Ecological state of marine pelagic communities near the Verbyanaya spit (Temryuk Gulf, Azov Sea)

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Abstract. Phytoplankton, ciliates, holoplankton, meroplankton, ichthyoplankton, ctenophores were studied in the vicinity of the Verbyanaya spit in the Temryuk Gulf during the month of June, which is the most productive month. It has been discovered that formation of net zooplankton structure was largely determined by predator-prey trophic relations. Leanness of ichthyoplankton and mosaic distribution of holoplankton and meroplankton is evidently explained by the abundance of large carnivorous comb jellies Mnemiopsis leidyi. Dominant holoplankton complex was represented with marine euryhaline species of copepods – Acaritia tonsa (93–98% of the total holoplankton amount). Larvae of Mytilaster lineatus bivalves, comprised a significant part of net zooplankton (43.5%). A total biomass of zooplankton on average reached 537.4 mg/m³, at that, ciliates comprised 38% of this amount. Abundance of zooplankton in the area of research is largely explained by a high level of trophicity of waters coming with the flow of the Kuban River. The obtained results offer an insight into ecological state of marine pelagic communities in protected zones of eastern Azov region and may be useful for subsequent monitoring of the region.

1. Introduction

In the eastern part of the Temryuk Gulf there is a Verbyanaya spit, forming a specific natural ecosystem. It is an enormous spit of detritic sand with shelly ground and an admixture of fine sand with a width of 25–30 m, separating liman-reed bed area of Azov overflow land (delta of the Kuban River) from the Sea of Azov. In the vicinity of the spit, at the shelf of the Sea of Azov and in the liman-reed bed lands between the rivers Kuban and Protoka, there are oil reconnaissance works and exploration works for oil pipeline construction. Waters and marshes of this deposit are protected under the Ramsar Convention, which creates great responsibilities for operation of this area.

The purpose of the study is to evaluate the ecological state of marine pelagic communities in eastern Sea of Azov, in the vicinity of the Verbyanaya spit.

2. Materials and methods

Plankton samples were taken during June 2014, in the vicinity of the Verbyanaya spit (Temryuk Gulf, Sea of Azov) (Figure 1). During the sampling, water temperature at the surface was 25.5°C. Phytoplankton was collected from the surface with a plastic bucket and fixed with a neutral formalin to a final concentration of 1–2%. The samples were condensed on core filters with a diameter of 1–2
using a Sorokin funnel for inverse filtering. Some samples were studied in the living unfixed state [1]. The samples were studied in Nageotte counting chamber under a microscope. Cells were counted in 0.1–0.05 of the concentrated sample volume depending on cell abundance. Algae biomass was calculated according to a volumetric method using original and reference data for volumetric measurements for each species [2].

Plankton ciliates were collected by complete catching with a Juday small-sized net (aperture size 40 μm) in the topmost half meter layer of water (qualitative analysis). Shelled ciliates (tintinnids) were collected from the surface with a plastic bucket (quantitative analysis). Samples with a volume of 1500 ml were fixed with a solution of glutaric aldehyde (final concentration 2%) and were concentrated by a sedimentation method down to 10–20 ml. In order to determine the number of aloricate ciliates, non-fixed (living) samples were studied in Sorokin counting chamber. Holoplankton and meroplankton (crustaceans, larvae of benthos organisms, rotifers and other animals > 200–500 μm) were collected by complete catching through the water column using a medium-size Juday net with an inlet opening of 25 cm (aperture size 100 μm).

The material was fixed with a 2–4% solution of neutral formaldehyde and processed in the laboratory following a common method. Biomass calculations were performed using size and mass tables composed by Petipa [3]. Comb jellies, fish eggs and larvae were collected using MNT and Bogorov-Ras nets (80/114 cm, aperture size 500 μm, inlet opening area 0.5 m²). The samples were collected by horizontal 10-minute sweeping from a vessel moving with a speed of up to 2 knots and by vertical catching from the bottom to the surface. Identification of ichthyoplankton, identification of development stages and state of fish eggs and larvae of pelagic fish were according to [4].

Figure 1. Schematic map of sampling stations

3. Results and Discussion
In the Sea of Azov, June is the most productive month of a year, characterized by maximum species diversity of feed zooplankton and its largest abundance. In July, under the pressure of Mnemiopsis leidyi, a pelagic carnivorous comb jelly, the numbers of zooplankton drastically reduces and by August–September plankton samples include only individual plankton animals [5, 6].

Phytoplankton. In June, phytoplankton of the studied area includes 44 taxa of plankton algae, among them 18 are dinophytes, 10 are chlorophyceans [7]. The proportion of dinophytes in the total count of phytoplankton was 34%, chlorophyceans were 32% and blue-green algae were 21%. Diatoms amounted to 46% of the total phytoplankton biomass, dinophytes amounted to 42%. In the surface layer of water, phytoplankton count was twice higher than in bottom waters (597 000 cells/liter). Biomass had comparable values (222 mg/m³).

Ciliates and rotifers. Among ciliates, 28 taxa were identified: 25 aloricate forms and 3 shelled forms (tintinnids) (Table 1).
Table 1. Taxonomical composition of ciliates and rotifers

| Ciliophora                      | Strombidium conicoides (Leeg.) Kahl +++ |
|---------------------------------|-----------------------------------------|
| LOXODIIDAE                      | S. dalum Lynn at al ++                  |
| Loxodes sp.+                    | S. emergens (Leeg.) Kahl +++            |
| PRORODONTIDAE                   | S. vestiium (Leeg.) Kahl ++             |
| Urotricha spp.+++               | S. viride Stein ++                      |
| HOLOPHRIIDAE                    | Strombidium sp. +                       |
| Holophrya sp. +                 | Rimostrombidium conicum (Kahl) +++      |
| COLEPIDAE                       | Rimostrombidium sp.+++                  |
| Tiarina fusus (Clap. Et Lach.)+ | Loxmaniella oviformis (Leeg.) +++       |
| Coleps hirtus (O.F. Müller) Nitzsch ++ | Pelagostrobilidium spirale (Leeg.) +++ |
| Euplopidae                      | STROBILIDIIDAE                          |
| Euplotes sp.+                   | CODONELLIDAE                            |
| DIDINIIDAE                      | Tintinnopsis beroidea Stein++           |
| Didinium sp.+                   | Tintinnopsis cylindrica Daday            |
| MESODINIIDAE                    | Tintinnopsis minuta Wailes++            |
| Myrionecta rubra (Lohm.) +++    | Brachionus quadridentatus Herm.+        |
| Mesodinium pulex (Cl. et Lach.)+++ | Brachionus plicatilis Muller++          |
| Askenasia stellaris Leeg. ++    | Brachionus diversicornis (Daday) +      |
| Pleuronematidae                 | Pelagostrobilidium spirale (Leeg.) +++  |
| Pleuronema sp.+                 | Keratella cochlearis (Gosse) ++         |
| Vorticellidae                   | Keratella quadra (Mull.) +              |
| Vorticella anabaena Still ++    | Keratella valga (Ehrb.) +               |
| Zoanthamium sp.+                | Synchaeta sp. ++                        |
| HALTERIIDAE                     | Filinia longiseta Ehr.+                 |
| Halteria grandinella (Muller) Dujardin +++ | Asplanchna priodonta Gosse ++        |
| STROMBIDIIDAE                   | Trichocerca marina (Daday.) +           |

Note. “+” – found species, “++” – common species, “+++” – abundant species

Tintinnids amounted to ≤ 5% of the total ciliates count. The dominant complex of aloricate ciliates included Myrionecta rubra, Mesodinium pulex, Loxmaniella oviformis, Strombidium conicoides, S. emergens, Halteria grandinella, Rimostrombidium conicum, Rimostrombidium sp., Pelagostrobilidium spirale. Urotricha sp. In bottom waters Urotricha sp., Loxodes sp., Euplotes sp. were registered. Ciliates count varied between 4.5 and 7.2 million ind/m³, biomass varied from 159 to 252 mg/m³, with average values of 6.1 million ind/m³ and 204.2 mg/m³ respectively (Figure 2). Ciliate biomass was large and amounted to 38% of the total zooplankton biomass. Their abundance in bottom waters was on average 12–13 times lower.

Holoplankton. Within holoplankton, 4 taxa were identified, among them Ctenophora – 1, Rotatoria – 11, Cladocera – 1, Copepoda – 2. Among rotifers, Keratella cochlearis, Synchaeta sp. and carnivorous Asplanchna priodonta dominated. The abundance of holoplankton varied between 7.5–62.8 thousand ind/m³, biomass amounted to 92.4–301.9 mg/m³, with average values of 28.8 thousand ind/m³ for abundance and 201.1 mg/m³ for biomass (Figure 3). Copepoda dominated holoplankton (99% of the total count). Among copepods, calanoid copepos Acartia tonsa showed absolute domination with 22.8 thousand ind/m³, biomass 202.6 mg/m³.
Figure 2. Numerical density and biomass of ciliates in the area of investigation and their share of the total zooplankton biomass

The share of A. tonsa in the total holoplankton counts reached 98%, their share in the total biomass reached 93%. Active spawning was registered in the population of A. tonsa. Population structure of this species consisted of 68.6% nauplii and 21.5% younger copepodites. Calanoid copepods Centropages ponticus and cladocerans Pleopsis polyphemoides were rare at 220 – 320 ind/m$^3$.

Figure 3. Numerical density and biomass of holoplankton in the area of investigation and the share (%) of dominant species in the total count (A) and biomass (B) of holoplankton

Meroplankton. Among meroplankton, 7 taxa were identified, among them Polychaeta – 1, Cirripedia – 1, Decapoda – 1, Bivalvia – 2, Gastropoda – 2. The share of meroplankton in the zooplankton total amounted to 43.5%. The density of meroplankton varied between 1.5 – 48.9 thousand ind/m$^3$, biomass amounted to 20.3 – 234.1 mg/m$^3$, with average values of 21.6 thousand ind/m$^3$ for abundance and 112.9 mg/m$^3$ for biomass. Foundational pool of bottom invertebrates was formed by larvae of bivalves Mytilaster lineatus – 19.4 thousand ind/m$^3$ (90% of the total meroplankton count), larvae of bivalves Cerastoderma sp. – 0.8 thousand ind/m$^3$, barnacle larvae Amphibalanus improvisus – 1.3 thousand ind/m$^3$ (Figure 4). Larvae of bristle worms fam. Spionidae Polydora cornuta, gastropods Chrysallida sp., Hydrobia acuta and decapods Rhitropanopeus harrisii.
tridentata were rare. In horizontal catches with a Bogarov-Rass net, the abundance of decapod *R. harrisii tridentata* larvae amounted to 15.9 ind/100 m$^3$. Maximum count of crab larvae amounted to 46.2 ind/100 m$^3$.

**Figure 4.** The share (%) of dominant species in the total count (A) and biomass (B) of meroplankton

*Ichthyoplankton.* Among the components of ichthyoplankton at a water temperature of 25.5°C, fish eggs of migrating Azov anchovy (a subsp. of European anchovy, fam. Engraulidae) *Engraulis encrasicholus maeoticus* [5] have been recorded. They differ from Black Sea anchovy *E. encrasicholus ponticus* by a lighter color and smaller size. The length of the Azov anchovy is usually 8–9 cm with a maximum of 10–11 cm. In the Sea of Azov this fish spends the summer by actively feeding, spawning (in June-July) and fattening the young fish. In autumn, Azov anchovy of all ages leaves the Sea of Azov through the Kerch Strait to the Black Sea and moves to depth. In our investigations, the count of anchovy fish eggs was low. In vertical catches with a Bogorov–Rass net it varied from 0 to 8 ind./m$^2$ with an average of 4.4 ind./m$^2$. In horizontal catches with an MNT net, the average count of anchovy fish egg was 7.2 ind./100 m$^3$ (maximum 21.9 ind./100 m$^3$) (Table 2).

**Table 2.** Taxonomic composition and numerical density of neuston (ichthyoplankton, ctenophores and meroplankton) (ind./100 m$^3$) in horizontal catching

| Taxa                      | Stations |
|---------------------------|----------|
|                           | 1        | 2       | 3       | 4       | 5       |
| **PISCES**                |          |         |         |         |         |
| *Engraulis encrasicholus maeoticus* | 1.3      | 21.9    | 2.4     | 2.5     | 7.9     |
| **CTENOPHORA**            |          |         |         |         |         |
| *Mnemiopsis leidy*        | 22.2     | 4.6     | 6.7     | 55.4    | 43.5    |
| **DECAPODA**              |          |         |         |         |         |
| *Rhitropanopeus harrisii tridentata* | 14.5     | 46.2    | 7.2     | 6.6     | 5.3     |

*Ctenophores.* In vertical catches with a Bogorov–Rass net, the abundance of carnivorous comb jellies *Mnemiopsis leidy* with a body length of 1.5–6 mm varied from 4 to 46 ind/m$^2$ with an average value of 12 ind/m$^2$. In horizontal catches with an MNT net, the abundance of *M. leidy* with a body length of 2–5.5 mm was in the range of 6.7 to 55.4 ind/100 m$^3$ with an average value of 26.5 ind/100 m$^3$ (Table 2). A half of individuals had their stomach full with larvae of bivalves, *Acartia* and fish
eggs. In catches with a medium Juday net, the average abundance of comb jelly larvae with a size of 0.25 mm amount to 209 ind/m$^3$, biomass amounted to 104.4 mg/m$^3$. The total abundance of the population of *M. leidyi* taking into account its larva stage amounted to 269 ind/m$^3$, biomass 3.84 g/m$^3$. According to [5] in case of early entry, biomass of *M. leidyi* may reach 13.0 g/m$^3$. Thus, as of June 2014, comb jelly did not reach its peak density. Thus, in plankton there were large numbers of copepods *A. tonsa*, bivalve *M. lineatus* larvae. According to [5], the comb jelly was first accounted for in the Sea of Azov in late 1980s.

Every year this carnivorous animal migrates from the Black Sea to the Sea of Azov in May-June, develops until November and then disappears. According to the early entry scenario, comb jellies appear in the open sea in May and develop en masse in June. According to the late entry scenario, individual comb jellies appear in the open sea in June and large masses of the animal appear in July. In both cases, by mid-summer, this carnivore covers almost the entirety of the Sea of Azov, excluding its western part. By August, the animal expands through the whole water area, leading to degradation of holoplankton and meroplankton.

Since the appearance of this invasive species, seasonal dynamics of zooplankton has completely lost its traditional summer maximum. Under the early entry scenario, the intensive development of zooplankton in the open sea is in May, under the late entry it is in May-June. June is the most productive month of a year, characterized by maximum species diversity of feed zooplankton and its largest abundance. According to our date [6, 8], the average abundance of comb jelly larvae with a size of 0.25 mm amount to 209 ind/m$^3$, biomass amounted to 104.4 mg/m$^3$. The abundance of holoplankton varied between 7.5 – 62.8 thousand ind/m$^3$, biomass amounted to 92.4 – 301.9 mg/m$^3$, with average values of 28.8 thousand ind/m$^3$ for abundance and 201.1 mg/m$^3$ for biomass.

4. Conclusion
It has been discovered that formation of net zooplankton structure in June was largely influenced by predator-prey trophic relations. Leanness of ichthyoplankton and mosaic distribution of holoplanktron and meroplankton is evidently explained by the abundance of large carnivorous comb jelly *Mnemiopsis leidyi*.

Dominant holoplankton complex was represented with marine euryhaline thermophile species of copepods – *Acartia tonsa* (93–98% of the total holoplankton amount). Larvae of *Mytilaster lineatus* bivalves comprised a significant part of net zooplankton (43.5%). A total biomass of zooplankton on average reached high values (537.4 mg/m$^3$), at that, ciliates comprised 38% of this amount. Allochtonous organics coming with the flow of the Kuban River, evidently, forms a stock of food available to zooplankton in the form of particulate matter and dissolved fraction. These sources may facilitate high productivity of the Verbyanaya spit of the Temryuk Gulf, where during the long research period [8] there were significant concentrations of zooplankton.

The obtained results offer an insight into ecological state of marine pelagic communities in protected zones of eastern Azov region and may be useful for subsequent monitoring of the region.

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