Ecologica Montenegrina
ISSN 2336-9744 (online) | ISSN 2337-0173 (print)

Ecologica Montenegrina 11: 42-48 (2017)
This journal is available online at: www.biotaxa.org/em

Article

Microbial control of live/dead zooplankton ratio in Sevastopol Bay

VLADIMIR S. MUKHANOV & DARIA LITVINYUK

Kovalevsky Institute of Marine Biological Research of Russian Academy of Sciences, 2, Nakhimov ave., Sevastopol 299011, Russia
Corresponding author: Vladimir S. Mukhanov. E-mail: *v.s.mukhanov@gmail.com*

Received: 5 June 2017 | Accepted by *V. Pešić*: 16 July 2017 | Published online: 26 July 2017.

Abstract
To explain higher fraction of live zooplankton in heavily polluted and eutrophic Sevastopol Bay comparing with cleaner adjacent waters, a hypothesis has been proposed and tested experimentally that more intensive bacteria-driven decomposition of dead organisms in the bay reduced their pool and, as a result, increased the live-to-dead zooplankton ratio. In the experiment, a heat-killed batch culture of the copepod *Calanipeda aquaedulcis* was used as a substrate for decomposition by natural microbial communities from the waters of different pollution status. Bacterioplankton abundance and *in situ* decomposition rate of copepod carcasses were shown to be about 3-fold higher in the bay (1.3×10^6 cells ml^{-1} and 0.13 day^{-1}, respectively) while an approximation of zooplankton non-predatory mortality rates gave equal values for both the sites (about 0.046 day^{-1}). These findings call for revising the ways of interpreting the results of zooplankton viability assays in their relation to water pollution status.

Key words: zooplankton, viability, mortality, copepod, carcasses, decomposition, microbial hotspots, Sevastopol bay.

Introduction

Zooplankton have been widely shown to suffer from non-predatory mortality owing to starvation, diseases, injuries, parasites, harmful algal blooms, environmental stresses (Carpenter et al., 1974; Murtaugh, 1981; Byron et al., 1984; Burns, 1985; Ianora et al., 1987; Kimmerer, McKinnon, 1990; Hall et al., 1995; Delgado, Alcaraz, 1999; Gomez-Gutierrez, 2003; Tang et al., 2006; Dubovskaya, 2009; Elliott, Tang, 2011). However, a linkage between the mortality (and its derivative – live/dead organisms ratio) and pollution status of aquatic environments is still poorly understood.

Recent studies of live and dead planktonic copepods in Sevastopol bay and adjacent coastal waters (Litvinyuk et al., 2011) have revealed a surprising pattern: average annual fraction of live organisms (FLO) in highly polluted and eutrophic waters of the bay was significantly higher than one in the cleaner offshore area (Fig. 1). This result contradicted the conclusions made earlier by other authors (e. g. Pavlova et al., 2001; Pavlova, Melnikova, 2005) who explained higher necrozooplankton abundances and low FLO by adverse environmental conditions. In this study, bacterial degradation of copepod carcasses was considered a key factor controlling the 'dead pool' and, hence, the value of FLO in the studied area. Higher microbial activity in the polluted bay was hypothesized to accelerate copepod decomposition and, as a result, shift the ratio of live-to-dead organisms to higher values. The hypothesis was tested experimentally and a simple

model was used to understand how the pool of dead organisms forms in the water column and how the balance between their inflow (via non-consumptive mortality) and outflow (via decomposition) is maintained in time.

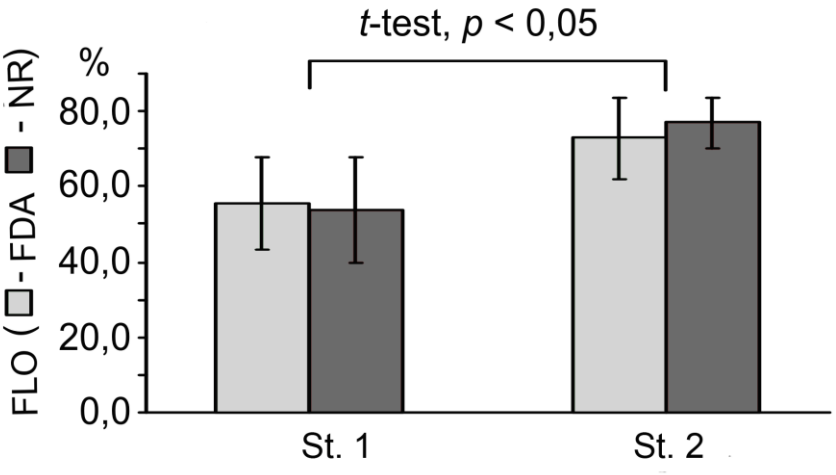

Figure 1. Fluorescein diacetate- (FDA) and neutral red (NR) -based estimates of the average annual FLO in the open coastal waters (St. 1 in this study) and the polluted bay (St. 2 in this study) in 2010 – 2011. Calculated from the data presented in Litvinyuk et al. (2011).

Material and Methods

To estimate microbial abundances and conduct decomposition experiments *in situ*, seawater samples were collected at two stations in Sevastopol Bay and adjacent, open waters (Fig.2). The water area was characterized by pronounced gradients of water salinity, contamination and eutrophic state from the mouth of the Black River in the apex of the bay (close to St. 2) to the bay mouth and the open sea outside the bay (St. 1). The concentration of biogenic elements in the river outflow was 2 to 10 times higher than in the bay (Ovsyanyi et al., 2007), resulting in formation of stable concentration gradients of nitrite-N (from 0.9 μg L^{-1} at St. 1 to 2.3 μg L^{-1} at St. 2), nitrate-N (3.2 and 13.3 μg L^{-1}, respectively), ammonium-N (17.4–23.1 μg L^{-1}), phosphate-P (7.9–8.8 μg L^{-1}), and silica (57–81 μg L^{-1}) (V. Gubanov, unpublished data). The average E-TRIX eutrophication indices (Vollenveider, 1998) were 4.7 (St. 1) and 5.5 (St. 2) (Gubanov et al., 2015). The degree of chronic contamination by petroleum products and heavy metals increased from the open sea outside the bay to the bay center. Thus, the content of oil hydrocarbons in the bottom sediments of St. 1 was ~30 mg 100 g^{-1}, increasing to 180 mg 100 g^{-1} at St. 2 (Osadchaya et al., 2004).

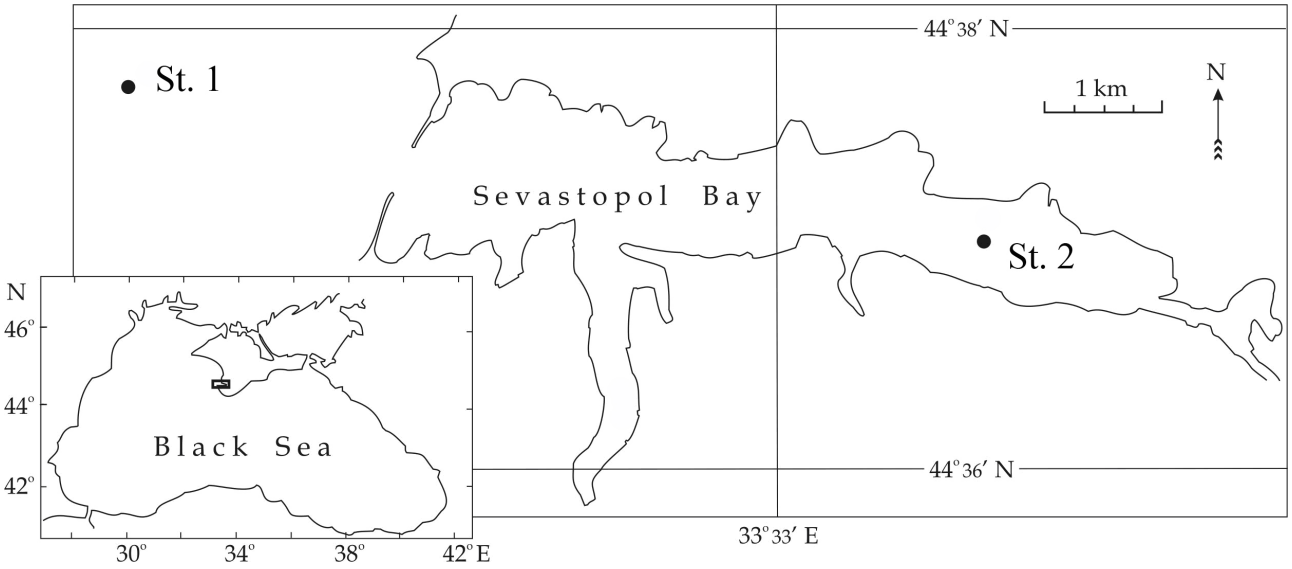

Figure 2. Sampling sites in Sevastopol Bay and adjacent coastal waters.

The rates of degradation and decomposition of dead 'model' organisms (the copepod *Calanipeda aquaedulcis* Kritchagin, 1873) by natural microbial communities from marine waters of different pollution status (St. 1 vs St. 2) were measured experimentally and compared. A batch culture of the copepods fed microalgal mixture (Bacillariophyceae: *Phaeodactylum tricornutum*; Chlorophyceae: *Chlorella vulgaris*, *Dunaliella salina*; Dinophyceae: *Prorocentrum cordata, Prorocentrum micans*; Prymnesiophyceae: *Isochrysis galbana*). The copepods were killed by heat (85 °C for 10 min.), trapped on 100-μm nylon mesh, rinsed and resuspended in native seawater freshly collected at the two stations (June 2012) and prefiltered through 100-μm nylon mesh to remove mesozooplankton. The final concentration of the dead organisms in the experimental flasks was about 300 ind. L^{-1}. The flasks were prepared in triplicates for each of the locations, submersed to the sea (down to about 2-m depth) and incubated under *in situ* temperature (22 °C) and light conditions for 4 days that was an expected time period for decomposing the bulk of the carcasses (Tang et al., 2006). Then, decomposition of the copepods was examined by light microscopy.

130 carcasses of nauplii, copepodites and adults were analyzed visually and photographed in every replicate for identifying visual signs of disintegration and decomposition of the carcasses and classifying the extent of their degradation to the five categories: I – no visual signs of any body injuries or internal decomposition, II – partial postmortal decomposition of the body with light 'spots' and cavities, III – distinct visual signs of internal decomposition of up to 50% of the body volume, IV – body injuries and more than 50% internal decomposition, cephalothorax deformation and degradation, V – fragmented and/or empty carcasses with no tissue inside (Fig. 3). The carcasses at the stage V were defined as completely decomposed.

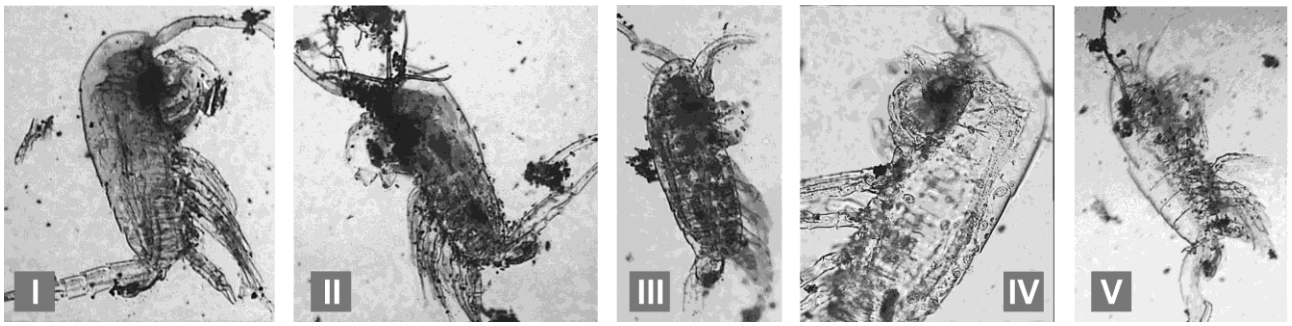

Figure 3. Stages of decomposition of copepod carcasses. Explanations are in the text.

To compare average annual bacterioplankton abundances at St. 1 and St. 2, seawater samples were collected in the surface layer bimonthly over 2010-2011. These data and the data on zooplankton FLO in Sevastopol Bay published earlier (Litvinyuk et al., 2011) were both obtained simultaneously at the same stations (as a part of Sevastopol Bay Time Series), thus allowing their inter-comparison.

Bacterial abundances in the samples and in the experimental flasks were estimated by flow cytometry. Counts were performed using a Beckman Coulter flow cytometer (Cytomics FC 500) equipped with an air-cooled blue laser (15 mW, 488 nm) and the standard filter setup. Aliquots of 1ml water samples previously fixed with formaldehyde (2% final conc.) were stained with SYBR Green I (Molecular Probes Inc.), following the procedures described by (Marie et al., 1997; Gasol, Giorgio, 2000). SYBR-Green I fluorescence at the green channel FL1 (525 nm) was considered proportional to intracellular nucleic acid content and interpreted as a measure of bacterial cell-specific metabolic activity according to (Servais et al., 2003). Consequently, the integral metabolic activity of the community was estimated as FL1 × N (in relative units), where N is the total bacterial abundance in the sample.

To analyze the field and experimental results, a simplified model (ignoring sedimentation) has been used, describing dynamics of live and dead organisms and the balance between their pools:

$$dN_L/dt = (\mu - m) \times N_L \quad (1),$$

$$dN_D/dt = m \times N_L - d \times N_D \quad (2),$$

$$FLO = (N_L/(N_L+N_D)) \quad (3),$$

where N_L and N_D are abundances of live and dead organisms, respectively; μ is specific growth rate; m is non-consumptive mortality rate; d is decomposition rate of zooplankton. Under steady-state conditions ($\mu = m$, constant N_L) in the model population, FLO depends on N_D and, hence, on the ratio between the non-consumptive mortality rate (m) of live zooplankton (inflow to the pool of dead organisms) and the decomposition rate (d) of copepod carcasses (outflow from the pool). In the decomposition experiment, the equation 2 was used to calculate d at $N_L = 0$, $\mu = 0$, $m = 0$, and FLO = 0.

Statistical analyses were performed using software STATISTICA ver. 10 (StatSoft, Inc., USA).

Results and Discussion

By fourth day of the exposition, about 20% (St. 1) and 50% (St. 2) of the copepods were decomposed completely, reaching the stage V (Fig. 4). Frequency distributions of the copepod decomposition stages differed significantly in the locations, indicating faster process in the bay. The rates of decomposition (d) approximated from these data amounted 0.05 day^{-1} in the bay (St. 1) and 0.13 day^{-1} outside it (St. 2), thus supporting the hypothesis of microbial control of live/dead zooplankton ratio in Sevastopol bay.

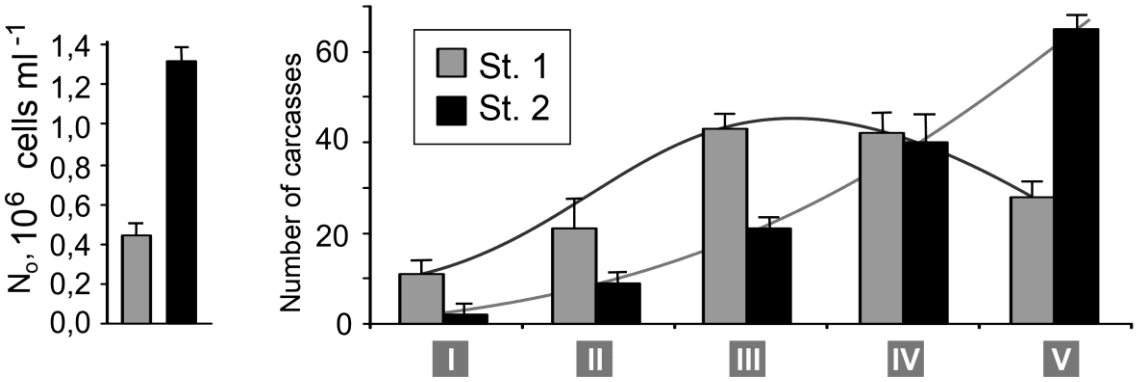

Figure 4. Initial bacterial abundances in the experiment (N_o, left plot) and frequency distribution of the copepod decomposition stages on the fourth day of exposition at St. 1 and 2 (right plot). Means and standard deviations are presented.

Disintegration and decomposition of zooplankton carcasses was earlier shown to be driven by external, not internal, bacteria (Harding, 1973; Lee and Fisher, 1992; Reinfelder et al., 1993), hence, indicating a direct link between FLO and microbial activity in the water column. The initial bacterial abundances in the experimental flasks differed significantly, 0.4×10^6 cells ml^{-1} at St. 1 versus 1.3×10^6 cells ml^{-1} at St. 2 (Fig. 4). Approximately the same difference between the locations maintained over the year – average annual abundance of bacterioplankton proved to be about 2.5-fold higher in Sevastopol Bay as against the adjacent waters (Fig. 5).

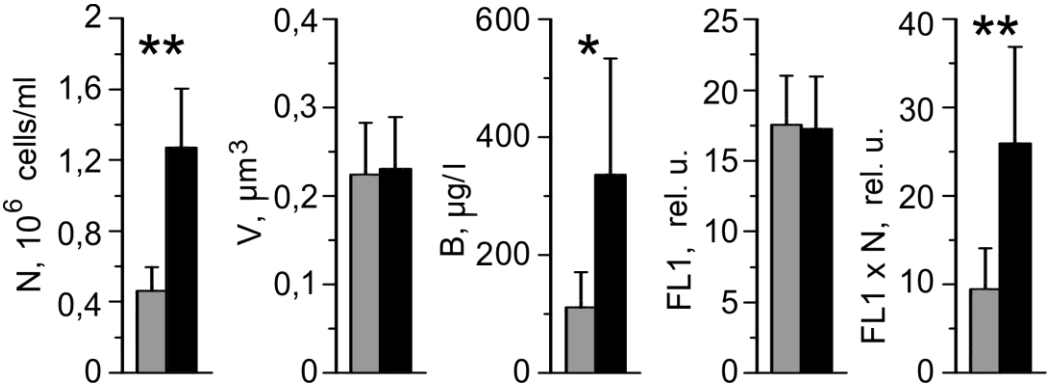

Figure 5. Bacterioplankton average annual (2010 - 2011) abundance (N), cell volume (V), biomass (B), intracellular nucleic acids (FL1) and integral metabolic activity (FL1 × N) (± 95% CI) at St. 1 (grey) and St. 2 (black). Significant differences are marked (* $p < 0.05$, ** $p < 0.01$).

According to the seasonal microbiological data obtained in 2010-2011, no significant difference between the stations has been revealed in terms of bacterial cell volumes and intracellular nucleic acid contents (FL1 signal). However, the integral metabolic activity measured as FL1×*N* was 2.5- to 3-fold higher in the bay community (Fig. 5).

Thus, both the experimental results and microbiological data have provided an evidence of a linkage between FLO and bacterioplankton. There was an increase in FLO at higher bacterial abundances and high FLO variability at fewer bacteria (Fig. 6). The latter seemed to be a result of contribution of other important factors to the FLO control (like sedimentation or elimination by detritivores).

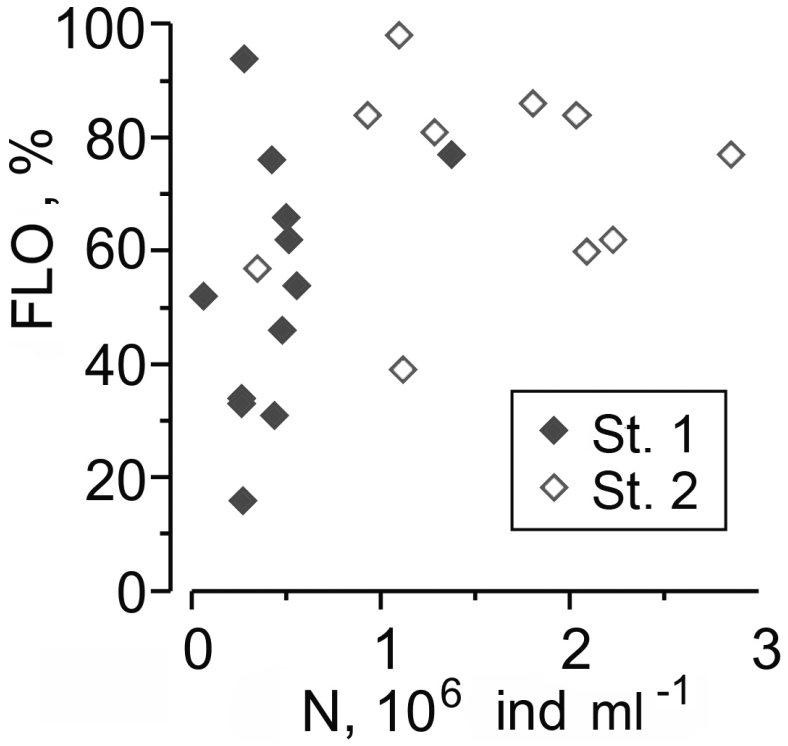

Figure 6. Fraction of live organisms (FLO) in zooplankton versus bacterioplankton abundance (N). Data on FLO (2010-2011) are from Litvinyuk et al. (2011).

Given constant N_L and equality of μ and m (losses are compensated for by reproduction) under stationary conditions, the value of FLO depends only on the d/m ratio irrespective of the initial N_L, m and d (Fig. 7). The dependence has allowed a calculation of the d/m ratios from the field data on the FLO annual averages (54% and 77%, Litvinyuk et al., 2011), which amounted about 1.2 and 3.0 at St. 1 and 2, respectively (Fig. 6). Thus, according to the model, the rates of copepod mortality and decomposition were roughly equal in the open waters while in the bay, decomposition occurred three times faster. Combining these calculations with the experimental results gives nearly equivalent rates of zooplankton non-consumptive mortality at the St. 1 and 2 (0.045 and 0.047 day^{-1}, respectively) which are within the range (0.01 to 0.1 day^{-1}) commonly reported for marine zooplankton (see review in Tang et al., 2014).

In summary, it can be concluded from the present findings that higher FLO values observed in more polluted and eutrophic marine coastal waters were not necessarily a result of higher zooplankton mortality but rather a consequence of higher abundance of heterotrophic bacteria and more intensive bacteria-driven decomposition of dead organisms in the detrital food web. Unexpectedly, the rates of non-consumptive mortality of copepods in polluted Sevastopol Bay were approximated to not differ significantly from those in the less polluted adjacent waters. Thus, results of zooplankton viability assays should be interpreted carefully in their relation to water pollution status. Higher pollution status of an area does not necessarily imply higher zooplankton mortality and/or lower FLO values.

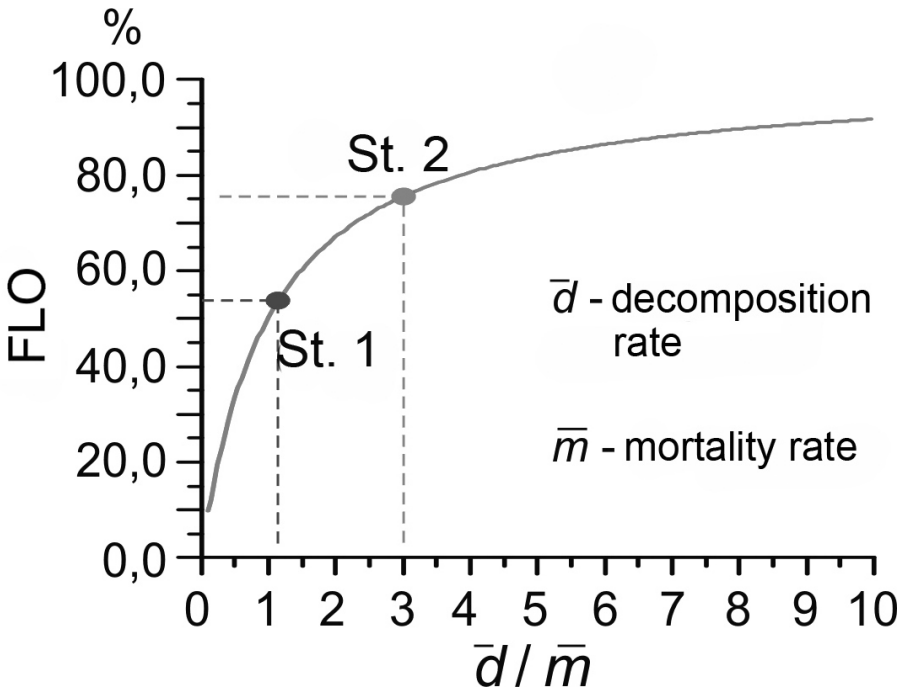

Figure 7. Fraction of live organisms (FLO) as a function of the decomposition-to-mortality ratio (d/m) in the model under steady-state conditions (mortality and specific growth rates are balanced, μ = m) and projections of natural zooplankton communities (St. 1 and 2) onto the model curve.

Acknowledgements

The study was partly funded by RFBR research project № 16-34-01020.

References

Byron, E.R., Folt, C.L. & Goldman, C.R. (1984) Copepod and cladoceran success in an oligotrophic lake, *Journal of Plankton Research,* 6, 45-65. doi.org/10.1093/plankt/6.1.45

Burns, C.W. (1985) Fungal parasitism in a copepod population: the effects of Aphanomyces on the population dynamics of *Boeckella dilatata* Sars, *Journal of Plankton Research,* 7, 201-205. doi.org/10.1093/plankt/7.2.201

Carpenter, E.J., Peck, B.B. & Anderson, S.J. (1974) Survival of copepods passing through a nuclear power station on northeastern Long Island Sound, USA, *Marine Biology*, 24, 49-55. doi: 10.1007/BF00402846

Cherepanov, O.A. & Pavlova, Y. V. (2008) Estimating mortality rate of zooplankton using net tows and differentiation to live and dead fractions, *Sistemi kontrolya okruzhayuschey sredi. Sredstva i monitoring. Proc. of MHI NASU*, Sevastopol: EKOSI-Gidrophizika, , 406–410, (*in Russian*).

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), *Journal of Plankton Research,* 21, 2361-2371. doi.org/10.1093/plankt/21.12.2361.

Dubovskaya, O.P. (2009) Non-predatory mortality of planktonic crustaceans, its possible causes (a review). *J. General Biol.*, 70, 2, 162–185 (in Russian).

Elliott, D. T. & Tang, K. W. (2011) Spatial and temporal distributions of live and dead Copepods in the lower Cheasapeake Bay (Virginia, USA), *Estuaries and Coasts,* 34, 103–1048. doi: 10.1007/s12237-011-9380-z

Gasol, J.M. & Giorgio, P.D. (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Scientia Marina,* 64, 197–224.

Gomez-Gutierrez, J., Peterson, W.T., De Robertis, A. & Brodeur, R.D. (2003) Mass mortality of krill caused by parasitoid ciliates, *Science,* 301, 339. doi: 10.1126/science.1085164.

Gubanov, V.I., Gubanova, A.D. & Rodionova, H.Yu. (2015) Diagnosis of trophicity of the Sevastopol Bay and the Sevastopol coastal water, *Proc. Conf "Aktual'nye problemy akvakul'tury v sovremennyi period" (Focal Problems of Aquaculture at the Modern Period),* Rostov-on-Don, pp. 64–67.

Hall Jr., L.W., Ziegenfuss, M.C., Anderson, R.D. & Killen Jr., W.D. (1995) Use of estuarine water column tests for detecting toxic conditions in ambient areas of the Chesapeake Bay watershed, *Environmental Toxicology and Chemistry,* 14, 267-278. doi: 10.1002/etc.5620140212

Hirst, A.G. & Kiørboe, T. (2002) Mortality of marine planktonic copepods: global rates and patterns, *Marine Ecology Progress Series,* 230, 195–209.

Ianora, A., Mazzocchi, M.G. & Scotto di Carlo, B. (1987) Impact of parasitism and intersexuality on Mediterranean populations of Paracalanus parvaus (Copepoda: Calanoida), *Diseases of Aquatic Organisms,* 3, 29-36.

Kimmerer, W.J. & McKinnon, A.D. (1990) High mortality in a copepod population caused by a parasitic dinoflagellate, *Marine Biology,* 107, 449-452. doi: 10.1007/BF01313428

Koval, L. G., (1984) *Zoo- and Necrozooplankton of the Black Sea.* Naukova Dumka, Kiev, pp. 127, (in Russian).

Litvinyuk, D.A., Altukhov, D.A., Mukhanov, V.S. & Popova, E.V. (2011) Dynamics of live Copepoda in plankton of Sevastopol bay and open coastal waters (the Black sea) in 2010-2011, *Morskii Ekologichniy. Zhurnal,* 10, 56–65 (in Russian).

Marie, D., Partensky, F., Jacquet, S. & Vaulot, D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR green I. *Applied and Environmental Microbiology,* 63, 186–193.

Murtaugh, P.A. (1981) **.**Inferring properties of mysid predation prom injuries to Daphnia, *Limnology and Oceanography,* 26, 811-821.

Osadchaya, T.S., Alemov, S.V., & Shadrina, T.V. (2004) Ecological quality of the Sevastopol Bay bottom sediments: retrospective and modern state, *Ekologia Morya,* 66, 82–87.

Ovsyanyi, E.I., Artemenko, V.M., Romanov, A.S. & Orekhova, N.A. (2007) Flow of the Chernaya river as a factor in formation of the water-salt regime and ecological state of the Sevastopol Bay, *Ekologicheskaya bezopasnost' pribrezhnoi i shel'fovoi zon (Ecological Safety of the Coastal and Shelf Zones),* 15, 57-65 (in Russian)

Pavlova, Y. V. & Melnikova, Y. B. (2005) Variability of live zooplankton abundances in Sevastopol Bay in 1998–1999 (Crimea, the Black Sea), *Hydrobiological Journal*, 41, 6, 3–12, (in Russian).

Pavlova, Y.V., Murina, V.V. & Kuphtarkova, E.A. (2001) Hydrochemical and biological studies in Omega bay (the Black Sea, Sevastopol shelf), *Ecological safity of coastal and shelf zones and complex exploration of shelf resources,* 2, 159–176, (in Russian).

Servais, P., Casamayor, E. O., Courties, C., Catala, P., Parthuisot, N. & Lebaron, P. (2003) Activity and diversity of bacterial cells with high and low nucleic acid content. *Aquatic microbial ecology*, 33, 1, 41-51. doi:10.3354/ame033041

Tang, K.W., Freund, C. S. & Schweitzer, Ch. L. (2006) Occurrence of copepod carcasses in the lower Chesapeake Bay and their decomposition by ambient microbes, *Estuarine, Coastal and Shelf Science*, 68, 499–508. doi.org/10.1016/j.ecss.2006.02.021

Tang, K. W, Gladyshev, M.I., Dubovskaya, O.P., Kirillin, G. & Grossart, H.-P. (2014) Zooplankton carcasses and non-predatory mortality in freshwater and inland sea environments, *Journal Plankton Research*, 36, 3, 597–612. doi.org/10.1093/plankt/fbu014

Vollenveider, R., Giovanardi, F., Montanari, G., & Rinaldi, A. (1998) Characterization of the trophic conditions of marine coastal waters, with special reference to the NW Adriatic Sea, *Environmetrics*, 9, 329–357.