircus*. When the brood pouch of these
Cladocera contains developing eggs and young
embryos, the osmolarity of the marsupial fluid
equals that of the haemolymph (Fig. 8, 9). But
when the brood pouch contains embryos in the
final stages of development with a nuchal (neck)
organ or epipodites, the marsupial fluid rises in
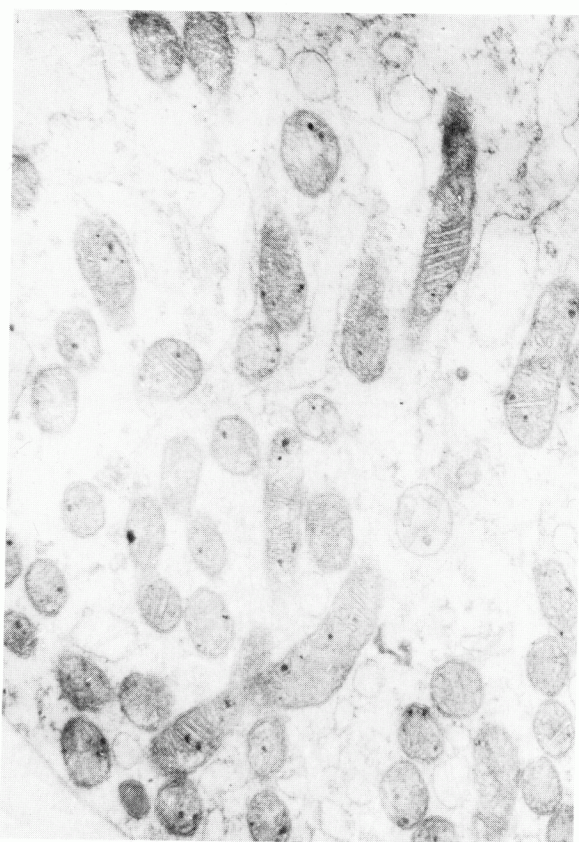concentration to that of the surrounding sea or

brackishwater (Fig. 10, 11). The ability to regulate
the embryonic environment and so protect the
early developmental stages from extremes of
osmotic stress is one of the important physiologi-
cal adaptations that permit some Cladocera to
inhabit the oceans and highly mineralised con-
tinental water-bodies (Khlebovich & Aladin,
1976; Aladin, 1978, 1982a, 1982, 1983).

The Aral sea, formerly the world's fourth lar-
gest lake in area, is disappearing. Between 1960
and 1989, its level dropped nearly 14 meters, its
area decreased by 40%, and its volume decreased
by 60% (Fig. 12) (Micklin, 1988; Micklin &
Bond, 1988). Recession of the water level has
resulted from reduced inflow caused primarily by
withdrawals of water for irrigation. Because of
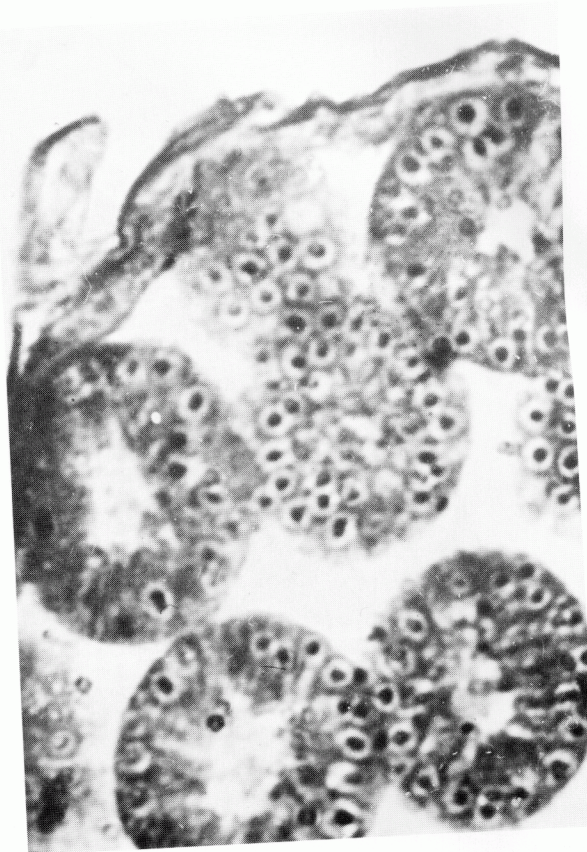desiccation of the Aral sea the salinity has in-

Fig. 8. Closed brood pouch of *Evadne anonyx* containing developing eggs (blastula). a – low magnification, total parthenogenetic female, b – high magnification, sagittal section of closed brood pouch contains developing eggs (blastula)
1 – developing eggs (blastula)

creased over the last 30 years from 8–10‰ to 26–28‰, and this had led to changes in flora and fauna.

Serious changes have take place in the Cladocera fauna. In the early 1960s, 14 species inhabited the Aral sea: *Diaphanosoma brachyurum*, *Chydorus sphaericus*, *Alona rectangula*, *Bosmina longirostris*, *Daphnia longispina*, *Ceriodaphnia reticulata*, *Ceriodaphnia cornuta*, *Ceriodaphnia pulchella*, *Moina mongolica*, *Moina micrura*, *Podonevadne camptonyx*, *Podonevadne angusta*, *Evadne anonyx* and *Cercopagis pengoi aralensis* (Mordukhai-Boltovskoi, 1974). Some Cladocera, such as *Alona rectangula*, *Ceriodaphnia reticulata*, *Moina mongolica*, *Podonevadne camptonyx*, *Po-*

donevadne angusta and *Evadne anonyx* lived in the open sea with a salinity over 8‰. Other Cladocera, such as *Diaphanosoma brachyurum*, *Chydorus sphaericus*, *Bosmina longirostris*, *Daphnia longispina*, *Ceriodaphnia cornuta*, *Ceriodaphnia pulchella*, *Moina micrura* and *Cercopagis pengoi aralensis*, inhabited the north and south parts of the Aral sea near the Syrdarya and Amudarya river deltas with a salinity less than 8‰.

The negative influence of increasing salinity on Cladocera started in 1971, when the salinity in the open Aral sea exceeded 12‰. By the middle 1970s, when the salinity in the open Aral sea exceeded 14‰, nearly all Cladocera of freshwater origin had already died. In the summer of 1980

Fig. 9. Sagittal section of closed brood pouch of *Penilia avirostris* containing developing eggs (blastula)

Fig. 10. Closed brood pouch of *Evadne anonyx* containing embryos at final stages of development with nuchal (neck) organ

during an expedition all over the Aral sea, only 5 species of Cladocera were found. At that time the salinity in the open Aral sea was more than 17‰. But in the north and south parts of the Aral sea near the deltas of the Syrdarya and Amudarya rivers, the salinity was about 16‰. In the open Aral sea only the podonids *Podonevadne camptonyx*, *Podonevadne angusta*, *Podonevadne trigona* and *Evadne anonyx* were found. In the southern part of the Aral sea near the Amudarya river delta *Cercopagis pengoi aralensis* was found only at two stations with a salinity of 15,92‰ (Aladin & Andreev, 1984). It is important to note that in 1980 *Podonevadne trigona* was caught in the Aral sea for the first time. Possibly this podonid became acclimatized in the Aral sea during introduction of fishes from the Caspian sea.

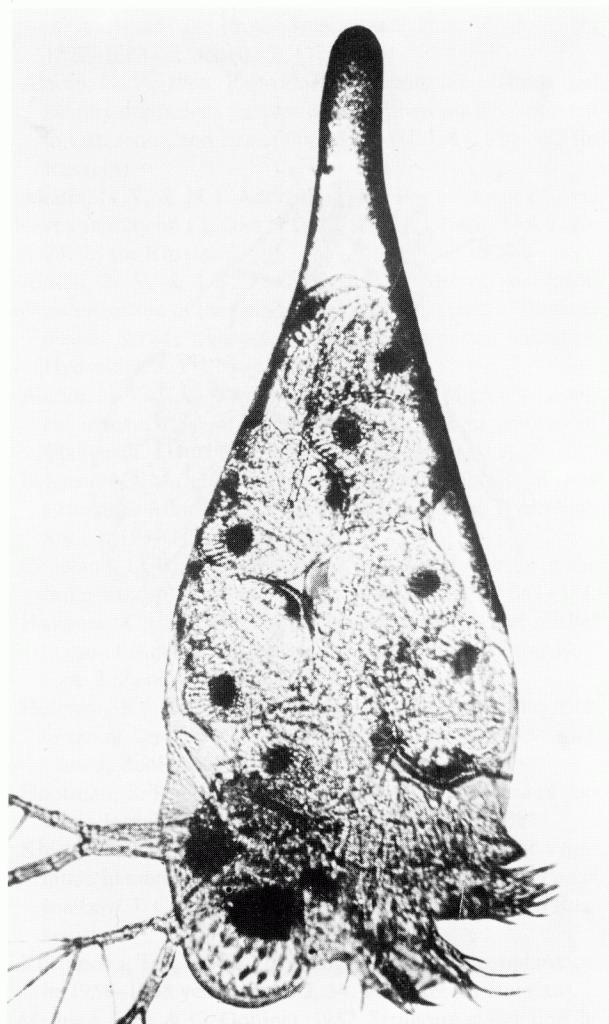Regular observation during the last ten years in the north and south part of the Aral sea and in the open Aral sea around Barsakelmes Island show that now at a salinity of 26–28‰ only the 4 species of Cladocera *Podonevadne camptonyx*, *Podonevadne angusta*, *Podonevadne trigona*, and *Evadne anonyx* still live there. Until the middle 1980s when the salinity was less than 24‰, all these 4 species were numerous. But when the salinity exceeded 24‰, only *Podonevadne camptonyx* and *Evadne anonyx* remained numerous.

The serious changes that took place in the Cladocera fauna of the Aral sea during its desiccation and salinization are in agreement with
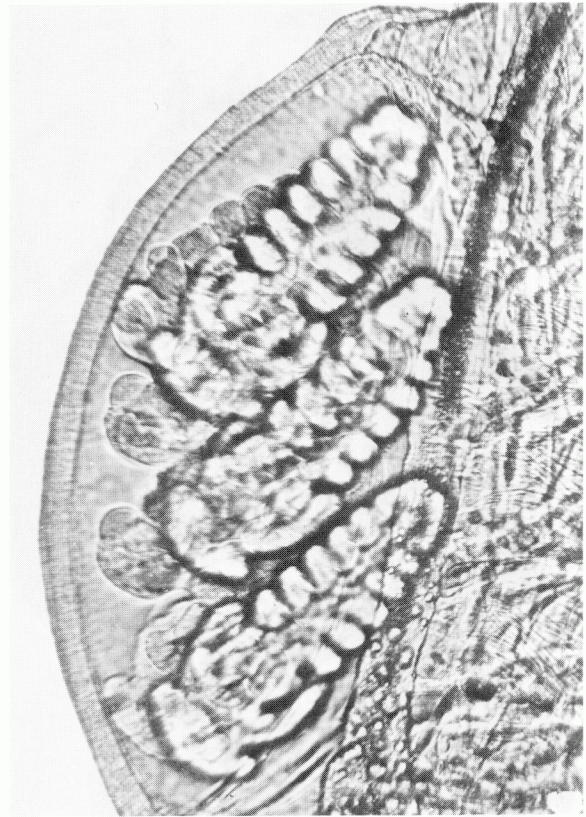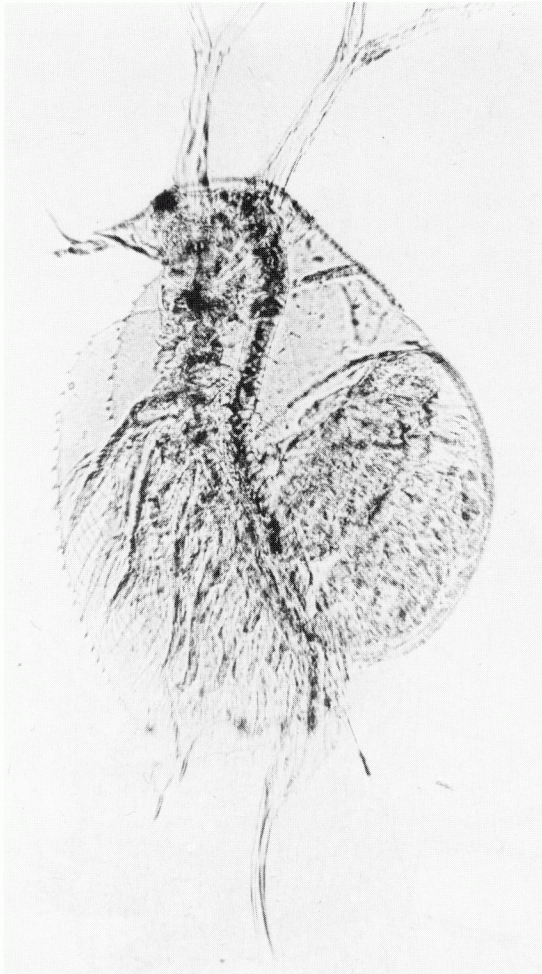
Fig. 11. Closed brood pouch of *Pinilia avirostris* containing embryos at final stages of development with epipodites. a – low magnification, partenogenetic female; b – high magnification, saggittal view on closed brood pouch containing embryos at final stages of development with epipodites.

osmoregulation capacities of Cladocera. Now only Podonidae live in the Aral sea because these Cladocera are able to hypoosmotically regulate their haemolymph by means of the nuchal (neck) organ and thus can protect the early development stages of eggs and embryos in their closed brood pouch from extremes of osmotic stress.

Other cladocerans (except *Moina mongolica*) that are not able to hypoosmotically regulate and thus cannot protect the early developmental stages of eggs and embryos died. *Moina mongolica* is able to hypoosmotically regulate by means of special epipodite cells and thus can protect early developmental stages of eggs and embryos in their closed brood pouch, but this species was last seen in the Aral see in 1974 when the salinity was about 14‰. The main reason for the disappearance of *Moina mongolica* was the acclimatization of plankton eating fishes (Mordukhai-Boltovskoi, 1974; Kortunova, 1975; Aladin & Andreev, 1984).

In the Kiziltash estuary *Moina mongolica* is found at salinities between 40,7 and 63,8‰, and reproduction occurs even at 97,14‰ (Sukhanova, 1971). In laboratory experiments *Moina mongolica* was acclimated to 88‰ of Aral sea water (Aladin, 1983). At present *Moina mongolica* lives around the Aral sea in salt lakes and former lagoons and gulfs now separated from the Aral sea. It is very possible that in the near future *Moina mongolica* will naturally reacclimatize in the open Aral sea.
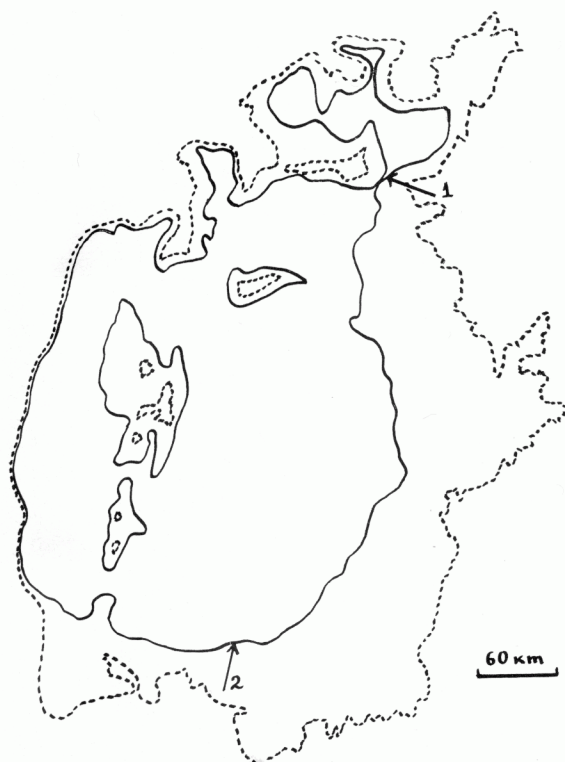
Fig. 12. The Aral sea scheme
Dotted line – the shore line in 1960
Unbroken line – the shore line in 1989
Arrow 1 – Syrdarya river delta
Arrow 2 – Amudarya river delta

References

Aladin, N. V., 1978. Hypoosmotic regulation in the marine Cladocera *Penilia avirostris*. J. Comp. Biochem. Physiol. 14: 599–601 (in Russian).

Aladin, N. V., 1982a. Salinity adaptations and osmoregulation abilities of the Cladocera. Forms from open Seas and oceans. Zool. J. 61: 341–351 (in Russian).

Aladin, N. V., 1982b. Salinity adaptation and osmoregulation abilities of the Cladocera. Forms from Caspian and Aral seas. Zool. J. 61: 507–514 (in Russian).

Aladin, N. V., 1982c. Salinity adaptations and osmoregulation abilities of the Cladocera. Forms from brackish and fresh water. Zool. J. 61: 851–860 (in Russian).

Aladin, N. V., 1983. Amphiosmotic regulation in the euryhaline Cladocera *Moina mongolica* Daday. Hydrobiol. J. 19: 76–81 (in Russian).

Aladin, N. V., 1984. The influence of temperature on the osmoregulation abilities of the Branchiopoda and Ostracoda. Zool. J. 63: 1158–1163 (in Russian).

Aladin, N. V., 1987. Salinity adaptation and osmoregulation in Ostracoda and Cladocera from continental water bodies of Australia and from Seyshelles Island. Zool. J. 66: 1822–1828 (in Russian).

Aladin, N. V., 1988. Reprodactive salinity adaptations and salinity dependent features of the embryonic development in Ostracoda and Branchiopoda. Zool. J. 67: 974–982 (in Russian).

Aladin, N. V. & N. I. Andreev, 1984. The influence of Aral sea salinity on Cladocera fauna changes. Hydrobiol. J. 20: 23–28 (in Russian).

Aladin, N. V. & I. S. Plotnikov, 1985. Microcryoscopical investigation of the liguid from maxillary gland of *Daphnia magna* Straus during acclimation to different salinities. Hydrobiol. J. 21: 62–65 (in Russian).

Aladin, N. V. & R. S. Villar Valdivia, 1987. Microcryoscopical investigation of the liquid from egg and embryo of Cladocera. Hydrobiol. J. 23: 93–97 (in Russian).

Belyaev, G. M., 1950. Osmoregulation abilities of low Crustacea from inland water. Trans. allunion. Hydrobiol. Soc. 2: 194–213 (in Russian).

Copeland, D. E., 1967. A study of salt-secreting cells in the brine shrimp (*Artemia salina*). Protoplasma 63: 363–384.

Halcrow, K., 1982. Some ultrastructural features of nuchal organ of *Daphnia magna* Straus (crustacea: Branchiopoda). Can. J. Zool. 60: 1257–1264.

Halcrow, K., 1985. A note on the significance of the neck organ of *Leptodora kindtii* (Focke) (*Crustacea, Cladocera*). Can. J. Zool. 63: 738–740.

Hootman, S. R. & F. P. Conte, 1975. Ultrastructure of Artemia larval neck organ. J. Morphol. 145: 371–385.

Khlebovich, V. V. & N. V. Aladin, 1976. Hypotonic regulation in marine Cladocerans *Evadne nordmanni* and *Podon leuckarti*. J. Comp. Biochem. Physiol. 12: 591–592 (in Russian).

Kortunova, T. A., 1975. On changes in Aral sea zooplankton in 1959–1968 years. Zool. J. 54: 657–669 (in Russian).

Meurice, J. Cl. & G. Goffinet, 1982. Structure et fonction de l'organe nucal des Cladoceres marins gymnomeres. C. R. Acad. Sci. Paris. 29(D: 693–695.

Meurice, J. Cl. & G. Goffinet, 1983. Ultrastructural evidence of the ion-transporting role of the adult and larval neck organ of the marine gymnomeran Cladocera (*Crustacea, Branchiopoda*). Cell. Tissue Res. 234: 351–363.

Micklin, P. P., 1988. Desiccation of the Aral sea: A water management disaster in the Soviet Union. Science. 241: 1170–1176.

Micklin, P. P. & A. R. Bond, 1988. Reflections on environmentalism and river diversion projects. Sov. Econ. 4: 253–274.

Mordukhai-Boltovskoi, F. D., 1974. Subclass Branchiopoda. In F. D. Mordukhai-Boltovskoi (ed.) Atlas of the Aral sea invertebrates. Moscow: 112–134 (in Russian).

Potts, W. T. W. & Durning C. T., 1980. Physiological evolution in the Branchiopoda. Comp. Biochem. Physiol. 67b: 475–484.

Sukhanova, E. R., 1971. *Moina microphthalma* (Cladocera, Daphniidae) under ultrahaline conditions in the North Caucasus. Zool. J. 50: 285–287 (in Russian).