COPEPODS COLONISING ITALIAN SPRINGS

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Introduction

The Copepoda, a subclass of the Maxillopoda, are one of the largest and most diversified group of crustaceans, including over 13,000 species (Boxshall & Defaye 2006), nearly half of them living in symbiotic relationships with other organisms. At present copepods comprise 10 orders (Boxshall & Halsey 2004), four of which (Calanoida, Cyclopoida, Gelyelloida and Harpacticoida) include free-living freshwater representatives.

Copepods are typically small and fragile organisms that do not fossilise well. The first true fossils found were reported by Palmer (1960); these include few cyclopoids and harpacticoids discovered in North and South America in Miocene and Pleistocene lake deposits. The recent discovery of *Kabatarina pattersoni* Cressey & Boxshall, 1989, a parasite found on the gills of a fossil fish from Brazil, extends considerably the known fossil records of copepods to the Lower Cretaceous (110 to 120 MYA: Huys & Boxshall 1991).

During their long evolutionary history, copepods spread over all the continents and successfully colonised almost all of the available water habitats. They successfully colonised all salinity regimes, from very soft freshwaters, like small glacier pools on metamorphic rocks, to hyperhaline coastal basins, and all temperature regimes, from subzero polar waters to hot thermal springs (Huys & Boxshall 1991). Copepods occur from depths of over 10,000 metres in the Marianas Trench to an altitude of over 6000 metres up in the Himalayan glaciers (Kikuchi 1994). Freshwater habitats range from large lakes to ephemeral water bodies, including tree-holes, leaf axils, wet mosses and very small containers (Reid 2001), benthic substrates in running waters (Dole-Olivier *et al.* 2000), as well as every kind of subterranean habitat (Galassi 2001). Moreover, free-living cyclopoids and harpacticoids may form an important component of the cryptozoic fauna in moist forest litter (Fiers & Ghenne 2000) and wet campos (Reid 2001).

Stoch F., 2007 - Copepods colonising Italian springs. In: Cantonati M., Bertuzzi E. & Spitale D., *The spring habitat: biota and sampling methods*. Museo Tridentino di Scienze Naturali, Trento: 217-235 (Monografie del Museo Tridentino di Scienze Naturali, 4).

Copepods, as well as several other aquatic organisms, can be easily transported, either actively or passively, often as resting stages (Dahms 1995); up to now, resting stages are not known for stygobionts and most of spring dwelling species. For this reason, species exclusively inhabiting crenal and groundwater have usually a low dispersal power and a high rate of endemism (Stoch 2005; Berera *et al.* 2005).

Only two orders (Cyclopoida and Harpacticoida) have been detected so far in Italian spring habitats, and will be considered herein; they can be the most abundant and species rich groups in spring meiobenthic communities (Stoch 2003). Up to now, 99 cyclopoid species (Stoch 2005) are recorded from Italian inland waters, together with several subspecies of doubtful validity; they belong to the family Cyclopidae. Excluding exclusively brackish water species, six families and about 160 species of Harpacticoida are recorded so far from Italian freshwater habitats (Berera *et al.* 2005); nearly half of them belong to the family Canthocamptidae (Figs 1-2).

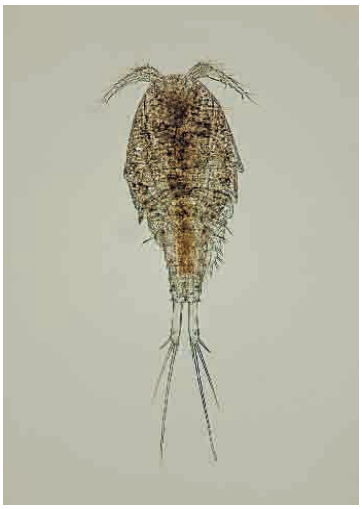

Fig. 1 - Cyclopoid copepod (Paracyclops imminutus).

Fig. 2 - Harpacticoid copepods (mating couple of Bryocamptus tatrensis*).*

General characteristics

Morphology

The general characteristics and morphology of copepods are reported in Huys & Boxshall (1991) and Boxshall & Halsey (2004); the morphology of freshwater meiobenthic species was examined in detail by Dole-Olivier *et al.* (2000).

The reader is referred to the above mentioned texts for a detailed morphological description; only a brief synthesis is reported herein to help in specimen identification.

Free-living freshwater harpacticoids and cyclopoids range from 0.2 to 4.0 mm in length. They are podoplean copepods, i.e. there is a major articulation between the fourth and fifth pedigerous somites, which divides distinctly the body into two parts, termed prosome and urosome.

The prosome comprises the cephalosome (including the acron, five cephalic somites and the first thoracic somite fused together), and four thoracic, pedigerous somites. The first pedigerous somite is usually partly or totally fused to the cephalosome, forming the cephalothorax. The cephalosome bears six paired appendages: antennules (A1), antennae (A2), mandibles (Md), maxillules (Mx1), maxillae (Mx), and maxillipeds (Mxp); each pedigerous somite bears a pair of swimming legs (P1-P4) (Fig. 3).

The urosome consists of the sixth and seventh thoracic somites (bearing respectively the fifth pair of swimming legs, P5, which may be reduced or absent, and a rudimentary P6), three abdominal somites lacking appendages and the anal somite, which bears the caudal rami (furca). In males, the urosomites are distinct, whereas, in females, the last thoracic somite and the first abdominal somite are usually fused to form a genital double-somite.

The caudal rami bear seven setae, some of which may be reduced or secondarily absent.

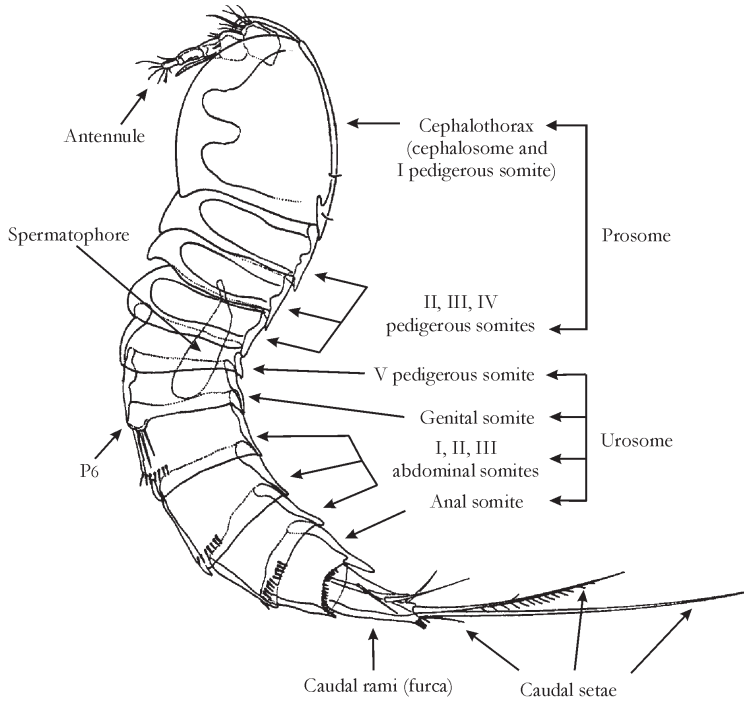

Fig. 3 - General body morphology of the harpacticoid copepod Moraria alpina.

Reproduction and development

Copepod reproduction is bisexual, but parthenogenesis has been demonstrated in three harpacticoid species (*Elaphoidella bidens*, *Epactophanes richardi*, *Canthocamptus staphylinus*), which are sometimes found in springs; its adaptive role is still unclear because a reduced embryonic survival of the offspring was discovered in a parthenogenetic population of *C. staphylinus* (Sarvala 1979a). Fertilisation of eggs requires the attachment of the spermatophore by the male to the copulatory pore of the female; after copulation, sperm is stored in the female seminal receptacle, located in the genital double-somite. The fertilised eggs are usually extruded into egg sacs (usually two in cyclopoids, one in harpacticoids); some stygobiotic species do not produce egg sacs, and after fertilisation eggs are directly released in the water, or at a time on the substratum, such as the genus *Parastenocaris*. Epigean benthic species produce a higher number of eggs than subterranean species (Dole-Olivier *et al.* 2000) and brood size varies with temperature and habitat specialisation, ranging from 1-2 (such as species living in subterranean habitats or high altitude, cold springs) to 100 or more eggs (Fig. 4).

Among crustaceans, copepods exhibit one of the most complete examples of metamorphosis. The eggs hatch into a larva called nauplius. There are six naupliar stages (N1-N6); after the fifth moult, the nauplius develops all the oral appendages, becomes segmented and begins to look like the adult, becoming a copepodid. Six copepodid stages (C1-C6) are recognised, the C6 being the sexually mature adult, which stops moulting. While much information is available on development and life cycles of planktonic species (Dussart & Defaye 1995), little is known of freshwater benthic copepods (Dole-Olivier *et*

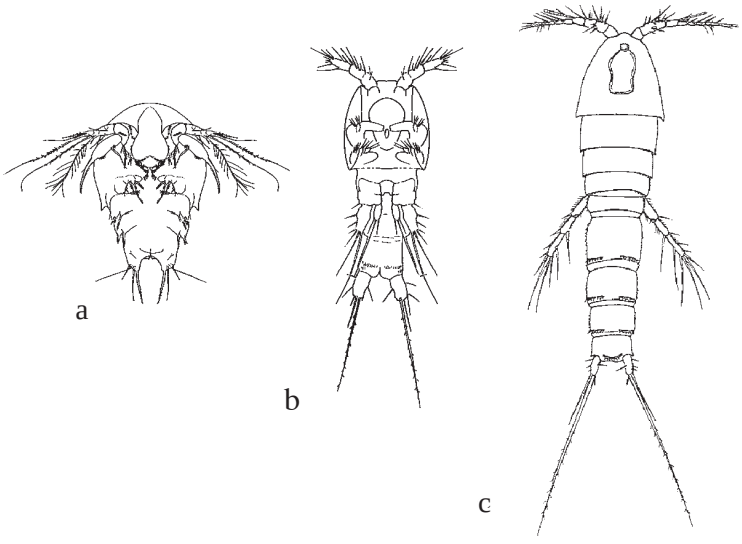

*Fig. 4 - Development of a harpacticoid copepod (*Canthocamptus staphylinus*). a) nauplius; b) copepodid; c) adult female.*

al. 2000), especially spring dwelling species. The rate of development is strongly affected by temperature and food supply. Among the species studied by Sarvala (1979b), the fastest development time was observed in the cyclopoid *Paracyclops fimbriatus*, a common inhabitant of Italian springs at lower altitudes (egg development times: 5 days at 11.5 °C; post-embryonic development from eggs to adult: 65 days at 11.5 °C). Among harpacticoids, the whole postembryonic development of the benthic, *Canthocamptus staphylinus*, requires 30 to 44 days at 12 °C (Sarvala 1979b); stygobiotic harpacticoids generally develop more slowly (Rouch 1968). Therefore, spring copepods show development rates which fall between those of epigean benthic species and stygobionts. Benthic freshwater copepods have one or more generations per year; data on spring species are lacking.

Feeding

Little is known on copepods feeding in spring habitats, as well as generally in benthic and interstitial habitats (Dole-Olivier *et al.* 2000). Some large cyclopoids occasionally present in springs (mainly of the genera *Macrocyclops*, *Acanthocyclops*, *Megacyclops*) are known to be predatory, eating rotifers, oligochaetes, crustaceans, chironomid larvae and even fish fry (Dussart & Defaye 1995). Most of them are probably omnivorous, as the species of *Tropocyclops*, *Paracyclops* and large *Diacyclops*. Species of the genus *Eucyclops* should be mainly phytophagous. The main food source for the smaller interstitial cyclopoids (like the genera *Diacyclops*, *Speocyclops*, *Graeteriella*), as well as for harpacticoids, is coarse and fine particulate organic matter, including the associated microbial biofilm (Dole-Olivier *et al.* 2000). Predation is rarely found in freshwater harpacticoids, however, *Phyllognathopus viguieri* was observed feeding on nematodes (Lehman & Reid 1992).

Methodology

Sampling methods

A detailed illustration of collecting methods for copepods was reported by Huys & Boxshall (1991) and Reid (2005). Collection of spring meiofauna, including cyclopoid and harpacticoid copepods, is exhaustively illustrated by Crema *et al.* (1996), Gerecke *et al.* (1998, 2005), Stoch (2004a). Finally, Malard *et al.* (2002) described the most suitable techniques to collect stygobiotic species in karstic springs.

Springs are complex environments, which provide multiple microhabitats that may be colonised, either temporarily or permanently, by distinct copepod species (Crema *et al.* 1996; Gerecke *et al.* 1998; Galassi *et al.* 2001; Stoch 2003). Moreover, copepods may be found in aquatic or semi-terrestrial microhabitats contiguous to the spring outlet, including bed sediments, dead organic materials such as woody debris and leaf litter, hygropetric layers on rocky surfaces and mosses.

Considering the high, within and between, microhabitat heterogeneity, the application of a single standardised sampling method for spring meiofauna would likely underestimate copepod species richness. For this reason, a combination of methods is suggested as a function of spring morphological structure and hydrological regime (Fig. 5).

Stones, gravel and sand. Copepods living in the benthic layer can be collected by washing the sediments and filtering the water immediately downstream of the disturbed area using a simple pond net (mesh size 60-100 μm). Water-saturated sediments that accumulate near the mouth of rheocrene or rheohelocrene springs can also be sampled using the Karaman-Chappuis method (Delamare-Deboutteville 1960). This consists of digging a hole in the sediments near the water edge, and straining the water that collects in the hole through a plankton net. This method is less damaging to the animals than other methods suitable for collecting interstitial fauna, like the Bou-Rouch pump (Gerecke *et al.* 2005), moreover, it is easier to apply in remote and high elevation sites. In alpine springs, especially above 1500 m a.s.l., interstitial fauna was considered very scarce (Crema *et al.* 1996; Gerecke *et al.* 1998). However, recent discoveries of new interstitial species of harpacticoids (Stoch 1998b; Cottarelli *et al.* 2002, 2005; Stoch unpublished data) at high altitudes (even close to 3000 m a.s.l.) suggests that further research is needed in this field.

Dead organic materials and mud. The detritus, including leaves, roots, dead wood and aquatic vegetation can be removed from the spring and washed in a bucket before filtering the washed water through the net. In springs with sandy, muddy, or thickly vegetated sediments, a simple hand-held plastic corer may be effective for quantitative studies (Reid 2005), however, the number of samples to be collected

Fig. 5 - Sampling copepods in an Alpine spring using a hand net.

to estimate overall species richness may be quite high due to the low density of specimens in mountain springs.

Mosses. Mosses are one of the most copepod-rich habitats, due to the complex spatial structure and the high availability of food resources. Even wet mosses located on hygropetric rivulets or on the ground near the spring mouth may be very rich in harpacticoid copepods. Small amounts of mosses can be removed from the spring and squeezed, collecting the water in the net. Submerged mosses can be squeezed *in situ*, filtering the water downstream, without damaging the microhabitats of the smaller springs.

Drift. In most cases, springs may be viewed as ecotones between groundwater and surface water, and can be considered as access points that can be used to collect the groundwater fauna living in the aquifer feeding the spring (Malard *et al.* 2002; Di Lorenzo *et al.* 2005). Springs fed by karstic aquifers usually exhibit sudden increases in discharge during rainfall events which result in the drift of numerous stygobionts; therefore, a drift net can be placed at the outlet of the spring at the beginning of the high discharge period. The number of individuals that enter the drift can be so impressive that Rouch (1970) introduced the term "haemorrhage" to describe this phenomenon. The size and design of the net depends on the discharge of the spring (Malard *et al.* 2002. An outer net with a larger mesh-size (500-1000 μm) can be used to protect the inner net (mesh size 60-100 μm); the same net can be used to prevent large predators (like water beetles, stonefly nymphs or amphipods) to enter the device and feed on copepods. Depending on discharge and the amount of suspended sediment, the net has to be removed regularly (every 15 minutes in some karstic springs, every 24 hours or every week for high altitude, non karstic springs) to collect the fauna. A longer filtering period damages the animals, promotes clogging, and increases the risk of losing the net. Drift nets are suitable for rheocrenes and rheohelocrenes, because the structure of most helocrenes and limnocrenes prevents the proper positioning of the net at the spring outlet. This method gave exceptionally interesting results even for spring brooks fed by glacier melting waters (Cottarelli *et al.* 2005).

Artificial substrates. During low water periods, artificial substrates can be lowered into the mouth of large and deep limnocrene springs, which cannot usually be sampled with any other method except diving. This promising technique was never applied in investigations on spring-dwelling copepods of alpine springs.

Fixation, staining and storage

Meiofaunal samples should be fixed within 5 minutes after collection and fixed with a 3-5% formalin buffered solution with available compounds to reduce acidity (usually sodium tetraborate or sodium carbonate) (Huys & Boxshall 1991; Reid 2006). The pH of the fixative should be raised to about 8-8.2. Formaldehyde should be used with caution (this chemical is toxic and is classified as a probable

human carcinogen), moreover, specimens fixed with formalin are not suitable for molecular studies. Alternatively, 70% ethanol is also a good fixative and yields more relaxed specimens. Unfortunately, the high amount of fixative required makes ethanol suitable only for small samples, moreover, it is a flammable substance.

Staining samples using Rose Bengal, before or during fixation with formalin, helps with the visual separation of specimens from sediment or detritus-filled samples using the light microscope. However, staining must be avoided if the specimens are to be used for taxonomic studies (for example if they are to be examined by Nomarski Differential Interference Contrast (DIC) microscopy).

Samples fixed with formalin can be sorted, even after several years; unfortunately, specimens can become brittle. Sorting techniques for meiofauna are quite tedious, and require a careful examination of the sample under a stereomicroscope (20 to 50x); the specimens can be individually extracted using a Pasteur pipette fitted with a flexible rubber bulb. Alternatively, faunal extraction may be done by elutriation/decantation (which brings copepods into the supernatant, that is subsequently filtered through a net), or density-gradient centrifugation (Dole-Olivier *et al.* 2000). Unfortunately, centrifugation can damage tiny specimens preventing detailed taxonomic studies.

Storage media are buffered formalin (recommended by Huys & Boxshall 1991) or 70% ethanol (recommended by Reid 2006). In both media, copepods can become brittle, however, specimens preserved in ethanol for more than one century have been dissected without problems (personal observation on Lilljeborg collection), and for this reason ethanol is suggested as the preferred storage medium. Glycerine (1-5%) can be added to maintain flexibility of specimens and avoid evaporation. Glycerine is not recommended if specimens are to be examined by electron microscopy (Reid 2006).

Specimens may be stored in small polyethylene containers placed in a glass or plastic tube and properly labelled. The contact between tiny copepods and cotton is to be avoided, because small harpacticoids may be lost in the cotton caps.

Dissection and mounting on slides

Dissection is required for a precise identification to genus and species levels. Before dissection it is important to observe body size, shape, cuticular ornamentation, length of antennules in relation to body length, size and antennular morphology in males.

For manipulating copepods, clean Pasteur pipettes or Irwin loops are useful to transfer individual specimens to a slide or a Petri dish without transferring unnecessary amounts of medium. Irwin loops are available commercially, or can be made by electrolysing tungsten wire to a tapered point, then bending the wire in a loop with fine forceps (Reid 2006). For manipulating, dissecting and transferring tiny parts (like mouthparts), the use of a fine (0.2 mm thickness) stainless steel entomological pin (commercially known as 'minute pin') mounted on

a holder is recommended. Alternatively, some scientists use tungsten needles (sharpened by electrolysing one end in a 6 Volt circuit) or micro-scalpels (Reid 2006), i.e. standard (non-stainless) insect pins (size 00 or 000) sharpened on two sides on a fine stone with a little mineral oil.

Dissection is conducted in glycerine or lactic acid. Specimens can be dissected in lactic acid if they are to be mounted in polyvinyl lactophenol or simply in lactophenol as recommended by Huys & Boxshall (1991). Glycerine should be used if the mounting medium is Faure's or Hoyer's liquid, glycerine jelly, glycerine itself or other aqueous media.

Dissection is performed under a stereomicroscope (range of magnifications up to 100x or more). Substage illumination providing transmitted light is almost essential for the best contrast. Dissection sequences have been discussed by Huys & Boxshall (1991).

A recommended mounting medium is glycerine; several small cover slips can be used to mount separately on the same slide the appendages and the abdomen. Slides using glycerine should be sealed using commercial epoxids to prevent evaporation and deterioration of the medium. Specimens mounted in glycerine were studied without any problem after 50 years (for example, Kiefer's collection). Larger specimens may be mounted in Faure's medium, and the cover slip can be sealed using Canada balsam. This medium may remain stable and

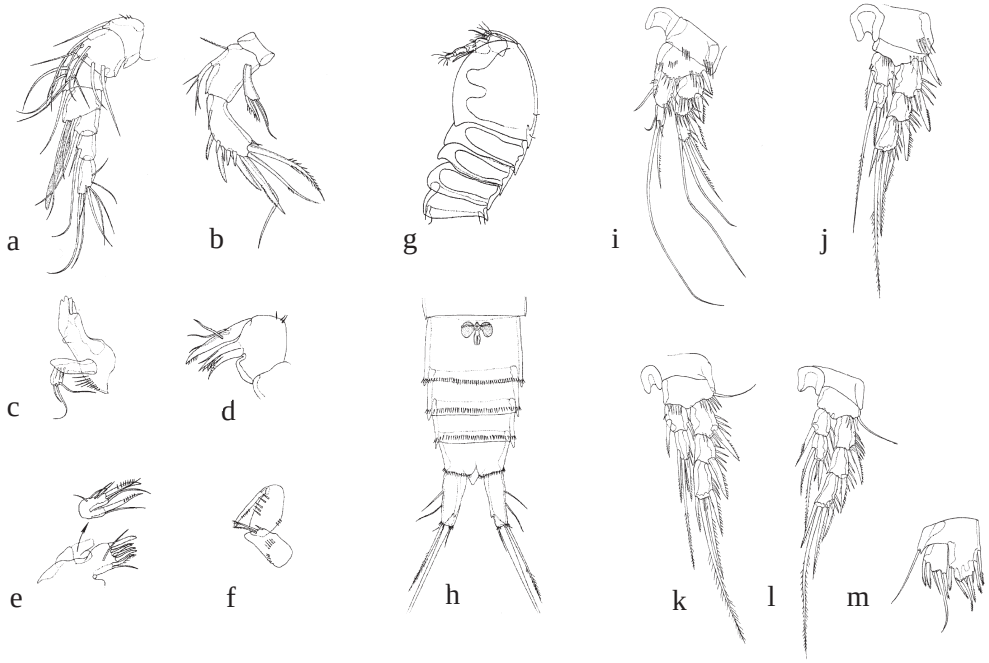

*Fig. 6 - Dissection of a harpacticoid copepod (*Moraria alpina*, female): a) antennule; b) antenna; c) mandible; d) maxillule; e) maxilla; f) maxilliped; g) prosome; h) urosome and furca; i) P1; j) P2; k) P3; l) P4; m) P5.*

provide good viewing conditions for 20 years or more, however, it must be protected from light and, if not properly sealed, may develop crystals damaging the specimens. This problem can be avoided reducing the amount of Arabic gum in the medium. Other media are not recommended for taxonomic collections, especially polyvinyl lactophenol should be avoided (Huys & Boxshall 1991). Polyvinyl lactophenol usually forms needle-like phenol crystals under the coverslip and obscures the material within about 10 years.

Identification

The Cyclopoida, with a broad cephalothorax, are usually easily distinguished from the Harpacticoida, with few exceptions (some interstitial cyclopoid species, like *Graeteriella unisetigera*, have a harpacticoid body shape).

The most recent identification key for distinguishing families and genera of freshwater copepods is the guide by Dussart & Defaye (1995).

Unfortunately, the taxonomy of larval stages of copepods is only at the beginning, and most nauplii and copepodids cannot be identified even at the genus level.

The most important taxonomic characters for species identification, apart from body shape, cuticular microsculptures, and shape of seminal receptacle in female, are the segmentation and setation of body appendages and the shape and ornamentation of caudal rami. Recent developments in copepod taxonomy require careful examination of minute characters, usually overlooked in the older keys. The use of Nomarski DIC microscopy is necessary to properly study these characters at 1000x magnification.

The most recent taxonomic keys useful for identifying spring copepods in Europe are the monographs by Einsle (1993) for cyclopoids and by Janetzky *et al.* (1996) for harpacticoids (Fig. 6). These keys need to be updated using more recent monographs (especially Karaytug 1999, for the genus *Paracyclops*) and papers. Stoch (2005) and Berera *et al.* (2005) give a taxonomically updated list of all the Italian freshwater cyclopoid and harpacticoid copepods, with details on their ecology and distribution.

Copepods in springs

General overview

Springs may be viewed as ecotones between groundwater and surface water (Williams 1991).

The small area around the spring mouth and associated net of branched rivulets and pools (i.e. the eucrenal *sensu* Gerecke *et al.* 1998) harbour copepod assemblages consisting of at least three ecological groups of species: a) benthic species that colonise springs coming from

the brooks downstream (epirhithral), b) stygobionts, coming from the groundwater, which may live in the interstitial habitat of gravel and sand near the spring mouth, and c) species that characteristically occur only in springs (crenobionts, *sensu* Gerecke *et al.* 1998).

Copepods found in springs are usually not true crenobionts (Stoch 1998a), contrary to water mites (Gerecke & Di Sabatino 2007. Most of the spring-dwelling species also occur in the epirhithral, in the littoral zone of lakes, in semiterrestrial habitats (like wet mosses and moist soil), or in groundwaters (Gerecke *et al.* 1998; Jersabek *et al.* 2001; Galassi *et al.* 2002; Stoch 1998a, 2000, 2003, 2006). The species collected in some Italian spring areas, and the other habitats which harbour them, are listed in table 1.

Tab. 1 - Representative species of copepods in the Alpine springs of Italy.

Harpacticoida	*Attheyella (Attheyella) crassa*	(Sars, 1863)
	Attheyella (Attheyella) wierzejskii	(Mrázek, 1893)
	Bryocamptus (Arcticocamptus) alpestris	(Vogt, 1845)
	Bryocamptus (Arcticocamptus) cuspidatus	(Schmeil, 1893)
	Bryocamptus (Arcticocamptus) rhaeticus	(Schmeil, 1893)
	Bryocamptus (Limocamptus) echinatus	(Mrázek, 1893)
	Bryocamptus (Limocamptus) hoferi	(Van Douwe, 1907)
	Bryocamptus (Rheocamptus) pygmaeus	(Sars, 1863)
	Bryocamptus (Rheocamptus) tatrensis	Minkiewicz, 1916
	Bryocamptus (Rheocamptus) typhlops	(Mrázek, 1893)
	Bryocamptus (Rheocamptus) zschokkei	(Schmeil, 1893)
	Canthocamptus (Canthocamptus) staphylinus	(Jurine, 1820)
	Echinocamptus pilosus	(Van Douwe, 1915)
	Epactophanes richardi	Mrázek, 1893
	Hypocamptus brehmi	(Van Douwe, 1922)
	Maraenobiotus zschokkei	Kreis, 1920
	Maraenobiotus vejdovskyi	Mrázek, 1893
	Moraria alpina	Stoch, 1998
	Moraria poppei	(Mrázek, 1893)
	Moraria stankovitchi	Chappuis, 1924
	Paracamptus schmeili	(Mrázek, 1893)
Cyclopoida	*Acanthocyclops vernalis*	(Fischer, 1853)
	Diacyclops sp. gr. *languidoides*	(Lilljeborg, 1901)
	Eucyclops serrulatus	(Fischer, 1851)
	Graeteriella (*Graeteriella*) *unisetigera*	(Graeter, 1908)
	Paracyclops fimbriatus	(Fischer, 1853)
	Paracyclops imminutus	Kiefer, 1929
	Speocyclops demetiensis	(Scourfield, 1932)

Patterns of species richness

Ecological and historical factors shape species richness patterns of copepod assemblages in springs, the most important being altitude and the effect of glaciation (Galassi *et al.* 2001, 2002; Stoch 1998a, 2000, 2003). It is well known that copepod species richness decreases with elevation in Alpine lakes (Jersabek *et al.* 2001), and the same happens for springs. In high altitude environments (above 2700 m a.s.l.), close to the glacier borders on the Alps, copepod assemblages are usually composed by 1-2 harpacticoid species of the genera *Hypocamptus* and *Maraenobiotus* (personal observations). At lower altitudes (between 1100-2700 m a.s.l.), species richness increases; Stoch (1998a) listed 12 species for the Adamello-Brenta massif, Trentino. Finally, in the Musi massif (Julian Pre-Alps, altitude of springs between 350 and 1100 m a.s.l.), 16 species were reported by Stoch (2003, 2004a).

A high species richness has been observed in low elevation springs in the Apennines, for example, the spring complex of Tirino (Abruzzo, 330-340 m a.s.l.) harbour 25 copepod species (Galassi *et al.* 2001). This is a remarkably high number, considering that the whole copepod fauna of 63 South Tyrol springs consists of only 17 species (Stoch 2006). To provide some examples for other European countries, 6 species were identified in the alpine Park of Berchtesgaden (Gerecke *et al.* 1998: 19 springs), and 17 species in Luxembourg (Gerecke *et al.* 2005: 41 springs). Lower values were reported for northern European countries, for example from Finland (Särkkä *et al.* 1998) (Fig. 7).

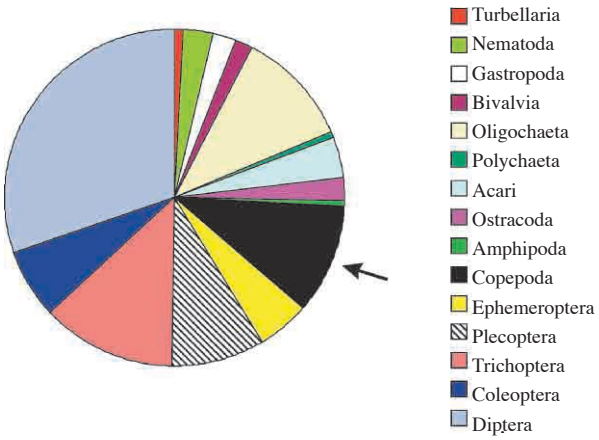

Fig. 7 - Percent composition of the benthic assemblages of pre-alpine springs in northeastern Italy (Musi massif); percentages are based on the number of species.

These differences may be explained considering the rarity or absence of stygobionts in high-altitude mountain springs and in previously glaciated areas, which contribute significantly to copepod species richness in springs located at lower altitudes (Crema *et al.* 1996; Galassi *et al.* 2001; Stoch 1998a, 2000, 2003). Although stygobionts may be present in areas covered by the Quaternary glaciers as demonstrated for Italy in Trentino (Stoch 2000) and in the Carnic Alps (Stoch

2004b), their presence is limited to marginal areas or refuge massifs. The copepod fauna of previously glaciated areas like those cited for Italian central Alps, Germany, or Luxembourg, located far away from the southern Quaternary glacier borders, is very poor and comprises few or none stygobionts. The absence of stygobiontic copepods is also reflected in the absence of strictly endemic species in previously glaciated areas. On the contrary, endemics may be well represented in low elevation spring assemblages, especially in the Apennines, where several Tertiary and glacial relicts were reported as well (Galassi & De Laurentiis 1997; Galassi *et al.* 2001; Di Lorenzo *et al.* 2005; Fiasca *et al.* 2005) (Fig. 8).

Fig. 8 - Percent composition of the meiofaunal assemblages of pre-alpine springs in northeastern Italy (Musi massif); (left): percentages based on the number of species; (right): percentages based on the number of specimens.

The low copepod species richness of glaciated areas may be due to the low dispersal power of spring copepods. In fact, Acari, which have a high dispersal power through phoresis, are well represented in the springs located in these areas, that is, 19 species are reported for the Adamello-Brenta massif, 28 for South Tyrol, 23 for Berchtesgaden, and 38 for Luxembourg (Gerecke & Cantonati 1998; Gerecke *et al.* 1998, 2005; Gerecke 2006). Although the ecological factors responsible for the high species richness and the high level of crenobiosis displayed by spring-dwelling water mites compared to copepods are far from being clear, Gerecke & Di Sabatino (2007) suggest as key factors the presence of a diversified dipteran fauna, including a large set of potential hosts for mite larvae, as well as the stability of flow and oxygenation of spring waters. A diversified insect fauna may negatively influence copepod diversity through predation; moreover, copepods and insect species may display different habitat preferences in springs. Glazier (1991) pointed out that spring assemblages of temperate regions can be distinguished in non-insect and insect communities, non-insect taxa being dominant in hard, alkaline waters. Furthermore, harpacticoid copepods may be the dominant taxon, both in terms of abundance and species richness, of the meiofaunal assemblages of low elevation karstic springs (Stoch 2003), especially in the case of fluctuating discharge and environmental parameters, like conductivity, dissolved oxygen and temperature. In these environments Acari may be very scarce or absent.

Finally, another factor that may influence copepod distribution

in springs (Galassi *et al.* 2001; Fiasca *et al.* 2005) is the geological substratum. For example, in the Adamello-Brenta massif only 5 species out of 12 were found in springs with low conductivity flowing out from metamorphic rocks, while 11 species where recorded in the dolomitic area (Stoch 1998a).

Copepods as biological indicators

Gerecke *et al.* (1998) noticed that species poor copepod faunas in Alpine springs do not allow for a separation between rheocrenic and helocrenic assemblages, indicating that most of the species which recolonised previously glaciated areas have a wide ecological tolerance. On the contrary, copepods in species rich areas are good indicators of spring typology (Stoch 2003). Moreover, creno-stygobiontic species may show a fine-scale microhabitat partition (Galassi *et al.* 2001; Fiasca *et al.* 2005), while some species of the genera *Moraria*, *Epactophanes*, *Arcticocamptus*, *Nitocrella*, *Parastenocaris*, *Speocyclops* and *Diacyclops* selectively segregate in different microhabitats, for example hygropetric rivulets and mosses, or sediment patches with different granulometric composition (Stoch 2003, 2004a; Fiasca *et al.* 2005). Habitat partition in karstic springs is poorly known, especially for high-discharge basal springs fed by deep aquifers. In these cases, the copepod fauna collected are predominantly located in the capacitive annexed systems of the aquifer, and only rarely living in the conductive ones (Di Lorenzo *et al.* 2005; Galassi *et al.* 2007).

The remarkable degree of habitat specialisation suggests that copepod assemblages may be used as biological indicators of environmental quality and aquifer vulnerability, at least at low elevation sites. Investigations on this topic are still in their embryonic phase, but the first results are promising. Di Lorenzo *et al.* (2003) performed a taxocenotic analysis using copepod assemblages to infer the isolation degree (and consequently the vulnerability) of karstic springs, which supply drinking water in central Apennines. Stygoxene copepods were used as markers of the presence, density, and dimension of the preferential paths of fast infiltration representing the main risk for water quality, which could not be assessed only through chemical, physical and microbiological criteria.

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