CrossMark

ORIGINAL PAPER

Non-predatory mortality in Mediterranean coastal copepods

Iole Di Capua[1] (ORCID) · **Maria Grazia Mazzocchi[1]**

Abstract Mortality affects the dynamics of zooplankton populations with important effects on trophic interactions and biogeochemical fluxes in marine environments, but is still one of the processes least investigated in the field. In the present study, the non-predatory mortality in copepod assemblages and species was investigated by applying the neutral red staining method to identify and quantify copepod carcasses throughout an annual cycle in a Mediterranean coastal site (station LTER-MC in the inner Gulf of Naples). Carcasses accounted on average for 10.3% (±9.7%) of total copepod abundance and were most abundant in spring, summer and autumn. Carcasses were represented predominantly by copepodites (78.9 ± 22.0%) and occurred more frequently and abundantly in calanoids than in other copepod orders, with interspecific differences in their abundance and temporal patterns. Using carcass abundances from field data and decomposition times from laboratory observations, we estimated non-predatory mortality rates of key calanoids that are common and abundant in Mediterranean coastal waters. Non-predatory mortality rates averaged 0.13 day^{-1} in *Paracalanus parvus*, 0.07 day^{-1} in *Clausocalanus* spp., 0.06 day^{-1} in *Temora stylifera* and 0.04 day^{-1} in *Acartia clausi*. Non-predatory mortality rates in these populations were not correlated with temperature, salinity or chlorophyll *a.*

Responsible Editor: X. Irigoien.

Reviewed by D. L. Calliari and undisclosed experts.

✉ Iole Di Capua
  iole.dicapua@szn.it

[1] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

Introduction

Mortality is an inherent process of life that affects population dynamics and eventually community structure in both terrestrial and aquatic ecosystems. Despite its acknowledged importance, mortality is still one of the most neglected aspects of zooplankton biology (Ohman and Wood 1995; Hirst and Kiørboe 2002). Studies addressing mortality in marine zooplankton are limited and discouraged by a belief that the problem is intractable (Ohman 2012). Estimating mortality rates in situ is challenging indeed, and the critical issues that should be addressed to render the problem tractable have been examined recently (Ohman 2012). Quantifying mortality is fundamental for understanding the development of populations under different conditions (Carlotti et al. 2000). Field and modeling studies show that mortality varies among species and stages, and plays a major role in determining zooplankton population dynamics (e.g., Ohman and Hirche 2001; Eiane and Ohman 2004; Mazzocchi et al. 2006), though its causes remain generally undefined. The main cause of mortality in zooplankton is generally attributed to predation (Genin et al. 1995), but many other factors can be responsible, such as disease (Delgado and Alcaraz 1999), parasites (Kimmerer and McKinnon 1990; Burns 1985; Ohtsuka et al. 2004; Duffy et al. 2005), environmental stress of physical and/or chemical origin (Carpenter et al. 1974; Hall and Alden 1997; Roman et al. 1993), starvation (Tsuda 1994), and senescence (Ceballos and Kiørboe 2011; Saiz et al. 2015). The relative role played by each of these factors in pelagic communities is still to be clarified, but it is expected to vary in different assemblages, environments, and conditions.

Mortality in planktonic copepods can be estimated in field samples from the occurrence of carcasses, which can be distinguished easily from the exuviae generated by molting

between developmental stages. The empty exoskeletons derived from ecdysis differ from carcasses, which contain traces of tissues even after an extended period of decomposition (Tang et al. 2006). The number of studies that have quantified copepod carcasses at sea is still limited, although the non-predatory mortality has been estimated to account for a remarkable fraction (1/4–1/3) of copepod mortality (Hirst and Kiørboe 2002). Copepod carcasses contribute to the downward fluxes of carbon and nitrogen and to fueling the benthic system in oligotrophic areas (Frangoulis et al. 2011; Sampei et al. 2009, 2012). Copepod carcasses occur everywhere in the water column and can be abundant in epipelagic layers (Genin et al. 1995), although their numbers generally increase with depth (Wheeler 1967; Böttger-Schnack 1995, 1996). In the upper layer, carcasses account for a large fraction of total particulate organic carbon flux, which can exceed the contribution of fecal pellets outside of the phytoplankton bloom period (Sampei et al. 2009, 2012). In the mixed layer, carcasses can remain in suspension for days, up to five times the average duration owing to turbulence (Kirillin et al. 2012). In shallow turbulent coastal waters, the presence of copepod carcasses may be important and failing to take them into the account can lead to incorrect estimates of zooplankton abundance and carbon fluxes.

Investigations of coastal zooplankton are generally based on preserved samples and no distinction is made between live and dead individuals. However, dead copepods can be identified by visual discrimination in live and preserved samples and by staining methods (Table 1 in Daase et al. 2014 and references therein). The most appropriate stain for copepods is neutral red (NR), a vital stain that is incorporated into the lysosomes of live cells; the lack of NR uptake corresponds to loss of cell viability and indicates a dead organism (Dressel et al. 1972). Following the first publication of the laboratory protocol to sort dead from live marine copepods based on their differential uptake of NR (Dressel et al. 1972), the method was later refined for a broader application in field studies to quantify copepod carcasses (Tang et al. 2006; Elliott and Tang 2009, 2011a, b). Yet, in spite of that, the method has been applied at a limited number of locations so far, e.g., Chesapeake Bay (Tang et al. 2006; Elliott and Tang 2011a; Elliott et al. 2013), the Gulf of Mexico (Kimmel et al. 2009, 2010), the Chilean coast (Yanez et al. 2012), and Sevastopol Bay in the Black Sea (Litvinyuk et al. 2011; figure 3 in Tang and Elliott 2014). In the Mediterranean Sea, copepod carcasses have been taken into account only in few studies (Frangoulis et al. 2010, 2011) and their differentiation using staining methods was reported only recently in the Levantine Sea (eastern Mediterranean) (Beşiktepe et al. 2015).

The present study estimates non-predatory mortality in copepods by applying the NR method in the inner Gulf of Naples (southern Tyrrhenian Sea, western Mediterranean),

where the dynamics of zooplankton is being monitored since 1984 in a long-term time series (Ribera d'Alcalà et al. 2004). Various aspects of community and population dynamics have been examined using this time series, and particular attention has been paid to the copepod assemblages (Mazzocchi and Ribera d'Alcalà 1995; Di Capua and Mazzocchi 2004; Mazzocchi et al. 2006, 2011, 2012), but the contribution of copepod carcasses has not been considered thus far. The aims of the present study are to (1) enumerate the copepod carcasses to evaluate their contribution to copepod abundance during an annual cycle, (2) estimate the non-predatory mortality rates in copepod species from carcass abundance, and (3) evaluate the temporal patterns of specific non-predatory mortality rates in relation to the population seasonal cycle and the environmental conditions.

Materials and methods

Study site and environmental variables

The present study was carried out from 28 January 2013 to 23 January 2014 in the inner Gulf of Naples at station LTER-MC (40°48.5′N; 14°15′E; ~75 m depth), site of the long-term time series *MareChiara* ongoing since January 1984 (http://szn.macisteweb.com). Temperature and salinity profiles from the surface to 70 m depth were obtained with an SBE911 CTD mounted on a Rosette sampler equipped with 12, 5 L Niskin bottles. Total chlorophyll *a* (chl *a*) concentration was determined at seven selected depths using a spectrofluorometer. The mixed or stratified structure of the water column was represented by the mixed layer depth (MLD, Z_{ml}). Between the two groups of criteria generally used to define MLD from in situ observations of temperature and salinity profiles (Dong et al. 2008), we applied the density gradient ($\Delta\gamma_\theta$) criterion over a 5 m depth interval. The bottom of the mixed layer was the depth below which the gradient was ≥0.05 density unit m^{-1}.

Zooplankton sampling, staining and taxonomical identification

Zooplankton samples for the time series were collected at almost weekly frequency from 50 m depth to the surface by vertical tows with a Nansen-type net (113 cm mouth diameter, 200 µm mesh) equipped with a 1 L filtering cod-end and hauled at low speed (0.7–1.0 m s^{-1}) (see Mazzocchi et al. 2011 for further details). For the present study, 47 fresh samples were stained with NR ($C_{15}H_{17}ClN_4$) solution before proceeding with counting as described below.

Immediately after collection, the sample was concentrated in a 500 ml plastic jar and 750–1500 µL of NR solution (Sigma-Aldrich, Fluka Neutral Red) was added

according to zooplankton abundance, for a final NR concentration of 0.15–0.3%. The sample was then placed in a cooler in the dark at in situ water temperature and transported to the laboratory in 30–40 min (incubation time). In the laboratory, the sample was concentrated on a 100 μm mesh Nitex screen and rinsed briefly with filtered seawater to remove excess stain. The concentrated sample was then re-suspended in a graduated vessel filled with filtered seawater up to a volume of 200 mL. At least two replicated sub-samples of equal volume were taken with a large-bore graduated pipette after carefully mixing to homogenize the organism distribution. Each sub-sample was analyzed in a mini-Bogorov chamber (10 mL). The analyzed fractions varied from 1/8 to 1/20 of the entire sample and the number of copepods counted in each sample ranged from 187 to 3260 individuals, according to the organism abundance. The counts were performed as soon as possible (as recommended by Elliott and Tang 2009) and always within 4 h after the incubation phase. The samples were analyzed under a Leica M165 C stereomicroscope with dark and light background. Stereomicroscope lighting is important for distinguishing between live and dead copepods. The animals alive at the time of staining appear entirely or partly bright red, while dead animals remain completely unstained. Adult copepods were identified to species level and gender with the exception of *Calocalanus* and *Oithona* males which were identified to genus level. All copepodite stages (efficiently retained by 200 μm mesh net from CII or CIII, according to species) were pooled in a single category for each species or genus (e.g., *Calocalanus*, *Oithona*, *Corycaeus*). Oncaeid males and juveniles were identified to family level.

Method preliminary assessment

At the beginning of this study, the staining protocol outlined above was applied to samples collected at station LTER-MC in different seasons to test it at different zooplankton abundances and under different conditions of temperature (14–22 °C) and salinity (36.6–38.3) at the in situ pH of 8. Changes in seawater salinity and incubation temperature (in situ seawater temperature ±2 to 3 °C) did not show any visible effect on stain uptake and efficiency. However, we observed good staining results at zooplankton abundance <600 ind. m^{-3} and lower staining uptake at zooplankton abundance >600 ind. m^{-3}, resulting in a light-pink coloration of the copepods. In case of higher abundances, the quantity of NR solution added to samples was doubled. The occurrence of carcasses in zooplankton samples highlighted by the NR staining method was mainly assessed for copepods (e.g., Dressel et al. 1972; Tang et al. 2006), because other groups require a staining time that is inconveniently

long for field application (Crippen and Perrier 1974; Zetsche and Meysman 2012). The method and the dye concentration used here also proved to be the most appropriate for copepods, and therefore only this taxonomic group was considered here.

In mixed live copepod assemblages, we observed that cyclopoids and harpacticoids showed a different and less intense stain uptake than calanoids. Their bodies appeared partially colored indicating the uptake of the stain and therefore their vital condition. The absorption of NR was more effective and visible in the antennules for *Oithona*, in the urosome for *Oncaea* and *Euterpina*, and in the eyes and furcal rami for *Corycaeus* and *Farranula*. This partial absorption seems to indicate that these genera may require longer time than calanoids for complete stain adsorption and retention.

Mortality rates

To estimate the non-predatory mortality rates from the occurrence of carcasses, laboratory observations were conducted to follow the decomposition process of dead copepods in a selection of taxa which were most abundant as carcasses during the annual field study. Females, males and copepodites of *Clausocalanus* spp., *Acartia clausi, Paracalanus parvus* and *Temora stylifera* were sorted from live zooplankton samples collected at station LTER-MC and killed by thermal shock (20 min at −30 °C), which maintained their body intact. Dead copepods were transferred individually and separately to 200 mL flasks containing 10 μm filtered seawater and incubated in the dark at different temperatures, which corresponded to the temperatures recorded at the respective carcass peaks (Table 1). At regular intervals, ranging from 1 to 6 h, all individual carcasses were checked under a stereomicroscope (Leica M165 C) and their images recorded by a digital camera (Leica IC 80 HD). The stages of decomposition were identified according to the criteria assigned by Tang et al. (2006: fig. 4). The non-predatory mortality rates were estimated as $m = D/[t(1 − D)]$, where D is the fraction of carcasses (dead copepods) in the sample and t is the time (days) necessary for complete body decomposition at a given temperature (Tang et al. 2006). This simplified approach, which allows a rough estimate of non-predatory mortality based on live and dead copepod abundances as acquired during the present study, is based on the assumptions that the copepod populations were in a steady state and that decomposition was the main significant loss term.

The influence of environmental variables on copepod non-predatory mortality was estimated with the Pearson correlation test applied to the mortality rates and the surface values of temperature, salinity and chl *a*.

Table 1 Decomposition stages and times (in hours) of copepod carcasses in four key calanoid taxa in the laboratory (see "Materials and methods" for details)

Taxa	No. of specimens	Experimental temperature (°C)	In situ temperature (°C)	Decomposition time (h)			
				Initial stage	Early stage	Mid-stage	Late stage
Clausocalanus spp.							
Females	15	13 ± 3	15 ± 2	10	46	70	170
Males	8			24	46	70	170
Copepodites	11			10	46	70	170
Acartia clausi							
Females	6	22 ± 2	21 ± 3	6	24	46	105
Males	3			10	32	56	142
Copepodites	2			6	24	46	105
Paracalanus parvus							
Females	6	22 ± 2	20 ± 4	24	32	56	79
Males	3			32	46	56	79
Copepodites	2			24	32	56	79
Temora stylifera							
Females	6	26 ± 1	23 ± 2	6	32	52	148
Males	3			32	46	56	148
Copepodites	1			6	32	52	148

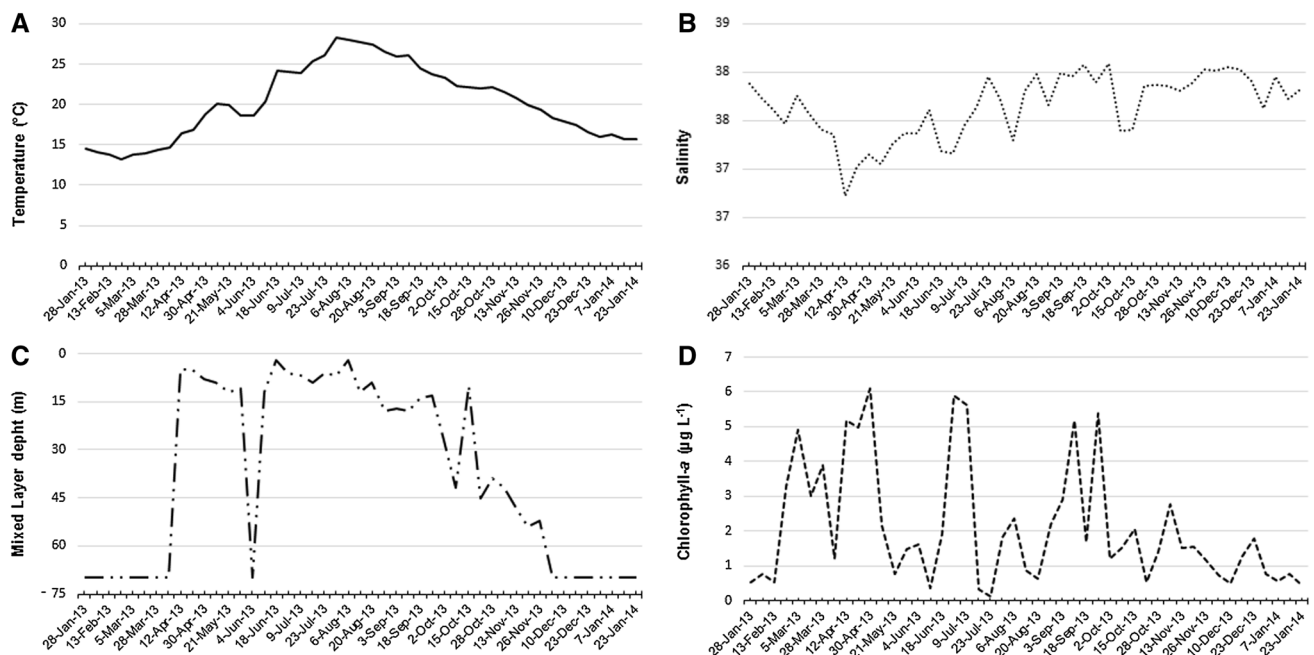Decomposition stages according to Tang et al. (2006)

Fig. 1 Temporal patterns of **a** temperature (°C), **b** salinity, **c** mixed layer depth (m) and **d** chlorophyll *a* at station LTER-MC in the inner Gulf of Naples during the year of the present study

Results

The hydrographic conditions at station LTER-MC showed surface temperatures ranging from 13.25 °C in late February to 28.23 °C in late July (Fig. 1a), and surface salinity varying between 36.13 in mid-April and 38.09 in early October, with ample weekly fluctuations (Fig. 1b). The water column was mixed from January to March; the seasonal stratification of the upper 2–15 m depth layer commenced abruptly in April and lasted until late September, followed by a gradual deepening of the mixed layer during the autumn (Fig. 1c). The surface chl *a* concentration showed oscillations during the year, ranging from a minimum of 0.11 µg L^{-1} on 23 July to a maximum of 6.08 µg L^{-1} on 30 April, with peaks

of similar amplitude recorded in May and late June–early July (Fig. 1d).

Copepods accounted for 54.7 ± 26.2% (mean ± SD) of total zooplankton abundance, ranging from a minimum of 75 ind. m^{-3} in December to peaks of 1304 ind. m^{-3} in April and 1418 ind. m^{-3} in August (Fig. 2). Calanoids made up 75.4 ± 13.2% of total copepod abundance, with the highest contribution (96.4%) in May; they were followed by cyclopoids (21.7 ± 11.9%), which increased their relative importance in winter and late autumn, and by harpacticoids, which contributed very low percentages throughout the whole year (2.9 ± 2.1%) (Fig. 2). Calanoids were represented by 77 species, but the records were dominated by only a few species, i.e., *Temora stylifera*, *Paracalanus parvus*, *Acartia clausi,* and *Centropages typicus*, which, together with *Clausocalanus* spp. juveniles, accounted for 80% of total calanoid abundance during the present study.

The abundance of dead copepods showed a median value of 25.7 carcasses m^{-3}, but overall a large temporal variability (Fig. 3). Carcasses were most abundant in May, August (peak of 258 carcasses m^{-3}) and October; they were negligible from mid-June to late July and in November, and completely absent on a few scattered occasions. Carcasses contributed a mean of 10.3 ± 9.7% (range 0–33.8%) to total copepod abundance and were predominantly represented by copepodites (78.9 ± 22.0%) (Fig. 3).

The majority of copepod carcasses were calanoids (99.4 ± 2.3%) and for this reason the analysis was focused on this group. The abundance of calanoid carcasses was frequently lower in correspondence of higher abundance of live calanoids (Fig. 4); however, no linear relationship was found between the two categories even when carcasses were lagged by one or two sampling dates after the live calanoids. Lack of relationship was found also between dead and live juveniles, though juveniles prevailed over adults in both calanoid carcasses (mean 78.9%) and in live calanoids (61.6%). Carcasses were found mostly for *Clausocalanus* spp., *Temora stylifera*, *Paracalanus parvus* and *Acartia clausi* and showed a clear temporal succession (Fig. 5). *Clausocalanus* spp. carcasses predominated from January to March (79.3 ± 6.3%) and from October to January (53.4 ± 33.3%); *A. clausi* and *P. parvus* made up most of the carcasses from April to June, accounting for 43.7% (±31.9%) and 40.2% (±23.8%), respectively; from July to September, *T. stylifera* carcasses predominated (48.9 ± 32.5%).

The experiment to determine the decomposition time necessary to estimate the non-predatory mortality rates showed differences in the four target copepod species (Table 1). The times necessary for a complete decomposition of carcasses were longer for *Clausocalanus* spp. at 13 °C (7 days) and *T. stylifera* at 26 °C (6 days) than for *A. clausi* and *P. parvus* at 22 °C (about 4 and 3 days, respectively). Decomposition times did not differ between adults and copepodites, with the exception of *A. clausi* males, which had longer decomposition time (6 days) than females and juveniles.

In *Clausocalanus* spp., non-predatory mortality rates based on decomposition time and fraction of carcasses in the sample were generally higher in periods of low population
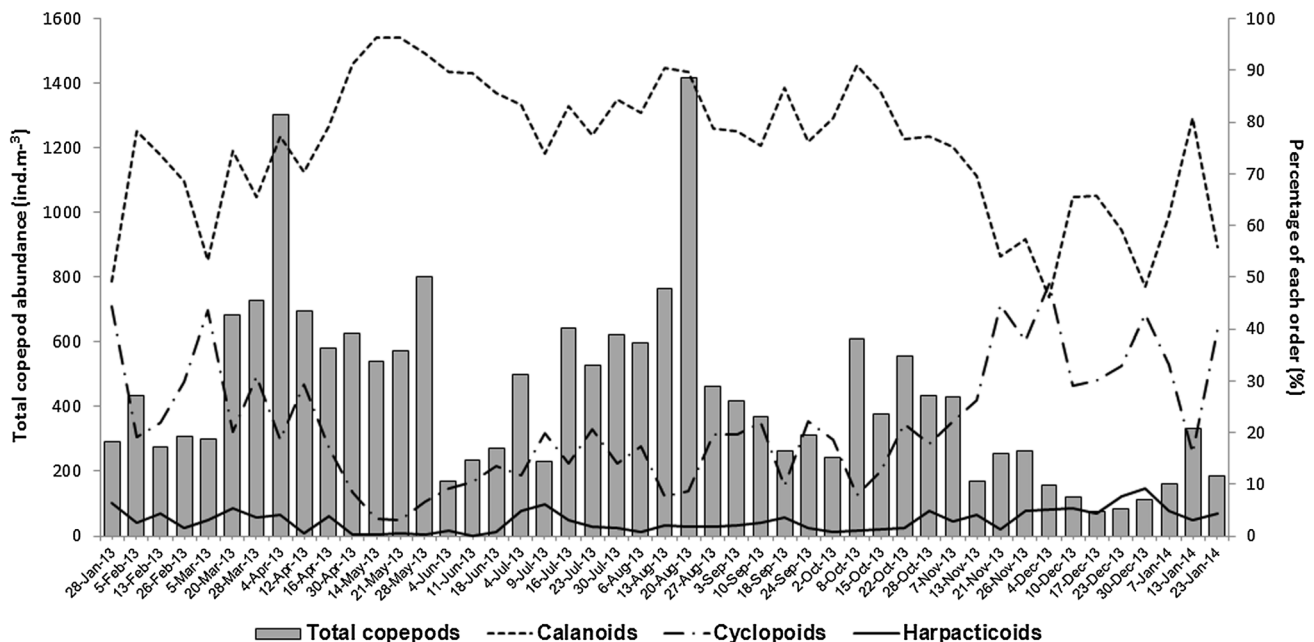

Fig. 2 Abundance of total copepods (ind. m^{-3}) at station LTER-MC (*bars*) and relative contribution (%) of the three main copepod orders (*lines*)
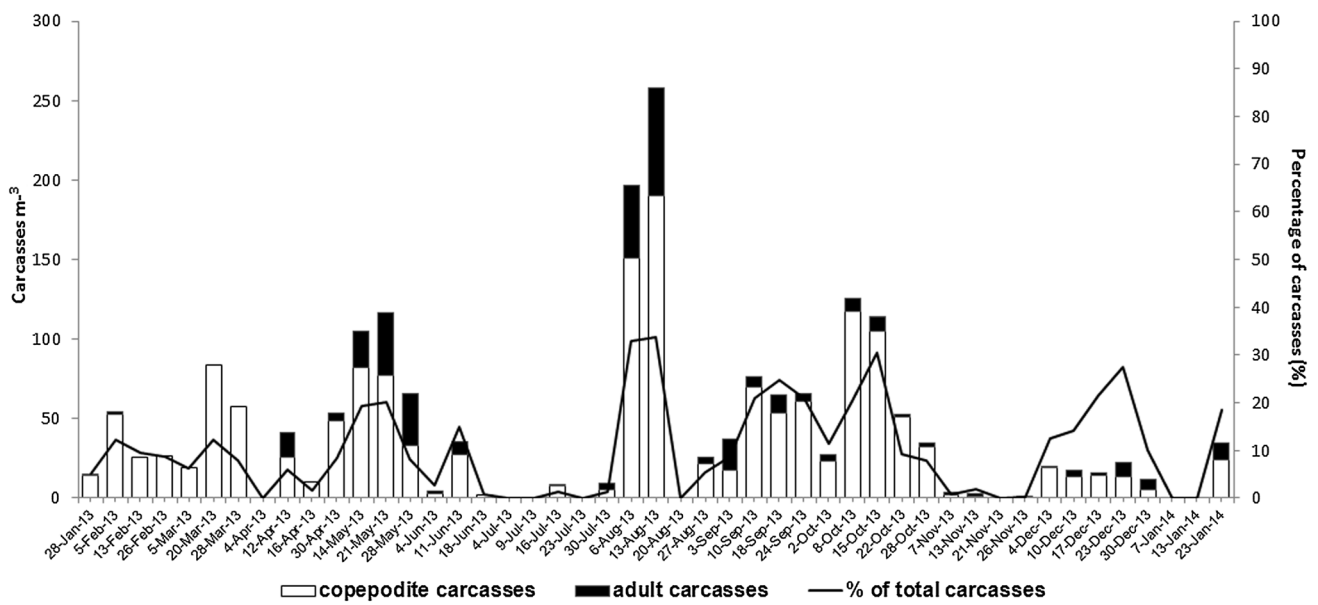
Fig. 3 Abundance of copepod carcasses (adults and copepodites, *bars*) and percentage contribution of carcasses (*line*) to total copepod abundance at station LTER-MC
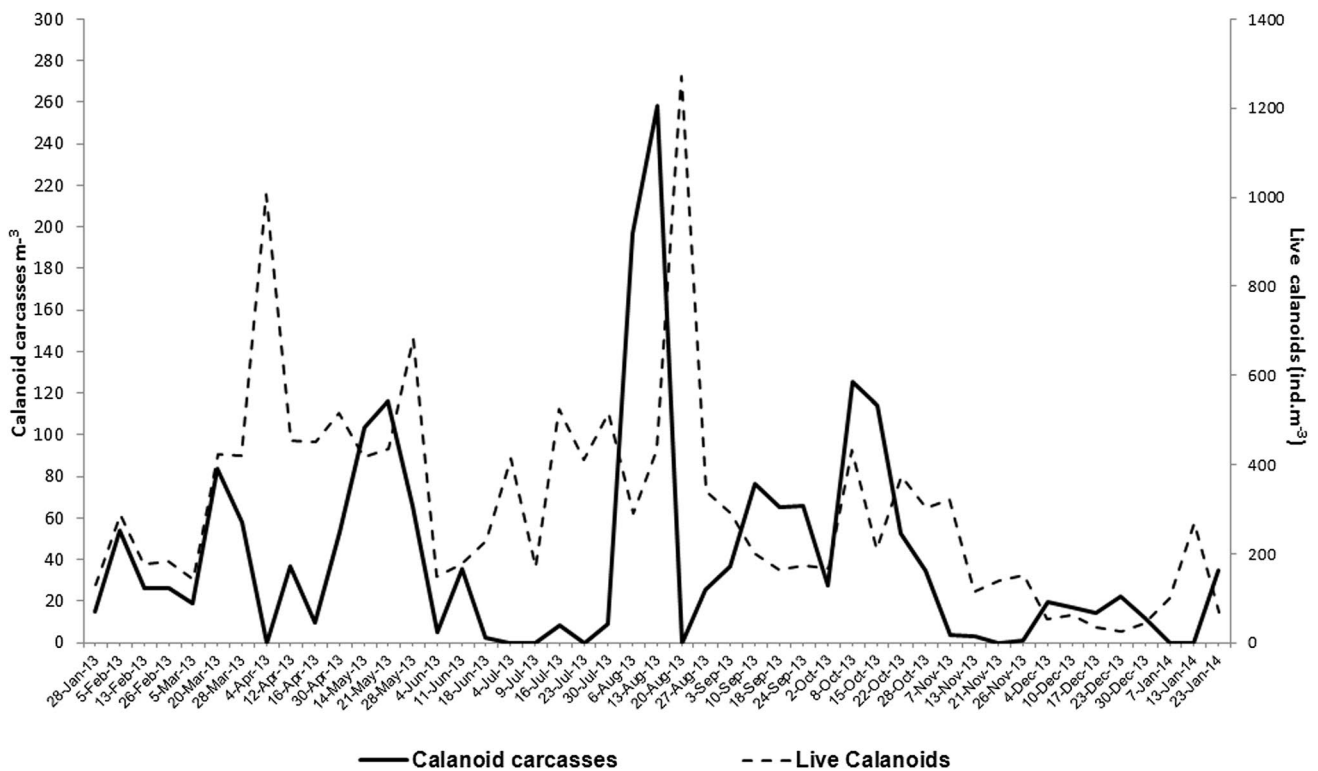

Fig. 4 Annual cycle of calanoid abundance (ind. m^{-3}) at station LTER-MC: carcasses (*solid line*) and live individuals (*dashed line*)

abundance, as in mid-May, mid-August and December (Fig. 6a). In *A. clausi*, mortality increased with the decrease of the population abundance in May and peaked at the end of the population seasonal cycle in mid-July (Fig. 6b). In *P. parvus*, mortality was highest after the spring population peak and in January 2014, corresponding with very
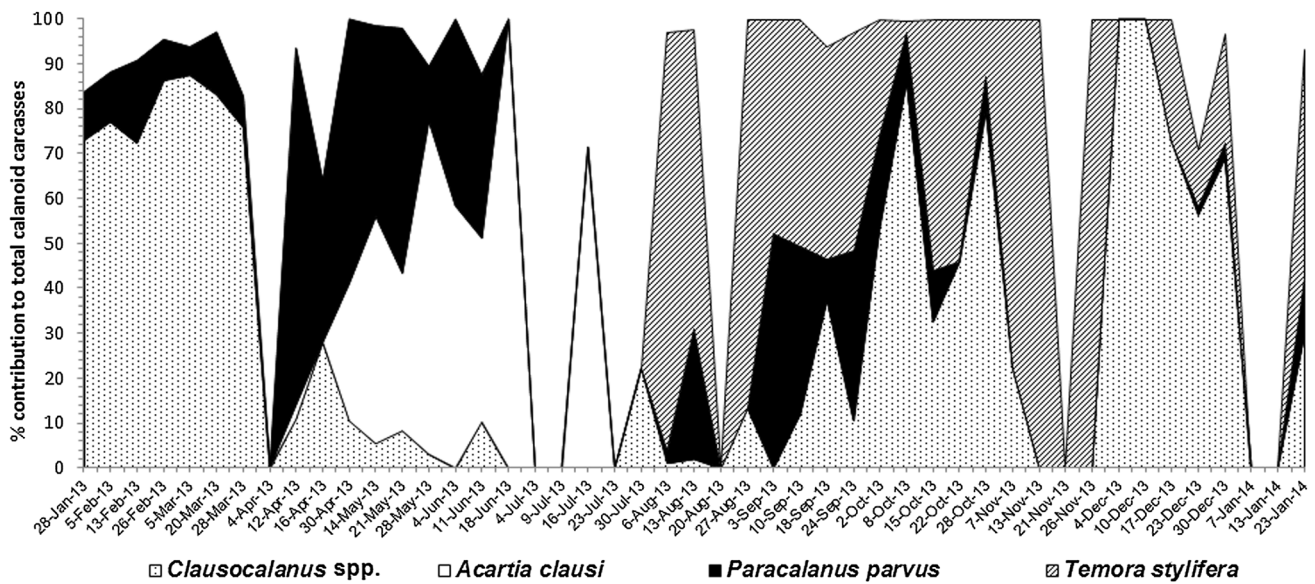
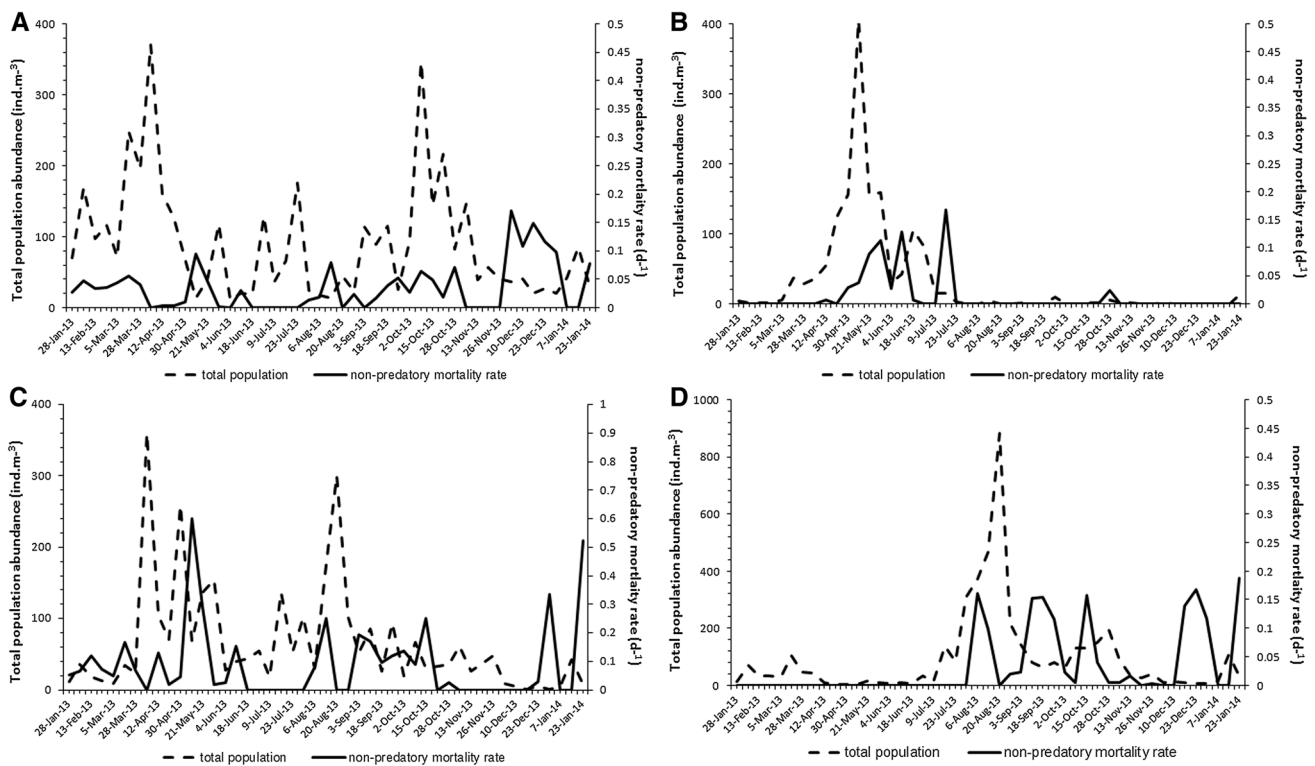**Fig. 5** Percentage contribution of four calanoid taxa to total calanoid carcasses at station LTER-MC

Fig. 6 Annual cycle of total population abundance (ind. m^{-3}, *dashed line*) and non-predatory mortality rates (day^{-1}, *continuous line*) of **a** *Clausocalanus* spp., **b** *Acartia clausi*, **c** *Paracalanus parvus* and **d** *Temora stylifera*

low total abundances; the period following the summer peak of abundance was characterized by continuous and quite high mortality rates (Fig. 6c). In *T. stylifera*, mortality rates showed similar high values in different phases of the population cycle, i.e., before the primary (August) and

secondary (October) peaks of abundance as well as during the following periods of low abundance (September, December, January). In this species, mortality was zero during the long period (January–July) that preceded the start of the steep population increase in summer (Fig. 6d).

Table 2 Non-predatory mortality rates (day^{-1}) in four key calanoid taxa at station LTER-MC

	First quartile	Median	Third quartile	Max	Mean
Clausocalanus spp.					
Females	0.00	0.00	0.02	0.10	0.02
Males	0.00	0.00	0.00	0.00	0.00
Copepodites	0.05	0.07	0.14	0.76	0.12
Acartia clausi					
Females	0.00	0.01	0.13	0.18	0.06
Males	0.00	0.01	0.07	0.11	0.03
Copepodites	0.01	0.04	0.08	0.17	0.05
Paracalanus parvus					
Females	0.00	0.00	0.03	0.33	0.04
Males	0.00	0.00	0.00	0.35	0.01
Copepodites	0.03	0.11	0.25	0.60	0.14
Temora stylifera					
Females	0.00	0.02	0.11	0.22	0.06
Males	0.00	0.01	0.07	0.25	0.06
Copepodites	0.00	0.02	0.15	0.28	0.07

Table 3 Pearson correlation coefficient calculated between environmental variables and non-predatory mortality rates of the key copepod taxa (total population) at station LTER-MC

	Clausocalanus spp.	*Acartia clausi*	*Paracalanus parvus*	*Temora stylifera*
Temperature	0.08	−0.24	−0.07	0.18
Salinity	−0.21	0.25	−0.19	0.25
Chlorophyll *a*	−0.22	−0.23	−0.07	−0.02

Overall, the non-predatory mortality rates differed among species and varied between genders and stages within species (Table 2). The rates were higher in copepodites than in adults in *P. parvus* and *Clausocalanus* spp.; in *A. clausi*, mortality rates were similar in adult females and copepodites and were slightly higher than in adult males; in *T. stylifera*, the mortality rates were similar in adults and juveniles (Table 2). There were no significant correlations between non-predatory mortality rates in these four calanoids and any of the environmental variables taken into consideration (Table 3).

Discussion

The present study provides the first estimate of copepod non-predatory mortality in the coastal waters of the western Mediterranean Sea by assessing the number of copepod carcasses using the neutral red method. This staining technique can be used easily and reliably following a standardized

protocol (Elliott and Tang 2009) and was shown to be the most robust method to establish the vital state of zooplankton larger than 50 μm in a comparative test among three procedures for determining plankton viability (Zetsche and Meysman 2012). Yet, this method has not become widespread, in contrast to the more laborious visual discrimination (Daase et al. 2014). The present study, conducted in the frame of the long-term zooplankton monitoring in the inner Gulf of Naples (Mazzocchi et al. 2011, 2012), is one of the few investigations estimating copepod non-predatory mortality during an entire annual cycle of the entire copepod assemblage, as well as its component species.

The percentage contribution of dead individuals to total copepod abundance was highly variable, as also recorded in other coastal, estuarine and oceanic regions by using different methods (Daase et al. 2014, their Table 1; Tang et al. 2014; Besiktepe et al. 2015). The mean percentage of copepod carcasses at our sampling station (10.3%) was very close to the lower limit of the range (11.6–59.8%) reviewed for marine zooplankton by Tang et al. (2014, their fig. 1). It was higher than the carcass contribution reported at a coastal site of the Levantine Sea in the eastern Mediterranean (2.6%, Besiktepe et al. 2015), but very similar to the average percentage observed in open waters of the same basin (10.6%, Besiktepe et al. 2015). The wide range in the proportion of dead copepods reported in these studies likely reflects the large variety of natural conditions, including differences in season, local hydrology and stressors that may affect copepod assemblages.

The causes of non-predatory mortality that generate copepod carcasses in the field are numerous and varied, but difficult to ascertain. Only in a few cases have factors been identified, such as, in *Acartia tonsa*, hypoxia for nauplii (but not copepodites) (Elliott et al. 2013), or increasing water temperature for both larval and juvenile stages (Elliott and Tang 2011b). The occurrence of dead copepods has been generically attributed to adverse hydrological conditions in an upwelling system (Weikert 1977) and in deep tropical waters (Weikert 1982). No significant relationship between hydrographic variables and carcasses was found either in the Levantine Sea (Besiktepe et al. 2015) or in Chesapeake Bay for copepods in a summer study (Tang et al. 2006) and in *A. tonsa* during 2 years of monthly sampling (Elliott and Tang 2011a). We cannot rule out the possible effects of advection on the abundances of live and dead copepods recorded at our site, which lies at the border between the coastal and offshore systems (Ribera d'Alcalà et al. 2004) in an area that is affected by intense vessel traffic characteristic of a large port city. We recorded the peaks of carcass abundance in summer, when the wind-driven circulation in the Gulf of Naples is much weaker (Pierini and Simioli 1998; Gravili et al. 2001) and would favor the retention of populations within the coastal area. Summer is also the season of the

most intense vessel traffic in the area and vessel-generated turbulence may contribute to increases in carcass numbers, as reported in Chesapeake Bay where field sampling and laboratory experiments demonstrated that turbulence increased mortality in *A. tonsa* (Bickel et al. 2011). The most frequently suggested source of copepod carcasses seems to be the incomplete consumption by predators as reported by Daase et al. (2014). However, during our study, copepod carcasses did not show visible injuries that would indicate feeding attacks as a possible cause of death. A mortality factor in adult copepods could be aging, which is associated with an increase in oxidative damage and a deterioration of vital rates, though the adult life span, as monitored in the laboratory, can be long and even prolonged in conditions of caloric restriction (Saiz et al. 2015). In our sampling site, the abundance of adult carcasses was low during most of the year and non-predatory mortality affected the copepodite stages more than adults, particularly in *Paracalanus parvus* and *Clausocalanus* spp. Differences in the percentage occurrence of carcasses during the life cycle were also recorded in other regions, with developmental stages, in particular nauplii, being generally more vulnerable to non-predatory mortality (Elliott and Tang 2011a; Besiktepe et al. 2015), although conflicting evidence was reported by Martinez et al. (2014). In our study, the predominance of copepodite carcasses did not simply reflect the predominance of juveniles in the assemblage and we infer that it was probably due to the vulnerability of these developmental stages to either internal (physiological) or external factors that we could not identify.

The complex interplay of different factors that may act as possible causes of non-predatory mortality in copepod populations overlaps with the seasonal changes of population structure and abundance and leads to the remarkable variability in the temporal pattern of dead copepods during the year. The temporal occurrence of carcasses belonging to copepod species that are always abundant at station LTER-MC mirrored the seasonal pattern of these species distribution (Mazzocchi et al. 2011, 2012). In winter, calanoid carcasses were dominated by *Clausocalanus* spp. copepodites. In that season, only *C. paululus* occurred among dead adults (not shown), reflecting the numerical importance of this species in the winter zooplankton associations (Mazzocchi et al. 2011). Mortality in the genus remained more or less constant during that season until it dropped rapidly to very low rates in spring, when carcasses of *Clausocalanus* spp. decreased in numbers corresponding to an increase in *C. pergens* abundance (unpublished data). In contrast, during the autumn season, when *C. furcatus* was among the most abundant copepods at station LTER-MC (unpublished data), dead *Clausocalanus* spp. occurred during both peak and low abundance periods of the population cycle; the highest mortality rates recorded in late autumn might be responsible for

the low abundance of the genus in that period. The seasonal differences in the patterns of *Clausocalanus* spp. carcasses and mortality rates suggest different causes of death in this genus (particularly in juveniles) and we cannot exclude that these patterns reflect specific characteristics of congeners that occupy different ecological niches at local (Mazzocchi and Ribera d'Alcalà 1995; Peralba and Mazzocchi 2004) and latitudinal (Peralba et al. 2016) scales. In the spring species *Acartia clausi*, carcasses appeared after the rise of the population and mortality rates peaked during and after the declining phase of population abundance, suggesting a role of non-predatory mortality in the recurrent late-summer fading of this species in the area (Mazzocchi et al. 2012). The patterns of carcass occurrence in *P. parvus* also suggest that non-predatory mortality contributes to shaping the temporal distribution of this species in the inner Gulf of Naples (Mazzocchi et al. 2012), as observed for *A. tonsa* in lower Chesapeake Bay (Elliott and Tang 2011b). In *Temora stylifera,* the non-predatory mortality rates show, starting from the rise of the population in summer, regular oscillations between lows and peaks of similar amplitude, which suggest that this process is an intrinsic feature independent of the phases of the population cycle. A high percentage of carcasses in a population after its abundance peak does not necessary indicate a senescent population; in our study area, all these species continue to reproduce during the period of lowest abundance at the end of their seasonal cycle, as shown by the notable contribution of juveniles to total population numbers (unpublished data) and carcasses.

The non-predatory mortality rates in the populations of *Clausocalanus* spp., *A. clausi*, *P. parvus* and *T. stylifera* at station LTER-MC were compared to the global rates and patterns reviewed by Hirst and Kiørboe (2002) at temperatures similar to those recorded in the Gulf of Naples. The mortality rates estimated for *Clausocalanus* spp. fall within the ranges reported for sac spawners by Hirst and Kiørboe (2002) and those estimated for *P. parvus*, within the ranges reported for broadcast spawners. The rates estimated for *A. clausi* and *T. stylifera* were lower than the rates reported by Hirst and Kiørboe (2002) for broadcast spawners at the temperatures of their respective seasons. The method we have applied to estimate the non-predatory mortality rates from the in situ occurrence of carcasses and the times of their bacterial decomposition in the laboratory (Tang et al. 2006) represent a simplified approach that is easy to apply for monitoring data. This method assumes that the copepod populations are in a steady state, a condition hardly verifiable at sea, but that can be inferred in our study, given the short time interval (mostly a week) from two successive sampling events and the observation that the duration of certain trophic states in the system is in excess of a week (D'Alelio et al. 2015, 2016). Elliott and Tang (2011b) propose a more accurate model to derive non-predatory mortality from carcass data,

but the staged abundances of nauplii and copepodites necessary for that model are not available for the present study. In addition, bacterial decomposition is likely an important loss process in the field, but others, for instance, advection and sinking out of our sampling depth (50 m) may also play a role in the disappearance of carcasses, contributing to their notable temporal variability in abundance.

Assessing mortality rates in copepod populations is important for gaining a better understanding of temporal zooplankton dynamics, and accurate carcass identification and enumeration is an easy method for estimating the non-predatory component of mortality at sea. Moreover, non-predatory mortality in copepod species and developmental stages is a crucial term for a better tuning of population dynamics in individual-based models, which help understanding the population cycles in relation to possible factors that cause individual loss (e.g., Mazzocchi et al. 2006). Knowing the temporal variations of non-predatory mortality allows quantification of the potential contribution of copepod carcasses to the downward flux of organic detritus (Frangoulis et al. 2010, 2011; Ivory et al. 2014). Copepod and other zooplankton carcasses can represent a substantial amount of downward carbon flux at sea (Frangoulis et al. 2010) and contribute to fuel the plankton–benthos coupling in the form of organic material that can be used by benthic detritivores, especially in winter when copepod carcasses may persist for a week before complete degradation (our "Results"). Moreover, enumerating carcasses allows a better quantification of the trophic relationships in the coastal planktonic food webs, where the calanoid species analyzed in the present study play a conspicuous role (D'Alelio et al. 2015).

The easy applicability of the neutral red method and the results of the present annual study foster efforts to quantify the abundance of copepod carcasses and investigate the impact of non-predatory mortality in the copepod assemblages as part of the routine sampling in long-term time series. The occurrence of dead copepods showed recurrent features in Chesapeake Bay during a 2-year study, suggesting that carcasses may be a persistent feature in an area (Elliott and Tang 2011a), although the low number of studies that have quantified copepod carcasses at sea prevent us from generalizing the results obtained so far. Long-term monitoring of copepod carcasses at sea in relation to environmental conditions would allow exploration of interannual variability in their occurrence in response to climate change and episodic events, contributing to a better understanding of the causes of non-predatory mortality and its influence on the population dynamics and fluxes at sea.

References

Beşiktepe Ş, Tang KW, Mantha G (2015) Seasonal variations of abundance and live/dead compositions of copepods in Mersin Bay, northeastern Levantine Sea (eastern Mediterranean). Turk J Zool 39:494–506. doi:10.3906/zoo-1405-23

Bickel SL, Hammond JDM, Tang KW (2011) Boat-generated turbulence as a potential source of mortality among copepods. J Exp Mar Biol Ecol 401:105–109

Böttger-Schnack R (1995) Summer distribution of micro- and small mesozooplankton in the Red Sea and Gulf of Aden, with special reference to non-calanoid copepods. Mar Ecol Prog Ser 118:81–102

Böttger-Schnack R (1996) Vertical structure of small metazoan plankton, especially non-calanoid copepods. I. Deep Arabian Sea. J Plankton Res 18:1073–1101

Burns CW (1985) Fungal parasitism in a copepod population: the effects of *Aphanomyces* on the population dynamics of *Boeckella dilatata* Sars. J Plankton Res 7:201–205

Carlotti F, Giske J, Werner F (2000) Modelling zooplankton dynamics. In: Harris RP, Wiebe PH, Lenz J, Skjodal HR, Huntle M (eds) ICES zooplankton methodology manual. Academic Press, London, pp 571–667

Carpenter EJ, Peck BB, Anderson SJ (1974) Survival of copepods passing through a nuclear power station on northeastern Long Island Sound, USA. Mar Biol 24:49–55

Ceballos S, Kiørboe T (2011) Senescence and sexual selection in a pelagic copepod. PLoS One 6(4):e18870. doi:10.1371/journal.pone.0018870

Crippen RW, Perrier JL (1974) The use of neutral red and Evans blue for live-dead determination of marine plankton (with comments on the use of rotenone for inhibition of grazing). Stain Technol 49:97–104

Daase M, Øystein V, Falk-Petersen S (2014) Non-consumptive mortality in copepods: occurrence of *Calanus* spp. carcasses in the Arctic Ocean during winter. J Plankton Res 36:129–144

D'Alelio D, Libralato S, Wyatt T, Ribera d'Alcalà M (2016) Ecological-network models link diversity, structure and function in the plankton food-web. Sci Rep 6:21806. doi:10.1038/srep21806

D'Alelio D, Mazzocchi MG, Montresor M, Sarno D, Zingone A, Di Capua I, Franzè G, Margiotta F, Saggiomo V, Ribera d'Alcalà M (2015) The green–blue swing: plasticity of plankton food-webs in response to coastal oceanographic dynamics. Mar Ecol 36:1155–1170

Delgado M, Alcaraz M (1999) Interactions between red tide microalgae and herbivorous zooplankton: the noxious effects of *Gyrodinium corsicum* (Dinophyceae) on *Acartia grani* (Copepoda: Calanoida). J Plankton Res 21:2361–2371

Di Capua I, Mazzocchi MG (2004) Population structure of the copepods *Centropages typicus* and *Temora stylifera* in different environmental conditions. ICES J Mar Sci 61:632–644

Dong S, Sprintall J, Gille ST, Talley L (2008) Southern Ocean mixed-layer depth from Argo float profiles. J Geophys Res 113:C06013. doi:10.1029/2006JC004051

Dressel DM, Heinle DR, Grote MC (1972) Vital staining to sort dead and live copepods. Chesap Sci 13:156–159

Duffy MA, Hall SR, Tessier A et al (2005) Selective predators and their parasitized prey: are epidemics in zooplankton under top–down control? Limnol Oceanogr 50:412–420

Eiane K, Ohman MD (2004) Stage-specific mortality of *Calanus finmarchicus*, *Pseudocalanus elongatus* and *Oithona similis* on Fladen Ground, North Sea, during a spring bloom. Mar Ecol Prog Ser 268:183–193

Elliott DT, Tang KW (2009) Simple staining method for differentiating live and dead marine zooplankton in field samples. Limnol Oceanogr Methods 7:585–594

Elliott DT, Tang KW (2011a) Spatial and temporal distributions of live and dead copepods in the lower Chesapeake Bay (Virginia, USA). Estuar Coasts 34:1039–1048

Elliott DT, Tang KW (2011b) Influence of carcass abundance on estimates of mortality and assessment of population dynamics in *Acartia tonsa*. Mar Ecol Prog Ser 427:1–12

Elliott DT, Pierson JJ, Roman MR (2013) Copepods and hypoxia in Chesapeake Bay: abundance, vertical position and non-predatory mortality. J Plankton Res 35:1027–1034

Frangoulis C, Psarra S, Zervakis V, Meador T, Mara P, Gogou A, Zervoudaki S, Giannakourou A, Pitta P, Lagaria A, Krasakopoulou E, Siokou-Frangou I (2010) Connecting export fluxes to plankton food-web efficiency in the Black Sea waters in flowing into the Mediterranean Sea. J Plankton Res 32:1203–1216

Frangoulis C, Skliris N, Lepoint G, Elkalay K, Goffart A, Pinnegar JK, Hecq J-H (2011) Importance of copepod carcasses versus faecal pellets in the upper water column of an oligotrophic area. Estuar Coast Shelf Sci 92:456–463

Genin A, Gal G, Haury L (1995) Copepod carcasses in the ocean. II. Near coral reefs. Mar Ecol Prog Ser 123:65–71

Gravili D, Napolitano E, Pierini S (2001) Barotropic aspects of the dynamics of the Gulf of Naples (Tyrrhenian Sea). Cont Shelf Res 21:455–471

Hall LW, Alden RW (1997) A review of concurrent ambient water column and sediment toxicity testing in the Chesapeake Bay watershed: 1990–1994. Environ Toxicol Chem 16:1606–1617

Hirst AG, Kiørboe T (2002) Mortality of marine planktonic copepods: global rates and patterns. Mar Ecol Prog Ser 230:195–209

Ivory JA, Tang KW, Takahashi K (2014) Use of neutral red in short-term sediment traps to distinguish between zooplankton swimmers and carcasses. Mar Ecol Prog Ser 505:107–117

Kimmel DG, Boicourt WC, Pierson JJ, Roman MR, Zhang X (2009) A comparison of the mesozooplankton response to hypoxia in Chesapeake Bay and the northern Gulf of Mexico using the biomass size spectrum. J Exp Mar Biol Ecol 381:S65–S73

Kimmel DG, Boicourt WC, Pierson JJ, Roman MR, Zhang X (2010) The vertical distribution and diel variability of mesozooplankton biomass, abundance and size in response to hypoxia in the northern Gulf of Mexico USA. J Plankton Res 32:1185–1202

Kimmerer WJ, McKinnon AD (1990) High mortality in a copepod population caused by a parasitic dinoflagellate. Mar Biol 107:449–452

Kirillin G, Grossart HP, Tang KW (2012) Modeling sinking rate of zooplankton carcasses: effects of stratification and mixing. Limnol Oceanogr 57:881–894

Litvinyuk DA, Altukhov DA, Mukhanov VS et al (2011) Dynamics of live Copepoda in plankton of Sevastopol Bay and open coastal waters (the Black Sea) in 2010–2011. Mar Ecol J (in Russian) 10:56–65

Martinez M, Espinosa N, Calliari D (2014) Incidence of dead copepods and factors associated with non-predatory mortality in the Río de la Plata estuary. J Plankton Res 36:265–270. doi:10.1093/plankt/fbt106

Mazzocchi MG, Ribera d'Alcalà M (1995) Recurrent patterns in zooplankton structure and succession in a variable coastal environment. ICES J Mar Sci 52:679–691

Mazzocchi MG, Buffoni G, Carotenuto Y, Pasquali S, Ribera d'Alcalà M (2006) Effects of food conditions on the development of the population of *Temora stylifera*: a modeling approach. J Mar Syst 62:71–84

Mazzocchi MG, Licandro P, Dubroca L, Di Capua I, Saggiomo V (2011) Zooplankton associations in a Mediterranean long-term time-series. J Plankton Res 33:1163–1181

Mazzocchi MG, Dubroca L, Garcia-Comas C, Di Capua I, Ribera d'Alcalà M (2012) Stability and resilience in coastal copepod assemblages: the case of the Mediterranean long-term ecological research at Station MC (LTER-MC). Prog Oceanogr 97–100:135–151

Ohman MD (2012) Estimation of mortality for stage-structured zooplankton populations: what is to be done? J Mar Syst 93:4–10

Ohman MD, Hirche H-J (2001) Density-dependent mortality in an oceanic copepod population. Nature 412:638–641

Ohman MD, Wood SN (1995) The inevitability of mortality. ICES J Mar Sci 52:517–522

Ohtsuka S, Hora M, Suzaki T, Arikawa M, Omura G, Yamada K (2004) Morphology and host-specificity of the apostome ciliate *Vampyrophrya pelagica* infecting pelagic copepods in the Seto Inland Sea, Japan. Mar Ecol Prog Ser 282:129–142

Peralba À, Mazzocchi MG (2004) Vertical and seasonal distribution of eight *Clausocalanus* species (Copepoda: Calanoida) in oligotrophic waters. ICES J Mar Sci 61:645–653. doi:10.1016/j.icesjms.2004.03.019

Peralba À, Mazzocchi MG, Harris RP (2016) Niche separation and reproduction of *Clausocalanus* species (Copepoda, Calanoida) in the Atlantic Ocean. Prog Oceanogr. doi:10.1016/j.pocean.2016.08.002

Pierini S, Simioli A (1998) A wind-driven circulation model of the Tyrrhenian Sea area. J Mar Syst 18:161–178

Ribera d'Alcalà M, Conversano F, Corato F, Licandro P, Mangoni O, Marino D, Mazzocchi MG, Modigh M, Montresor M, Nardella M, Saggiomo V, Sarno D, Zingone A (2004) Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. Sci Mar 68(Suppl. 1):65–83

Roman MR, Gauzens AL, Rhinehart WK, White JR (1993) Effects of low oxygen waters on Chesapeake Bay zooplankton. Limnol Oceanogr 38:1603–1614

Saiz E, Calbet A, Griffell K, Guilherme J, Bersano F, Isari S, Solé M, Peters J, Alcaraz M (2015) Ageing and caloric restriction in a marine planktonic copepod. Sci Rep 5:14962

Sampei M, Sasaki H, Hattori H, Forest A, Fortier L (2009) Significant contribution of passively sinking copepods to the downward export flux in Arctic waters. Limnol Oceanogr 54:1894–1900

Sampei M, Sasaki H, Forest A, Fortier L (2012) A substantial export flux of particulate organic carbon linked to sinking dead copepods

during winter 2007–2008 in the Amundsen Gulf (southeastern Beaufort Sea, Arctic Ocean). Limnol Oceanogr 57:90–96

Tang K, Elliott D (2014) Copepod carcasses: occurrence, fate and ecological importance. In: Seuront L (ed) Copepods: diversity, habitat and behaviour. Nova Science Publishers, pp 255–278

Tang KW, Freund CS, Schweitzer CL (2006) Occurrence of copepod carcasses in the lower Chesapeake Bay and their decomposition by ambient microbes. Estuar Coast Shelf Sci 68:499–508

Tang KW, Gladyshev MI, Dubovskaya OP, Kirillin G, Grossart HP (2014) Zooplankton carcasses and non-predatory mortality in freshwater and inland sea environments. J Plankton Res 36:597–612. doi:10.1093/plankt/fbu014

Tsuda A (1994) Starvation tolerance of a planktonic marine copepod *Pseudocalanus newmani* frost. J Exp Mar Biol Ecol 181:81–89

Weikert H (1977) Copepod carcasses in the upwelling region south of Cap Blanc, N.W. Africa. Mar Biol 42:351–355

Weikert H (1982) The vertical distribution of zooplankton in relation to habitat zones in the area of the Atlantis II Deep, central Red Sea. Mar Ecol Prog Ser 8:129–143

Wheeler EH (1967) Copepod detritus in the deep sea. Limnol Oceanogr 12:697–701

Yanez S, Hidalgo P, Escribano R (2012) Natural mortality of *Paracalanus indicus* (Copepoda: Calanoida) in coastal upwelling areas associated with oxygen minimum zone in the Humboldt current system: implications for the passive carbon flux. Rev Biol Mar Oceanogr 47:295–310

Zetsche E-M, Meysman FJR (2012) Dead or alive? Viability assessment of micro- and mesoplankton. J Plankton Res 34:493–509