J. Biol. Res. 2009 n. I - Vol. LXXXII - Rubbettino - Soveria Mannelli

Ultrastructural Evidence of Luminescent Gland Openings in *Metridia gerlachei* Giesbrecht, 1902 (Copepoda: Calanoida)

P. Romano[1], M. Feletti[2], G.L. Mariottini[3], L. Pane[4]

[1] Dipartimento di Medicina Sperimentale, Università di Genova, I-16132 Genova, Italia
[2] Regione Liguria, Dipartimento Agricoltura - Protezione Civile - Ufficio Produzioni Agroalimentari, I-16100 Genova, Italia
[3] Dipartimento di Biologia, Università di Genova, Viale Benedetto XV 5, I-16132 Genova, Italia

KEYWORDS: *Zooplankton, Copepods, Metridia gerlachei, Antarctica, Luminescence*

Abstract

The luminescence is a feature of several marine planktonic organisms, such as bacteria, algae, cnidaria, ctenophores, crustaceans and pyrosomes; in particular, a lot of pelagic crustacea have got luminescent organs (Ghirardelli, 1981, Gruner, 1994) useful for trophic, reproductive or defense purposes. Several calanoid copepods are luminescent and possess variously distributed epidermal glands. In this paper the occurrence of luminescent gland openings in adults and copepodids of Metridia gerlachei sampled during the Italian Oceanographic Campaign in Antarctica 1987-88 in Terra Nova Bay (Ross Sea) is described by using scanning electron microscopy (SEM) techniques. The openings of cephalothorax luminescent gland has been observed and described with the aim to complete what is known about this subject (Piñero de Verdinelli, 1981); the occurrence of a gland opening, not previously described, has been reported. Considering the extreme characteristics of the environment in which Metridia gerlachei lives and considering also that this species seems to prefer to live in low-light waters (Carli et al., 1990, 1992a) as the constant light conditions during the Antarctic summer restrict its occurrence in surface waters, luminescent structures could have an adaptive significance both for reproduction and defence.

Introduction

The luminescence is a phenomenon occurring in several organisms living in different environments (Hastings, 1996) and it can provide ecological and biological advantages, for example in inter- and intra-specific relationships, in the reproductive behaviour, in predation and in defence activities.

In the marine environment the luminescence is typical not only of deep-water organisms, but also of several beings living in intermediate and surface waters: light emission is known in the bacteria *Photobacterium* spp. and *Vibrio* spp. which live in association with zooplankton and fish (Boyd, 1992; Haygood and Distel, 1993), in *Vibrio fisheri* symbiotic of squids (Boettcher and Ruby, 1990; Lee and Ruby, 1992; Ruby and McFall-Ngai, 1992), in several strains of bacteria isolated from cultured prawns (Liu et al., 1996). The occurrence of bioluminescent bacteria has been also considered as a marker to recognize the pollution state of the water (Ramaiah and Chandramohan, 1993).

Bioluminescent phenomena are also recorded in several unicellular algae: luminescent dinoflagellates are abundant in epipelagic oceanic zones (Tett, 1971) and in eterotrophic species the emission of light seems to be affected by starvation (Buskey et al., 1992) and by food quality and concentration (Buskey, 1995).

Several higher marine organisms, such as cnidarians, ctenophores, cephalopods, crustaceans (ostracods, copepods, amphipods, mysids, euphausiids, decapods), and tunicates (pyrosomes) show bioluminescence. Light production due to the zooplankton (siphonophores, ctenophores, euphausiids) emission was observed in sound scattering layer (Clarke and Backus, 1956; Kampa and Boden, 1956; Barham, 1963, 1966; Sameoto, 1980; Greene et al., 1988; Widder et al., 1992). All luminescent crustaceans are marine and almost all pelagic (Gruner, 1994); some of them produce secretions forming a luminescent cloud, probably to frighten predators (Ghirardelli, 1981). Luminescent organs of Crustaceans consist of epidermal cells or glands, but also parts of the epatopancreas can produce light.

Among Copepods, luminescent species belong mostly to calanoids (Clarke et al., 1962; Gruner, 1994). Light production in copepods involves epidermal glands distributed all over the body and in the appendages according to a definite scheme. The occurrence of light glands, fluorescent glands and luminous pores is also known in calanoids such as *Gaussia princeps* (Barnes and Case, 1972).

In the Antarctic region the study of the luminescence is a subject of particular concern to recognize morphological, biochemical and physiological processes and to explain evolutionary events and adaptive strategies accomplished in such peculiar and extreme ecosystem. Among Antarctic copepods *Metridia gerlachei* is a particularly interesting autochtonous species; it inhabits the waters around the whole continent (Farran, 1929) and was recognized as a frequent species in the Ross Sea (Carli et al., 1990, 1992a, 1992b; Zunini Sertorio et al., 1990, 1992; Pane et al., 2004). In the family Metridinidae luminescent glands consist of two spindle-shaped cells differently coloured, debouching by a common pore; it has been supposed that the first one produces luciferin and the other one luciferase; the ejected secretion forms a bright cloud or a luminous green-bluish point (Gruner, 1994). Among Metridinidae *Metridia longa* was indicated to be the most luminescent organism in the marginal ice zone of the Greenland Sea (Buskey, 1992). As at present the ultrastructural morphological characteristics of *Metridia gerlachei* have been scarcely studied (Romano et al., 1999; Mariottini et al., 2000), aim of this work is to describe the occurrence of luminescent gland openings in the adult female of *Metridia gerlachei* by means of scanning electron microscopy (SEM) techniques.

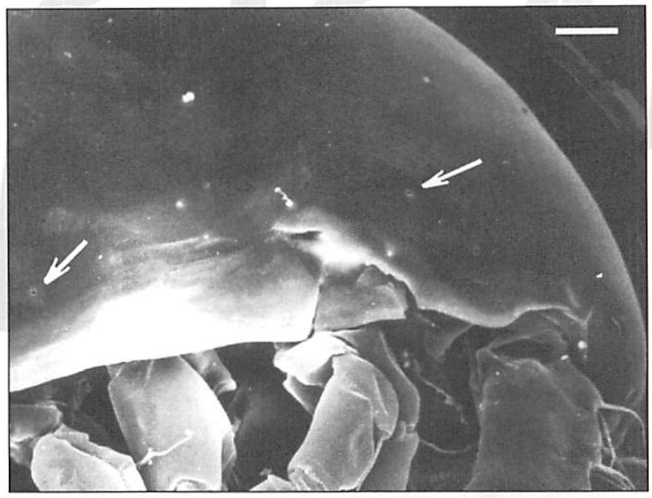

Fig. 1 – Metridia gerlachei adult female. Gland openings (white arrows) in the right side of prosome. Bar: 100 μm.

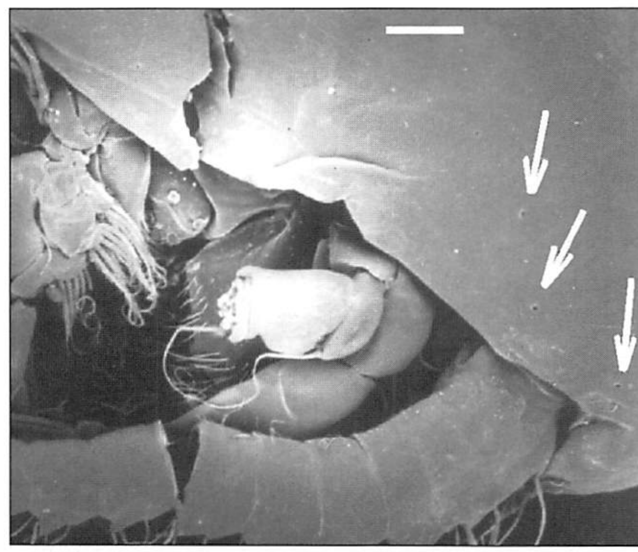

Fig. 2 – Metridia gerlachei adult female. Particular of the right anterior part of prosome. White arrows sign some gland openings. Bar: 100 μm.

Materials and methods

Metridia gerlachei specimens were sampled between December 1987 and February 1988 during the Italian Oceanographic Campaign in Antarctica in the Terra Nova Bay (Ross Sea) by means of a BIONESS electronic multinet (Guglielmo et al., 1990).
Specimens have been maintained in 4% formalin; in the laboratory the taxonomic identification was carried out by means of a Wild M3C stereomicroscope, a Leitz Diaplan microscope and a Zeiss ID02 invertoscope.
Samples utilized for the SEM observation were treated according to Cohen (1979); briefly, they were rinsed twice in 2.5% glutaraldehyde in cacodylate buffer 0.1M at 4°C for 24 hours and subsequently post-fixed in 1% OsO_4 in cacodylate buffer at 4°C for 30 minutes, rinsed twice in the same buffer, rinsed ones in distilled water, dehydrated in ethanol of increasing concentration up to 100%; critical point dried from liquid CO_2.
Specimens were mounted with small pieces of double-sided adhesive tape on aluminium stubs, coated with a 20 nm gold layer in an argon atmosphere flow discharge sputter coating-unit (Polaron E 5100) and examined by means of a ISI SS-40 scanning electron microscope operated at an accelerating voltage of 10-20 Kw.

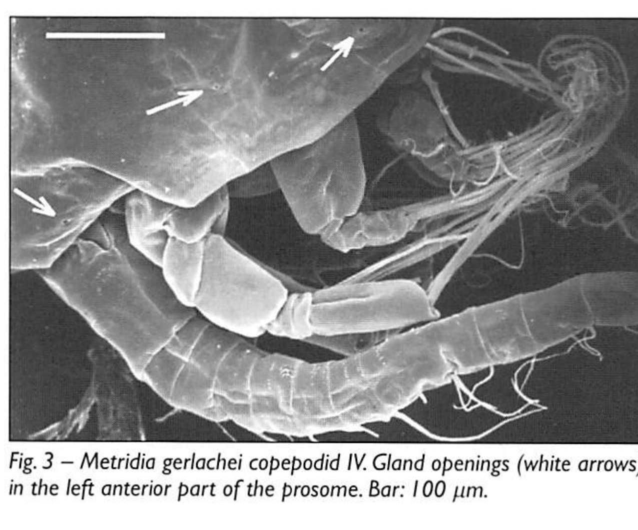

Fig. 3 – Metridia gerlachei copepodid IV. Gland openings (white arrows) in the left anterior part of the prosome. Bar: 100 μm.

Results

The arrangement of luminescent gland openings placed in the cephalothorax has been observed considering the work of Piñero de Verdinelli (1981). Scanning electron images show the occurrence of some openings near the emergence of the antennae and above the emerging of mouth appendages of the adult female (figs. 1, 2) and of the copepodid IV (fig. 3); some openings have been also observed in the appendages and in the abdomen of copepodid IV (fig. 4), and in the end part of prosome of the adult female (figs 5, 6). A detailed picture of an opening is shown in fig. 7.

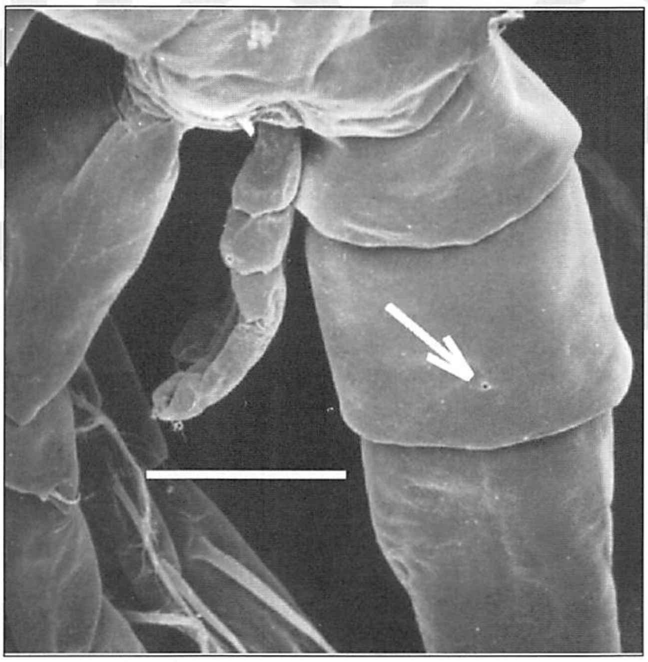

Fig. 4 – Metridia gerlachei copepodid IV. Opening (white arrow) in the abdominal segment. Bar: 100 µm.

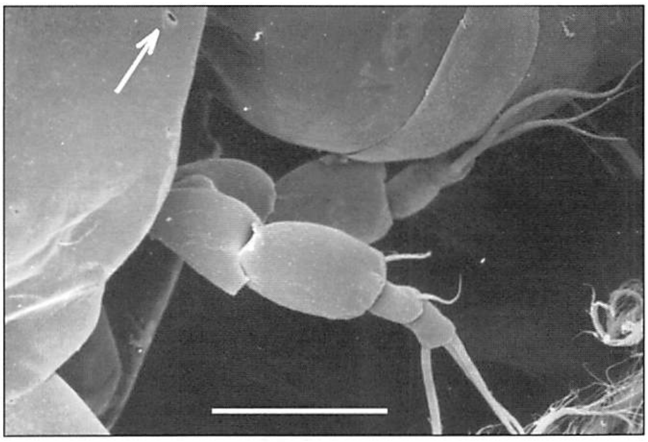

Fig. 5 – Metridia gerlachei adult female. Gland opening (white arrow) in the end part of the prosome. Bar: 100 µm.

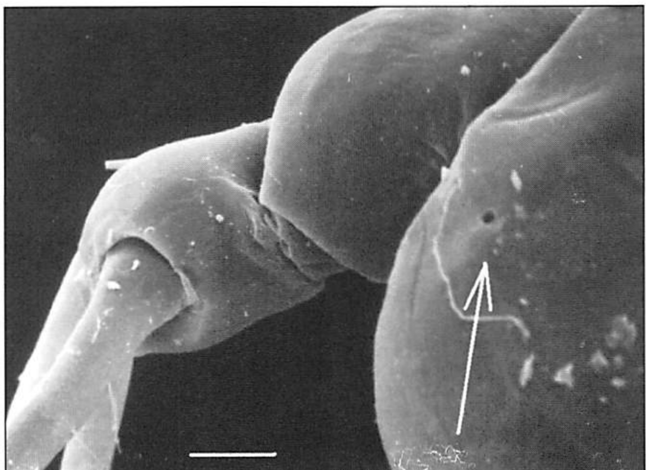

Fig. 6 – Metridia gerlachei adult female. Gland opening (white arrow) in the end part of the prosome. Bar: 10 µm.

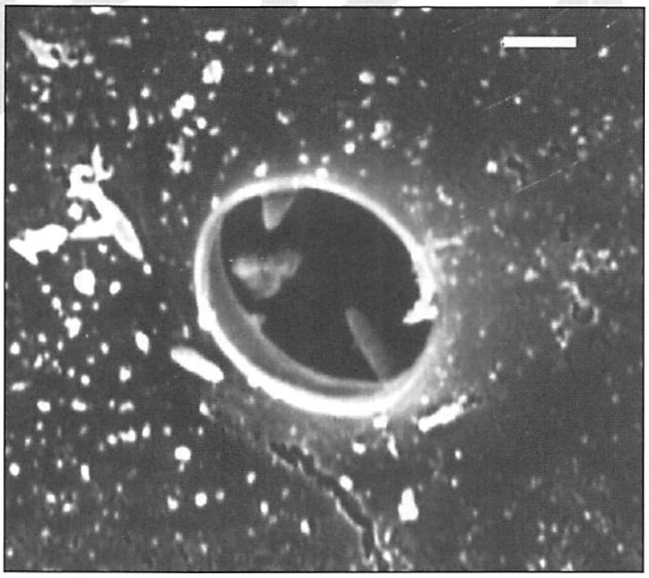

Fig. 7 – Particular of a gland opening of Metridia gerlachei. Bar: 1 mm.

Therefore, the description and distribution of lateral openings and partly of frontal and posterior ones observed by Piñero de Verdinelli (1981) are confirmed by the here reported observations.
Nevertheless, during this study also the occurrence of other openings in the portion of cephalothorax placed between the anterior and the middle-hind part of cephalothorax, at mandibular level, and in abdomen and appendages has been observed.

Discussion

Metridia gerlachei is a typical Antarctic copepod, which greatly occurs in the Ross Sea (Bradford, 1971; Carli et al., 1990, 1992a, 1992b; Zunini Sertorio et al., 1990, 1992). Although its morphological description using light microscopy has been carried out by several scientists (Giesbrecht, 1902; Wolfenden, 1908; Tanaka, 1960; Bradford, 1971; Ramirez and Dinofrio, 1976; Piñero de Verdinelli, 1981), the available ultrastructural observations are scant; thus, specific studies could allow to better characterize the species and improve the morphological knowledge. Furthermore, on the whole the knowledge about luminescence of Crustaceans, and particularly of Copepods, is still scarce, since a number of luminescent species live in meso- and bathypelagic waters; therefore, the occurrence, distribution, biological and physiological significance of luminescent glands in Antarctic species is to date greatly unknown. On the other hand, only by electron microscopy it is possible to obtain detailed informations about this matter. Several hypotheses exist on the aptitude of pelagic marine organisms to produce light, but direct research and observations are very scarce, so the explication of such phenomenon is often theoretical. However, it was supposed that the ability to produce bioluminescence provides with several biological and ecological advantages for the organisms in the intraspecific relationships and also as a response to the environment (Gruner, 1994).

It's well known that luminescent organs of Crustaceans are formed differently in the different taxonomic groups; among Copepods it is possible to observe such structures particularly in Calanoids, although studies concerning luminescence have been carried out also on *Oncaea* and *Corycaeus* (Poecilostomatoida), *Oithona* (Cyclopoida), and *Macrosetella* (Harpacticoida); the species belonging to these genera are characteristic of low-light zones and make vertical diel migrations, inhabiting deep waters with daylight and rising to the surface during the night (Gruner, 1994).
In Copepods luminescent glands are epidermic and disposed in well defined locations; as concerns Metridinidae each gland consists of two spindle-shaped and differently stained cells, debouching together with a pore (Clarke et al., 1962); it has been supposed that one of them produces luciferin, and the other one luciferase (Gruner, 1994). After stimulation, the "mixture" secreted is ejected in the water, where it forms a diffuse light cloud or a well defined light point, which can shine even for one minute. Subsequently, Copepods go away from the emission swimming actively; in this case the luminescent reaction is an escape defensive behaviour. Previous descriptions pointed out the typical green-blue colour of the light. The secretion is clearly sticky and traces often remain on the body near the pores. The luminescence can be also harmful for the emitting organism, owing to the possible attraction of specific predators, nevertheless, luminescent specimens have probably developed still greatly unknown fine neurophysiological adaptations and behaviours.
Piñero de Verdinelli (1981) described in *Metridia gerlachei* small (sensilla), mean (not defined glands) and large openings (luminescent glands); the location on prosome of luminescent gland openings was exhaustively described and these structures have been divided into three groups: the first one includes 10 openings located in the anterior region, the second is composed by 1 pair of openings for each side in the middle-hind region; finally, the third group includes 6 openings located in the hind region; luminescent glands are present also in the last thoracic segment, one on the left and two on the right side. The above mentioned arrangement has been confirmed by SEM, at least as concerns lateral openings; further studies will attempt to furnish scanning images also of dorsal structures. Nevertheless, the occurrence of other openings has been indicated.
It's noticeable that, as the above-mentioned luminescent and diel migrating species (Gruner, 1994), also *Metridia gerlachei* is a species living in low-light waters (Carli et al., 1990, 1992a) and its occurrence in surface waters during the Antarctic summer is highly restricted by the nearly constant light conditions. In this connection, luminescent structures could have an adaptive significance both as concerns reproductive strategies and defence behaviour.

Acknowledgements

This research was supported by the Italian PNRA (Programma Nazionale di Ricerche in Antartide).

References

Barham. G. 1963. Siphonophores and the deep scattering layer. Science, 140: 826-828.

Barham E.G. 1966. Deep scattering layer migration and composition: observations from a diving saucer. Science, 151: 1399-1403.

Barnes A.T., Case J.F. 1972. Bioluminescence in the mesopelagic copepod *Gaussia princeps* (T. Scott). J. Exp. Mar. Biol. Ecol., 8: 53-71.

Boettcher K.J., Ruby E.G., 1990. Depressed light emission by symbiotic *Vibrio fischeri* of the sepioid squid *Euprymna scolopes*. J. Bacteriol., 172(7): 3701-3706.

Boyd R.F. 1992. Microbiologia generale. Medical Books, Palermo, 984 pp.

Bradford J.M., 1971. Pelagic Copepoda. In: The fauna of the Ross Sea. Part 8. N.Z. Oceanogr. Inst., Mem.59: 9-31.

Buskey E.J. 1992. Epipelagic planktonic bioluminescence in the marginal ice zone of the Greenland Sea. Mar. Biol., 113: 689-698.

Buskey E.J. 1995. Growth and bioluminescence of *Noctiluca scintillans* on varying algal diets. J. Plankton Res., 17(1): 29-40

Buskey E.J., Strom S., Coulter C., 1992. Bioluminescence of heterotrophic dinoflagellates from Texas coastal waters. J. Exp. Mar. Biol. Ecol., 159: 37-49.

Carli A., Mariottini G.L., Pane L. 1990. Contribution to the study of Copepods collected in Terranova Bay (Ross Sea). Nat. Sc. Com. Ant., Ocean. Camp. 1987-88, Data Rep., II: 129-159.

Carli A., Feletti M., Mariottini G.L., Pane L. 1992a. Contribution to the study of Copepods collected during the Italian Oceanographic Campaign in Antarctica 1989-90. Nat. Sc. Com. Ant., Ocean. Camp. 1989-90, Data Rep. II: 179-210.

Carli A., Feletti M., Mariottini G.L., Pane L. 1992b. Distribuzione di *Metridia gerlachei* Giesbrecht, 1902 (Copepoda, Calanoida) nella Baia di Terranova (Mare di Ross). Atti 9° Congr. A.I.O.L., S.Margherita Ligure 20-23/11/1990: 623-633.

Clarke G.L., Backus R.H. 1956. Measurements of light penetration in relation to vertical migration and records of luminescence of deep-sea animals. Deep-Sea Res., 4: 1-14.

Clarke G.L., Conover R.J., David C.N., Nicol J.A.C. 1962. Comparative studies of luminescence in copepods and other pelagic marine animals. J. Mar. biol. Ass. U.K., 42: 541-564.

Cohen, A.L. 1979. Critical point drying. Principles and procedures. Scanning Electron Microsc., 2: 303-322.

Farran G.P., 1929. Copepoda. British Antarctic Terra Nova Expedition 1910; Nat. Hist. Rep., Zoology, 8: 203-306, 4 pls.

Ghirardelli E. 1981. La vita nelle acque. UTET, Torini, 610 pp.

Giesbrecht W., 1902. Copepoden. Expedition Antarctique Belge. Résultats du Voyage du S.Y. Belgica en 1897-1898-1899. Zoologie, 49 pp., 13 pls.

Greene C.H., Wiebe P.H., Burczynski J., Youngbluth M.J., 1988. Acoustical detection of high-density krill demersal layers in the submarine canyons off Georges Bank. Science, 241: 359-361.

Gruner H.E. 1994. Organes lumineux et luminescence. In Grassé P.P., Crustacés. Traité de Zoologie. Anatomie, Systématique, Biologie. VII(I). Masson Ed., Paris: 411-434.

Guglielmo L., Costanzo G., Manganaro A., Zagami G. 1990. Spatial and vertical distribution of zooplanktonic communities in the Terranova Bay (Ross Sea). Nat. Sc. Com. Ant., Ocean. Camp. 1987-88, Data Rep., I: 257-398.

Hastings J.W. 1996. Chemistries and colors of bioluminescent reactions: A review. Gene, 173(1): 5-11.

Haygood M.G., Distel D.L. 1993. Bioluminescent symbionts of flashlight fishes and deep-sea anglerfishes form unique lineages related to the genus *Vibrio*. Nature, 363 (6425): 154-156.

Kampa E.M., Boden B.P., 1956. Light generation in a sonic-scattering layer. Deep-Sea Res., 4: 73-92.

Lee K.H., Ruby E.G. 1992. Detection of the light organ symbiont, *Vibrio fischeri*, in Hawaiian seawater by using lux gene probes. Appl. Environ. Microbiol., 58(3): 942-947.

Liu P., Lee K., Yii K., Kou G., Chen S. 1996. Isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicus*. Curr. Microbiol., 33(2): 129-132.

Mariottini G.L., Feletti M., Romano P., Cadoni A., Carli A. 2000. An ultrastructural study of the Antarctic Calanoid Copepod *Metridia gerlachei* Giesbrecht, 1902. Adult female. J. Biol. Res. - Boll. Soc. It. Biol. Sper., 76: 73-80.

Pane L., Feletti M., Francomacaro B., Mariottini G.L. 2004. Summer coastal zooplankton biomass and copepod community structure near the Italian Terra Nova Base (Terra Nova Bay, Ross Sea, Antarctica). J. Plankton Res., 26 (12): 1479-1488.

Piñero de Verdinelli M.E., 1981. Sistemática e ecologia das espécies de *Metridia* (Copepoda, Calanoida) do Mar de Weddell, com considerações sobre a hidrografia e o fitoplâncton. Thesis Doutor em Ciências, Inst. Oceanográfico da Universidade de São Paulo, 196 pp.

Ramaiah N., Chandramohan D., 1993. Ecological and laboratory studies on the role of luminous bacteria and their luminescence in coastal pollution surveillance. Mar. Pollut. Bull., 26(4): 190-201.

Ramirez F.C., Dinofrio E.O. 1976. Resultados plantológicos de la Campaña Oceantar I. III. Copepodos. Instituto Antartico Argentino, Contr. N° 196, 49 pp.

Romano P., Feletti M., Mariottini G.L., Carli A. 1999. Ecological and nutritional implications of the mandibular structure in the Antarctic calanoid copepod *Metridia gerlachei* Giesbrecht, 1902: an ultrastructural study. Polar Biol., 22: 7-12.

Ruby E.G., McFall-Ngai M.J. 1992. A squid that glows in the night: Development of an animal-bacterial mutualism. J. Bacteriol., 174(15): 4865-4870.

Sameoto D.D. 1980. Quantitative measurements of euphausiids using a 120-kHz sounder and their in situ orientation. Can. J. Fish. Aquat. Sci., 37: 693-702.

Tanaka O., 1960. Pelagic Copepoda. Biological results of the Japanese Antarctic Research Expedition, 95 pp.

Tett P.B. 1971. The relationship between dinoflagellates and the bioluminescence of sea water. J. Mar. Biol. Assoc. U.K., 51: 183-205.

Widder E.A., Greene C.H., Youngbluth M.J. 1992. Bioluminescence of sound-scattering layers in the Gulf of Maine. J. Plankton Res., 14(11): 1607-1624.

Wolfenden R.N. 1908. Crustacea. VIII Copepoda. National Antarctic Expedition 1901-1904. Natural History 4, Zoology, 46 pp., 7 pls.

Zunini Sertorio T., Salemi Picone P., Bernat P., Cattini E., Ossola C. 1990. Copepods collected in sixteen stations during the Italian Antarctic Expedition 1987-1988. Nat. Sc. Com. Ant., Ocean. Camp. 1987-88, Data Rep., II: 67-125.

Zunini Sertorio T., Licandro P., Ricci F., Giallain M. 1992. A study on Ross Sea Copepods. Nat. Sc. Com. Ant., Ocean. Camp. 1989-90, Data Rep., II: 217-246.