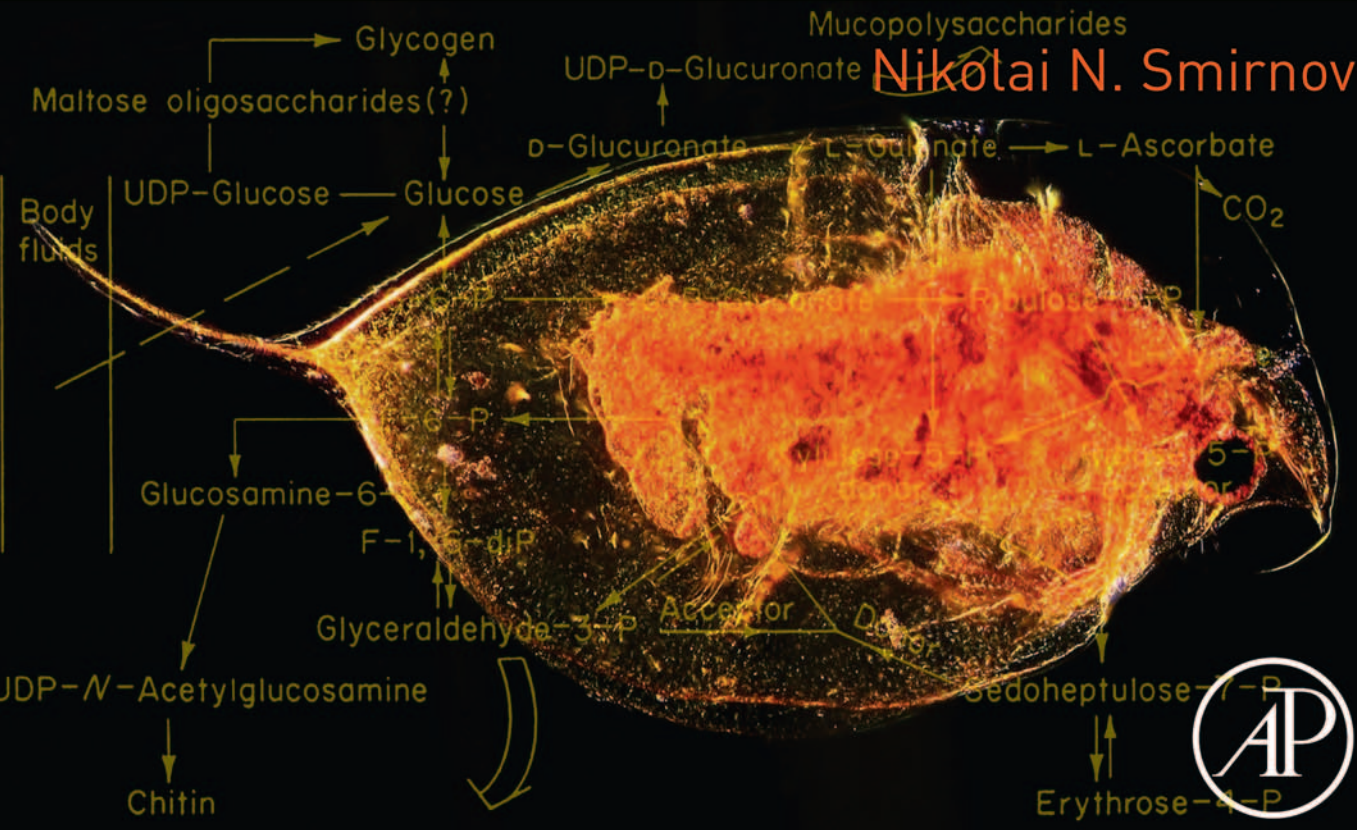
Second Edition

Physiology of the CLADOCERA

Nikolai N. Smirnov



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With Additional Contributions

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18. Notes on the Physiology of Embryogenesis

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Preface

Increasing and universal environmental pollution stimulated numerous recent investigations in the pathological physiology of the Cladocera that brought forth abundant information discussed in the present edition. In addition, studies in normal physiology progressed in various ways.

The structure of the previous edition is only slightly changed. However, new sections are added and numerous new findings are introduced throughout the text. As well, misprints and errors are corrected. The present edition is supplemented mainly with data from sources for recent years.

Reviewing the results of the worldwide investigations one may note that they remain mostly confined to daphnids which live in open water, especially to *Daphnia*, whereas representatives of other families coping with different life conditions are still awaiting investigation. The vast and specific world of littoral and bottom-living Cladocera is still waiting for due physiological assessment. Some data available for such species are discussed as well. Transformations of functions and the related structures in different families would be highly instructive and revealing. Probably, the present review will indicate what and how should further be studied in these animals which live very differently from pelagic species. Especially demonstrative would be, e.g., data on transformations of the system of muscles and of the involved skeletal structures performing different kinds of locomotion in representatives of various genera.

Another special point of studies in Cladocera physiology is that they are still fragmentary: some issues are now clarified and some are almost unknown.

Over 700 species of Cladocera (Crustacea: Branchiopoda) are known and representatives of this group are often dominant in the freshwater fauna, sometimes occurring in enormous quantities. They live in both small and large water bodies from arctic to tropical latitudes, in open water, on the bottom, in mud, among inshore vegetation, in acid pools on bogs, in small accumulations of water in epiphytic plants, in narrow aquatic spaces between moist sand grains. A few species even left the water and live in moist moss-like growth on tree trunks in tropical cloud forests. Some species are specialized for life in saline lakes and in the sea.

Cladocerans, especially *Daphnia* species, belong to the commonest animals in hydrobiology. They are counted, measured, weighed, cultured, their species lists are composed, distribution in space and time described. Their role is recognized as the food resource of fish and as water quality indicators. Species of Cladocera are described and redescribed nowadays in excellent morphological detail.

Usually, little more is taken into consideration than a cladoceran as a living object with individual, mostly external, traits that permit species recognition. There follows an attempt to summarize information showing that the Cladocera possess complicated and special metabolism and behavior which deserve knowing, as these data may explain how and why Cladocera species successfully live in their various media. The following summary is an attempt to contribute to a more profound understanding

of how and why they participate in the processes developing in inland waters. Particular chapters of cladoceran physiology are still covered very unevenly. Recently, numerous and more in-depth data on pathological physiology are being accumulated, comparing with older data that were confined mostly to longevity and amount of progeny as affected by xenobiotics.

Comparative physiological investigations of representatives of various families with their different ecology are urgently desirable and would make a promising field. Of course, sometimes more questions are raised than answers supplied. The present review of this vast field is rather an attempt at systematic assembling of the available data and demonstration of specificity of this group of crustaceans. Within the present context, the main attention is paid to data demonstrating which and how the metabolic links are influenced by particular natural and anthropogenic factors in the hope of revealing the reasons of this impact.

Investigations of aquatic invertebrates frequently endeavor to obtain answers why a certain species is present or absent, why it is abundant, why it lives in a certain habitat. Part of explanation is supplied by morphology (structural traits), e.g., thin skeletons, swimming appendages, oil drops, sometimes the presence of slime, etc., in planktonic forms. However, physiology makes a wide field which can be used for understanding causative relationships.

Daphnia is more and more frequently used in water-quality testing and as a model organism supposed to represent situations in natural or artificial ecosystems (Lampert, 2011; Seda and Petrusek, 2011).

Along with special discussions, introductory remarks are made whenever it seemed to be necessary to make the matter useful both for specialists and for nonspecialists.

The present review comprises studies made in the period starting from the second half of the 19th century. Completeness of information was checked against Zoological Record, part 10 (Crustacea), vols 1−150 (for 2015) and Russian Referativnyi Zhurnal (Biology, Zoology) (1992−2015). Some earlier sources are also added. Any incompleteness herein is because some fields are not yet investigated, some literature has not been found, and some points might have escaped the author's attention.

Acknowledgments

The present volume is an attempt at making a summary of work of many experts throughout the world in various fields using special methods. A substantial contribution to physiology of the Cladocera has recently been made by toxicologists.

Special thanks are due to Dr. M.J. Burgis (United Kingdom) who generously used her time and experience to make the manuscript of the first edition acceptable.

The present review is motivated by the author's observations on living, mostly littoral, cladocerans. Both the initial training and subsequent work of the author implied that hydrobiology is impossible without physiological data. Some recent observations are made at the Hydrobiological Station "Lake Glubokoe" (Russia). The author is grateful to his immediate colleagues from the "Cladocera team" for help and discussions: O.S. Boikova, N.M. Korovchinsky, A.A. Kotov, E.I. Bekker, and A.Y. Sinev. Dr. Kotov critically read the draft manuscript, suggested numerous useful additions, and used his skill for preparation of the manuscript and figures.

The author is obliged to many teachers at school and at the Linguistic University (Moscow) for training in European languages sufficient for direct understanding of original texts. Many librarians, mostly personally unknown to the author, retrieved numerous publications in different languages and times. Their care and labor are appreciated, including those of Ms. N.I. Gotovskaya and Ms. E.V. Morozova the Biological Department Library of the Russian Academy of Sciences (RAS). The facilities and library of the Moscow Society of Naturalists were very useful, especially for earlier sources. Dr. V.R. Alekseev and Ms. N.M. Sukhikh were very helpful in work with resources of the library of the Zoological Institute RAS (St. Petersburg). I am sincerely grateful to Professor G.A. Boxshall (FRS; UK) for help in getting rare publications.

My wife, L.A. Smirnova, Ph.D. (cited here as L.A. Luferova) is tolerant (mostly) toward using a big part of my time for such ventures as this.

Formulation of ideas included in this book and its composition was much favored by creative environment at the Institute of Ecology and Evolution of the RAS, and by personal attention of academicians D.S. Pavlov and Yu.Yu. Dgebuadze.

The authors of Chapters 16–18 described their special fields and made the subject much more advanced and complete.

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Abbreviations and Units

Standard abbreviations and units are used.

ACh	Acetylcholine
ATP	Adenosine triphosphate
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DW	Dry weight
EPA	Eicosapentaenoic acid
FAs	Fatty acids
GST	Glutathione S-transferase
h	Hour
Hb	Hemoglobin
HUFAs	Highly unsaturated fatty acids
IUs	International units
MF	Methyl farnesoate
mg%	mg per 100 g
min	Minute
MUFAs	Monounsaturated fatty acids
NADH	Nicotinamide adenine dinucleotide plus hydrogen
PCBs	Polychlorinated biphenyls
PUFAs	Polyunsaturated fatty acids
RNA	Ribonucleic acid
RQ	Respiratory Quotient
SAFAs	Saturated fatty acids
TBT	Tributyltin chloride
UVR	Ultraviolet radiation
WW	Wet weight

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CHAPTER

1

General

1.1 SYSTEMATIC POSITION

It is now thought that over 700 species of the order Cladocera exist in the world fauna, many of which develop enormous populations and thus play a big role in the biosphere. New species are still being described.

The Cladocera belong to the subclass Phyllopoda of the class Crustacea. Most Cladocera belong to the orders Anomopoda and Ctenopoda. Anomopoda principally comprise the families Daphniidae (e.g., the genera *Daphnia*, *Ceriodaphnia*, *Simocephalus*, and *Scapholeberis*), Moinidae (*Moina* and *Moinodaphnia*), Ilyocryptidae (*Ilyocryptus*), Macrothricidae (e.g., *Macrothrix* and *Streblocerus*), Acantholeberidae (*Acantholeberis*), Ophryoxidae, Eurycercidae (*Eurycercus*), Chydoridae (e.g., *Chydorus* and *Pleuroxus*), Bosminidae (*Bosmina*, *Bosminopsis*). Ctenopoda comprise the families Sididae (e.g., *Sida*, *Pseudosida*, and *Diaphanosoma*) and Holopedidae (*Holopedium*).

Others belong to the order Onychopoda (the freshwater *Polyphemus*, as well as a few marine and brackish-water species) and the order Haplopoda with the family Leptodoridae (*Leptodora*, with two species). Both anomopods and ctenopods produced species living on various substrata and planktonic species. Onychopods and haplopods comprise fewer species, all of which are planktonic predators.

For reliable identification of the subjects in physiological investigations, the keys to the worldwide fauna of Cladocera are available: "Guides to the Identification of the Microinvertebrates of the Continental Waters of the World" issues 1, Macrothricidae (Smirnov, 1992); 3, Ctenopoda (Korovchinsky, 1992), 11, Chydorinae (Smirnov, 1996); 17, *Simocephalus* (Orlova-Bienkowskaja, 2001), 13, The predatory Cladocera (Rivier, 1998); 21, *Daphnia* (Benzie, 2005); 22, Ilyocryptidae (Kotov and Štifter, 2006), and 25, *Eurycercus* (Kotov and Bekker, 2016). There are also newer, general worldwide resources for ctenopods, created by Korovchinsky (2004); *Leydigia* (Chydoridae), by Kotov (2009); and *Eurycercus*, by Bekker et al. (2012); as well as recent regional keys. As investigations into Cladocera are actively developing, the aforementioned summaries are rapidly becoming incomplete, and literature that is more recent should be considered.

1.2 GENERAL MORPHOLOGICAL BACKGROUND

As animal functions are linked to their form, some comments on the body structure and organs of Cladocera are provided here. Most of the animals attributed to the order Cladocera have the same principal structure, with various modifications present in different species. Investigations into comparative and functional morphology (such as, those by Fryer, 1968,

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1974, 1991, etc.) have revealed exciting data on particular species, permitting a better understanding of their lifestyles.

Cladocerans have inherited from their ancestors a weakly segmented body covered with a chitinous, mostly bivalved, shell and bearing few pairs of appendages—antennules, antennae (biramous, with the single exception of female *Holopedium*), mandibles, maxillulae, maxillae (may be completely reduced), mandibles, and five or six pairs of thoracic limbs (Figs. 1.1–1.4). Cladocerans are mostly oval, compressed from the sides, but many are spherical. In the case of *Graptoleberis*, there is a curious and unique combination of lateral and dorsoventral compression. The appendages have numerous, but organized, setae. The posterior end of the body (postabdomen) is bent at a right angle to the abdomen, or may even be reversed. On the proximal dorsal side of the postabdomen, a pair of setae are present, traditionally termed setae natatoriae (shown, e.g., in Figs. 1.3 and 13.1 right). All structures tend to undergo morphological radiation, and

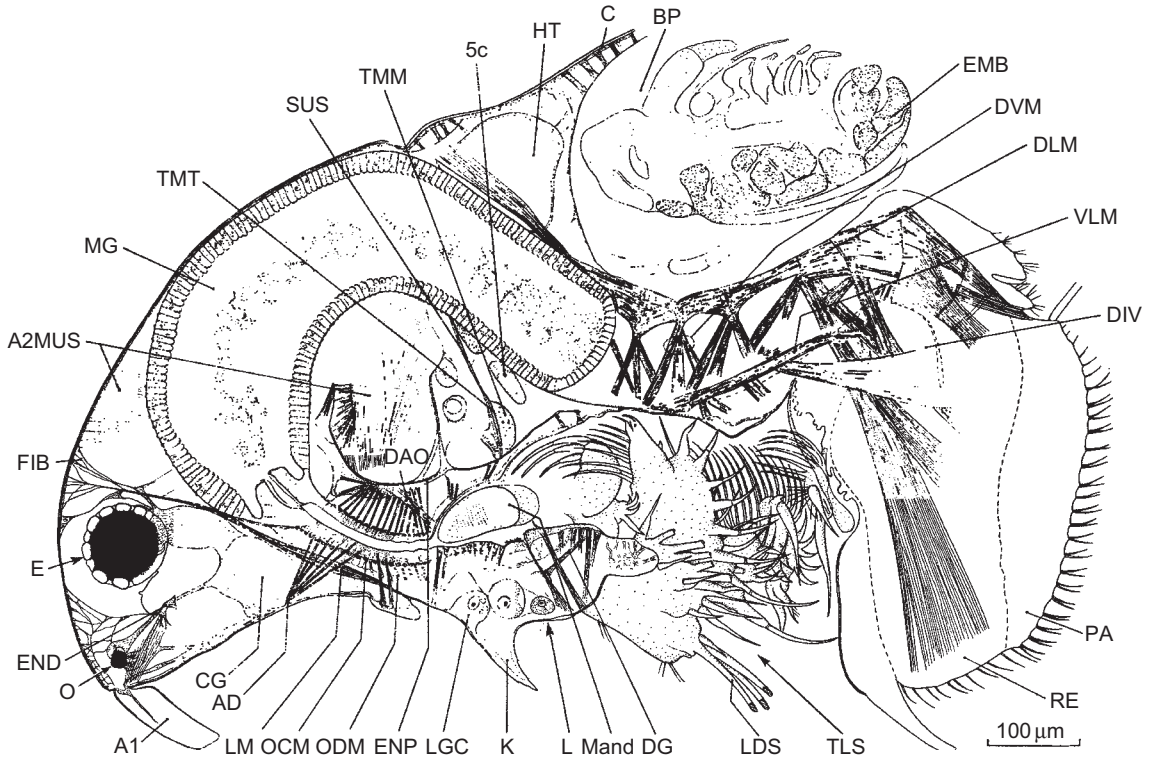

FIGURE 1.1 General anatomy of *Acantholeberis curvirostris*. *A1*, antennule; *A2MUS*, antennary muscles; *AD*, apodeme; *BP*, brood pouch; *C*, carapace; *CG*, cerebral ganglion; *DAO*, dilator muscle of atrium oris; *DG*, duct of labral glands; *DIV*, diverticulum; *DLM*, dorsal longitudinal muscles; *DVM*, dorso–ventral trunk muscles; *E*, compound eye; *EMB*, embryo; *END*, endoskeleton; *ENP*, endoskeletal plate; *FIB*, fibrils; *HT*, heart; *K*, keel of labrum; *L*, labrum; *LDS*, long distal setae of outer distal lobe of limb 1; *LM*, levator muscle of labrum; *Mand*, mandible; *MG*, midgut; *O*, ocellus; *OCM*, esophageal constrictor muscles; *ODM*, esophageal dilator muscles; *PA*, postabdominal lamella; *RE*, rectum; *SUS*, suspensory ligament; *TLS*, trunk limbs; *TMM*, 5c, transverse muscle of mandible; *TMT*, transverse mandibular tendon; *VLM*, ventral longitudinal trunk muscles. *From Fryer, G., 1974. Evolution and adaptive radiation in the Macrothricidae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philosophical Transactions of the Royal Society of London, B: Biological Sciences 269 (898), 137–274.*

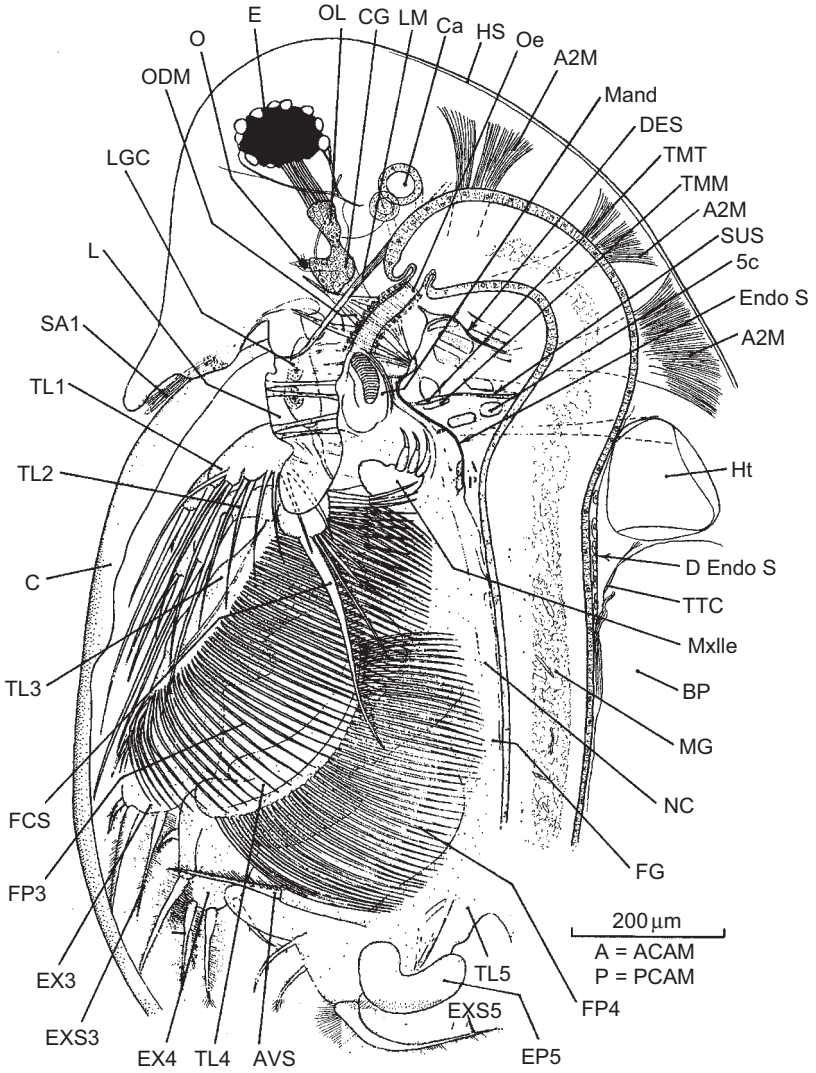

FIGURE 1.2 General anatomy of *Daphnia longispina*. *A*, anterior carapace adductor muscle; *A2M*, antennal muscles; *AVS*, anterior vertical seta of trunk limb 5; *Ca*, caecum; *D Endo S*, dorsal endoskeletal sheet; *DES*, dorsal extension of ventral endoskeletal sheet; *EndoS*, endoskeletal sheet; *EP5*, epipodite of trunk limb 5; *EX3, 4*, exopod of trunk limbs 3, 4; *EXS5*, exopod seta 5; *FCS*, filter-cleaning spine of trunk limb 2; *FG*, food grove; *FP3*, gnathobasic filter plate of trunk limb 3; *FP4*, gnathobasic filter plate of trunk limb 4; *Ht*, heart; *HS*, head shield; *LGC*, labral gland cells; *MxIIe*, maxillule; *NC*, nerve cord; *Oe*, esophagus; *OL*, optic lobe of cerebral ganglion; *P*, posterior carapax adductor muscle; *SA1*, sensory seta of antennule; *TL1, 2, 3, 4, 5*, trunk limbs 1, 2, 3, 4, 5; *TTC*, thickened trunk cuticle. Other abbreviations as in Fig. 1.1. *From Fryer, G., 1991. Functional morphology and adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda). Philosophical Transactions of the Royal Society of London, B: Biological Sciences 331 (1259), 1—99.*

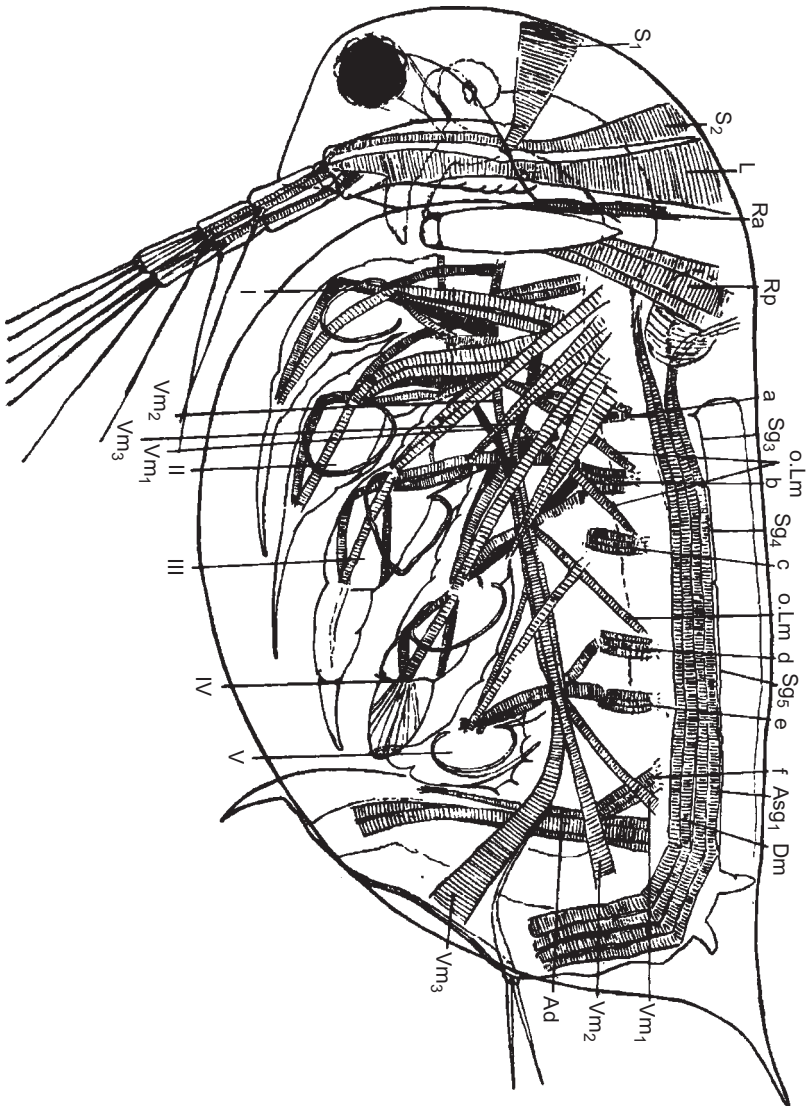

FIGURE 1.3 Muscles of *Daphnia magna*. *From Binder, G., 1931. Das Muskelsystem von Daphnia. International Review of Hydrobiology 26, 54–111.*

homologous structures may occur in different species in various forms, from the ancestral state to their complete disappearance or, in contrast, enlargement, and specialization.

The body in most species is covered by the head shield and valves. The outer surface of the chitinous shell may be smooth, reticulated, ciliated, or variously honeycombed (see, e.g., Kotov, 2013). The head shield of most species exhibits head pores (Frey, 1959; Olesen, 1996), leading to an organ the function of which is probably ion exchange. Littoral cladocerans, which live

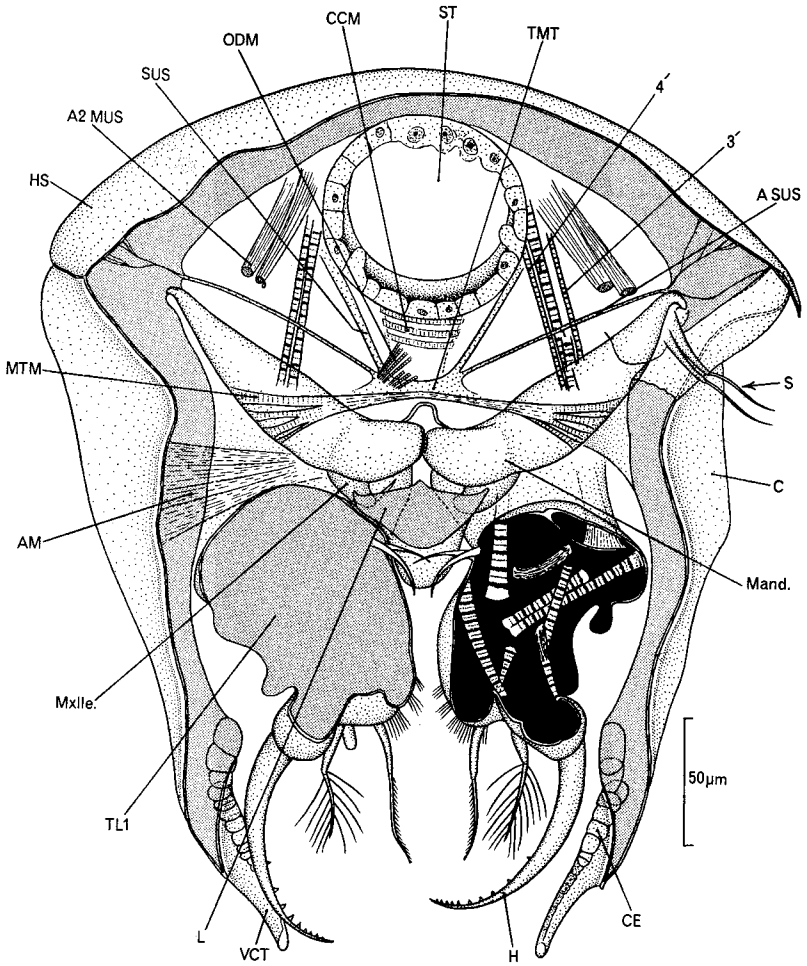

FIGURE 1.4 Transverse section of *Anchistropus emarginatus*. *3′*, promotor roller muscles; *4′*, remotor roller muscles; *AM*, adductor muscle of carapace; *ASUS*, accessory suspensory ligament; *CCM*, circular esophageal constrictor muscles; *CE*, chitinous elaboration within carapace; *HS*, cuticle of head; *MTM*, major transverse muscles of mandibles; *Mxille.*, maxillule; *S*, sensory setae of antenna; *ST*, stomach; *SUS*, suspensory ligament; *TL1*, trunk limb 1; *TMT*, transverse mandibular tendon; *VCT*, ventral carapace tooth. Other abbreviations as in Fig. 1.1. *From Fryer, G., 1968. Evolution and adaptive radiation in the Chydoridae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philosophical Transactions of the Royal Society of London, B: Biological Sciences 254, 221–385, Fig. 115 on p. 341.*

among organic and mineral particles and require protection, are supplied with thick chitinous shells, most with sculpturing; this increases the durability of their shells. In pelagic species, the integuments are thin.

The dorsal space under the shell is the brood chamber into which eggs are laid.

The trunk segments are not numerous and the outer structure is rather simplified.

The inner organs are situated within the body rather loosely. The intestine may be straight or convoluted. Muscles do not form compact masses and most of them can be seen individually (Figs. 1.1–1.4). The largest muscles are

longitudinal bands stretching along the gut. Groups of muscles allow motion of the thoracic limbs and antennae. Small muscles rotate the eye and move the labrum and antennules. The intestine is supplied with circular muscles. The ovary (or testis) is paired and situated ventrally along the gut; this is also where the fat body is situated (Fig. 4.13), in contact with the ovaries (Jaeger, 1935).

There are two paired remains of the coelom: the antennal gland and the maxillary gland (shell gland; Fig. 7.1). The latter is the organ of excretion, whereas the antennal gland has no duct and no outer orifice.

The nervous system comprises a double chain of ganglia, with the brain located in the cephalic region. Nerve fibers reach all structures, including remote ones. Sense organs comprise the unpaired eye, the unpaired ocellus (Figs. 1.1, 1.2, and 13.2), sensory papillae situated on the antennule and on some thoracic limbs (Fig. 13.8), and numerous tactile setae. The eye or the ocellus, or both, may be absent in some species.

Each homologous structure in representatives of various genera is a result of morphological radiation, ranging from the ancestral state to enlargement and specialization or to reduction (sometimes complete disappearance) (Smirnov and Kotov, 2009, 2010). On the basis of the general structural scheme, three kinds of specialization are formed: one used for collecting food from substrata (Fig. 1.1), another for filter feeding in open water (Fig. 1.2), and the last, predatory.

Further information on Cladocera may be obtained from www.cladocera-collection.cz, Lampert (2011); Kotov (1913).

1.3 GEOGRAPHIC DISTRIBUTION

Clear intercontinental differences exist in the composition of the Cladocera fauna. More precisely, zoogeographic regions are discerned as formed due to geologic history (see, e.g., Darlington, 1957). In a general way, they are: Palearctic (including North Africa), Nearctic, Oriental (South Asia), Australian (see Smirnov and Timms, 1983; Van Damme et al., 2007a,b), Ethiopian (Africa south of Sahara), and Neotropical regions. The Cape region is also clearly discerned (Smirnov, 2008). There are good reasons (eight endemic Cladocera species) to discern the Baikal region, as made by Starobogatov (1970) with reference to Mollusca.

With reference to ctenopods, Korovchinsky (2004) suggests the Boreal region with the Palearctic and Nearctic subregions, the Mediterranean–Asian region with the Mediterranean–West Asian and East Asian subregions, the Paleotropical region comprising the South Asian and Australasian subregions, the Central American–South American region comprising the Neotropical and Patagonic–Chilean subregions. Peripheral limits of geographic ranges of particular species are mostly unknown.

Some species are very widely distributed, whereas others are restricted to very small geographic areas (e.g., some endemics of the Cape region). Navigation resulted in cases of transcontinental transfer of Cladocera, for example, of *Bythotrephes* from Europe (Lake Ladoga) to North American Great Lakes (Bur et al., 1986). Circumtropical species obviously prefer high temperature, whereas some northern species do not expand their ranges to tropical latitudes.

Obvious differences exist in the intercontinental and latitudinal distribution of Cladocera species. Some species are clearly circumtropical and occur in latitudes where the limiting factor is a high water temperature. High water temperature is sometimes combined with slight salinity. Some species are confined to northern latitudes or occur in the area of minimum winter temperatures. Differences in the geographic ranges, obvious for many Cladocera species, may be confronted with the geochemical or hydrochemical provinces. It seems that little is done in this promising line.

Studies of the Quaternary history of Cladocera communities by skeletal remains in bottom deposits shed light on their state in the past and trends of development (Frey, 1959, 1962; Berglund, 1986; Smol et al., 2001; Smirnov, 2010; Desellas et al., 2011).

1.4 SPECIES-SPECIFIC EFFECT OF XENOBIOTICS

Different sensitivity to toxic substances was reported for different Cladocera species. Immobilization by copper tested for 44 species was different: EC_{50} (effective concentration determined in 48 h) was from 5.3 for *Scapholeberis mucronata* to 70.6 μg Cu/L for *Disparalona rostrata* (Bossuyt and Janssen, 2005). Comparative tests of Cd and Zn on *Daphnia magna*, *Daphnia pulex*, *Daphnia ambigua*, and *Ceriodaphnia dubia* demonstrated that *D. magna* is significantly more tolerant to these metals, or their combinations, than other daphnids (Shaw et al., 2006).

It was shown that *Moina macrocopa* was twice more sensitive than *D. magna* in 7-day toxicity test to perfluorooctane, sulfonic acid, and perfluorooctanoic acid (Ji et al., 2008). The highest sensitivity to the same concentrations of carbaryl and methomyl (carbamate insecticides) was manifested by *Ceriodaphnia reticulata*, the lowest—by *M. macrocopa* and *Scaphleberis kingi* (Mano et al., 2010). *D. magna* is more severely affected than *D. pulex* by diflubenzuron (Duchet et al., 2011). Sensitivity to insecticides imidacloprid and fipronil was different (in descending order): in *Ceriodaphnia*, *Moina*, and *Daphnia* (Hayasak et al., 2012).

D. magna was less sensitive than *Daphnia curvirostris* to veterinary antibacterials (Bona et al., 2014).

Mechanisms of reaction to toxic blue-green algae were found to be different in *D. magna* and *D. pulex* (Asselman et al., 2014).

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CHAPTER

2

Methods

Methods of investigating the physiology of Cladocera range from direct observation of living specimens to recording the physical and chemical manifestations of particular physiological processes.

2.1 METHODS OF COLLECTION

Cladocera may be collected with planktonic nets and dip nets in the littoral and pelagic zones of large and small water bodies. They should be looked for in ponds, pools, puddles, temporary pools, roadside ditches, acid bogs, fountains, all kinds of artificial basins, moist moss, and even in moist, moss-like growth on tree trunks. Usually, a catch of a planktonic net contains many specimens belonging to several genera. However, sometimes Cladocera may be absent or rare. The latter situation occurred, for example, in water bodies of the Yucatan; though the faunal list was rather long, large volumes of water had to be screened to collect a few specimens (Smirnov and Elias-Gutierrez, 2011). Quantitative sampling (per unit volume or unit surface) with special devices is used in limnology.

2.2 OBSERVATION OF LIVING SPECIMENS

Detailed examination of preserved or living specimens supplies useful information on their anatomical background and function, as well as on the composition of food in the intestine. Intravital staining aids observation of functioning in living cladocerans. Various kinds of intravital staining of the organ systems of Cladocera were originally elaborated by Fischel (1908). The salivary gland can be stained using neutral red and Bismarck brown (Cannon, 1922). In thoracic limb IV of *Eurycercus*, there is a slime gland the secretions of which are stained bright blue with Mallory's stain (Fryer, 1962, 1963). Gut contents have been stained with eosin, Congo red, methyl red, neutral red, and uranine for the determination of pH (Lavrentjeva and Beim, 1978). External slime may be distinguished by placing a cladoceran in diluted Indian ink. Histochemical techniques have been used in the analysis of *Holopedium* slime (Brown, 1970).

2.3 CULTIVATION

Cultures started from a single female are called clonal cultures and provide relatively uniform material. Culture methods, mostly for *Daphnia* and *Moina*, have been described by various authors either alone (e.g., Biotechnics of *Daphnia* Culture at Fish Farms, 1958; Dewey and Parker, 1964; Ivleva, 1969; Parker and Dewey, 1969; Bogatova, 1973, 1980; Lampert, 1975; Ten Berge, 1978; Goulden et al., 1982; Dodson et al., 1991) or in descriptions of particular

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experiments. Cultured pelagic Cladocera should be fed with algae and adequate algal cultures should be maintained for this purpose. Some species (*Daphnia magna* and *Daphina pulex*) are more easily cultivated than others.

Gajewskaja (1940, 1948) suggested a method of separating large-scale cultures of Cladocera and algae because cultivation of Cladocera and algae requires conflicting conditions. Since then, methods of large-scale culture have been developed further (Yalynskaya, 1961; Ivleva, 1969; Lampert, 1975; Bogatova, 1992). The combined culture of *Daphnia* with other cladocerans was suggested by Bogatova (1963).

A method was described for cultivation of *Pleuroxus hamulatus* individually in small vessels, frequently supplied with fresh food (Galtsoff et al., 1937, p. 220). A similar method was used for littoral Cladocera by Smirnov (1964, 1965a). Fresh detritus was given at least every other day (actually, a specimen was transferred to a dish containing fresh detritus). Organic debris (detritus) was collected at shore bottoms and screened to remove foreign animals.

Artificial detritus prepared from plants was also used in cultivation of Cladocera (see, e.g., Rodina, 1963; Esipova, 1969, 1971; Dekker et al., 2006). Sterilization was used to demonstrate the role of bacteria in the alimentation of Cladocera (Gajewskaja, 1938; Rodina, 1963; Esipova, 1969, 1971). The latter author used a diluted Lugol's solution to minimize the quantity of bacteria in food offered to Cladocera.

Suspensions of latex beads have also been used in investigations of feeding behavior (Burns, 1968b, 1969; Hessen, 1985).

Preferences for environmental factors, and for food, may be ascertained by experiments on living specimens. Examination of food composition is made easier by dissolving the soft tissues of cladocerans with 3% sodium hypochlorite (Infante, 1978). This treatment may also be useful for other purposes.

2.4 IMMOBILIZATION AND ATTACHING

Most cladocerans are very agile; therefore, high-speed photography has been used (e.g., Storch, 1929; Zaret and Kerfoot, 1980) to study them. Alternatively, methods to inhibit their movement have been devised. To retard quick motion, Fryer (1968) immersed living cladocerans in an nontoxic viscid medium ("cellulose nitrate"). Immobilization by narcotization is discussed in more detail in Section 12.5. Early techniques included attaching specimens to a substrate on a glass slide using wax dissolved in alcohol (Scourfield, 1900b; Peñalva-Arana et al., 2007). Porter and Orcutt (1980) used silicone grease to fix *D. magna* by its head shield to observe its feeding. For the purposes of his study, Jacobs (1980) attached *Daphnia* by the caudal spine to a plasticine bed. Specimens thus immobilized could be observed and the next instar was released into free water from the attached exuvium.

Using cyanoacrylate glue, Onbé (2002) attached *Pseudevadne* and *Evadne* to the tip of a glass capillary held in place by a stand to facilitate video recording. Peñalva-Arana et al. (2007) reported unbiased observations using computer recording combined with the immobilization system.

Methods of attachment were recently described by Seidl et al. (2002) and used by Pirow et al. (2004) for investigating oxygen transport processes in *D. magna*. The latter authors immobilized fasting animals by gluing their posterior apical spine with histoacryl to a bristle, which was then fixed to a coverslip with plastilin. Dye was then microinjected into the circulatory system from the dorsal side into the space "directly downstream of the heart." The coverslip then formed the base of a thermostated perfusion chamber in which an immobilized daphnid was able to freely move its antennae.

Ivanova and Klekowski (1972) achieved immobilization of *Simocephalus* by placing it into the bulb of a Cartesian diver (used for determining oxygen consumption) and leaving no free space around the animal, that is, it was too small to allow movement. Photographic recording of the heart rate and movement of thoracic limbs has been used (e.g., Kolupaev, 1988). Further on, computer recording of behavior is now used (Peñalva-Arana et al., 2007).

2.5 MICROSCOPY

Various kinds of microscopy, including scanning electron microscopy (SEM), can be used in investigations of Cladocera (Kotov, 2013). A variation of cladoceran preparation for SEM was suggested by Laforsch and Tollrian (2000). Modern video microscopy and digital image processing methods take advantage of the transparency of cladocerans (Colmorgen and Paul, 1995). Fluorescence analysis was originally used by Pravda (1950).

Surgical methods can be applied in investigations of regeneration, vision, and neurosecretion. These are described by Ermakov (1927), Angel (1967), and in Chapters 10 and 13.

2.6 BIOCHEMICAL AND SPECIAL PHYSIOLOGICAL METHODS

In biochemical and metabolic investigations, special procedures, such as homogenization (e.g., Guan and Wang, 2004b) and radiotracer or chemical methods, have been used. There are various respirometric methods (Fig. 2.1. See also Chapter 5). Studies on homogenates using modern sensitive methods have opened up the possibility of investigating metabolic pathways. Special techniques may be found in descriptions of the original investigations.

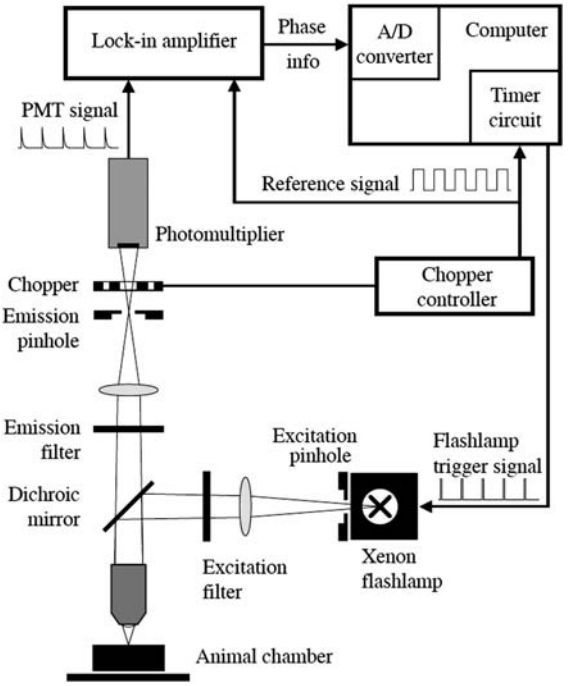

FIGURE 2.1 The microscopic setup for partial pressure of oxygen (pO_2) imaging. *A/D*, analog/digital; *PMT*, photomultiplier tube. *From Pirow, R., Wollinger, F., Paul, R.J., 1999a. The importance of the feeding current for oxygen uptake in the water flea Daphnia magna. Journal of Experimental Biology 202 (5), 553–562, Fig. 2.1 on p. 555.*

Spectrophotometry has been used for the identification of particular compounds, including hemoglobin (Hb) (Karnaukhov et al., 1986). Initially, Fox (1948) suggested that a quantitative estimation of Hb content in *Daphnia* in arbitrary units could be obtained against a wedge-shaped standard prepared from the worker's blood. Following this, cladoceran Hb was investigated using spectral (Hildemann and Keighley, 1955; Hoshi et al., 1968) and chemical (Hoshi, 1963a, 1963b; Smirnov, 1970) methods.

Recent toxicological studies have assessed the effects of xenobiotics on particular physiological processes.

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CHAPTER

3

Chemical Composition

3.1 LABILITY OF CHEMICAL COMPOSITION

The chemical composition of the Cladocera is labile. In their body, relative quantities of physiologically important constituents, those of no such importance, or of xenobiotics vary depending on the composition of the environment and of food. Cladocera may accumulate useless or toxic substances. Furthermore, the chemical composition fluctuates in the course of the molting cycle.

3.2 MOISTURE CONTENT AND CALORIFIC VALUE

The moisture content and calorific value of some Cladocera spp. are shown in Table 3.1. Moisture content is usually within 80–90% and calorific value within 3–6 kcal/g dry weight (DW). According to Sushchenya et al. (1990), the dry matter content in *Daphnia magna* ranges from 7.4% to 10.6%, increasing at higher temperatures and higher food concentrations.

The calorific value (kcal/g) is: *Daphnia hyalina*, 6.3; *Bosmina coregoni*, 6.3; *Chydorus sphaericus*, 6.1; and *Leptodora kindtii*, 5.8 (Vijverberg and Frank, 1976). Variations in the calorific value obviously depend mostly on the fat content. Thus, the calorific value of "lean" *D. magna* is only 60% of that of *Daphnia* containing more fat (Chalikov, 1951).

The caloric content also changes in cladocerans exposed to xenobiotics. Thus, it somewhat decreased in *Daphnia schodleri* exposed to hexavalent chromium (as potassium dichromate) due to restructuring of its chemical composition (Arzate-Cárdenas and Martínez-Jerónimo, 2012).

3.3 PRINCIPAL CONSTITUENTS

There have been various determinations of the chemical composition of some Cladocera (daphnids, *Moina*, *Bosmina*, and *Chydorus sphaericus*). The general chemical composition of some Cladocera is shown in Table 3.2. The protein content ranges from 30% upward, the fat content is 1–20%, and the carbohydrate content is 10–30%. Far more informative are reports on the dynamics of chemical composition, which depend on seasonal changes in a number of factors, on starvation, or on other particular factors. Often, scattering of the data indicates dependence on specific factors. Indeed, if arranged by season, chemical constituents demonstrate clear composition changes, as shown, e.g., for *Daphnia pulicaria* (in Fig. 3.2, from Heisig-Gunkel and Gunkel, 1982). The chemical composition of *Daphnia pulex* generally confirms these data but depends on the period of starvation, with relative quantities of carbohydrate and fat decreasing, and those of protein and ash increasing (Fig. 3.1) (Lemcke and Lampert,

http://dx.doi.org/10.1016/B978-0-12-805194-8.00003-9

TABLE 3.1 Moisture Contents and Calorific Values of Some Cladocera spp.

Species	Moisture Content (%)	Calorific Value (kcal/g DW)	Ash (% DW)	References
Bosmina longirostris	89	6.5	–	Sherstyuk (1971)
Bosmina longispina	–	9.9	4.8	Romanova and Bondarenko (1984)
Bythotrephes longimanus	84.7	8.6	5.2	Romanova and Bondarenko (1984)
Ceriodaphniaaffinis, adults	89.2	–	–	Stepanova (1967)
Ceriodaphnia affinis, juveniles	90.4	–	–	Stepanova (1967)
Ceriodaphnia pulchella	70–82	4–4.7	–	Sherstyuk (1971)
Ceriodaphnia quadrangula	–	4.9	4.6	Riccardi and Mangoni (1999)
Ceriodaphnia reticulata	87–96	2.4–5.5	7.2–23	Bobiatyńska-Kwok (1970) and Bogatova et al. (1971)
Daphnia cucullata	–	5.1–5.4	10.6–14.0	Riccardi and Mangoni (1999)
Daphnia hyalina	–	4.9–5.0	12.0–12.5	Riccardi and Mangoni (1999)
Daphnia longispina	82.6	4	22	Romanova and Bondarenko (1984)
Daphnia magna	86.4–95.6	2.4–5.6	–	Karzinkin (1951), Ostapenya et al. (1968), Schindler (1968), Bogatova et al. (1971), Stepanova (1968), Stepanova et al. (1971), Mityanina (1980) and Sushchenya et al. (1990)
Daphnia pulex	89–95	2.8–4.9	7.6–25	Birge and Juday (1922), Ivlev (1939), Karzinkin (1951), Malikova (1953), Ostapenya et al. (1968) and Stepanova (1974)
Bythotrephes longimanus	–	5.2	5.6	Riccardi and Mangoni (1999)
Leptodora kindtii	97.2	4.6	–	Sherstyuk (1971)
Moina macrocopa	95	5	–	Bogatova et al. (1971)
Moina spp.	87–88	4–4.3	–	Ostapenya et al. (1968), Stepanova (1974), and Zhao et al. (2006a,b)
Polyphemus pediculus	80–86	4.4–4.9	–	Sherstyuk (1971)
Sida crystallina	84	5.3	–	Sherstyuk (1971)
Simocephalus vetulus	82–92.4	3.65–4	–	Sherstyuk (1971), Stepanova (1968), and Stepanova et al. (1971)
Eurycercus lamellatus	80–88	4.1–4.3	–	Sherstyuk (1971)

TABLE 3.2 Composition of Some Cladocera spp. in % DW

Species	Carbon	Hydrogen	Nitrogen	Carbohydrate	Protein	Lipid	Author
Ceriodaphnia quadrangula	49.1	7	9.8	–	54.4	5.3	Riccardi and Mangoni (1999)
Daphnia cucullata	53.3–54.5	7.8–8	10.1	14.6	57.0–62.8	19.8–22.7	
Daphnia hyalina	50.2–52.3	7.6	11	21.3–22.9	62.2–64.0	13.1–16.5	
Daphnia pulex	–	–	–	3.3–10.9	36.4–61.7	2.8–27.9	Birge and Juday (1922)
D. pulex	–	–	–	1.13	60.4	21.8	Malikova (1953)
D. pulex	–	–	–	6.4	63.4	8.6	Stepanova (1968)
Moina brachiata	–	–	–	8.2	63.4	17.5	
Simocephalus vetulus	–	–	–	16.7	52.3	11.5	
Bythotrephes longimanus	51.2	7.6	11.1	22.0	63.9	14.1	Riccardi and Mangoni (1999)

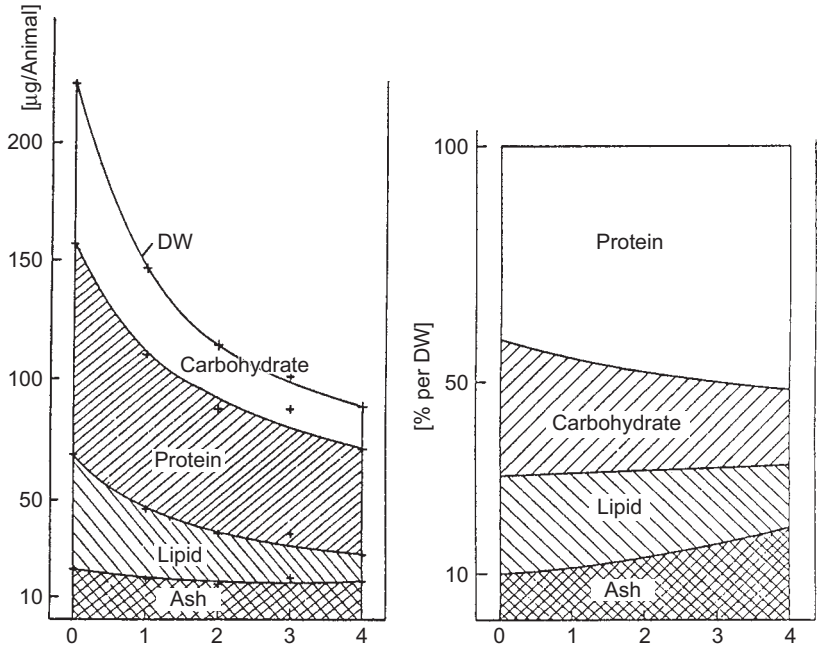


FIGURE 3.1 Chemical composition of *Daphnia pulex* and changes caused by starvation (left, per animal; right, % DW). Horizontal axis, days of starvation. *From Lemcke, H.W., Lampert, W., 1975. Changes in weight and chemical composition of* Daphnia pulex *during starvation. Archiv. für Hydrobiologie 48 (1), 108–137.*

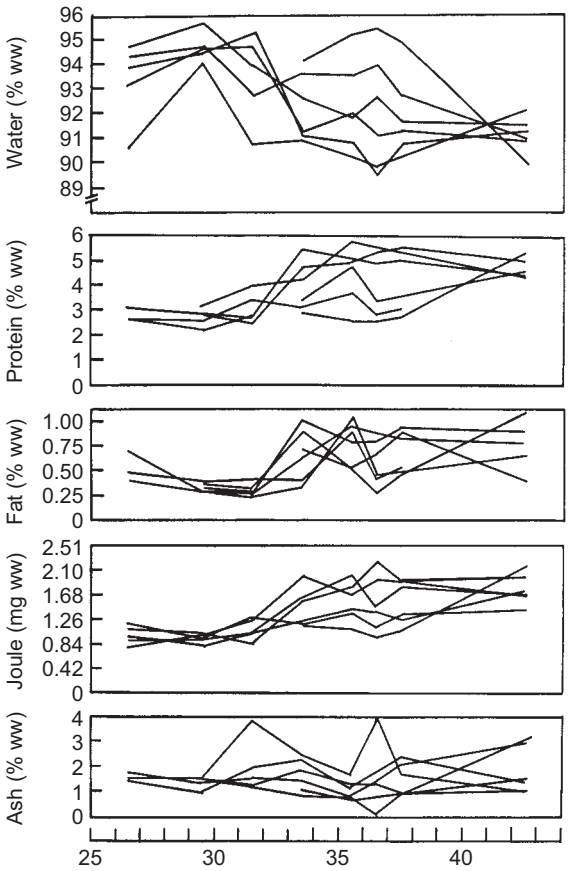


FIGURE 3.2 Seasonal changes of chemical composition of *Daphnia pulicaria* in six ponds, beginning from June. *From Heisig-Gunkel, G., Gunkel, G., 1982. Distribution of a herbicide (atrazin, s-triazine) in* Daphnia pulicaria*: a new approach to determination. Archiv für Hydrobiologie, Supplement 59 (4), 359–376.*

1975). Seasonal variations in the biochemical composition of *D. magna* (in Luxembourg) were studied by Cauchie et al. (1999). The variation (in mg/g DW) was: protein from c. 140 to 400; lipids, 120–180; chitin, 40–70; carotenoids, 70–230; and ash, 100–340.

To understand these variations, data on the dynamics of the chemical constituents in relation to growth are also useful. McKee and Knowles (1987) studied the levels of protein, glycogen, lipid, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) during the growth of *D. magna*. They found that the protein content (percentage DW from the first to the 21st day of life) varied within the range of 48–62% (maximal on day 8); glycogen content steadily increased from 2.4% to 7.5%; lipid content varied from c. 18% to c. 15%, decreasing to 6.4% on day 21. In addition, RNA content varied from 8.6% to 6.6%, decreasing to 4% on day 21, and DNA content generally decreased from 0.20% to 0.14%.

The chemical composition of *D. magna* is much influenced by the chemical composition of its food (Stepanova et al., 1971). Thus, the highest content of protein, fat, and carbohydrate was found in *Daphnia* fed on yeast.

3.3.1 Protein

The protein content of *Daphnia* and *Ceriodaphnia* increases with increased protein in their natural food (Guisande et al., 1991). It has also been shown that during culture the protein and lipid contents of *Moina macrocopa* decreased in comparison with those of the initial culture, from about 25 to 0.56 mg/g WW to about 18 and 0.26–0.33 mg/g WW, respectively (Romanenko et al., 2004).

The protein content was shown to decrease during chronic exposure of *D. magna* to chlordecone (an organochloride insecticide) (McKee and Knowles, 1986).

Amino Acids. The amino acid content of various Cladocera is shown in Table 3.3. According to Malikova (1953, 1956), the content of amino acids in *D. pulex* is (% of total protein): tyrosine 4.27, tryptophan 3.62, arginine 10.92, histidine 2.69, cystine 1.17, methionine 3.45. The amino acid composition of *D. magna*, *Ceriodaphnia reticulata*, and *Chydorus sphaericus* was determined by Sadykhov et al. (1975).

In the carotenoprotein complexes, the following predominant amino acids were found:

TABLE 3.3 The Content of Different Amino Acids in Some Cladocera spp.

Amino Acid	*Bosmina longirostris* (% protein) (Verbitsky, 1990)	*Daphnia magna* (mg/100 g WW) (Stepanova and Naberezhnyi, 1972)	*Daphnia pulex* (% total amino acids per DW) (Dabrowski and Rusiecki, 1983)	*Daphniopsis tibetana*, (g/100 g protein) (Zhao et al., 2006a)	*Ceriodaphnia* spp. (% total amino acids per DW) (Dabrowski and Rusiecki, 1983)	*Simocephalus vetulus* (mg/100 g WW) (Stepanova and Naberezhnyi, 1972)	*Moina mongolica* (g/100 g protein) (Zhao et al., 2006a,b)
Phenylalanine	1.3	228–833	3.76	1.79	3.82	107–157	3.40
Tyrosine	3.1	–	4.05	3.55	3.81	60	2.80
Leucine	3.3	222–725	3.74	5.76	4.61	425–625	5.30
Isoleucine	2.1	–	2.24	3.68	2.43	–	3.40
Methionine	–	–	1.44	3.64	1.40	–	1.50
Valine	2.9	–	3.71	3.78	3.49	–	3.90
Alanine	2.8	157–438	3.95	6.72	4.57	283–342	4.30
Glycine	2.6	194–239	2.85	3.91	2.69	236–296	3.20
Proline	2.2	211–358	2.53	4.53	2.76	100–250	2.70
Glutamic acid	5.0	317–458	5.46	7.40	6.29	522–619	8.00
Serine	1.8	205–357	2.57	3.27	3.07	255–285	3.00
Threonine	2.6	121–279	2.93	4.00	2.99	121–164	3.20
Aspartic acid	3.2	365–474	5.22	7.46	5.94	318–371	6.40
Arginine	1.9	200–401	3.40	2.85	3.45	244–348	4.30
Histidine	1.5	–	1.25	1.64	1.46	–	1.20
Lysine	3.8	–	3.78	4.78	4.36	–	3.40
Cystine	0.7	275–423	0.71	0.49	–	100–127	0.80
Tryptophan	–	–	–	0.43	–	–	1.20

in *D. magna*, alanine, glutamine, glycine, and leucine (Czeczuga, 1984); in *Moina micrura*, asparagine, glutamine, and glycine (Velu et al., 2003). The presence of free intracellular amino acids in *D. magna* was shown by Gardner and Miller (1981).

It was determined that in *Daphnia*, the amino acid composition during ontogeny is rather constant (Brucet et al., 2005). However, it may vary rather widely depending on the culture conditions, as has been shown for *D. magna* (Stepanova and Naberezhnyi, 1970, 1972) and *Moina* spp. (Kokova, 1982). In well-fed *Daphnia*, the content of amino acids is 6.29 g% WW (i.e., 6.29 g/100 g of wet weight), whereas in "lean" *Daphnia* it is 3.12–3.35 g% WW.

Amines. The following amines are found in *D. magna* (Ehrenström and Berglind, 1988):3,4-dihydroxyphenylalanine, dopamine, noradrenaline, adrenaline, tyramine, epinine, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid, L-tryptophan, 5-hydroxytryprophan, 5-hydroxytryptamine, and 5-hydroxyindolacetic acid. Diurnal variations of the contents are recorded for 3,4-dihydroxyphenylalanine, dopamine, and 3,4-dihydroxyphenylacetic acid (the catechols). The variations are recorded in *Daphnia* exposed to 12 h of continuous illumination.

3.3.2 Carbohydrates

Carbohydrates comprise glycogen and chitin.

Glycogen. Glycogen is a polysaccharide consisting of glucose bound to protein. Most likely, Smith (1915) was the first to demonstrate distribution of glycogen stained with neutral red over the gut and thoracic limbs of *Daphnia* females, in contrast to fat (Fig. 3.4). The content of glycogen was determined by Blazka (1966) (% of total composition, WW): 23% in *Bosmina longirostris*, 53% in *Ceriodaphnia reticulata*, 1–36% in *Daphnia* spp., and 33% in *Simocephalus* sp. Glycogen is present in developing embryos. The glycogen content in *Simocephalus vetulus*

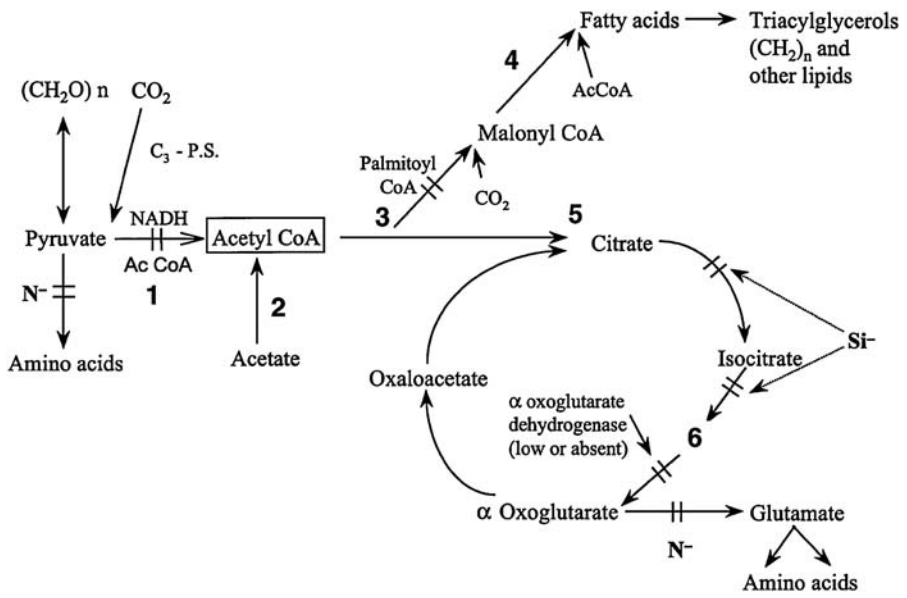


FIGURE 3.3 Schematic pathway from initial photosynthetic saccharides to lipids and amino acids in algae. C_3-*P.S.*, photosynthesis. *From Arts, M.T., Robarts, R.D., Evans, M.S., 1997. Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake. Freshwater Biology 38 (3), 525–537.*

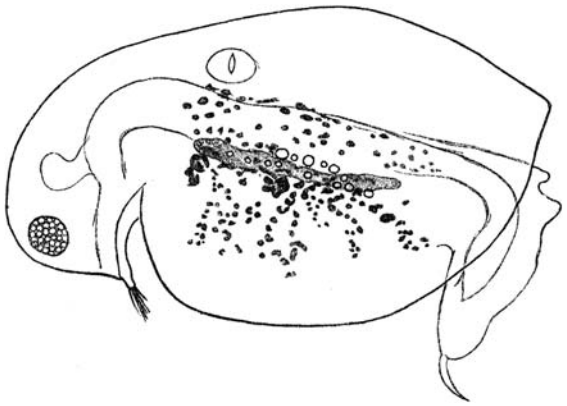


FIGURE 3.4 Glycogen in the body of *Daphnia* stained red (shown as dark patches). Fat globules are shown as circles. *From Smith, G., 1915. The life-cycle of Cladocera, with remarks on the physiology of growth and reproduction in Crustacea. Proceedings of the Royal Society of London B 88, 418–435, Fig. 3.5 on p. 425.*

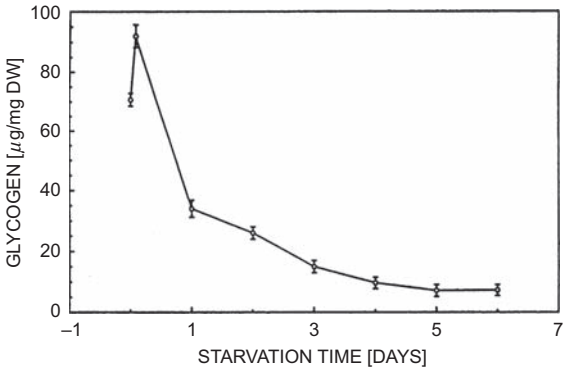


FIGURE 3.5 Change of the glycogen content of *D. magna* during starvation. *From Elendt, B.P., 1989. Effects of starvation on growth, reproduction, survival and biochemical composition of* Daphnia magna. *Archiv für Hydrobiologue 116 (4), 415–433, Fig. 3.7a on p. 423.*

was determined to be 0.7% in the gastrula, 0.91% in the nauplius, 0.71% in released young, 1.2% in the third instar, and 2.23% in the fifth instar (Hoshi, 1953).

In *D. magna* exposed to tetradifon at 0.44 mg/L during 120 h the content of glycogen was 0.24 µg/individual versus 0.64 in the control (Villarroel et al., 2009).

Chitin. Chitin is a major structural component of arthropods. The polysaccharide chitin is a polyacetylglucosamine [β-(1-4)-linked homopolymer of *N*-acetyl-D-glucosamine]. The acetamide group CH_3CONH is present in the chitin molecule. The chitinous covers of cladocerans are strong and nonwettable. In Cladocera, like in other Crustacea (Hohnke and Scheer, 1970), chitin is formed and secreted by the hypodermis underlying the shell. Secretion of chitin in the wound healing was studied by Anderson and Brown (1930) who found that chitin secretion starts 60% of the way through the intermolt period.

In resting eggs of *Ceriodaphnia quadrangula* 16–17% DW of chitin and 11% DW of chitosan are found (Kaya et al., 2014). Chitosan is a derivative of a linear polysaccharide, the macromolecules of which consist of randomly bound β-(1–4)-D-glucosamine units and *N*-acetyl-D-glucosamine.

Cladocera, being abundant in nature, produce enormous quantities of chitin. The content of chitin in the body of *Daphnia* is approximately 15% (Chalikov, 1951) or 7% DW (Andersen and Hessen, 1991). In *D. magna*, it is 2.9–7% DW (Cauchie et al., 1995) or c. 30–70 mg/g DW (Cauchie et al., 1999). The total annual chitin production by various cladocerans in Europe is estimated as: *D. magna*, 11.5 g/m^2 (4.6 g/m^3); *Daphnia galeata*, 3.2 g/m^2 (0.16 g/m^3), *Daphnia hyalina* and *Daphnia cucullata* combined, 0.14–0.30 g/m^2 (0.09–0.2 g/m^3) (Cauchie et al., 1995).

In contrast to chitin from copepods, chitin from dead Cladocera is not decomposed in bottom sediments (or at least is not fully decomposed). After their death, it accumulates at the bottom of water bodies, sometimes forming a high proportion of the total mass of the sediment. Bottom sediment with dominant chitinous remains was termed *chitin gyttja* by Lundqvist (1927).

Chitin is not derived directly from food: it must be formed by the process of metabolism. Although chitin is specific to arthropods and abundantly accumulated by cladocerans, the metabolic pathways of chitin formation were not clearly described. For insects, Kuznetsov (1948, p. 336) noted that "there are no actual data on metabolism yielding chitin from the cycle of transformations; even more, there are no data on the metabolism intermediate as to chitin." It was noted by Hackman (1964, p. 499) that "the biological synthesis of chitin in insects (or other animals and plants) has received little attention."

In other crustaceans, chitin is formed from glucosamine chiefly derived from glycogen, with the acetyl group furnished by oxidation of fatty acids (Vonk, 1950; Hohnke and Scheer, 1970). The latter authors suggested a general scheme of crustacean carbohydrate metabolism yielding, inter alia, chitin (Fig. 3.6).

An inhibitor of chitin synthesis, larvicide diflubenzuron is highly toxic for *D. magna*, Median Effective Concentration at 48 h exposure is 0.06 μg/L (Abe et al., 2014).

3.3.3 Mucopolysaccharide (Slime)

Slime may abundantly surround the animal (as is the case with common forms of *Holopedium*) or make a thin layer over the body of a cladoceran (except antennae) (as in *Sida* and *Ophryoxus gracilis*) (Montvillo et al., 1987). The chemical composition of slime was determined for the jelly capsule of *Holopedium* (Brown, 1970). Sulfated mucopolysaccharide was found to be present, as well as mucopolysaccharide modified by carboxyl groups. It has been suggested that the jelly capsule is produced by the mechanism that produces the exoskeleton at each molt.

Slime is also produced by salivary glands situated within the labrum (Fig. 4.1) and stored in

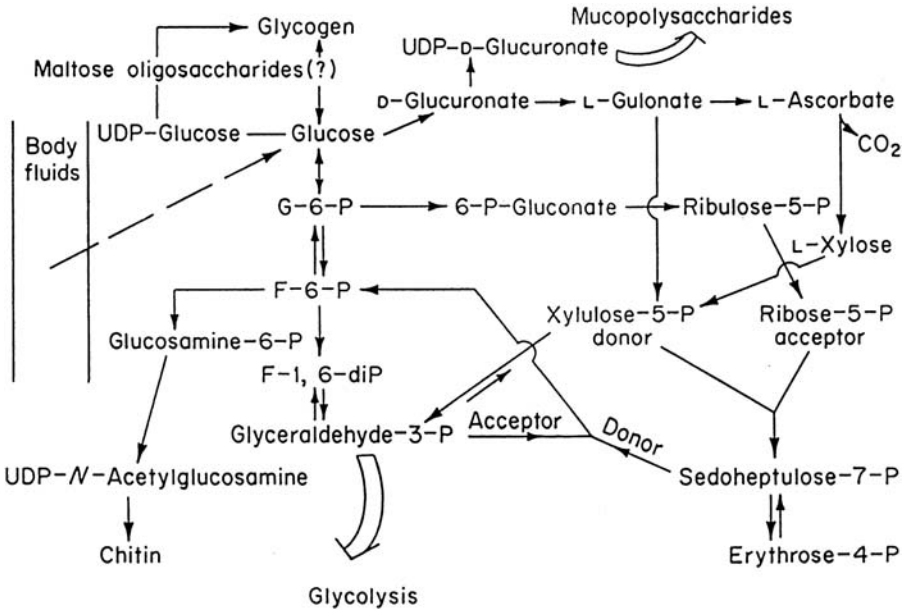


FIGURE 3.6 Carbohydrate metabolism in Crustacea. *UDP*, uridine diphosphate. *From Hohnke, L., Scheer, B.T., 1970. Carbohydrate metabolism in Crustacea. Chemical Zoology, vol. V. Arthropoda, Part A. Academic Press, New York, London, pp. 147–197.*

paired reservoirs letting the secretion into the lumen of the esophagus. Slime glands are found also in some thoracic limbs (Fig. 4.2), as has been shown for *Eurycercus* (Fryer, 1962, 1963) and for *Alonopsis elongata* (Fryer, 1968), but not yet for other cladocerans. The salivary glands may be shown by intravital staining with neutral red or Bismarck brown (Cannon, 1922).

3.3.4 Phosphorus-Containing Substances

The content of phosphorus in *D. pulex* was determined as 1.49% DW (Malikova, 1953). In *D. galeata*, 35–69% of the total phosphorus content is associated with nucleic acids (Andersen and Hessen, 1991; Vrede et al., 1999). Nucleic acids (RNA, DNA) are P-containing high molecular weight compounds (polynucleotides). The RNA content was determined to be c. 2–6% DW in *Daphnia* spp. and the phosphorus content to be c. 0.7–1.5% DW (Kyle et al., 2006). The nucleic acid content comprises 4.7–5.2% of the DW of *Moina* spp. (Kokova, 1982), below 3% in *Alona affinis* (Bullejos et al., 2014). In *D. magna*, RNA content varied from 8.6% to 6.6% DW, decreasing to 4% on day 21 of its growth; and DNA content generally decreased from 0.20% to 0.14% (McKee and Knowles, 1987).

Ribosomal DNA contains a significant fraction (c. 49%) of total body phosphorus.

(Acharya et al., 2004). The growth of Cladocera involves the requirement for a greater amount of ribosomal RNA, and especially of phosphorus (Main et al., 1997).

In juvenile *D. pulex*, an elevated DNA content was measured in the postmolt period, followed by an increase in RNA during the intermolt and premolt periods (Gorokhova and Kyle, 2002). It was found that the ratio of RNA:DNA in *D. galeata* increased in response to an increase of P:C ratio in its food (Vrede et al., 2002). Use of this ratio has been suggested (Markowska et al., 2011) for evaluating the condition of *D. pulex*, assuming that higher values correspond to a "good" condition. Specimens with a lower RNA:DNA ratio have lower metabolic rates and greater longevity. The RNA:DNA ratio is more than 8 times higher in Cladocera than in Copepoda (Bullejos et al., 2014).

Accumulation of messenger RNA (mRNA) products in daphnids was the highest under induction by piperonyl butoxide, chlordine, 4-nonyphenol, Cd, chloroform (Hannas et al., 2011). It was also found that presence of dissolved humic substances in the environment caused methylation of DNA in *D. magna* and *M. macrocopa* (Menzel et al., 2012).

3.3.5 Introductory Remarks About Lipids

Lipids are a major and complex group of organic compounds controlling biological processes at cellular, tissue, organismal, and cenotic levels. Recent investigations have supplied abundant new information on lipids in freshwater organisms [Lipids in Aquatic Ecosystems (2009)].

Oil droplets are often clearly seen in the bodies of cladocerans. According to Jordão et al. (2015) the core of a such droplet consists of neutral lipids (triacylglycerols and cholesterylesters), it is surrounded by a monolayer of phospholipid and cholesterol with associated specific proteins.

It is notable that the fat content is especially variable. Up to 17.5% DW was measured in *Moina rectirostris* (syn. *Moina brachiata*) (Stepanova and Vinogradova, 1970), 17–22% in *D. magna*, 10–40% in *D. pulex*, 10–19 in *Bythotrephes cederstroemi* (Bilkovic and Lehman, 1997). Variations in the content of lipids in Cladocera are shown in Figs. 3.1 and 3.5. It was found that the content of triacylglycerols decreased with growth in immature *D. magna* (Bychek and Gushchina, 1999). The fatty acid composition of Cladocera spp. is indicated in Tables 3.4–3.8; that of *Ceriodaphnia quadrangula* was reported by Farhadian et al. (2012), of

TABLE 3.4 The Total Content of Lipid and Percentage Lipid Components in Some Cladocera spp.

Species	Total Lipid (%DW)	Phospholipid (%)	Triglyceride (%)	Cholesterol (%)	Cholesterol Esters (%)
Bosmina obtusirostris	30.6	70	13	3	12.8
Holopedium gibberum	52.4	57	8.7	18.8	17
By. cederstroemi	16.4	55	11.3	5	11.3

DW, dry weight.
From Lizenko, E.I., Bushman, L.G., Nefedova, Z.A., 1977. Content of lipids in plankton of some Karelian lakes. Gidrobiologicheskii Zhurnal 13 (3), 74–80.

TABLE 3.5 Content of Fatty Acids (as % of Total Fatty Acid Content)

Fatty Acids	*Daphnia* spp.	*Daphniopsis*	*Bosmina*	*Holopedium*	*Leptodora*	*Bythotrephes*
SATURATED						
C12:0	0.7–2.3		–	0.8	0.1–2.1	0.4
C14:0	4–10	2.92	8.9	12.0	2.2–7.4	4.4
C15:0	0.6–3	0.99	1.1	0.9	0.6–2.8	0.9
C16:0	21–36	11.4			24–36	22–26
C17:0	20.6	0.64	17.3	15.4		19.1
C18:0	0.3–9.9	2.57	–	–	6.5–17.6	6.7–9
MONOUNSATURATED						
C16:1ω7	6.7	5.81	4.1	3.5		2.9
C18:1ω6 + 18:1ω9	9.2		12.3	8.1		10.3
C18:1ω7	4.1		5.0	8.1		6.0
C20:1ω9	–		1.2	2.3		–
POLYUNSATURATED						
C18:3ω3	6.8	26.5	7.6	6.7		5.3
C18:4ω3	11.8		10.4	11.0		4.1
C20:5ω3	11.8	1.41	14.4	17.5		23.0
C22:6ω3	0.9		2.6	2.1		2.1
C18:2ω6	4.6		5.3	4.1		3.6
C18:3ω6	1.6		0.6	0.8		0.6
C20:4ω6	3.5	0.63	4.7	6.8		9.3

–, not detected.
Daphnia *spp.*, Bythotrephes longimanus *from Bychek, E.A, Guschina I.A., 2001. The transfer of fatty acids in a freshwater planktonic foodweb of the Kuibyshevskoe Reservoir (middle reaches of the Volga). Hydrobiologia 442, 261–268;* Daphniopsis tibetana *from Zhao, W., Huo, Y.-Z., Gao, J, 2006. Analysis and appraisement of nut rient compositions for* Daphniopsis tibetana *Sars. Journal of Fishery Sciences of China 13 (3), 446–451;* Leptodora kindtii *from Bychek, E.A, Guschina I.A., 2001. The transfer of fatty acids in a freshwater planktonic foodweb of the Kuibyshevskoe Reservoir (middle reaches of the Volga). Hydrobiologia 442, 261–268;* Bosmina coregoni *and* Holopedium gibberum *from Bychek, E.A, Guschina I.A., 2001. The transfer of fatty acids in a freshwater planktonic foodweb of the Kuibyshevskoe Reservoir (middle reaches of the Volga). Hydrobiologia 442, 261–268.*

TABLE 3.6 Content of Fatty Acids (% DW)

Fatty Acids	*Daphnia cucullata*	*Daphnialongispina*	*Bosmina longirostris*	*Simocephalus vetulus*
C14:0	7.0	2.5	2.5	3.5
C14:1	0.8	3.0	2.3	3.8
C14:2	0.1	0.8	2.8	1.4
C16:0	16.4	11.7	14.0	12.8
C16:1	5.7	14.6	10.3	12.1
C16:2		0.8		
C18:0	6.8	3.7	7.4	5.1
C18:1	8.3	10.1	19.3	11.8
C18:2	15.5	4.2	4.2	6.0
C18:3	8.2	11.3	5.7	6.8
C18:4	6.3	15.8	2.8	7.8
C20:2	1.1	0.7	1.7	2.0
C20:4	6.7	1.3	5.4	6.6
C22:1		0.6		
C20:5	7.6	17.4	21.7	18.9
C22:5	5.4			0.4
C22:6	4.1	1.5		1.0

From Herodek, S., Farkas, T., 1967. Gas chromatographic studies on the fatty acid composition of some fresh-water crustaceans. Annales Instituti Biologici (Tihany) 34, 147–152.

TABLE 3.7 The Content of Phospholipid and Neutral Lipids in Three Age Stages of *Daphnia magna*

Types	Lipids	Newborn	3 Days Old	Mature
Phospholipids	Phosphatidylcholine	14.40	8.75	4.06
	Phosphatidylethanolamine	9.15	3.94	3.21
	Sphingomyelin	1.84	0.66	0.65
Neutral lipids	Triacylglycerols	566.2	283.2	452.1
	Diacylglycerols	Traces	Traces	74.1
	Free sterols	183.6	86.2	35.5
	Free fatty acids	146.9	128.0	60.8
	Wax esters	Traces	Traces	63.4

All values in μg/100 mg WW.
From Bychek, E.A., Guschina, I.A., 1999. The age changes of lipid composition in Daphnia. *Biokhimiya 64 (5), 652–655.*

TABLE 3.8 The Content of Fatty Acids (as % Total Fatty Acids) in Three Age Groups of *D. magna*

Fatty Acids	Newborn	3 Days Old	Mature
C14:0	0.9	2.3	3.5
C14:1	0.5	1.5	0.9
C14:2	0.6	1.7	2.2
C16:0	13.2	21.2	24.3
C16:1	4.7	4.7	4.5
C16:2	1.5	5.5	0.3
C16:3	1.2	3.4	7.7
C16:4	1.7	1.8	3.3
C17:0	1.2	Non det.	2.8
C18:0	3.4	7.7	5.8
C18:1	53.9	9.9	9.8
C18:2	6.7	22.0	18.9
C18:3	4.6	7.7	7.7
C20:4	2.8	6.1	3.8
C20:5	0.5	1.2	1.4

From Bychek, E.A., Guschina, I.A., 1999. The age changes of lipid composition in Daphnia. *Biokhimiya 64 (5), 652–655.*

D. pulex grown under different conditions was determined by Mims et al. (1991).

Cladocera synthesize de novo a minor fraction of total lipid which they obtain from food. As well, they possess a very limited capacity to transform lipids obtained with food.

Among the algae, diatoms are a prominent group that synthesize and store lipids: after photosynthetic production of a carbohydrate, they transform it into lipids and the stored lipid is seen as oil drops. The diatoms are one of the dominant groups on the bottom substrata and in phytoplankton. They are not the only lipid-producing group, but the metabolism of other algae is less well known. The lipid composition of algae is highly variable and depends on many environmental factors (Guschina and Harwood, 2009).

In temperate latitudes of the Northern Hemisphere, the spring peak of diatoms producing lipids as their reserve substance is followed by an accumulation of abundant oil drops by Cladocera. The content of particular fatty acids noticeably varies at different photoperiods, thus the content of saturated fatty acids and monounsaturated fatty acids in *Ceriodaphnia quadrangula* was maximum at 6Light:6Dark at feeding with *Chlorella* (Farhadian et al., 2013). In this species the content of α-linolenic acid (C18:3ω3) significantly increased during formation of resting eggs.

Due to their high-energy value, lipids are a prominent storage substance in Cladocera. Lipids are also used in the construction of biological membranes and hormones.

The lipid pathway in the aquatic environment starts with lipid formation by plants from initial products of photosynthesis (monosaccharide). According to Harwood and Jones (1989, p. 12), in algae "de novo synthesis requires the concerted action of acetyl-CoA carboxylase and a type II (dissociable) fatty acid synthase." The chain length of the product may then be elongated and unsaturated bonds may be introduced. Fig. 3.3 shows the schematic pathway from initial photosynthetic saccharides to lipids and amino acids in algae.

Lipids are present in water in both a particulate (i.e., within algae) and a dissolved state (Arts et al., 1997). In Cladocera, they are used as energy source, in the construction of cell membranes, and in metabolism; further, derivatives of lipids act as sex hormones. To understand the next sections, it is first necessary to state some basic ideas and definitions.

There are several classifications of lipids (e.g., Kucherenko and Vasilyev, 1985). In one of these, the following four groups are distinguished:

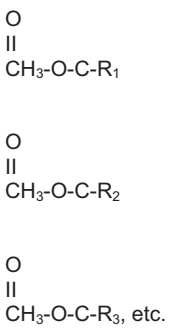
1. *Simple lipids*, which are esters of fatty acids with glycerol (fats) or aliphatic alcohols

(waxes). Waxes comprise true waxes and esters of cholesterol, vitamin A, or vitamin D.

2. *Complex lipids*, which are esters of fatty acids with other alcohols, e.g., phospholipids, glycolipids, sulfolipids, lipoproteins, or lipopolysaccharides.
3. *Lipid derivatives* (*lipoids*), which include fatty acids (saturated and unsaturated), monoglycerides, diglycerides, steroids alcohols with β ionic ring (vitamin A group), phosphatides, and carotenoids. Phosphatides are esters of polyatomic alcohols, fatty acids, and phosphoric acid. They comprise lecithins, which consist of radicals of glycerol, phosphoric acid, choline, and higher fatty acids (saturated or unsaturated).
4. *Various others*, including vitamins E and K, and aliphatic carbohydrates.

Functionally, the lipids are storage lipids (triglycerids and wax esters) and structural lipids (phospholipids and sterols) (Goulden and Place, 1993).

Generally, fat is represented as a triglyceride connected to various fatty acids (R):



The fatty acids may be saturated, i.e., containing no double bonds between the carbon atoms, and unsaturated, i.e., containing double bonds. The latter are described in the format *x:y* ω *z* (see, e.g., Ahlgren et al., 1990), in which *x* is the number of atoms, *y* is the number of double bonds (ω), and z is the position of the first double bond from the methyl end of the molecule. For example, 20:0 in which 20 is the number of carbon atoms and 0 (or a certain digit) is the number of double bonds in the molecule of a fatty acid. Some names of lipids corresponding to these designations are listed:

C14:0 myristic fatty acid
C16:0 palmitic acid
C16:1 palmitoleic acid
C18:0 stearic acid
C18:1 oleic acid
C18:2ω6 linoleic acid
C18:3ω3 α-linolenic acid
C18:3ω6 γ-linolenic acid
C18:4 octadecatetraenoic acid
C18:4ω3 stearidonic acid
C20:1 eicosanoic acid
C20:3 eicosatrienoic acid
C20:4ω6 arachidonic acid
C20:5ω3 eicosapentaenoic acid
C22:5 docosapentaenoic acid
C22:6ω3 docosahexaenoic acid

The following abbreviations are currently used; SAFAs—saturated fatty acids, MUFAs—monounsaturated fatty acids, PUFAs—polyunsaturated fatty acids, HUFAS—highly unsaturated fatty acids (the latter, namely C20: 4ω6, C20: 3ω3, C20:5ω3, C22:6ω3, overlap the range of PUFAs, i.e., they are also PUFAs).

Essential Fatty Acids

It is thought that almost all polyunsaturated fatty acids (PUFAs) are obtained by animals from plants and are not synthesized by animals. Thus, linoleic acid (C18:2ω6) and α-linolenic acid (C18:3ω3) are essential fatty acids, whereas eicosapentaenoic acid (20:5ω3) is not strictly essential. Becker and Boersma (2007, p. 463) arrived at the conclusion that "although dietary fatty acids can be used for energy purposes, specific fatty acids (namely, PUFAs) are required to build new biomass."

Lipids in Cladocera

Oil drops are easily observed in Cladocera and are often mentioned in the literature (e.g., Flückiger, 1951). They seem to be distributed throughout the body in some regular way, although this has not been sufficiently described for representatives of various genera living in different environments. They are mostly orange in color and their size ranges from quite small to very large. Tessier and Goulden (1982) recorded an abundance of oil globules in *Daphnia* by the visually estimated lipid index (LI). Hoenicke and Goldman (1987) estimated the lipid-ovary index in scores (based on visual scoring) in *Daphnia* and *Holopedium* and found that this index varies depending on the composition of their natural food. Dodson (1989) observed that in presence of predators the fat content in the body (LI) decreases, which might be related to defense from predators.

The following components are found in the fat of *D. magna* (Jaeger, 1935): butyric acid, sodium oleate, triolein, cholesterol oleate, cholesterol stearate, stearic acid, sodium stearate, tristearin, lecithin, linseed oil, and linoleic acid. Tessier et al. (1983) found that the major lipid types in *Daphnia* are triacylglycerols and wax esters. Arts et al. (1993) confirmed this prevalence and indicated that the next most dominant lipid class is phospholipids and sterols. It has been determined that Cladocera predominantly contain (12−23%) eicosapentaenoic acid, 20:5ω3 (EPA), a highly unsaturated fatty acid) (Persson and Vrede, 2006) (Fig. 4.5), in contrast to copepods. This difference is assumed to be a result of their phylogenetic origin.

It is likely that Goulden and Place (1993) were among the first to discuss the quantitative distribution of lipids in Cladocera. Taking into consideration that the fatty acids may be either derived from food or synthesized de novo in the body and that acetate (derived from the breakdown of carbohydrates or amino acids) is necessary for their synthesis, they used [^{14}C]acetate or $^{3}H_2O$ precursors and determined that the lipids synthesized by well-fed *Daphnia* (following incubation of up to 4 h) make up no more than 1.6% of the accumulated fatty acids.

Goulden and Place (1993) also indicated that lipids in daphnids consist of storage (triglycerides and wax esters) and structural (phospholipids and sterols) lipids, and that most of the lipids are transferred to the ovaries and then into eggs and used in the development of embryos.

Daphnia growth (in Schösee, Germany) was shown proportional to the available EPA (20:5ω-3) (Fig. 4.6) (Brett and Müller-Navarra, 1997) and is seasonally controlled by availability of EPA (Brzezińsky and von Elert, 2007). As algal polyunsaturated fatty acids make a major trophic resource for Cladocera, it was shown that in eutrophic lakes their growth and reproduction may be limited by EPA as was shown experimentally for *D. pulex* (Ravet et al., 2012). Limiting effect of EPA extends to predators. It was shown that *Bythothrephes longimanus* kept in the laboratory did not release broods as they were impoverished in EPA comparing with filed−collected specimens; having received EPA-enriched *Daphnia* as food they became heavier and had larger clutch size (Kim et al., 2014a,b).

It was found (Wacker and von Elert, 2001) that EPA (C20:5ω3) and α-linolenic acid (C18:3ω3) are not mutually substitutable resources for *D. galeata*, that their physiological functions are likely to be different, and that the former is not limiting for the growth of *D. galeata* cultivated on seston.

Bychek et al. (2005) found that *D. magna* is capable of high rates of de novo lipid radiolabeling; *D. magna* also makes direct use of dietary components (such as the PUFAs linoleate and α-linolenate). In addition, *D. magna* tolerates 24-h fasting with little change in lipoid metabolism. It has also been shown that *Daphnia* (specifically with reference to *D. magna*) cannot

synthesize linoleic acid (C18:2ω6) or α-linolenic acid (C18:3ω3) de novo (Persson and Vrede, 2006) (Fig. 4.5). Thus, they depend on fatty acids produced by plants (including essential fatty acids, e.g., EPA).

The content of fatty acids varies with age (Tables 3.7 and 3.8). In neonates of *M. macrocopa* percentage of myristic, palmitic, and stearic acids was 67% of the total fatty acids, in adults it decreased to 26% (Gama-Flores et al., 2015).

Having accumulated their fat reserves, *Daphnia* propagate, consume this resource, and then decline (Goulden and Hornig, 1980). Normally, the fat present in the body may be used by starving daphnias, as was observed in *D. magna* by Flückiger (1951), and short-term (24-h) starvation does not lead to profound changes in lipid metabolism (Bychek et al., 2005).

In addition to their basic trophic role, some lipid compounds may have exceptional properties. Pérez Gutierrez and Lule (2005) dried large numbers of *D. pulex* at room temperature, ground them, and produced 3 kg of fine powder. By extracting and fractionating it they obtained four glyceroglycolipids, all of which were found to be cytotoxic.

In *D. magna* exposed to tetradifon at 0.44 mg/L during 120 h the content of lipids decreased to 1.87 μg/ind. versus 18.36 in the control (Villarroel et al., 2009).

Further data are presented in Section 4B (Digestion).

3.3.6 Introductory Remarks About Steroids

Steroids are a major group of organic constituents of Cladocera controlling and channeling biological processes. They are derivatives of cyclopentano-perhydro-phenantrene. They comprise:

1. Sterols and their derivatives including ergosterol ($C_{28}H_{44}O$) and cholesterol ($C_{27}H_{46}O$). In the course of metabolism the latter is transformed into progesteron. Cholesterin also takes part in synthesis of ecdysons. Sterols are commented in detail by Martin-Creuzburg and von Elert (2009a,b);
2. Ecdysons (molt hormones; their antagonists are juvenile hormones), in the course of metabolism producing ecdysterone;
3. Steroid hormones—sex hormones: testosterone, androsterone (male hormones), estradiol, estron (progesterone) (female hormones), and hormones of carbohydrate metabolism—cortisol, hydrocortisone;
4. Vitamins of D group.

Ecdysteroid (ecd) concentration in whole *D. magna* is c. 200 pg ecd equivalent/mg DW (Bodar et al., 1990b) (see also Chapter 11).

In addition to PUFAs, sterols limit the growth, as Cladocera do not synthesize them de novo (Martin-Creuzburg and von Elert, 2009a,b). Sterols take part in formation of membranes and are precursors of steroid hormones, cholesterol being the most prominent. When *D. magna* obtain sterols in sufficient quantities for growth, eicosapentaenoic acid (EPA, 20:5ω3, a highly unsaturated fatty acid) becomes limiting (Martin-Creuzburg et al., 2008). Somatic growth of *D. magna* is mainly limited by the absence of sterols whereas egg production—by absence of long-chain PUFAs (Martin-Creuzburg and von Elert, 2009a,b).

In diatoms, cholesterol and C_{28} sterols are the major sterols (Soma et al., 2005). A low content of sterols in blue-green algae constrains C assimilation and cholesterol synthesis by *Daphnia* (von Elert, 2002, 2003; Martin-Creuzburg et al., 2008) and thus growth and reproduction (von Elert et al., 2002). Heterotrophic bacteria are scarce in sterols and thus limited growth, if fed with bacteria supplemented with cholesterol, *D. magna* demonstrated increased somatic growth (Martin-Creuzburg et al., 2011).

In newborn *D. magna*, the content of free sterols and phospholipids is high (phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin)

(Bychek and Gushchina, 1999). These authors also found that the content of triacylglycerols decreases with the growth of *D. magna* fed on *Chlorella*. Fatty acid desaturation is lower in newborns, and wax esters are only detectable in adults.

The threshold concentrations of sterols in food are found to be from 3.5 to 34.4 μg/mg C (Martin-Creuzburg et al., 2014). Phytosterols are differently efficient in supporting somatic growth of *D.magna* (fucosterol and brassicasterol being more efficient). The limiting sterol level was higher in *D. galeata* than in *D. magna* (Martin-Creuzburg et al., 2005). The cholesterol content in *D. magna* increased with the increasing dietary cholesterol, as well as at increasing temperatures (15, 20, and 25°C) Martin-Creuzburg et al. (2009). At higher temperature (25 vs. 20°C), the cholesterol content in eggs increased.

The dietary sterol conversion into cholesterol by *D. magna* is also demonstrated (Martin-Creuzburg et al. 2014).

Martin-Creuzburg and von Elert (2009b, p. 50) also mention presence in *D. galeata* of "an efficient C-24 dealkylating system".

3.3.7 Pigments

Pigments are derived from food or produced during the course of metabolism. In Cladocera, they comprise orange (carotenoid) pigments, red hemoglobin (Hb) or bacterial carotenoids in cases of infestation by *Spirobacillus cienkowski*, green (carotenoprotein in hemolymph), and dark (e.g., ommochromes of eyes, or tanned protein formed at high pH, i.e., melanins) (Green, 1966b, 1971).

Generally, littoral Cladocera are brownish, whereas planktonic species are colorless. Orange or red coloration is also observed in some species. Rarely is a species brightly colored. Blue spots occur in *Eurycercus lamellatus* on the postabdomen, on the dorsal side of the trunk, at the base of the mandibles, and on the esophagus (Weismann, 1878; Behning, 1941; Smirnov, 1971, 1974). *Pseudochydorus* has large brown spots on its valves. Newly molted *P. globosus* are colorless, but during the intermolt period a brown spot appears and increases in intensity. At excessive solar irradiation, Cladocera are blackish (melanistic) as are their ephippia containing latent eggs.

Leydig (1860, p. 56) noted that the blood of Cladocera may be colorless, yellowish, reddish, bluish, or greenish. Green (1957a,b) reported his observations of *Daphnia* with pale green blood, *Simocephalus* with green blood, and *Megafenestra aurita* with blue blood. These colors are caused by carotenoid proteins, as they produce an orange color when treated with desaturating agents.

The coloration of cladocerans (especially of parthenogenetic eggs) may depend on the coloration of the food consumed. Information on the pigments of Cladocera, as related to their different ecology, and the transformation of ingested pigments is rather scarce. This is an open field for further useful investigations.

Carotenoids. Carotenoids are lipid derivatives. The cladocerans receive carotenoids with algal food (Green, 1966a), which contains significant quantities: green algae, 7–51 mg% WW (i.e., 7–51 mg/100 g WW); cryptomonads, 17–162 mg% WW, blue-green algae, 14–52 mg% WW (Lavrovskaya, 1965). The content of carotenoids in *Daphnia* and *Bosmina* was determined as 0.45–2.1 mg% WW (Lavrovskaya, 1965).

With reference to *Simocephalus*, Green (1955) found carotenoids (orange) in a free state in fat globules, in the gut wall, in fat cells, linked to proteins in the cytoplasm, ovary, and eggs, and as carotenoprotein (green) in blood.

Green (1966b) reported the principal pathways of carotenoid transfer, with reference to *Simocephalus* (Fig. 3.7). Carotenoids obtained from food are passed into the blood, and from there to fat cells, to the carapace epidermis, to the ovary, and then to the eggs. A female may pass half of its total carotenoids to her eggs. In