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Diapause, a potent force in the evolution of freshwater crustaceans

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Abstract

After a brief historical review of the discovery of diapause in freshwater crustaceans, its dramatic nature in certain cyclopoid copepods, in which diapausing individuals may occur at densities of $>10^6$ per m^2, is used to illustrate the enormous ecological significance of the phenomenon. Some of the problems presented by diapause in cyclopoid copepods are noted, including the different behaviour in different lakes of what appears to be a single species. Different physiological cues or different genetic endowments are clearly involved.

The wider incidence of diapause in freshwater copepods and ostracods is noted.

Among freshwater crustaceans it it the Branchiopoda that have universally adopted diapause, always at the egg stage. Even such an ancient order as the Anostraca, perhaps the most primitive of all crustaceans, produces elaborately constructed resting eggs that are capable of cryptobiosis, can remain viable in a dry state for long periods, and can tolerate extreme conditions. The nature of branchiopod resting eggs is briefly reviewed. Of these, only those of the Anomopoda are protected by containers derived from the parental carapace. These are mechanically complex in the most advanced species but, as shown by fossils, are extremely ancient structures.

Factors initiating the onset and termination of diapause in branchiopods are briefly noted, and the process of hatching of resting eggs is outlined.

Introduction

Although diapause is important in the lives of many freshwater crustaceans and has played a key role in the adaptive radiation and ecological success of the three major groups (Branchiopoda, Ostracoda and Copepoda), the very existence of the phenomenon in these animals long escaped detection. For example, although the production of drought-resistant resting eggs is a characteristic feature of anostracans and notostracans, and although observations on these animals date back to the early years of the 18th century, this attribute was not immediately apparent because the eggs of at least some species sometimes hatch without being dried or undergoing an appreciable period of rest. This was clearly the reason why diapause for long remained unknown in *Chirocephalus diaphanus*, the earliest mention of which was made as long ago as 1709 by Petiver whose brief diagnosis is accompanied by the terse statement 'I have hatched these from their eggs'. The same is true of *Triops cancriformis* the hatching of whose eggs was described by Schaeffer (1756), the animal itself having been first described a few years earlier. Schaeffer gives a minute drawing which shows a nauplius emerging from an egg — the first illustration of a hatching branchiopod. Even in the mid 19th century the ability of the eggs of such animals to remain dormant for long periods and to withstand drought seems not to have been known.

Likewise the true nature of the ephippium of anomopods and its properties were not immediately apparent. Although *Daphnia* was described by Swammerdam as early as 1669, its ephippium was not mentioned until more than 80 years later when the remarkable Schaeffer (1755) gave a very small illustration of an ephippial female of *Daphnia magna*, noting that the individual exhibited a black patch posteriorly above the spine, but was unaware of its nature. In 1785 Müller not only gave what for that time was a reasonable description and illustration of the ephippium of *Daphnia* but bestowed on it the name by which it continues to be known. He had, however, nothing to say about its nature, which was to remain unknown until well into the next century. In 1820 Jurine, an excellent observer, also noticed ephippia but thought that animals which bore them, which he illustrated, were afflicted by a disease, and it was left to Straus (1819–1820) to recognise that the ephippium of *Daphnia* contained resting eggs that enabled the animal to withstand the northern winter, which its producer was unable to do. This appears to be the first genuine observation on diapause in a freshwater crustacean.

That the eggs of certain freshwater ostracods could not only become dormant but could withstand drought was known by the mid 19th century (Baird, 1850). The discovery of diapause in the Copepoda seems to have occurred some time later, and only a few species were known to have this ability until the second half of the present century. While some calanoids and at least one harpacticoid produce resting eggs, the discovery of diapause in cyclopoids and harpacticoids proved to involve stages not known to display the phenomenon in other groups — copepodid stages in a cyclopoid (Birge & Juday, 1908) and adults in a harpacticoid (Wolf, 1905; Lauterborn & Wolf, 1909). In both cases dormant individuals were encased in a gelatinous cyst, apparently with an outer layer of mud particles in the latter though this may not be an essential feature. Harpacticoid cysts were subsequently found by several workers but until the 1950s Birge & Juday's finding of encysted individuals of *Acanthocyclops (Diacyclops) thomasi* (= *A. bicuspidatus thomasi*) or *A. thomasi* remained the only known example of diapause in cyclopoids.

The dramatic nature of diapause in certain cyclopoid copepods

A personal reminiscence shows how dramatic diapause can be in cyclopoid copepods. In January 1953 I had in my room, core samples taken at a depth of c15m from the profundal zone of Esthwaite Water in the English Lake District. After these had stood overnight I noticed that numerous copepods, which proved to be copepodids, almost all stage 5, of *Mesocyclops leuckarti*, were swimming freely in the water overlying the mud. None had been seen at the time of collection. In Britain *M. leuckarti* is a summer species and is not found in the plankton of Esthwaite Water in winter. Where the vast number of individuals that frequent the lake in summer go in winter, was a complete mystery. These cores supplied the answer. Further cores were obtained, some being allowed to warm up to room temperature (c. 16–17 °C) from the 3 °C or so at which they were collected, others being kept in a refrigerator. Copepods soon appeared in the former, not in the latter. Observations were also made at other temperatures, details of which need not concern us here. These simple observations revealed where *M. leuckarti* goes in the winter time. The number of individuals concerned was enormous. From the number emerging in a single core it was easy to calculate the density per square metre of bottom, and this proved to be about 1 000 000. (Far fewer were found in shallow water deposits.) Much clearly depends on just where the corer strikes, and perhaps on the year of investigation, as subsequent work by a colleague (Smyly, 1961) revealed rather smaller, but still enormous, concentrations of dormant copepodids. On the other hand, when resting stages of *Cyclops scutifer* were discovered later by Elgmork (1962), densities as great as 3 800 000 per m^2 were recorded, so we are here dealing with a phenomenon that involves prodigious numbers of individuals.

Naturally the mud from which *M. leuckarti* emerged was searched for dormant individuals. In spite of their abundance these were not easy to find. Less than 0.9 mm long and transparent, each tended to be obscured by adhering mud particles, and not all lay at the same depth in the semi- liquid mud. The interesting feature of these copepods was that they lacked any trace of a mucilaginous coat, being different in this respect from *A. thomasi* and from those of *Microcyclops bicolor* found in shallow water in the English Lake District by my colleague Smyly. The abdomen of each was flexed ventrally approximately at right angles to the cephalothorax, the antennules lay parallel to the sides of the body, the antennae were folded back and the swimming legs directed forward as at the beginning of a propulsive thrust. No movements of the gut, so characteristic of cyclopoids, were to be seen and the animals remained inert, and to all appearances dead, and could be turned over with a needle. Revival is rapid, especially if the temperature is raised quickly, and animals may swim in as little as 5 h. Diapause is thus easily broken in response to a single stimulus — a rise in temperature. Similar diapausing individuals were found also in Windermere at depths of about 65 m. (For further details see Fryer & Smyly, 1954).

Subsequent work on the life cycle of *M. leuckarti* in Esthwaite Water and Windermere and on the behaviour of the copepodids carried out by Smyly (1961, 1962) extended our understanding of the events so briefly recapitulated here. His findings showed, for example, that in nature some copepodids decend to the bottom and enter diapause in autumn while water temperatures are still above 10 °C (whereas in Lago Maggiore *M. leuckarti* remains active all winter even though the temperature falls to c. 7 °C); that copepodids revive in spring while temperatures are still at the winter minimum of c. 4 °C; and that dormancy can not be re-induced in revived copepodids by lowering the temperature to 0–2 °C. As such stimuli as changes in light intensity and disturbance could be ruled out, he concluded that the length of the dormant period is primarily determined by some physiological means and that changes taking place with time ensure that dormancy cannot be prolonged indefinitely even if there is no rise in temperature. The different behaviour in different lakes of populations assigned to this species raises the possibility that different physiological races, or even cryptic species, may be involved.

Curiously, at the same time as the initial work was in progress, Ulomsky (1953) was studying populations attributed to *M. leuckarti* in Northern Siberia and also found that copepodids spend the winter on the bottom, but not, apparently, in diapause. This apparent anomaly seems to have been resolved by Kiefer's (1981) revelation that *M. leuckarti* is confined to Europe and the western parts of northern Asia and that the many records of this species from elsewhere in fact refer to other, closely related, species.

The wider incidence of diapause in freshwater copepods

Dormant copepodids without gelatinous cysts have now been found in other cyclopoids and the phenomenon is clearly widespread: by 1967 Elgmork was able to list 12 species that display this attribute and Smyly (1967) added another. More cases are now known. Elgmork (1959) has given a detailed account of the life cycle of *Cyclops strenuus* and shown, for example, that dormant copepodids sometimes penetrate soft mud to depths of as much as 30 cm. A so far unique situation prevails in some populations of *Cyclops scutifer* where individuals are remarkably long-lived and spend two consecutive winters in a state of diapause, the first as stage II or III copepodids, the second as stage IV or V, and may conceivably sometimes enter diapause a third time (Elgmork, 1981). Remarkably, one-year cycles are known in populations in other lakes with colder water, which again presents intriguing problems for physiologists, and geneticists. Whether such striking biological differences reflect the existence of cryptic species could perhaps be resolved by molecular techniques.

Some non-cyst-forming species use diapause as a means of overwintering, as does the cyst-forming *Microcyclops bicolor*; others employ it as a means of withstanding high summer temperatures and low oxygen concentrations as does the cyst-forming *Acanthocyclops thomasi.*

Various freshwater copepods are now known to be able not merely to undergo diapause but to withstand desiccation. It has been known since the early years of this century that the calanoid *Diaptomus castor* has drought-resistant eggs, whose hatching to produce swarms of nauplii shortly after the filling of the temporary pools in which it often lives, is a characteristic feature of its usual life cycle. More recent work, summarised by Champeau (1970) who made extensive studies in nature, revealed that in the Mediterranean region several calanoids produce drought-resistant resting eggs. Other calanoids which produce such eggs, or eggs that do not have to face this hazard but which tide their producers over periods of adverse conditions in permanent water bodies, have also been found elsewhere, e.g. in North America. Calanoids with such abilities are listed by Hairston & Cáceres (1996). Champeau has also shown that several cyclopoids can undergo diapause as late copepodid stages that can tolerate a considerable degree of drying. Although he found that excessive desiccation kills such diapausing individuals, *Acanthocyclops bicuspidatus odessanus* emerged from dried mud stored for 3 years, and he collected many stage IV copepodids of this species from a water body that had just refilled after being dry for eight years, though rain must have fallen on the area during this time.

Ability to withstand intense desiccation is shown by *Metacyclops minutus* which Rzóska (1961) found encysted in temporary pools in the Sudan where true drought resistance is necessary for survival. Rzóska describes how advanced copepodid stages were found 'struggling out of completely transparent cysts' about 10 hours after a pool filled.

Several freshwater harpacticoid copepods can also enter diapause as adults and some of these can withstand at least some degree of desiccation (see Champeau, 1970 for summary) and at least one species, *Bryocamptus arcticus*, can produce thick-walled resting eggs (Borutzky, 1929).

The widespread occurrence of diapause in freshwater ostracods

That many freshwater ostracods produce drought-resistant eggs often receives casual mention in general accounts of the group, but the ecological and evolutionary significance of this ability has not received the attention it deserves. The importance of the phenomenon in these animals can be emphasised simply by noting that G. O. Sars, who, from the 1880s onwards, reared many freshwater crustaceans from dried mud, hatched no fewer than 48 species of ostracods from such mud received from S. Africa (Sars, 1924). He also records that the annually added water to mud from a sample kept dry from the time of its collection. Each year up to the time of writing, 12 years in all, he hatched species of *Cypridopsis*, whose remarkable ability to remain dormant for long periods in a dry state was thus dramatically demonstrated. Even longer periods of dormancy are now known.

Diapause in the Branchiopoda: its exploitation and its evolutionary significance

Of all crustaceans it is the Branchiopoda that have universally adopted diapause, without which they could not have achieved their present eminence as freshwater animals. Recent discoveries (Walossek, 1993) have confirmed that, as long suspected, branchiopods existed in the Cambrian. Just when they first colonised freshwater is not known, but they probably did so very early in the Palaeozoic, and they have been exclusively freshwater animals for most of their history. The few present-day marine forms are derived from freshwater ancestors. Colonisation of freshwater was almost certainly facilitated by the early-acquired habit of feeding on fine particles of detritus, which must have been one of the few sources of food initially available in freshwater habitats.

Early branchiopods were slow-moving, largely defenceless animals (as are extant representatives of the most primitive forms), but predators were then few. With the advent of active predators, survival of primitive forms — Anostraca, Notostraca and the two 'conchostracan' orders — whose ancient nature is attested by fossil evidence as well as by their morphology, became completely dependent on the possession of drought-resistant eggs which enabled them to live in predator-free temporary ponds and, to a lesser extent, in extremely cold habitats. Such habitats have changed little for vast periods of time and temporary ponds are indeed probably the most permanent of all freshwater habitats (Fryer, 1985). Highly specialised physiological adaptations, again with the possession of resting eggs, have enabled some primitive branchiopods to colonise extreme environments, such as highly saline waters, where predators are also lacking.

But not all branchiopods are conservative types. This group has given rise to some highly successful forms that, by virtue of small size, rapid reproduction, largely by parthenogenesis, and exceedingly efficient feeding mechanisms, have been able to occupy a wide range of freshwater habitats, including those where predation is intense, and a few have secondarily invaded the sea. All these nevertheless retain diapausing eggs, the presence of which, both as a device for countering adverse conditions and as agents of dispersal, has played a key role in their success. The evolution of such eggs has involved considerable diversification.

The Anostraca is probably the most primitive order, not only of the Branchiopoda but of all crustaceans, but the resting eggs are highly specialised. All save a few parthenogenetically reproducing species of *Artemia* produce such eggs. Parthenogenesis is clearly a secondary development, a belief supported by genetic evidence (Abreu- Grobois & Beardmore, 1980).

Anostracan eggs are usually spherical, but flattened or disc- like in *Tanymastix* and tetrahedral in some species of *Streptocephalus*. The embryo is invested in what is often described as a thin, inner, chitinous cuticle, but which is three-layered (Garreau de Loubresse, 1974) and separates into two distinct membranes at the time of hatching. These membranes are apparently produced at different times by the embryo. Outside these there is a much thicker, non-chitinous shell, sometimes called the tertiary shell. Harder than the chitinous cuticle, it consists of an outer cortex and an inner alveolar layer (Kupka, 1940; Gilchrist, 1978). In some species these are separated in places by a sub-cortical space which copes with the irregular nature of the cortex. The alveolar layer, whose thickness differs in different species, is closely associated with the embryonic cuticle which it invests with what can be described as a layer of solidified foam with large air spaces. In *Thamnocephalus platyurus* it is 'double', having a distinct partition that divides it into inner and outer layers (De Walsche *et al.*, 1991). The cortex, often but not always, thinner and denser in texture than the alveolar layer, often has its external surface puckered or drawn out into ridges or ribs. In *Chirocephalus diaphanus* these slender ribs have a honeycomb- like appearance, remarkably similar to the ribs on the surface of the carapace of the so-called honeycomb species of anomopods of the genus *Chydorus*. These surface patterns and egg shapes are of taxonomic value (Mura & Thiery, 1986; Brendonck & Coomans, 1994a, b).

Various workers have studied the histochemistry of the egg shell (e.g. Linder 1960, Fautrey & Fautrez-Firlefyn, 1971) and shown it to be a complex lipo-protein with mucopolysaccharides and other substances and to be impregnated with haemoglobin. Thiery (1987) suggests that the presence of haemoglobin, a polymer of high molecular weight, might be able to explain the formation of the superficial ornamentation of the eggs and draws attention to the remarkably similar patterns that form on inflated and solidified polymers (Tanaka *et al.*, 1987) and to patterns obtained by Galwey *et al.* (1980) by crystallisation under partial vacuum of solutions of chrome alum that bear an astonishing resemblance to the eggs of *C. diaphanus*.

At the time of shedding, eggs appear usually to contain embryos at the gastrula stage of development. Embryos often seem to remain at this stage for some time while the eggs remain fully hydrated. Further development seems to take place during the dry phase. Khalaf & Hall (1975) suggest that in *C. diaphanus* this occurs while the mud on which they lie is still moist and aerobic, and it is hardly feasible for development to take place in dehydrated embryos.

Dried eggs are often truly cryptobiotic. Their contents contract to a cup-like disc that occupies only a small proportion of the egg as bounded by the inner (embryonic) membrane. As such they can remain dormant for long periods, certainly for several years.

Notostracan eggs are spherical and larger than those of the Anostraca but are similar in structure. The embryo is surrounded by what appears to be a single chitinous membrane, but of which there are certainly two at the time of hatching. The tertiary shell consists of a thin external cortex with a scarcely elaborated surface and a thicker robust alveolar layer, bounded internally, according to Tommasini *et al.*, (1989), by a thin layer, so there are three layers in all. There is no sub-cortical space but in at least one species, *Triops granarius*, the alveolar spaces are in communication by a number of fine pores (Thiery, 1985).

Eggs of the Laevicaudata are small and spherical: those of the Spinicaudata are also small and sometimes spherical, but in some species are of complicated shapes or cylindrical. Like those of the Anostraca and Notostraca, most of those investigated have basically similar inner cuticles produced by the embryo (Zaffagnini & Minelli, 1970) and outer, protective shells, but the spinicaudatan *Leptestheria compleximanus* lacks the thick shell as apparently does *Eocyzicus klunzinger*. The now extensive studies on the structure of the resting eggs of primitive branchiopods are summarised by Martin (1992).*

Eggs of primitive branchiopods are amazingly resistant. In nature those of the notostracan *Lepidurus arcticus* withstand freezing while those of *Triops granarius* tolerate extremely high temperatures. Carlisle (1968) found that, where atmospheric pressure was such that water boiled at 100 °C, eggs remained viable after 16 hours at 98 °C — but failed to hatch after only 15 min. at 102 °C, which would boil out the last trace of water from the eggs. When the pressure was increased to raise the boiling point to 105 °C, eggs remained viable after 16 hours at 103 °C, but were killed by only 30 minutes exposure to 75 °C when reduction of pressure lowered the boiling point to 70 °C. Some anostracan eggs can tolerate temperatures close to absolute zero for several hours, and eggs of *Artemia* have been fixed to the outside of space-craft and retained their viability after a flight (Planel *et al.*, 1980).

The animals long known as the Cladocera in fact comprise a polyphyletic assemblage of four distinct orders separated by many trenchant differences (Fryer, 1987a, b). These include deep-seated differences in the nature of the resting eggs. All four orders have adopted parthenogenesis as a means of rapidly populating environments that are often habitable for only limited periods, but all also employ diapause. The tactic employed for protection of the resting eggs by the Anomopoda is completely different from that of the other orders.

The Haplopoda, consisting only of *Leptodora*, and most of the Onychopoda, reproduce by parthenogenesis in summer but overwinter as sexually produced resting eggs. Some Caspian cercopagids may be obligate parthenogens. Resting eggs are spherical and lack any equivalent of the thick shell of the more primitive orders but the outer of the two embryonic membranes is often thick and confers mechanical protection.

Haplopods and onychopods are open-water, often planktonic, animals whose eggs are shed freely and fall to the bottom. Those of *Polyphemus pediculus*, however, which are sticky, are often attached to vegetation. In nature, especially in planktonic species, they must almost always remain fully hydrated, and indeed

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* See note added in proof (p. 14).

there seem to be no reports of drought-resistance except in the littoral *P. pediculus*. Makrushin (1985) says that the resting eggs of neither *Leptodora kindti* nor *Bythotrephes longimanus* can withstand drought and have never been hatched from dried mud. Those of *Leptodora* are indistinguishable on histological criteria from parthenogenetic eggs, having coarse-grained yolk and vacuoles whereas drought-resistant eggs have fine-grained yolk and lack vacuoles.

In the hydrated state resting eggs of *L. kindti* and *B. longimanus* continue to have measurable metabolic rates even at the low temperatures that prevail on the bottoms of north temperate lakes in winter (Andrew & Hertzig, 1984). The metabolic rate also falls with oxygen concentration (Andrew, 1993). This metabolic activity contrasts with the cryptobiotic nature of dried eggs of other branchiopods. Data produced by Andrew & Hertzig (1984) and Hertzig (1985) suggest that most eggs undergo diapause for one winter only, and this is supported by experiments using newly produced resting eggs (Hertzig, 1985), but some eggs collected in nature remained dormant for more than a year, in one case for 17 months. Eggs that sink deeply into sediments may also remain dormant for more than a year, but prolonged dormancy seems not to be possible. Hatching appears to be independent of either photoperiod or temperature.

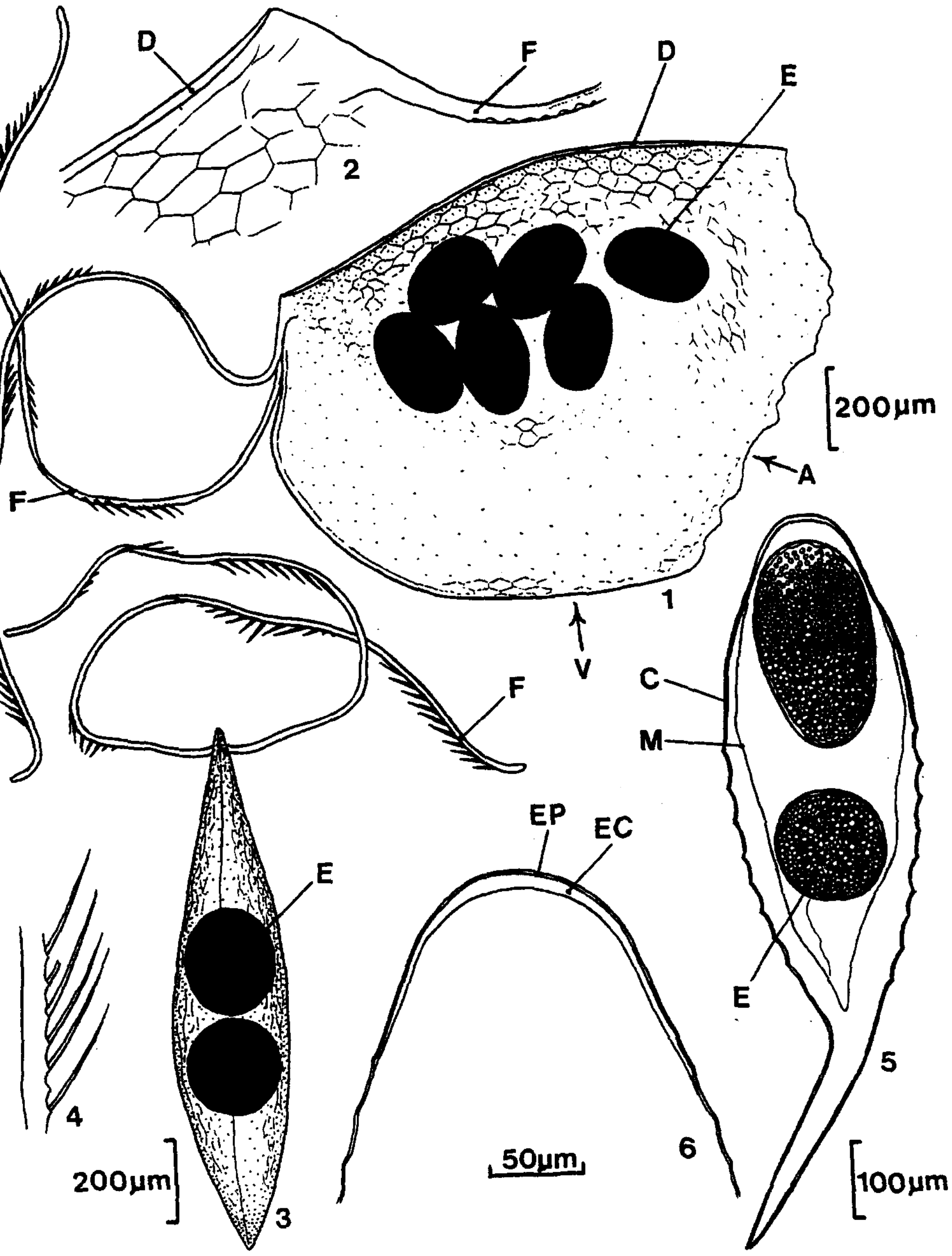
Resting eggs of the Ctenopoda are also shed freely. These also lack the thick outer shell so characteristic of the Anostraca and other primitive orders, but the outer of the two protective envelopes present is robust, sometimes has a rough surface, and sometimes bears small spines. In some cases (*Sida crystallina*, *Pseudosida variabilis* and some species of *Diaphanosoma*) the outer envelope is sticky and the eggs become attached to vegetation or other objects. Some species (e.g. *Latonopsis australis*) have been reared from dried mud so their eggs are clearly drought- resistant: those of others, such as *S. crystallina*, lose their viability after one day's drying (Makrushin 1981). Resting eggs of the pelagic *Holopedium gibberum* are also unable to withstand drying. (For differences in the nature of the yolk among ctenopods and the relevance of these differences to drought-resistance in these and other branchiopods see Makrushin 1978, 1985. For a general account of the resting eggs of ctenopods see Korovchinsky & Boikova, 1996).

At the time of hatching of ctenopod eggs the inner membrane swells in a distinctive manner, at least in *Holopedium*, *Sida* and *Diaphanosoma* (see below).

Resting eggs of the Anomopoda, usually the product of sexual reproduction, are protected in a manner unique to the order. Ovoid in shape and enveloped only in two embryonic membranes, these are protectyed by the carapace valves or parts thereof which close around them when the animal moults and sheds its clutch of resting eggs. This composite structure takes the name of an ephippium from the saddle-like structure of *Daphnia*, but primitive ephippia ('protoephippia') are much simpler than this. The most primitive situation is found in the Macrothricidae, but some members of the Chydoridae also have primitive ephippia. *Ophryoxus* (Figs 1–6) displays a very primitive condition. Here much of the carapace envelops the eggs (from one to at least 6 in number). Its only modification in connection with storage of the eggs is the development of a thickened rib along the mid-dorsal line. An almost equally primitive ephippium is found in *Acantholeberis* (Figs 7–10) which also holds a variable number of eggs. More specialised modifications of the carapace are seen in other macrothricids, e.g. in *Bunops* that part of the carapace which overlies the eggs is elaborated into closely packed blister-like swellings, and in several cases some means of attachment, sometimes elaborate, has been evolved. (Fryer, 1972; Makrushin, 1972; Makrushin & Markevitch, 1982).

Chydorids have thicker carapaces than macrothricids so their eggs are inevitably better protected. Dark pigmentation covers that part of the carapace which overlies the egg or eggs. Primitive chydorid ephippia, such as those of *Eurycercus* and *Saycia*, contain several eggs: more specialised types have reduced the number to two or, most commonly, a single egg. Descriptions of many of these and of the ephippia of the related family Bosminidae, were given long ago by Scourfield (1899, 1901, 1902). Some chydorid ephippia are attached to substrata by their producers at the time of shedding.

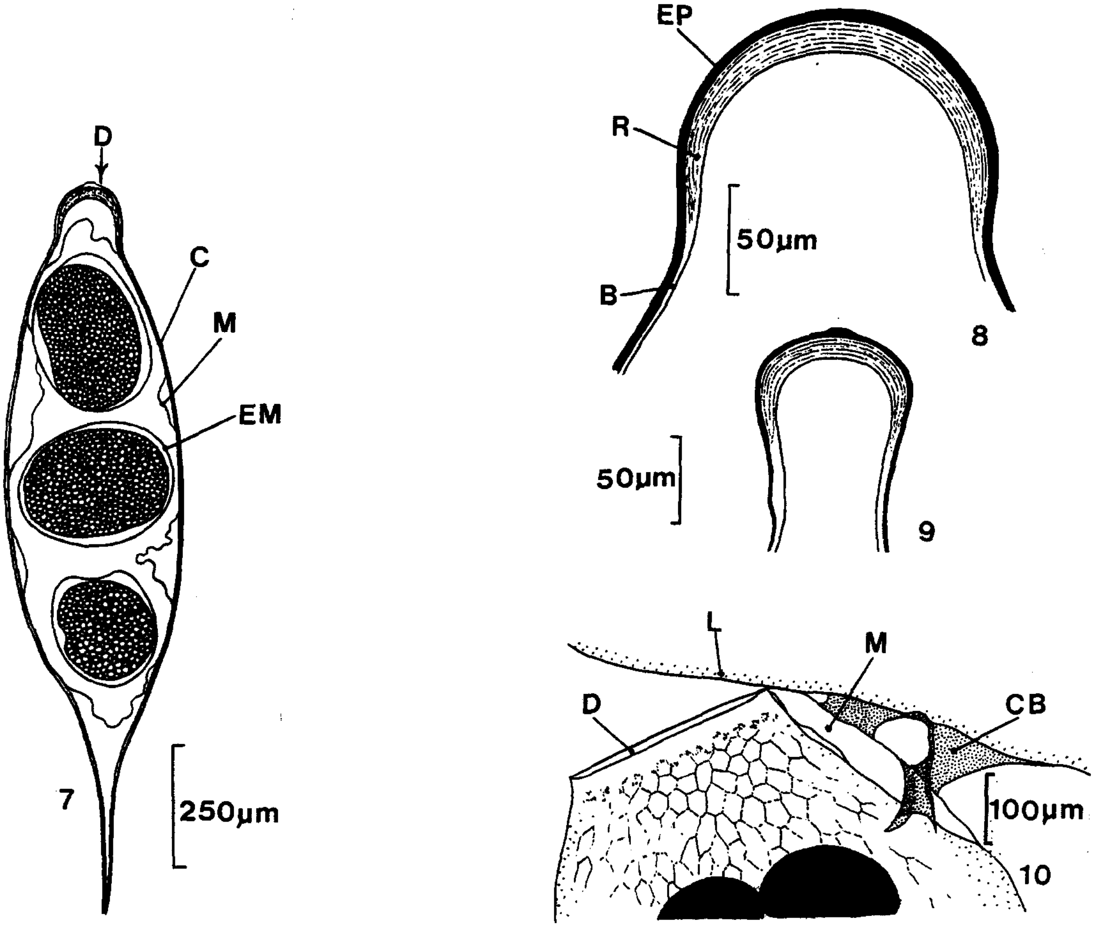
Daphniids, with which I include *Moina*, have the most specialised of anomopod ephippia. Here only part of the carapace is modified, and in very elaborate ways, to form a container for either one or two eggs according to genus. Several descriptions e.g. M. Wolff (1905), Zwack (1905) have long been available and Shultz (1977) provides information on fine structure. Much of the 'packing' that fills the gap between the inner and outer walls of the carapace is of a polygonal lattice nature that recalls the foam-like alveolar layer of anostracan and other primitive branchiopod egg shells, and fulfills a similar protective function. It is worth noting that although daphniids have the most



Figs 1–6. The ephippium of *Ophryoxus gracilis* 1. Ephippium, lateral. Note the long filaments (F) which represent the detached rims of the ventral carapace margins. 2. Posterior dorsal corner of the ephippium from the other side showing how the margins here remain attached. 3. Another ephippium containing only two eggs, ventral. 4. Part of one of the filaments. 5. Transverse section showing the very simple nature of the ephippium. The flexure of the delicate ventral portion is not a constant feature of the ephippium and has here probably been accentuated by the embedding process. 6. Transverse section of the dorsal part of the ephippium to show the thickened dorsal plate.

Abbreviations used in Figs 1–14.

A	Broken anterior margin
B	Blue-staining* (unsclerotized) endocuticle
C	Outer cuticular wall
CB	Cementing band
CC	Cast cuticle and cement
CH	Chorion
CM	Cement
CT	Cast cuticle
D	Dorsal plate
E	Egg
EC	Endocuticle
EM	Egg membrane
EMB	Embryo
EP	Epicuticle
F	Filament
L.	Leaf
M	Membrane derived from inner cuticular lining of carapace
PC	Moulted cuticle of post-abdominal claws
R	Red-staining* (somewhat sclerotized) endocuticle
V	Ventral margin of carapace from which the rim has become detached
VM	Vitelline membrane
VR	Ventral region of carapace which usually breaks free from ephippium
Z	Zone of attachment
*	With Mallory's triple stain

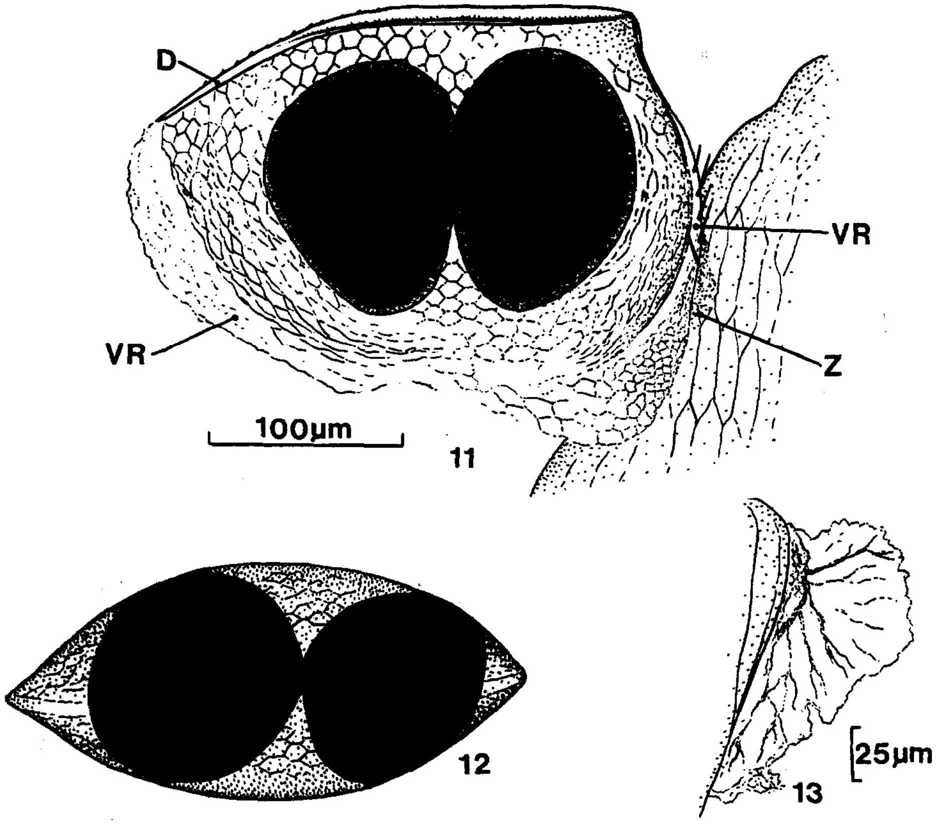


Figs 7–10. The ephippium of *Acantholeberis curvirostris* 7. Transverse section, cut square to the dorsal ridge and therefore somewhat obliquely dorso-laterally across the ephippium. 8. Transverse section through the dorsal ridge near the central portion. 9. The same near to one end. 10. Anterior end of an ephippium attached to a moss leaf, showing the cementing band which has become torn and detached at one point and hangs over the ephippium inclined towards the observer.

advanced ephippia they are nevertheless ancient structures, fossil ephippia assignable even to extant genera, being known from the Lower Cretaceous (Fryer, 1991; Smirnov, 1992).

Even the simplest anomopod ephippia can withstand drought. Although I was aware of this ability in daphniids and certain other anomopods, when I first dried the ephippia of *Eurycercus lamellatus* more than 30 years ago, I saw that the contained eggs simply shrivelled and I assumed that, in this case, they lacked this ability. However, when I immersed the ephippia in water the eggs rapidly hydrated, regained their original shape, and eventually hatched. This is true also of macrothricid eggs that are protected only by thin carapace valves. Resting eggs of the minute *Streblocerus serricaudatus* also shrivel and become grossly distorted when dried but quickly re-hydrate when immersed in water, become indistinguishable from untreated eggs, and often hatch. The same is true of resting eggs of *Acantholeberis curvirostris* that have been either dried or frozen. Though I was not aware of this at the time, eggs of daphniids, hidden in their stout ephippia, also shrivel, as do those of other branchiopods. For example Bishop (1968) found this in *Limnadia stanleyana* (Spinicaudata), and Belk (1982) removed the outer shell of *Eulimnadia antlei* to reveal shrivelled eggs within. Immersed in water, these hydrated and became perfectly spherical in one to three hours and proceeded to develop and hatch. Clearly the thick shell does not prevent desiccation.

Although able to withstand desiccation, resting eggs of *S. serricaudatus* (Figs 11–13) can also develop



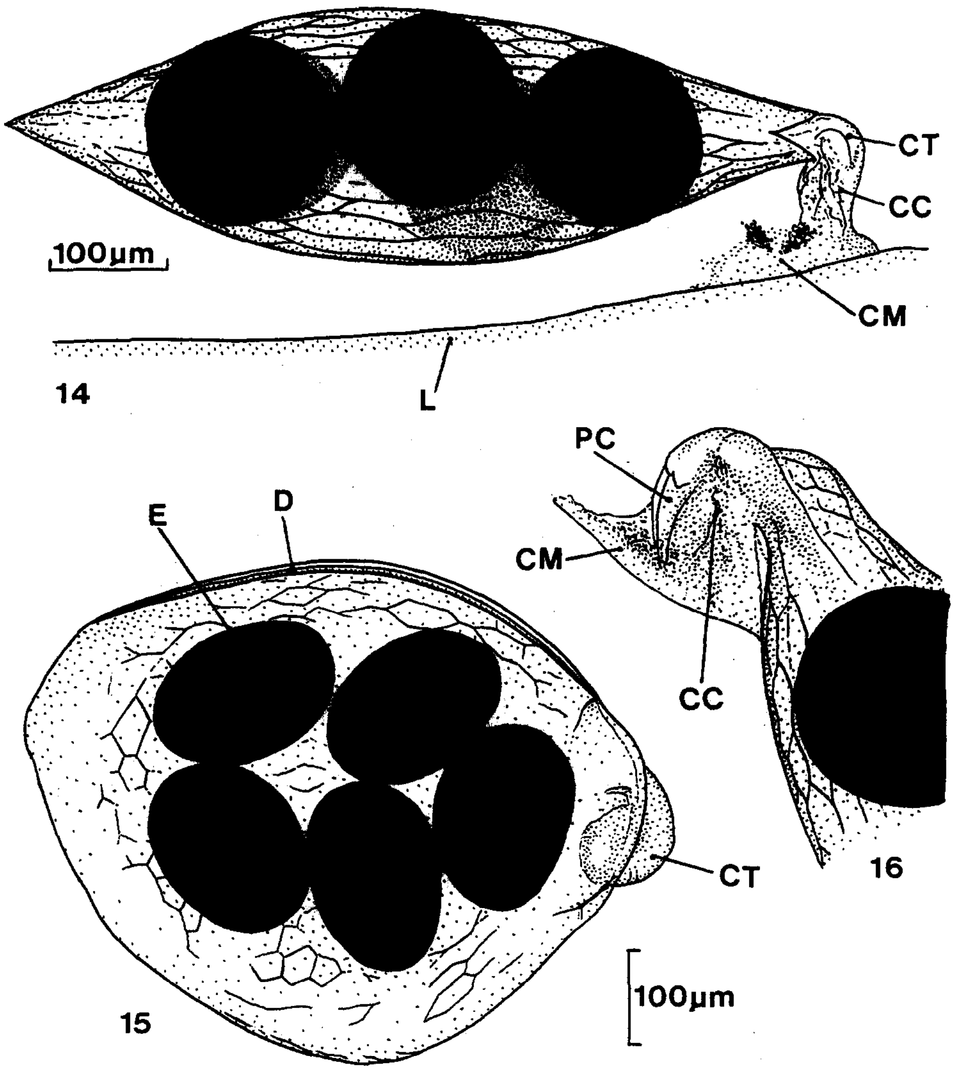
Figs 11–13. The ephippium of *Streblocerus serricaudatus.* 11. Ephippium, lateral, attached to a leaf of *Sphagnum.* 12. Ephippium from a slightly oblique dorsal aspect. Although the dorsal rib is conspicuous in the lateral view it can scarcely be made out from above. 13. The delicate disc by means of which one ephippium was attached to a cover slip.

without being dried. The habits of this species suggest that this is the most usual kind of diapause in nature. The same is true of other species that often deposit ephippia in permanent bodies of water, e.g. *E. lamellatus, O. gracilis* and *Lathonura rectirostris.*

Propagule protection and dispersal in branchiopods

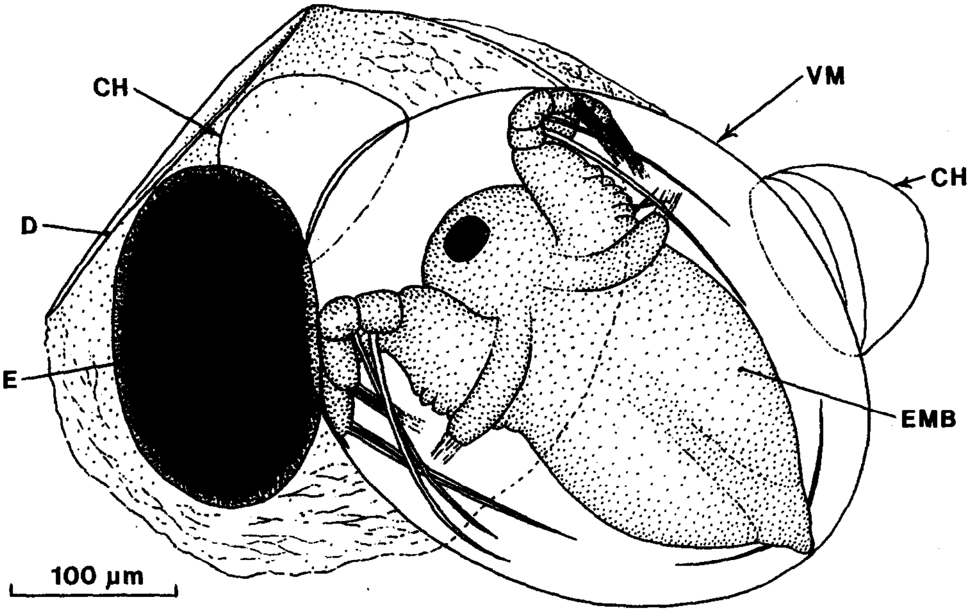
The outer covering of resting eggs and ephippia is obviously protective, but does not prevent the loss of water. It offers protection against abrasion and the attention of small predators. It is also evidently a good protection against the damaging effects of ultra-violet light. Belk (1970) showed that even one day's exposure to full sunlight was sufficient to kill all the eggs of *Eulimnadia antlei* from which the shells had been removed (an operation which does not in itself impair hatchability), but that more than half of those similarly exposed but covered by 1 cm thick glass, opaque to U.V., survived. It is probably significant that anomopod ephippia are often pigmented in the region overlying the eggs, those of daphniids being particularly well protected in this respect.

Drought resistant resting eggs are potential agents of dispersal, and sometimes undoubtedly fulfil this role, but many branchiopods have adopted the tactic of attaching these propagules to firm substrata, which effectively reduces the chances of dispersal. This suggests that it is often more advantageous to ensure persistence of a population in a habitat that has proved favourable than to subject eggs to the hazards involved in chance dispersal to suitable locations elsewhere. Eggs of notostracans are often firmly glued to substrata and some ctenopods and onychopods employ this tactic, but it is the Anomopoda that shows the greatest specialisation in this respect. Several chy-



Figs 14–16. The ephippium of *Lathonura rectirostris.* 14. Ephippium, ventral, cemented to a leaf. 15. The same ephippium, lateral: the leaf, to whose surface its lateral faces lie parallel, is omitted for clarity. 16. The cementing structure seen after detachment from the leaf. Note the moulted cuticle of the post-abdominal claws within the cement.

dorids and macrothricids firmly glue their ephippia to suitable objects, (e.g. see Figs 14–16 for *Lathonura rectirostris*) as first noticed by Schödler as long ago as 1846, and later by various workers. Others — the macrothricid *Ophryoxus gracilis* (Figs 1 and 3) and the chydorid *Leydigia acanthocercoides* — use the old carapace margins as hooks which entangle the ephippia in vegetation, a device employed also by certain species of *Daphnia*. Other daphniid ephippia are scattered freely. Free-shedding of eggs or ephippia is employed by planktonic branchiopods. These mostly sink, but some daphniid ephippia float and are sometimes washed up on the shore in large numbers. Eggs of these planktonic forms sometimes accumulate in vast numbers on the bottoms of lakes. In two small English lakes, Smyly (1977) found between 2 and 5 million eggs of *Holopedium gibberum* per m^2, but many of these were apparently infertile and probably represented the accumulation of several years.



Figs 17. A late embryo of *Streblocerus serricaudatus* which has developed from a resting egg and is near the point of hatching. The embryo is seen somewhat obliquely from the ventral aspect. Note the enormous extent of the osmotic swelling which has distended the vitelline membrane and forced apart the valves of the ephippium. Only the ventral margin of that part of the valve which overlies the embryo is indicated by a dotted line, the embryonated egg being shown as it in fact appears when seen as an optical section.

Induction and breaking of diapause and the hatching process in branchiopods

Although a detailed survey is beyond the scope of this account, brief reference may be made to the problems of what induces and terminates diapause in branchiopods. There are no universal answers. In many temporary pool species all the eggs shed are potentially capable of diapause and if the habitat dries out they enter deep, sometimes prolonged, dormancy, which is usually rapidly terminated when the eggs are again covered by water, though some eggs may not hatch on the first such occasion. Furthermore, eggs, which usually undergo desiccation, may remain hydrated, and enter diapause in this condition, but are easily stimulated to hatch by a rise in temperature. Some resting eggs are inhibited from hatching by darkness, e.g. those of different spinicaudatans (Belk, 1967; Bishop, 1972) and chydorid anomopods (Shan, 1976). In permanent habitats, more subtle stimuli may induce or break diapause though in such planktonic forms as *Leptodora*, *Bythotrephes* and *Holopedium* there may be endogenous rhythms. Thus not all eggs hatch at the same time or temperature and in some lakes where eggs lie in deep water they can scarcely be influenced by changes in light intensity or photoperiod. Another complication is provided by cases such as that of a population of *Holopedium gibberum* which Lampert & Krause (1976) found to produce resting eggs in spring as well as in autumn, which seems to rule out cues provided by temperature or day length. Likewise some populations of *Daphnia pulex* may enter diapause in spring at a time of long day-lengths and rising temperatures, and again in autumn when day lengths are shorter and temperatures falling. In some populations of *Daphnia* however, Stross & Hill (1965) showed a clearly defined relationship between photoperiod and the induction of ephippia-formation, but found that such photo-induction was conditional on population density. Genotypic differences in different populations may also be involved. Such problems are dealt with by Stross in a series of papers (e.g. 1969a, b), part of an extensive literature on the subject.

Hatching follows a basically similar pattern in the resting eggs of all branchiopods. As a result of the osmotic uptake of water, the volume of the space surrounding the nauplius or, in some orders, the juvenile, increases, stretching the inner, elastic embryonic membrane. This ruptures the inelastic outer membrane, often on what appears to be a pre-determined line of weakness (Fig. 17) and, if such is present, the outer egg shell. The ruptured outer embryonic membrane often sits like a pair of caps on the inner membrane but is sometimes readily lost, as for example is the case in

Lepidurus arcticus (Fryer, 1988), which may explain the apparent absence of an outer membrane reported in some species. Final escape by rupture of the inner membrane seems often to be facilitated by movements of the developing nauplius or juvenile.

Differences in details of hatching may be of phyletic interest. For example, the capsule formed by the inner membrane of the ctenopods *Sida*, *Diaphanosoma* and *Holopedium* assumes an elongate, cylindrical shape as it swells prior to hatching as compared with the ovoid shape assumed by its homologue in the Anomopoda. Investigation of other genera is necessary but this difference seems to emphasise the clear distinction of these orders.

Acknowledgments

I am much indebted to Dr N. M. Korovchinsky who kindly translated passages from publications written in Russian. The illustrations were originally prepared for a paper published in 1972. Unknown to the author until they appeared, however, these had been redrawn in a crude and inaccurate manner by a publisher's 'artist'. They are reproduced here from the original, accurate drawings.

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Note added in proof

Only recently did I see a paper by Roessler & Sanchez (1986) in a little known journal, of which a revised version by Roessler (1995) has also appeared. The spinicaudatan *Cyclestheria hislopi* produces an 'ephippium'. This resembles anomopod ephippia in several respects but differs in fundamental particulars and is clearly a parallel development of great interest.

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