A Note on Species Lists and Ecosystem Shifts: Black Sea Tintinnids, Ciliates of the Microzooplankton

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Abstract We investigated tintinnid (planktonic ciliate protists) in the Black Sea, a system which has undergone marked changes. Obvious shifts in the Black Sea began with eutrophication from the 1960's to the 1980's, followed by blooms of the carnivorous comb jelly M. nemiopsis in the late 1980's- early 1990's and finally de-eutrophication and the decline of the comb jelly since the mid-1990's. Here we document historical changes in apparent species inventories which correspond with ecosystem changes. Tintinnid ciliates have been studied in the Black Sea for over 130 years. Records were assembled by year of publication, ignoring all variability in sampling methods, geographic location and extent of sampling, season of collection, etc. Time lines were constructed for each species. The number of species reported increased steadily from the 1870's to the mid-1960's. With eutrophication and the damming of the Danube River, the frequency of new species records declined from the 1960's to the 1990's but with no apparent species losses. The 1990's to the present corresponds with rise and fall of blooms of the comb jelly and the collapse and recovery of the anchovy fishery. For this last period, we found an increase in the numbers of both new species records and 'apparent' losses of tintinnid species.
Our analysis suggests that abrupt changes in planktonic ecosystems may be detectable with a very crude metric of plankton community composition—lists of apparent species.

Introduction

Tintinnids are planktonic choreotrich ciliates, characterized by the possession of a shell or lorica. They are a component of the microzooplankton, and like most other planktonic ciliates, tintinnids are grazers on pico and nanoplankton, organisms both heterotrophic and autotrophic ranging in size from 0.2 - 20 µm in size. While ubiquitous in marine systems, tintinnids are generally a minor part of the ciliate plankton, representing 5 - 10% of total cell numbers of the ciliate microzooplankton (e.g. Dolan and Marassé 1995) although occasionally tintinnid ciliates can dominate the microzooplankton and be major consumers of pico and nanoplankton production (Karayanni et al. 2005). Copepods are typically identified as the dominant predators of tintinnids, in common with other ciliate microzooplankton, (e.g., Dolan and Gallegos 2001). However, in contrast to most taxa of the microzooplankton (ciliates as well as other groups), species are rather easily distinguished because identifications have long been based on distinctive features of the lorica or shell (e.g. Claparède and Lachmann 1858). There is a considerable amount of data on species compositions of tintinnids in different systems, which distinguishes them from most other groups of the microzooplankton.

In recent work on tintinnid ciliates in the Black Sea several species were found which, to our knowledge, had not been recorded as occurring in the Black Sea (Gavrilova 2005). Despite the fact that tintinnids have been studied in the Black Sea since the 1870's (e.g., Ulianin 1870), no checklist of Black Sea species exists. Consequently, we assembled the existing literature reports on tintinnids. Ordering the reports of occurrences chronologically, rather than by author, or by region, or season, revealed an apparent increase in numbers of species reported until the 1980's. Recent lists of species appeared more variable, listing not only species new to the Black Sea, but failing to note the presence of species which before had been more or less consistently found. Among marine systems, the Black Sea has undergone
perhaps the most notorious ecosystem shifts, and here we examine the historical trends in species lists of tintinnids in the light of ecosystem shifts summarized below.

In the Black Sea, a 'tipping point' in eutrophication was reached with the damming of the Danube River in 1972, which resulted in declines in the ratios of silicon to nitrogen and phosphorus (Humborg et al. 1997). The changes in nutrient input into the Black Sea corresponded with alterations in both the composition and quantities of planktonic algae. The post-damming nutrient ratios of the Black Sea favored small flagellates over the larger diatom (which posses a siliceous frustule) phytoplankton taxa. By the early 1980's 'abnormal phytoplankton blooms' occurred with increasing frequencies (Eiker et al. 1999, Prodanov et al. 2001). These shifts in the primary producer communities were followed by a spectacular change in the planktonic carnivore community.

In the late 1980's there were notable blooms of the comb jelly *Mnemiopsis*, a carnivorous species introduced from the East Coast of North America presumably via ballast water. High population densities of comb jellies accompanied a sharp decline in the abundance of copepod and other crustacean zooplankton (Shiganova and Bulgakova 2000) as well as a near complete collapse of the anchovy fishery (Kideys 1994). The proximate cause of the late-1980's shift in the plankton food web has been attributed to not only to the introduction of *Mnemiopsis* (Shiganova and Bulgakova 2000) but also to eutrophication (Prodanov et al. 2001) or over-fishing of top predators (Daskalov 2002). In recent years, with overall decreasing nutrient loads and the appearance of another comb jelly which feeds on *Mnemiopsis*, the Black Sea appears to be returning to a state resembling that of the early 1970's (Kideys 2002). Thus, the Black Sea has undergone remarkable shifts in the planktonic food web (Biloio and Niermann 2004) and against this background we examined changes in the reported inventories of tintinnid species.

We employed the very simple method of ordering species lists by time, ignoring all factors which are potentially important: locality within the Black Sea, season, temporal or geographic extent of sampling, methods used for sampling and sample analysis. The diversity of methods employed (nets of different sizes, whole water samples of different volumes, etc.) make direct comparison *a priori* impossible. Thus, the absence of a species from reports can not be taken as marking its
complete disappearance from the Black Sea no more than the listing of a species is evidence of a persistent and wide-spread occurrence. However, it should be noted that tintinnid communities are generally highly dominated, which is say that a few species (5 or fewer) account for the majority of individuals (80% or more), in systems ranging from a eutrophic estuary (Dolan and Gallegos 2001) to the oligotrophic central Pacific Ocean (Dolan et al. 2007). Thus, species lists may often represent little more than a catalogue of the dominant forms. Our historical compilation of a checklist of Black Sea tintinnids provided evidence that simple species reports may serve to mark alterations in planktonic food webs.

Methods

Literature records mentioning tintinnid ciliates were compiled, both primary reports and general reviews (e.g., the UN series "Black Sea Biological Diversity") which gave chronological data. A total of 23 reports were assembled covering the period 1870 - 2005 (Table I).

Nomenclature largely followed that of Kofoid and Campbell (1929; 1939). We recognize the problematic nature of the lorica-based taxonomy in tintinnids which ignores the infraciliature (e.g., Laval-Peuto and Brownlee 1986; Montagnes and Lynn 1991) and may, or may not, correspond with genetic groups (e.g., Agatha and Riedel-Lorjé 2005). Here, for the essential purpose of historical comparisons we consider 'morphospecies' to be species. However, we employed a conservative approach which is to say that certain species were grouped. Some taxa previously thought distinct are now known to be probable synonyms. For example, *Tintinnopsis kofoidi* and *T. davidofi* are likely the same as *Tintinnopsis cylindrica* (Agatha and Riedel-Lorjé 2005). Other taxa are now recognized as developmental variants, in particular those reported for *Favella ehrenbergii* (Laval-Peuto 1981, 1983) and *Tintinnopsis campanula* (Laval-Peuto and Brownlee 1986) and were grouped. Other groupings were made based on illustrations in the reports showing intermediate forms between species. For example, *Metacylis* species, considered distinct by
Kofoid and Campbell, appeared to be variants of *Metacylis mediterranea* based on the drawings of Rossolimo (1924). Some groupings were made based on our observations of intermediate forms such as those between *Tintinnopsis beroidea* and *T. karajacensis*, or *T. parvula*. These groupings, as well as common superceded names and spelling variants, are given in the species list (Table II). Each species was assigned a number in order of its chronological appearance in the Black Sea literature and data for a time line of occurrence collated.

**Results and Discussion**

Constructing time lines for each species as a means of obtaining a historical view of species lists has, to our knowledge, has not been previously attempted with regard to planktonic protistan organisms. It should be recognized that plankton studies are remarkably variable even today in terms of sampling means, effort and analysis; add the well-known historical changes in sampling and the chances of detecting a signal may be very small. Clearly then in time-lines we constructed, the absence or presence of a species is but weak evidence of a true absence or persistent or widespread occurrence. Changes in species lists are more likely to reflect shifts in dominance patterns. Despite then an apparent improbability of detecting historical shifts in planktonic protist communities using simple species lists, Black Sea tintinnids appear to have undergone changes. Firstly, one can consider large-scale patterns.

The major part of the tintinnid fauna appears to be Mediterranean; this corresponds with findings concerning the dinoflagellates of the Black Sea (Gomez and Boicenco 2007). The complete checklist of tintinnid species reported from the Black Sea appears in Table 2. With but a very few exceptions, all the species have been reported from the open Mediterranean (Jörgensen 1924; Dolan 2000; Pitta et al. 2001) or its sub-systems: the Catalan Sea (Margalef, 1957; Margalef and Morales 1960; Dolan and Marrasé 1995), the Ligurian Sea (Balech 1959; Gomez and Gorsky 2003; Posta 1963; Rampi 1948; Rassoulzadegan 1979), Sea of Marmasa (Balkis 2004), the Adriatic Sea (Krsinic 1980), the Aegean Sea (Balkis and Wasik 2005), the Gulf of Marseille (Travers and Travers 1971, Travers 1973), the coast of Lebanon (Abboud-Abi Saab 2002), Bay of Algers (Vitiello 1964), coastal waters of Sicily
(Moscatello et al. 2004; Sitran et al. 2007). Interestingly, Black Sea tintinnids not known from the Mediterranean are all species typical of productive, temperate, Atlantic waters: *Leprotintinnus pellucidus*, *Tintinnidium mucicolas*, *T. meurnie*, *T. subacuta*, *T. urnala*. Notably, most of these species were found from 1970's onward when the Black Sea became eutrophic (see Fig. 1: spp 14, 16, 18, 19).

INSERT FIGURE 1

It is tempting to speculate that apparent appearances of tintinnid species in the literature perhaps reflect changes in water transport, that is currents, between the Black and Mediterranean Seas or introductions via ballast water discharge (e.g., Pierce et al. 1997). However, habitat colonization by marine protists need not involve transport of water masses.

For example, the Black Sea tintinnid *Tintinnopsis subacuta*, not known in the Mediterranean but common in coastal water of the north Atlantic, has been found in an Egyptian salt lake in an arid region about 400 km from the Mediterranean Sea (Abdel-Malek and Ishak 1980). The contemporary flora and fauna of Lake Qarun are a clear testament to the dispersal capabilities of marine protists. Previously freshwater, Lake Qarun, became saline early in the 1900's and now has a plankton community, both zooplankton and phytoplankton, typical of eutrophic estuarine waters (Fathi and Flower 2005), as well as a diverse community of marine benthic foraminifera (Abu-Zeid et al. 2007). Among the forms of ciliates known to form cysts are tintinnids, notably species of *Tintinnopsis* (e.g., Kamiyama 1997) and cysts may serve as dispersal forms. Just as many types of protists appear to encounter few barriers to dispersal (e.g. Esteban et al. 2005; Lee et al. 2005) so may tintinnids.

We believe it is very likely that all of the Black Sea tintinnid species found were (and are) always present but not in easily detectable concentrations. The simplest explanation for changes in the Black Sea species lists is that temporal changes over the past few decades in the planktonic food web of the Black Sea resulted in changes in the relative abundances of tintinnid species. We considered decadal changes, relative to the previous decade from the 1960's onward (Fig. 2). The period encompassed 3 decades of eutrophication, (1960-1990), followed by the invasion
We found, despite the crudeness of using species reports, distinct patterns corresponding with the distinct periods or regimes in the Black Sea. Thus, the periods of eutrophication were characterized by the addition of new species, at a decreasing rate from the 1960's to the 1990's, with no apparent 'loss' of species. As mentioned above, the 'new' species were those typically found in productive temperate systems. The phytoplankton community, following the installation of the 'Iron Gates' dam, shifted with an increase in the relative importance of flagellates compared to diatoms (Humborg et al. 1997). Along with the change in phytoplankton composition was a net increase in chlorophyll concentrations (a measure of phytoplankton standing stock). The combination of the two may have simply allowed more tintinnid species to co-exist in higher concentrations.

The period 1990 - 2000, marked by spectacular blooms of the comb jelly and the collapse of the anchovy fishery, differed distinctly from the preceding decade. Several tintinnid species, previously unknown in the Black Sea but known from the Mediterranean Sea, were reported. There was an 'apparent' loss of the 3 species new from the 1970's (*Tintinopsis lobicanoi*, *T. subacuta* and *Leprotintinnus pellucidus*). The 'new' species of tintinnids (i.e., not previously recorded from the Black Sea) were all long-known from the Mediterranean Sea (e.g. Jörgensen 1924).

The larval stages of ctenophores are known to feed on (and perhaps require) protozoan microplankton such as tintinnids (Wagget and Sullivan 2006) and estimates of their feeding impact on the microzooplankton range in the Sevastopol Bay area range as high as 25% of the standing stock per day in recent years (Finenko et al. 2006). Thus, it is not unreasonable to hypothesize that ctenophores could exert a direct effect on tintinnid community composition. Alternatively, ctenophores, as predators on copepods could an exert an indirect effect on tintinnids as copepods feed on tintinnids.
The most recent period (2000-2007), characterized by the recovery of the anchovy fishery and improvements in water quality, appear also to be characterized by marked 'apparent' changes compared to the preceding decade. Species previously unnoted, again largely Mediterranean, have been documented and others appear absent. In contrast to the preceding decade, the 'apparent' lost species are Mediterranean, most of which had been new to the Black Sea in the preceding period.

We can not completely exclude the possibility that the changes in the species inventory we found reflect systematic changes in sampling, analytical methods, or taxonomic expertise. However, there is little evidence to support this view. For example, most of the studies were based on plankton net tow material, including some of the most recent (e.g., Türkoglu and Koray 2000). Technological improvements in microscopy are, we believe, an unlikely factor in the discovery of species new to the Black Sea. The drawings in the older monographs suggest that the microscopes used revealed a good deal of detail, largely comparable with contemporary microscopes (e.g., Rossolimo 1922) and none of the tintinnid species were described from the Black Sea.

Tintinnid ciliates are not alone in being characterized over long time periods. There are even fossil records for tintinnids, many of which strongly resemble living forms (e.g., Colom 1948) as there are for many other groups of protists. Thus, the approach we report here could be extended to other groups for which historical data exist such as dinoflagellates, radiolarians and foraminferia, etc.

Conclusions

We found changes in the species inventories of tintinnids that could be related to ecosystem changes. Admittedly our findings may be exceptional. The trophic position of tintinnids, as grazers on pico and nanoplankton and the prey of copepods, may render them especially sensitive to changes in the planktonic food web. Furthermore, the Black Sea has undergone rather extraordinary changes. However, it appears that the mining of old data, in the form of simple species lists, may be of value in documenting ecosystems shifts of the past. The corollary is that changes
predicted to occur in a system may reflected in qualities as crude as the composition of the microzooplankton species found.

Acknowledgements

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systematic groups. in: Biologiya severo-zapadnoy chast Chernogo morya, Naukova


summer period. Institute of Biology of the Southern Seas Sevastpol, 1992. Ms
Deposited 15.12.92 -3539-V92.
Table I. Literature sources for lists of tintinnid species for the Black Sea

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<th>Year</th>
<th>Reference</th>
<th>Year</th>
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<td>1870</td>
<td>Ulianin 1870</td>
<td>1975</td>
<td>Zaitsev &amp; Alexandrov 1998</td>
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<td>1879</td>
<td>Mereschkovsky 1879</td>
<td>1986</td>
<td>Petranu 1997</td>
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<td>1988</td>
<td>Petranu 1997</td>
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<td>1899</td>
<td>Minkevich 1899</td>
<td>1989</td>
<td>Petranu 1997</td>
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<td>1908</td>
<td>Markoff 1908</td>
<td>1990</td>
<td>Petranu 1997</td>
</tr>
<tr>
<td>1922</td>
<td>Rossolimo 1922</td>
<td>1991</td>
<td>Petranu 1997</td>
</tr>
<tr>
<td>1929</td>
<td>Nikitin 1929</td>
<td>1992</td>
<td>Zagorodnyaya</td>
</tr>
<tr>
<td>1936</td>
<td>Kosyakina 1936</td>
<td>1995</td>
<td>Türkoglu &amp; Koray 2000</td>
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<tr>
<td>1937</td>
<td>Galadgiev 1937</td>
<td>1999</td>
<td>Zagorodnyaya et al. 1999; Murzov et al. 1999</td>
</tr>
<tr>
<td>1940</td>
<td>Dogopolskaya 1940</td>
<td>2001</td>
<td>Gavrilova 2005</td>
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<td>1958</td>
<td>Petran 1958a,b</td>
<td>2002</td>
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<td>1964</td>
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<td>2004</td>
<td>Gavrilova 2005</td>
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<tr>
<td>1968</td>
<td>Morozovskaya 1968</td>
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Table II. Tintinnid species reported from the Black Sea. BSA denotes the Black Sea areas in which the species has been found, OS denotes the other systems in which the species has been found: M for Mediterranean, A for Atlantic. Note that most of the Mediterranean species occur also in the Atlantic.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species &amp; synonyms</th>
<th>BSA</th>
<th>OS</th>
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<td>Codonella lagenula</td>
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<td>M</td>
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<td>Dictyocysta mitra</td>
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<td>M</td>
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<td>Eutintinnus</td>
<td>Eutintinnis apertus (E. inequalis, Tintinnopsis inequalis)</td>
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<td></td>
<td>Eutintinnus lusus-undae</td>
<td>SW &amp; NW</td>
<td>M</td>
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<tr>
<td></td>
<td>Eutintinnus tubulosus</td>
<td>NW</td>
<td>M</td>
</tr>
<tr>
<td>Favella</td>
<td>Favella azorica</td>
<td>SW</td>
<td>M</td>
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<tr>
<td></td>
<td>Favella campanula</td>
<td>SW</td>
<td>M</td>
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<tr>
<td></td>
<td>Favella ehrenbergii (Coxiella helix, Cyttarocellus ehremb, Cyttarocellus helix)</td>
<td>throughout</td>
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<tr>
<td></td>
<td>Favella serrata (Parafavella denticulata)</td>
<td>SE</td>
<td>M</td>
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<td>Helicostomella</td>
<td>Helicostomella subulata</td>
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<td>Leprotintinnus</td>
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<td>Metacylis</td>
<td>Metacylis mediterranea (M. mereschkowskii, M. jörgensenii)</td>
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<td>Nolaclusilis</td>
<td>Nolaclusilis sp. (see fig. 3)</td>
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<td>Tintinnidium</td>
<td>Tintinnidium mucicola</td>
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<td>Tintinnopsis</td>
<td>Tintinnopsis baltica</td>
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<td>Tintinnopsis compressa</td>
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<td>Tintinnopsis cylindrica (T. kofoidi, T. fusiformis T. davidovi)</td>
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<td>Tintinnopsis minuta</td>
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<td>Tintinnopsis subacuta</td>
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<td>Tintinnopsis tubulosa (T. directa)</td>
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<td>Tintinnopsis urnula</td>
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<tr>
<td>Salpingella</td>
<td>Salpingella decurtata</td>
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Fig. 1. Time lines for each of the 31 species of tintinnids reported from the Black Sea. Species numbers follow the chronological order of the species appearance in literature reports listed in Table I.
Fig. 2. Changes in species from 1960 to 2007. Period "1" denotes eutrophication and the damming of the Danube River. Period "2" the blooms of the comb jelly and collapse of the anchovy fishery. Period "3" the decline of the comb jelly and recovery of the anchovy populations.
Fig. 3. Examples of old and new Black Sea tintinnid species. *Favella ehrenbergii* (a) found since the 1870's; the formalin-preserved specimen contains the lorica of *Tintinnopsis tubulosa*; the size bar = 50 microns. An apparently new species, tentatively identified as a *Nolaclusila* sp. as the lorica is flexible and collapsible (Synder and Brownlee 1991); 2 views of a single formalin-fixed individual, rotated with a micropipette (b & c); protargol-stained specimen (d); size bars = 10 microns.
The lorica of *Tintinnopsis tubulosa* (e), first reported in the 1920's. *Salpingella decurtata* (f), a common species in the Mediterranean Sea, not found in the Black Sea until recently; size bar = 10 microns.