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Exotopic protrusions and ellobiopsid infection in zooplanktonic copepods of a large, deep subalpine lake, Lago Maggiore, in northern Italy

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Exotopic protrusions were first recorded on zooplanktonic copepods of Lago Maggiore in 1992. They were classified into two types: (i) type I, the most abundant, dark, spherical and granular; (ii) type II, small, transparent and nongranular. They most commonly appeared on the lateral surface of adult Eudiaptomus padanus at the articulation of the second and third prosomal segments. Regular monitoring from 1994 to 2002 revealed the presence of additional, more complex protrusions, which may be later developmental stages of those already reported. In some instances, protrusions could be identified as successive stages of infection by ellobiopsids. The ellobiopsids are protists of uncertain taxonomic position, most probably achlorophyllous dinoflagellates, which during a phase of their life cycle parasitize zooplanktonic Crustacea. Originally described from marine organisms, the ellobiopsids have been reported from freshwater organisms only recently. They appear to herniate by puncturing the body of the host; this might explain the presence of host cells inside the cysts. Exotopic protrusions seem to represent a stable component of calanoid copepods from Lago Maggiore; however, they have been recently found to be more diverse in morphology and found to affect additional hosts, such as copepodites and nauplii of Cyclops abyssorum, which are the second most important copepod species of the lake.

INTRODUCTION

Exotopic protrusions were first identified in zooplanktonic copepods of Lago Maggiore, Italy, in 1992 (Manca *et al.*, 1996). Named 'cysts', they were classified into two types: (i) type I, the most abundant, dark, spherical and with a granular internal structure; (ii) type II, small, transparent and nongranular. They most commonly appeared on the lateral surface of adults or last-stage copepodites of *Eudiaptomus padanus*, at the articulation of the second and third prosomal segments. The structures on copepods from Lago Maggiore were similar to those reported from calanoid [*Diaptomus* and *Limnocalanus* (Omair *et al.*, 1999; Bridgeman *et al.*, 2000)] and cyclopoid [*Diacyclops bicuspidatus thomasi* (Messick *et al.*, 2004)] copepods of Lake Michigan and the nearby Lake Patterson, USA. Although histological analyses revealed the presence of necrotic tissue inside the protrusions, methods followed suggested that they were not a fixation artifact (Messick *et al.*, 2003).

Because exotopic protrusions were seen as a possible threat to the well-being of the food web, the prevalence of protrusions on copepods was regularly monitored in Lago Maggiore from 1994 to 2002, in parallel with observations carried out in Lake Michigan, as well as in other freshwater and marine sites from which protrusions were reported (Silina and Khudolei, 1994) and originally described (Crisafi and Crescenti, 1975).

This study was aimed at identifying the different types of protrusions, evaluating the degree of infection and the prevalence of protrusions during different developmental stages of the infested populations, as well as the potential damage of these structures at the population level.

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METHOD

The study was carried out in Lago Maggiore, a deep, oligomictic, subalpine lake in northern Italy. Located south of the Alps (latitude 45°57′ N, longitude 3°47′ W), it is the second largest Italian lake in surface area, depth and volume. It is at present oligotrophic. A gradual re-oligotrophication in the last 20 years was caused by phosphorus abatement after the implementation of sewage treatment and decrease of phosphorus content in detergents (Manca, 1993). However, the presence of xenobiotic organic compounds of recent origin has been reported, as a consequence of point-source contamination (Guzzella *et al.*, 1998).

Monthly zooplankton samples (integrated sinusoidal hauls in the upper 50 m) were collected between 1994 and 2002, at a station corresponding to the point of maximum depth (370 m), using a Clarke-Bumpus plankton sampler (76-μm nylon net). Samples were fixed in pure ethanol. Live animals were inspected on each collection date. For each sample, we estimated (i) the population density of the copepods, distinguishing the different copepod developmental stages, (ii) the proportion of infected/total number of organisms (degree of infection), and (iii) the percentage distribution of protrusions on the different species and/or developmental life stages (host prevalence).

RESULTS AND DISCUSSION

Nature of the infection

The most common protrusions were type I cysts (Manca *et al.*, 1996), which were dark, stalked and with a clear internal granular structure. Type II cysts (hyaline, with a more defined internal structure) were rarely observed (<5% of the total cysts). They were found almost exclusively on diaptomids, particularly of the species *E. padanus* (Fig. 1), the most abundant of the two calanoid copepods inhabiting the pelagic zone of the lake (the other species, *Mixodiaptomus laciniatus*, being rare). In some instances, they appear to be continuous with the gonad of infected animals (Fig. 1d and e).

Since 1998, in addition to the original cyst-like protrusions, it was also possible to identify more complex structures, continuous with the protrusions, which might be interpreted as successive stages of a parasite's infection (Fig. 2). Some of them were identified as infections by ellobiopsids (Fig. 3), a group of organisms of

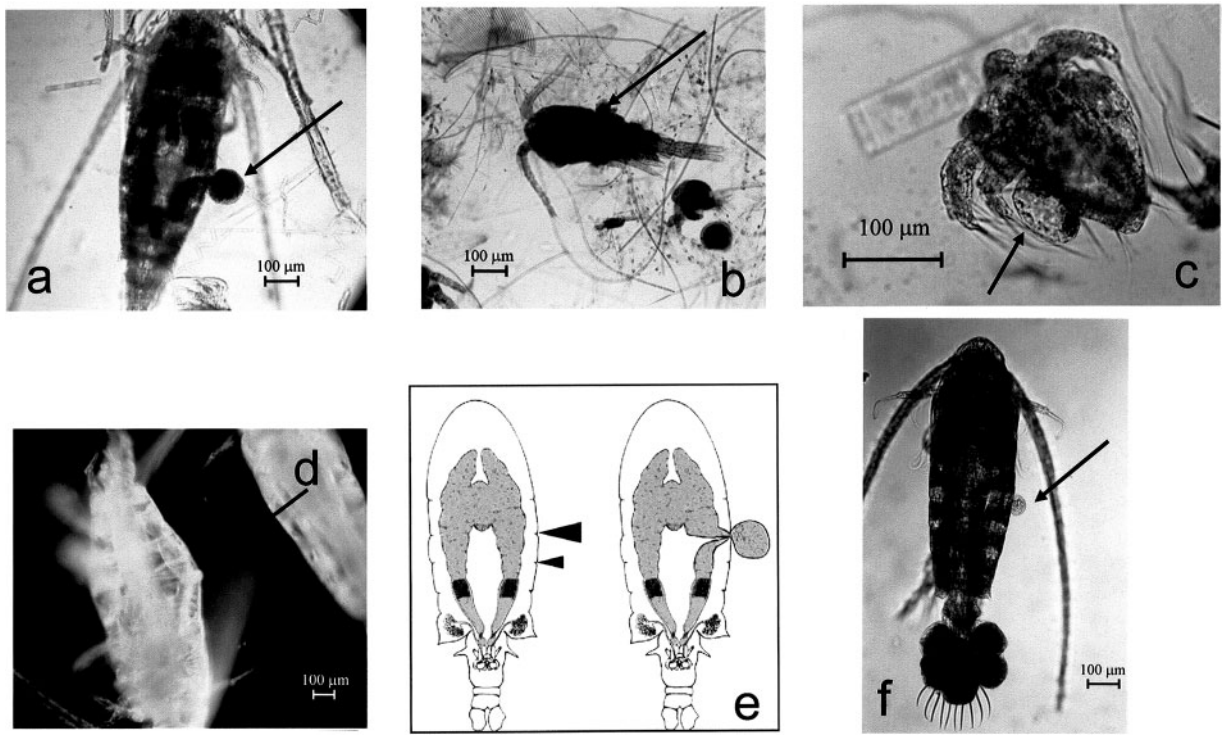

Fig. 1. Exotopic protrusions on copepods from Lago Maggiore; (**a**–**d**) protrusions of dark, spherical and a granular internal structure type (type I): (**a**) on a V copepodite of *E. padanus*; (**b**) on a copepodite and (**c**) on a nauplius of *Cyclops abyssorum*; (**d**) on a female of *E. padanus* in which a deviation of the gonad is visible, as schematically represented in (**e**), arrows referring to the usual location of the protrusions; (**f**) protrusion of the small, transparent and nongranular type (type II), on *E. padanus* ovigerous female.

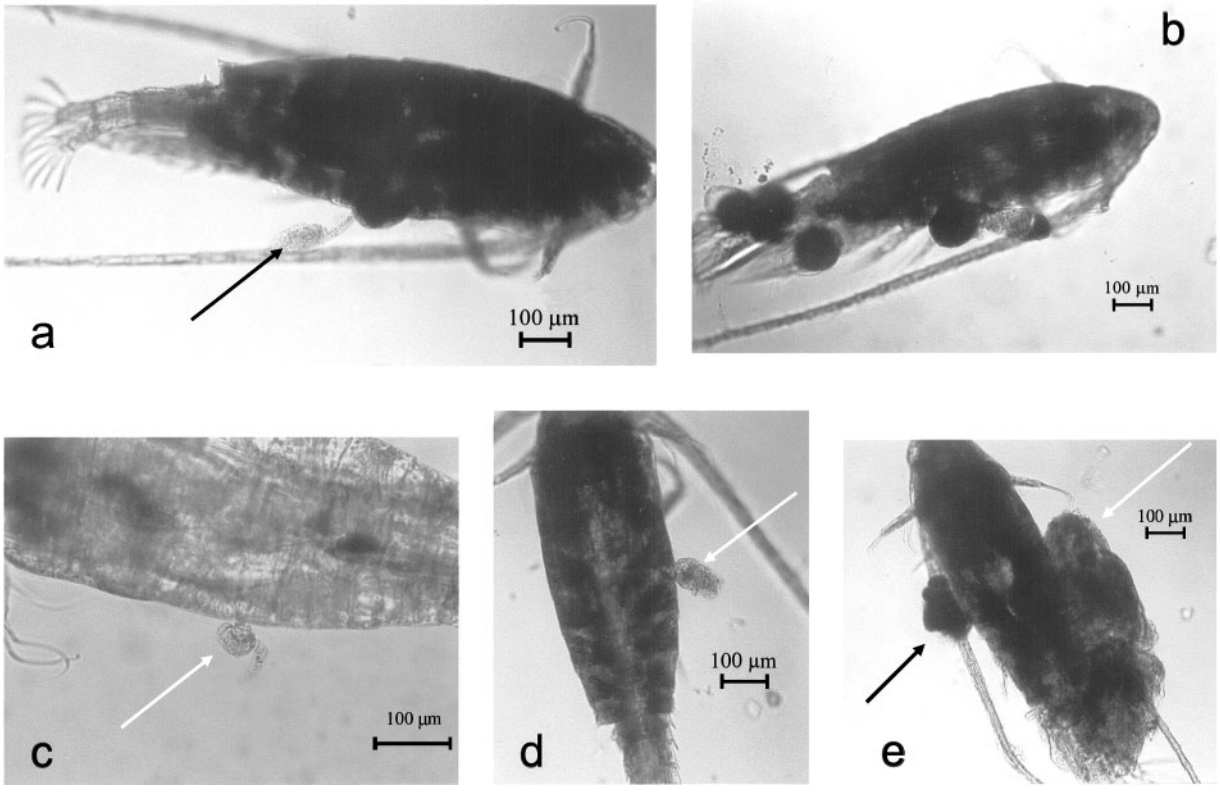

Fig. 2. Protrusions on V copepodites and adults of *E. padanus* from which parasites were found to grow; (**a**, **b**, **e**) type I protrusion; (**c**) type II protrusion; (**d**) parasite located on the same site where protrusions were found.

uncertain taxonomic position, recently defined as achlorophyllous dinoflagellates (Galt and Whisler, 1970), which during a stage of their life cycle parasitize marine crustacean zooplankters (Theodorides, 1989). Their life histories are heterogeneous and only partially understood.

Although the ellobiopsids were first described from marine organisms, only recently have they been reported from a few freshwater sites [Lake Midmar, South Africa (Rayner and King, 1986), and Lake Patterson, Michigan (Bridgeman *et al.*, 2000)]. Ellobiopsids consist of two parts: a trophomere, which is attached to a host's body; and a gonomere, in the distal portion, in which zoospores are produced. When mature, the zoospores are dispersed into the lake water. The ellobiopsids are most commonly located in the same region of the body where protrusions are found (Fig. 3); however, they are occasionally attached to other parts, namely, the mouth and the antennae. Occasionally, two to three very large parasites were found attached to the urosome of a single animal, and in one instance, up to five ellobiopsids were detected on a single host. In 1999 and 2003, the parasite was detected also on copepodites and nauplii of *Cyclops abyssorum* (Fig. 4a), the second most abundant copepod species of the lake and occasionally found free in the water sample (Fig. 4b).

Structures quite similar to the protrusions recently reported from freshwater sites all over the world were originally described from marine sites, particularly from Mar Grande and Mar Piccolo in the Taranto Sea, a heavily industrially polluted site in southern Italy (Crisafi and Crescenti, 1975). An inspection of live as well as fixed specimens collected in August 2002 (by M. Manca, with colleagues at the CNR, Istituto Ambiente Marino Costiero, sezione Talassografico, Taranto; five 126-µm nylon net vertical hauls in the upper 10 m, at different stations of Mar Grande and Mar Piccolo, Taranto) revealed that exotopic protrusions were abundant on calanoid copepods. In addition, several years ago, a colleague working at the Taranto Institute (G. Fanelli, CNR, IAMC, Taranto, Italy) found the same type of infection, which is at present attributed to ellobiopsids (Fig. 4c), on specimens of *Acartia latisetosa*. As in copepods from Lago Maggiore, the site of attachment of the parasite on a host's body was the same as that of the cyst-like protrusions, from which the protrusions may developmentally originate. It may be that, during an initial stage of infection, ellobiopsids puncture the host's body, thus provoking herniation of the tissue. This

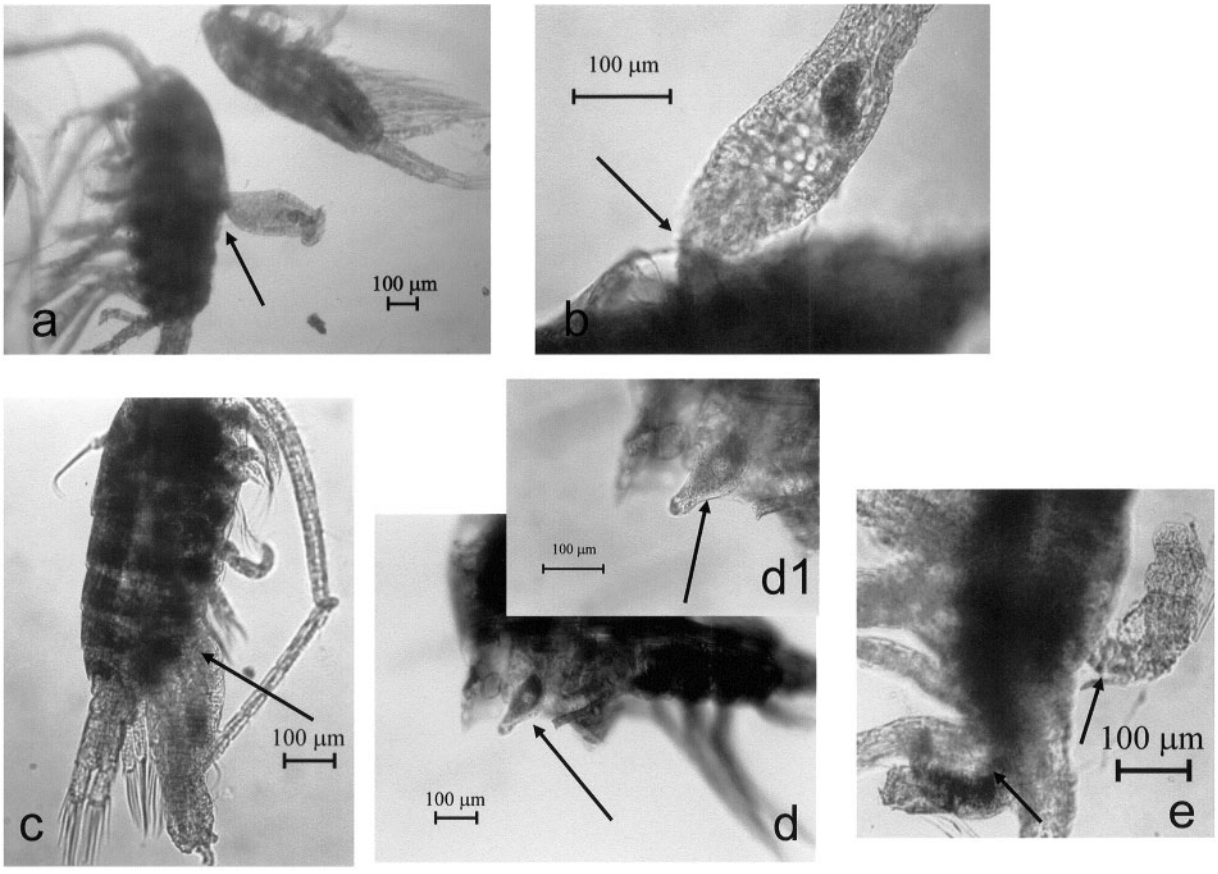

Fig. 3. Infection by ellobiopsids on copepodites and adults of *E. padanus* from Lago Maggiore; a trophomere and a sporulating gonomere are clearly distinguishable in the parasite's body. Most commonly located on the same site as protrusions, parasites can occasionally be found also on (**d**, **d1**) the thoracic appendages and (**e**) the urosome. Occasional sites of attachment were mouth appendages and antennae. Up to five parasites were found on the same host.

might also account for the presence of necrotic tissue inside the protrusions (cysts) (Messick *et al*., 2004).

The ellobiopsids from copepods of Lago Maggiore were clearly the same as those reported on *Tropodiaptomus spectabilis* from Lake Midmar, South Africa (*Ellobiopsis chattoni*) (Rayner and King, 1986).

Level of infection of the population

A clear seasonality was observed in the degree of infection (percentage of the infected/total number of individuals in the population) of *E. padanus*. In some years, there was a marked unimodal increase during the summer period, when up to 25% of the total individuals of the population were infected (Fig. 5). In other years, three main phases of increase were observed, the maximum always being reached in summer. The degree of infection seem to follow an increase in the proportion of adults and in the number of eggs per individual.

The average degree of infection in the different years was around 20%, a value comparable with that found for *T. spectabilis* from Lake Midmar, South Africa (Rayner and King, 1986) but lower than that reported for *Epischura lacustris* in Lake Michigan, USA (71%) (Bridgeman *et al*., 2000).

Occasionally, we observed differences in the horizontal distribution of infected animals: in October 1999, the level of infection of the population in a littoral station located in an area influenced by the effluent discharge of a factory was 2.5 times that of samples collected in the pelagic station located in the deepest area of the lake (M. Manca, personal observation). On the other hand, population density at the littoral station was 30% lower than that of the pelagic station.

Prevalence

As reported for cyst-like protrusions (Manca *et al*., 1996, 1999), adults, as well as last-stage copepodites of *E. padanus*, were the preferred hosts for ellobiopsids. Very few nauplii were found to be infected (Fig. 4a). In

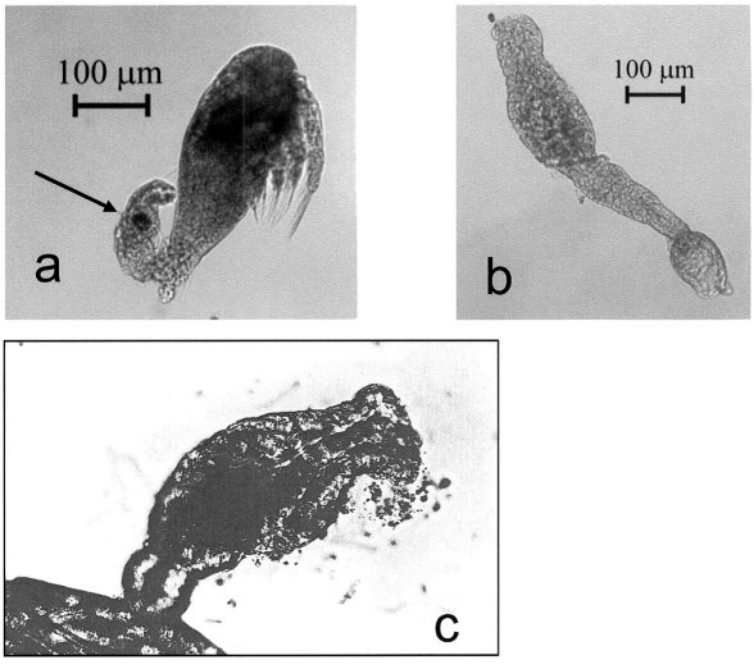

Fig. 4. Occasionally, ellobiopsids were found (**a**) on the caudal portion of nauplii or (**b**) were free in the water sample. To ellobiopsid infection should be ascribed some exotopic protrusions originally reported (Crisafi and Crescenti, 1975) from diaptomids of marine sites in Italy (**c**) (*A. latisetosa*, Mar Piccolo and Mar Grande di Taranto; photograph by G. Fanelli, CNR, IAMC, Taranto, Italy).

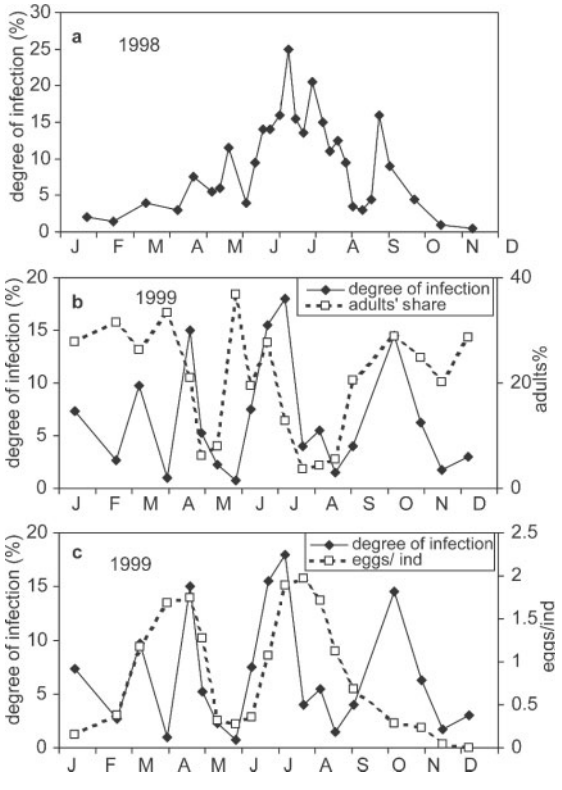

Fig. 5. (**a**) Seasonal changes in the level of infection of *E. padanus* population in Lago Maggiore, as well as (**b**) in the proportion of adults and (**c**) in the number of eggs per individual.

Lake Michigan (Bridgeman *et al.*, 2000), nearly opposite patterns of infestations were observed between calanoid and cyclopoid species. In calanoids, protrusions were most frequent in adults. Conversely, they were absent among adult cyclopoids but occurred frequently on nauplii cyclopoids, particularly of the species *D. bicuspidatus thomasi.* The protrusions most prevalent on nauplii were not necessarily ellobiopsids (G. Messick, Oxford, Maryland, personal communication): the ellobiopsids were rare and more prevalent in adults (Messick *et al.*, 2004).

In Lago Maggiore, although the prevalence of protrusions has remained more or less stable in the study period, we found that the protrusions have become more diverse than originally reported, in that more types of protrusions, as well as clearly defined ellobiopsids, are observed in the more recent period (2000–03), during which they seem to affect also early stages of cyclopoid copepods (Manca *et al.*, 2003).

Throughout spring and summer, protrusions were mainly found on adults, particularly on females (~70–90% of the total protrusions found), despite the fact that copepodites (of the different stages) were more abundant in the lake (Fig. 6).

The preference for adults might be related to gonadic maturation, possibly explaining the increase in the degree of infection in the population after an increase in the proportion of adults and the observation that gonads are often affected by this parasite.

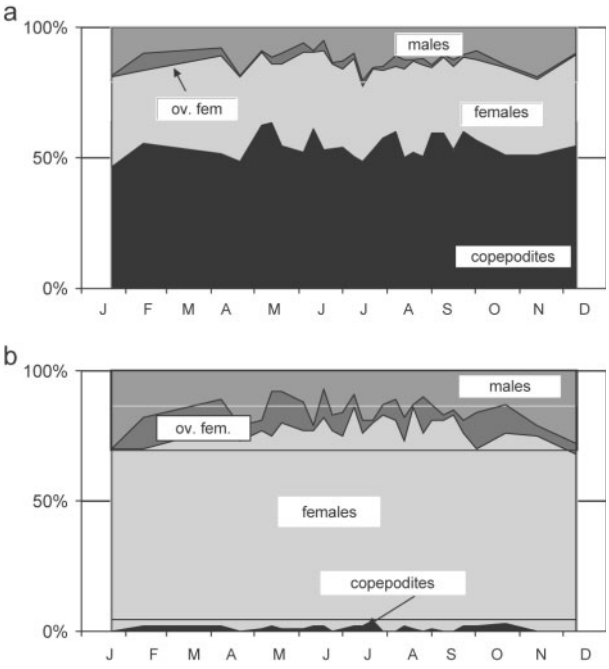

Fig. 6. Seasonal variations in the population structure of *E. padanus* (**a**) and in the prevalence of exotopic protrusions (**b**).

There were differences in host prevalence within the different components of adult population: an analysis of pluriannual data (for the period 1994–2002) showed that the mean prevalence was highest for non-ovigerous females and lowest for ovigerous females. Differences were statistically significant ($P < 0.001$; $n = 309$; Scheffé test for post-hoc comparison of means). A comparison between the infected and non-infected fraction of the population showed that prevalence in males was higher in the infected than in the non-infected ones. The differences were statistically significant ($P < 0.001$; $n = 204$; *t*-test for independent samples). As a consequence, during the entire study period, the ratio of females to males was lower in infected than in non-infected animals.

The prevalence in males had a clear seasonality, as values of the period between July and December were higher than those of the period between January and June. The differences between the two periods were statistically significant for the considered years ($P = 0.007$; $n = 84$; *t*-test for independent samples), with the exception of 1999, in which a complete vertical mixing of lake waters occurred in February (Manca *et al.*, 2000). Such an event, which used to occur in Lago Maggiore every 7 years on average, has become very rare in recent times (before that of 1999, it had been recorded only in 1981), as a consequence of warming and an increase in water column stability (Ambrosetti and Barbanti, 1999). Very severe winters, along with very low water temperatures and strong winds, are also known to alter the copepod's population structure (Manca *et al.*, 2000). In 1999, prevalence in males, between January and June, was double that of the period between July and December and three times that observed for the same period in the other years. Such differences might be explained by differences in prevalence between hypolimnetic and epilimnetic animals. However, this is an indirect estimate, and an analysis of the vertical distribution of infected/non-infected animals should be done to test this hypothesis.

Throughout the investigated period, the proportion of ovigerous females was higher in the infected than in the non-infected population; the differences were statistically significant (*t*-test for independent samples; $P < 0.001$; $n = 200$). However, this result might also be a consequence of sample manipulation, as protrusions were analysed before counting the samples. A correct estimation should be made based on live animals; these were inspected on several occasions but only with the aim of verifying that the protrusions were not a fixation artifact. Therefore, this point has to be further investigated.

Mean clutch size of ovigerous females tended to decrease with increasing levels of infection; however, the differences were not statistically significant on the pluriannual data set.

CONCLUSION

Exotopic protrusions are a stable component of calanoid copepods from Lago Maggiore, particularly of *E. padanus*, from which they were first described in freshwater. As originally observed, most protrusions were located on the prosome of adults, although, more recently, they were found on other sites of attachment and during early developmental stages, including nauplii and copepodites. Early copepodites of *C. abyssorum*, the second most important copepod species of the lake, were recently found to be infected. At least in some instances, the protrusions seem to represent an initial stage of infection by an ellobiopsid, which in 2002 was also found in one specimen in the adult stage.

Infestation of animals from Lago Maggiore appears to be of the same type that was originally reported from calanoid copepods of the Taranto Sea and of Lake Midmar in South Africa. However, further investigations will be necessary to confirm the exact nature of the infection; they should include histological analysis (currently being undertaken by G. Messick, at the Oxford Laboratory at NOAA, Oxford, MD) and culturing of infected animals.

Life-table experiments and an analysis of the population dynamics will be necessary to determine whether

infection by this parasite influences the value of the intrinsic rate of increase or of other life-history characteristics of the host. An important question to focus will be that of whether the population dynamics drives the infection dynamics or vice versa. However, there is no literature information from freshwater or marine sites on the population dynamics or behavior of individuals infected by ellobiopsids. As observed for other types of infections and for individuals heavily infested by epibionts, a high parasite load might increase susceptibility of copepods to visual predators also decreasing ability to escape predation (Weissman *et al.*, 1990; Xu and Burns, 1990; Manca *et al.*, 1996).

Ellobiopsids, originally described from marine organisms, of the species found in Lago Maggiore and in Lake Midmar might be regarded as invaders, similar to other protists which invaded the Great Lakes (USA and Canada) drainage from the Black Sea.

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REFERENCES

Ambrosetti, W. and Barbanti, L. (1999) Deep water warming in lakes. An indicator of climate changes. *J. Limnol.*, **58**, 1–7.

Bridgeman, T. B., Messick, G. and Vanderploeg, H. A. (2000) Sudden appearance of cysts and ellobiopsid parasites on zooplankton in a Michigan Lake: a potential explanation of tumor like anomalies. *Can. J. Fish. Aquat. Sci.*, **57**, 1539–1544.

Crisafi, P. and Crescenti, M. (1975) Conseguenze delle attività umane sullo zooplancton del Mare di Taranto. *Boll. Pesca Piscic. Idrobiol.*, **30**, 207–218.

Galt, J. H. and Whisler, H. C. (1970) Differentiation of flagellated spores parasite of marine Crustacea in *Thalassomyces marsupii*. *Ellobiopsis Microbiol.*, **71**, 295–303.

Guzzella, L., Patrolecco, L., Pagnotta, R. *et al.* (1998) DDT and other organochlorine compounds in the Lake Maggiore sediments: a recent point source of contamination. *Fresenius Environ. Bull.*, **7**, 79–89.

Manca, M. (1993) Recent changes in the zooplankton community structure of Lago Maggiore undergoing oligotrophication. Proceedings of the 5th International Conference on the Conservation and Management of Lakes, Stresa, 17–21 May, pp. 140–143.

Manca, M., Alemani, P. and Carnevale, A. (2003) Appearance of cysts on zooplanktonic copepods in a large, deep subalpine lake, Lago Maggiore (Northern Italy). Poster presented at the 46th Conference on Great Lakes, International Association of Great Lakes Research, De Paul University, Chicago, 22–26 June 2003.

Manca, M., Beltrami, M. and Sonvico, D. (1996) On the appearance of epibionts on the crustacean zooplankton of a large subalpine lake undergoing oligotrophication (Lago Maggiore, Italy). *Mem. Ist. Ital. Idrobiol.*, **54**, 161–171.

Manca, M., Morabito, G. and Cavicchioni, N. (2000) First observations on the effect of a complete, exceptional overturn of Lake Maggiore on plankton and primary production. *Int. Rev. Ges. Hydrobiol.*, **85**, 209–222.

Manca, M., Vanderploeg, H., Cavaletto, J. *et al.* (1999) Seasonal, spatial and global patterns of infestation of tumor-like anomalies on zooplankton. Zooplankton Tumor Workshop, 15–16 November 1999, Great Lakes Environmental Research Laboratory, Ann Arbor, MI.

Messick, G. A., Vanderploeg, H. A., Cavaletto, J. F. *et al.* (2004) Histological characteristics of abnormal protrusions on Copepods from Lake Michigan, USA. *Zool. Stud.*, **43**, 314–323.

Omair, M., Vanderploeg, H. A., Jude, D. J. *et al.* (1999) First observation of tumor like abnormalities (Exophytic lesion) on Lake Michigan zooplankton. *Can. J. Fish. Aquat. Sci.*, **56**, 1711–1715.

Rayner, A. N. and King, E. M. (1986) First record of a freshwater calanoid *Tropodiaptomus spectabilis* (Kiefer, 1929) (Crustacea, Copepoda) as host of an ellobiopsid parasite. *J. Plankton Res.*, **8**, 837–840.

Silina, N. I. and Khudolei, W. (1994) Tumorlike anomalies in planktonic copepods. *Hydrobiol. J.*, **30**, 52–55.

Theodorides, J. (1989) Parasitology of marine zooplankton. *Adv. Mar. Biol.*, **25**, 117–177.

Weissman, P., Cantrell, P. A. and Threlkeld, S. T. (1990) Epizoic euglenoid flagellates increase the susceptibility of some zooplankton to fish predation. *Limnol. Oceanogr.*, **35**, 954–959.

Xu, Z. and Burns, C. W. (1990) Effects of the epizoic ciliate *Epistylis daphniae*, on growth, reproduction and mortality of *Boeckella triarticulata* (Thomson) (Copepods: Calanoida). *Hydrobiologia*, **209**, 183–189.