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Historical trends in the species inventory of tintinnids (ciliates of the microzooplankton) in the Bay of Villefranche (NW Mediterranean Sea): Shifting baselines

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Abstract

In the Bay of Villefranche, tintinnid ciliates have been studied since 1879 yielding a unique time series. The species inventory, excluding likely synonymous records, numbers 108. Temporal increases in the inventory appear linearly related to sampling effort up until the 2000s with a cumulative sampling effort of about 200 dates. Subsequently, with a large increase in sampling to currently over 460 dates, the rate of increases in species numbers declines. Surprisingly, the inventory is not highly inflated by unique occurrences, as species found but once are only 17 out of the 108. However, in recent years many previously recorded taxa have not been seen. Missing from a species list derived solely from intensive sampling from 2013 to 2016 are 38 previously recorded species. Most (26 out of 38) were recorded from a single year and thus may have been temporary residents. However, 12 species were found in multiple years by different investigators suggesting relatively common occurrence in the past. The substantial effort required to adequately sample a locality and possible historical changes in species inventories suggest that claims of a species as 'new' to a locality should be made with caution & caveats.

Keywords: Biodiversity checklist; Plankton; Species discovery; Species diversity; Species richness

Introduction

Currently there is considerable interest in changes in species distribution related to climate change and the phenomenon of invasive species. Recent reviews include reports of 'new' marine protists in British waters (e.g. Minchin et al. 2013) and in the Adriatic Sea (Langer and Mouanga 2016; Pecarevic et al. 2013). With regard specifically to tintinnid ciliates, several species from the Mediterranean Sea have been catalogued as newly invasive species in the Black Sea (Nunes et al. 2014; Shiganova et al.2012), and conversely, as potential new introductions into the Adriatic from Black Sea ballast water (David et al. 2007). Apparent changes in distribution in the North Atlantic over the past 50 years have been reported for some species of tintinnids (Hinder et al. 2012).

Obviously identification of a species as new to a given area requires near complete knowledge of the species inventory of the system or locality under consideration. However, species checklists, at least with regard to planktonic protists, are generally based either on a single time series of samplings, generally 1–3 years maximum, conducted with a single sampling protocol and acknowledged as provisional (e.g. Hoppenrath 2004) or alternatively are based on a wide-ranging literature review encompassing reports both historical and current, and are assumed to be comprehensive (e.g. Gómez and Boicenco 2004). In neither case is the

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Table 1. Summary data of tintinnid studies in the Bay of Villefranche.

Year	# samplings	Method	Reference (Table 2 number)
1879	10	Net	Fol (1881) (1)
1880	10	Net	Fol (1883) (2)
1929	5	Net	Balech (1959) (3)
1938	1	Net	Balech (1959) (3)
1939	2	Net	Balech (1959) (3)
1948	5	Net	Balech (1959) (3)
1949	1	Net	Balech (1959) (3)
1951	2	Net	Balech (1959) (3)
1953	12	Net	Balech (1959) (3)
1960	6	Net	Posta (1963) (4)
1961	50	Net	Posta (1963) (4)
1962	40	Net	Posta (1963) (4)
1973	52	Net & bottle	Rassoulzadegan (1975) (5)
1998	6	Bottle	Cariou et al. (1999) (6)
2002	47	Bottle	Dolan et al. (2006) (7)
2003	18	Bottle	This study (8)
2009	1	Bottle	Bachy et al. (2013) (9)
2010	12	Bottle	Dolan and Stoeck (2011) (10)
2013	29	Net & bottle	Dolan (2016) (11)
2014	50	Net & bottle	Dolan (2016) (11)
2015	57	Net & bottle	This study (8)
2016	37	Net & bottle	This study (8)

possibility of historical changes addressed. To assess the possibility of a species being ‘new’ to a locality or indeed of any changes in species distributions a baseline for comparison is needed. To our knowledge, only reports of temporal changes in species inventories with regard to planktonic protists are that of Gavrilova and Dolan (2007) on Black Sea tintinnids based on reports from scattered locations and Modigh and Castalado (2002) for the Bay of Naples that compared recent sampling to that of Issel (1934). There appears to but a single study concerning historical trends from a single locale; it concerned macroinvertebrates from a large coastal embayment and concluded that human activity, in particular harvesting, had changed the species inventory (Trott 2016). Here I report on historical changes in species inventory of tintinnid ciliates in a single location, the Bay of Villefranche, arguably the best-studied location with regard to tintinnid ciliates.

Studies of tintinnid ciliates in Villefranche began with the classic work of Fol (1881, 1883) who described new species based on sampling in 1879 and 1880. Fol’s work, and the subsequent and current work summarized in Table 1, provides data allowing construction of a time-series of species lists and cumulative sampling effort (estimated as the number of samplings) of over 460 samplings over 137 years. Here I document the historical growth in the tintinnid species inventory, relate it to sampling effort, and examine apparent changes in community composition found in recent years compared to the historical record. The results suggest firstly that compiling a reasonable species inventory, i.e. species numbers approach a plateau with increased sampling effort, requires a very considerable sampling effort, and secondly that changes in species inventories with time apparently occur.

Material and methods

Data characteristics

Literature reports (see Table 1) were the primary source of data. However, some unpublished data, i.e. continuations of 2 published temporal series (i.e. Dolan et al. 2006; Dolan 2016), were included as well. Over the course of 137 years, sampling methods and sample analysis varied considerably and indeed in some instances protocols were not specified. Here the study methods are briefly described.

For the earliest reports (Fol 1881, 1883) samples were collected from unspecified locations in the bay using a custom made silk net, preserved with an iron perchloride solution, and examined using an immersion lens. According to the texts (Fol 1881, 1883), observations were made over 2 consecutive winters, 1879–1880 (Fol 1881) and 1880–1881 (Fol 1883). An arbitrary value of 10 dates sampled for each of 1879 and 1880 were assigned. The report of Balech (1959) concerns 28 net samples obtained from 1928 to 1953, without details on the type of net used, only notations as to month and year of the sample, and occasionally depth strata sampled. Balech gave no details as how samples were preserved nor analyzed. Posta (1963) analyzed samples obtained using a phytoplankton net (50 μm mesh) at Point B, the now standard Villefranche sampling location; the net tow is described only as ‘horizontal’.

Table 2. Species grouped as likely synonyms. Reference number refers to the Villefranche reports given in Table 1. Species description reference abbreviations: K. & C. 1929 for Kofoid and Campbell (1929); C. & L. 1858 for Claparède and Lachmann (1858).

Main species	Known & likely synonym (s)	Reference
Acanthostomella minutissima (K. & C. 1929)	*A. obtusa* (K. & C. 1929)	6
Amphorella laackmanni (Jörgensen 1924)	*Salpingella laminata* (K. & C. 1939)	6
Amphorides quadrilineata (C. & L. 1858)	*A. minor* (K. & C. 1939)	6
Climacocylis scalaria (Brandt 1906)	*C. elongata* (K. & C. 1929)	7
Codonellopsis morchella (Cleve 1900)	*C. schabi* (K. & C. 1929)	3,4,5,6,
	C. lata (K. & C. 1929)	6
Codonellopsis pusilla (Cleve 1900)	*C. inornata* (K. & C. 1929)	7
Cyttarocylis ampulla (Fol 1881)	*C. brandti* (K. & C. 1929)	5
	C. cassis (Haeckel 1873)	4,5
	C. eucrycephalus (Kofoid, 1912)	4
	C. magna (K. & C. 1929)	4
	Petalotricha major (K. & C. 1929)	4,5
Dadayiella ganymedes (Entz 1884)	*D. curta* (K. & C. 1929)	6
Dadayiella pachytoecus (Jörgensen 1924)	*D. cuspis* (K. & C. 1929)	6
Dictytocysta entzi (Jörgensen 1924)	*D. extensa* (K. & C. 1929)	3
Dictyocysta lepida (Ehrenberg 1854)	*D. polygonata* (K. & C. 1929)	8
	D. speciosa (K. & C. 1929)	6
	D. duplex (K. & C. 1929)	7
Dictyocysta mitra (Haeckel 1873)	*D. minor* (K. & C. 1929)	7
Favella ehrenbergii (C. & L. 1858)	*F. campanula* (Schmidt 1902)	3
Salpingella acuminata (C. & L. 1858)	*S. glockentogeri* (K. & C. 1929)	4,5
Salpingella attenuata (Jörgensen 1924)	*S. gracilis* (K. & C. 1929)	6
Steenstrupiella steenstrupii (C. & L. 1858)	*S. robusta* (K. & C. 1929)	7
	S. gracilus (Jörgensen 1924)	8
Tintinnopsis cylindrica (von Daday 1887)	*T. kofoidi* (Hada 1932)	6
	T. levigata (K. & C. 1929)	6

Net material was fixed with formol, concentrated by sedimentation, an aliquot poured into a Petri dish and transects examined until at least 100 cells were counted. No details on microscopy, sample volumes nor number of transects examined were reported. Data reported by Rassoulzadegan (1975) for the year 1973 was based on weekly net samples, using the same net (50 µm mesh) as Posta supplemented with whole water samples, both from Point B, fixed with Lugol's, settled in sedimentation chambers and examined using an inverted microscope. Sampling in 1998 (Cariou et al. 1999) consisted of 3 sets of whole water samples from 6 depths (0–75 m) collected on two dates from Point B. The water samples of 10 l each were concentrated using a 20 µm mesh concentrator and all of the concentrated material examined using an inverted microscope. Data from 2002 to 2003 (Dolan et al. 2006) were based on examination of all material from a 10 l integrated whole water sample made from 6 discrete depth samples (0–75 m) from Point B. Data for the 2009 sample was from examination of material from a single 10 l sample from 50 m depth at Point B (Bachy et al. 2013). Data for 2010 was from 12 samples of 10 l from 25 m depth at Point B (Dolan and Stoeck 2011). Data for 2013–2016 are from examination of phytoplankton net (50 µm, 57 cm diam.) material taken at about weekly intervals from Point B (0–75 m depth vertical tow). The net tow material of 1 l, assuming 100% filtration, represents 19 cubic meters sampled. Aliquots of net tow material, 1–3 ml, theoretically representing material from 19 to 58 l, was examined using an inverted microscope. In addition, whole water samples were examined. A 50 or 100 ml whole water sample from combining aliquots from 6 discrete depth samples (0–75 m) were examined using an inverted microscope. These samples were examined at weekly to monthly intervals. In the text here, the June 2013 to September 2016 samplings represent the intensive modern effort (144 samplings).

Taxonomic analysis

Accounting for synonymy is an important problem in compiling species lists, especially with regard to tintinnids. The classic monographs of Kofoid and Campbell (1929, 1939) cited in all the studies of Villefranche tintinnids save those of Fol, list about 725 species. However, a large fraction of about 25%, were, previous to Kofoid and Campbell (1929, 1939), considered as morphological variants of other species. Kofoid and Campbell raised to full species status these morphological variants based on "minute deviations in lorica shape and size" (Agatha and Strüder-Kype 2013). Conversely, some species which apparently differ little in lorica morphology, e.g. certain species of Tintinnopsis, can differ considerably in DNA sequences (Zhang et al. 2016). Different investigators

Table 3. Tintinnid species found in the Bay of Villefranche. First and last year denotes sample year of first and last records, respectively. First year found in the Mediterranean Sea shown as first Medit year with the corresponding reference. Species in bold are 'oncers', recorded only once. For a complete listing of all years found for each species see the Supplementary data file.

Villefranche species	First year	Last year	1st Medit year	1st Medit year ref.
Acanthostomella conicoides	1998	2015	1995	Pérez et al. (2000)
Acanthostomella lata	2002	2002	1950	Duran (1951)
Acanthostomella minutissima	1998	2010	1997	Modigh and Castalado (2002)
Amphorellopsis acuta	1998	1998	1998	Monti et al. (2012)
Amphorellopsis tetragona	2013	2015	1950	Duran (1951)
Amphorides amphora	1953	1953	1886	von Daday (1887)
Amphorides laackmanni	1998	2015	1886	von Daday (1887)
Amphorides quadrilineata	1952	2016	1886	von Daday (1887)
Ascampbelliella armilla	1998	2015	1950	Duran (1951)
Ascampbelliella oxyura	1998	2002	1909	Jörgensen (1924)
Ascampbelliella tortulata	1998	2016	1909	Jörgensen (1924)
Cantheriella pyrimidata	1998	2015	1909	Jörgensen (1924)
Climacocylis scalaria	2003	2016	1909	Jörgensen (1924)
Climacocylis scalaroides	1998	2016	1938	Rampi (1948)
Codonaria cistellula	1880	2016	1880	Fol (1883)
Codonella amphorella	2014	2016	1909	Jörgensen (1924)
Codonella aspera	1929	2003	1921	Issel (1934)
Codonella elongata	1998	2016	1886	von Daday (1887)
Codonella galea	1880	2014	1880	Fol (1883)
Codonella nationalis	1960	2016	1909	Jörgensen (1924)
Codonella perforata	2003	2015	1884	Entz (1884)
Codonellopsis lagenula	2002	2002	1886	von Daday (1887)
Codonellopsis morchella	1929	2016	1909	Jörgensen (1924)
Codonellopsis orthoceras	1929	2016	1909	Jörgensen (1924)
Codonellopsis pusilla	1929	2015	1909	Jörgensen (1924)
Dadayiella ganymedes	1929	2016	1884	Entz (1884)
Dadayiella pachytoeus	2002	2016	1909	Jörgensen (1924)
Daturella gaussi	2002	2016	2002	This study
Daturella stramonium	2014	2014	1993	Dolan and Marrase (1995)
Dictyocysta elegans	1960	2016	1886	von Daday (1887)
Dictyocysta entzi	1953	2009	1909	Jörgensen (1924)
Dictyocysta lepida	1880	2016	1880	Fol (1881)
Dictyocysta mitra	1938	2016	1909	Jörgensen (1924)
Epipliocylis acuminata	1929	2016	1909	Jörgensen (1924)
Epipliocylis undella	2002	2015	1909	Jörgensen (1924)
Eutintinnus apertus	1880	2016	1880	Fol (1883)
Eutintinnus elongatus	2002	2015	1938	Rampi (1948)
Eutintinnus fraknoi	1929	2016	1909	Jörgensen (1924)
Eutintinnus lusus-undae	1929	2016	1885	Entz (1885)
Eutintinnus macilentus	1929	2010	1950	Duran (1951)
Eutintinnus permintus	2013	2016	1938	Rampi (1948)
Eutintinnus pinguis	2015	2015	1984	Koray (1987)
Eutintinnus stramentus	2013	2016	1950	Duran (1951)
Eutintinnus tenuis	1998	1998	1993	Dolan and Marrase (1995)
Eutintinnus tubulosus	1953	2015	1950	Duran (1951)
Favella azorica	1948	2013	1909	Jörgensen (1924)
Favella ehrenbergi	1929	2016	1909	Jörgensen (1924)
Favella serrata	1929	2002	1909	Jörgensen (1924)
Helicostomella subulata	1973	1973	1886	von Daday (1887)
Metacylis jörgensenii	1953	2002	1930	Issel (1934)
Metacylis mediterranea	2002	2013	1886	von Daday (1887)
Metacylis mereschkowskii	2002	2002	1909	Jörgensen (1924)
Niemarshallia aperta	2002	2002	2002	This study
Ormosella bresslaui	2002	2002	2002	This study
Parundella aculeata	1953	2016	1909	Jörgensen (1924)

Table 3 (*Continued*)

Villefranche species	First year	Last year	1st Medit year	1st Medit year ref.
Parundella caudata	2002	2002	1909	Jörgensen (1924)
Parundella lohmanni	1939	1952	1909	Jörgensen (1924)
Petalotricha ampulla	1879	2016	1879	Fol (1881)
Proplectella amphora	2013	2015	2013	This study
Proplectella claparedei	1929	1998	1886	von Daday (1887)
Proplectella fastigata	2002	2016	1909	Jörgensen (1924)
Proplectella subacuta	2003	2003	1909	Jörgensen (1924)
Proplectella subcaudata	2003	2003	1909	Jörgensen (1924)
Protohabdonella curta	1998	2010	1909	Jörgensen (1924)
Protohabdonela simplex	2002	2002	1909	Jörgensen (1924)
Rhabdonella amor	2003	2003	1904	Enzt (1909)
Rhabdonella elegans	2003	2015	1909	Jörgensen (1924)
Rhabdonella spiralis	1879	2016	1879	Fol (1881)
Salpingella accuminata	1951	2016	1909	Jörgensen (1924)
Salpingella attenuata	1973	2016	1909	Jörgensen (1924)
Salpingella curta	2003	2016	1981	Balkis and Koray (2014)
Salpingella decurtata	1951	2016	1909	Jörgensen (1924)
Salpingella faurei	2002	2016	1996	Dolan et al., 1999
Salpingella minutissma	2002	2002	1993	Dolan and Marrase (1995)
Salpingella rotundata	1951	1953	1951	Balech (1959)
Steenstrupiella intumescens	2002	2003	1909	Jörgensen (1924)
Steenstrupiella steenstrupii	1953	2016	1886	von Daday (1887)
Stenosemella nivalis	1929	2016	1938	Rampi (1948)
Stenosomella ventricosa	1880	2016	1880	Fol (1883)
Tintinnopsis acuminata	2002	2016	2000	Moscatello et al. (2004)
Tintinnopsis beroidea	1952	2014	1909	Jörgensen (1924)
Tintinnopsis campanula	1879	2016	1879	Fol (1881)
Tintinnopsis compresa	1953	2002	1909	Jörgensen (1924)
Tintinnopsis cylinidrica	1929	2015	1887	von Daday (1887)
Tintinnopsis everta	2002	2002	1980	Abboud-Abi Saab (1989)
Tintinnopsis fimbriata	2003	2003	1992	Abboud-Abi-Saab (1992)
Tintinnopsis lobiancoi	1998	1998	1938	Rampi (1948)
Tintinnopsis major	2002	2002	2002	This study
Tintinnopsis minuta	2002	2002	1938	Rampi (1948)
Tintinnopsis nana	1952	1952	1952	Balech (1959)
Tintinnopsis nucula	1880	1880	1880	Fol (1883)
Tintinnopsis radix	1929	2016	1909	Jörgensen (1924)
Tintinnopsis tocantinensis	2002	2002	2002	Polat et al. (2002)
Tintinnopsis turbo	2003	2003	2003	This study
Tintinnopsis urnula	2003	2003	2003	This study
Undella attenuata	1973	1973	1909	Jörgensen (1924)
Undella clevei	1951	2015	1909	Jörgensen (1924)
Undella hyalina	1951	2016	1886	von Daday (1887)
Undellopsis marsupialis	1929	2016	1909	Jörgensen (1924)
Xystonella clavata	2009	2009	1909	Jörgensen (1924)
Xystonella lanceolata	2013	2016	1909	Jörgensen (1924)
Xystonella lohmanni	2010	2015	1909	Jörgensen (1924)
Xystonella longicaudata	1973	2016	1909	Jörgensen (1924)
Xystonella treforti	1938	2016	1886	von Daday (1887)
Xystonellopsis spicata	2016	2016	1909	Jörgensen (1924)
Xystonellopsis brandti	1973		1909	Jörgensen (1924)
Xystonellopsis paradoxa	1973	2016	1909	Jörgensen (1924)

may attribute different names to the same species depending upon their adherence or not to the species status attributed to many forms by Kofoid and Campbell as well their ability to recognize the ‘minute deviations’. Balech (1959) was notably critical of the some of the species designations of Kofoid and Campbell. Another complicating factor is that only in recent years has the phenomenon and magnitude of polymorphism in tintinnids become apparent (Dolan 2016) and some species found in the Bay of Villefranche do appear to be polymorphic. *Favella ehrenbergii* has been revealed to be capable of producing distinct lorica associated with several species of distinct genera (Laval-Peuto 1981, 1983). Bachy et al. (2012) found that several species of distinct genera and families had identical 18s RNA and ITS sequences justifying their fusion under one name, *Cyttarocylis ampulla*. To minimize artificial or inadvertent inflation of the species inventory, the species list was examined to identify probable synonymous species, forms likely to be recorded under multiple names, most of which were originally described as morphological varieties, not distinct species. Table 2 lists the 'species' grouped under a single name. The listing is by its nature subjective and in the light of future work will likely require revision.

Data analysis

For each species, the sampling year in which the species was first found and all the years subsequently found were recorded in a spreadsheet with rows as species and columns for years (see Supplementary data file). For species reported from a single year, that report was examined to determine if the occurrence was a unique event or if the species was found on more than one date within the year. A species found but once on a single date is herein termed a 'oncer', seen but once, and these species were noted as special cases. Sorting the entire listing by "sample year first found" allows plotting cumulative number of species by year of sampling. This was plotted along with cumulative number of samplings by year (see Table 1). The relationship between sampling effort and species discovery was examined by plotting cumulative number of samplings against cumulative number of species. It should be noted that the nature of the data, limited to species found by year and number of sampling within the year, precludes rarefaction analysis, which requires presence/absence data for each of the samplings. To identify species records as possibly new to the Mediterranean Sea, for each species a literature search was conducted to determine the first sample year the species was found in the Mediterranean Sea. The species list from recent intensive sampling (2013–2016) was compared to the species list derived from all previous sampling to identify differences, especially with regard to species recorded from multiple years.

Results

108 species, excluding probable synonyms have been found in the Bay of Villefranche in samples gathered over a span of 137 years (Table 3). Surprisingly, relatively few were encountered but one time, 17 out of the 108, and can so be considered as stray species. Other than the species first described by Fol (1881, 1883), the vast majority of species, 101, were found elsewhere in the Mediterranean Sea before being found in Villefranche. Thus not only have no species new to science been found since Fol's descriptions, but most were not even new records for the Mediterranean Sea. Of the few species first found in the Mediterranean Sea in Villefranche, 5 were also 'oncers', found but once, and likely members of the "accidental biosphere" out of their normal range due chance transport (e.g. Weisse 2014).

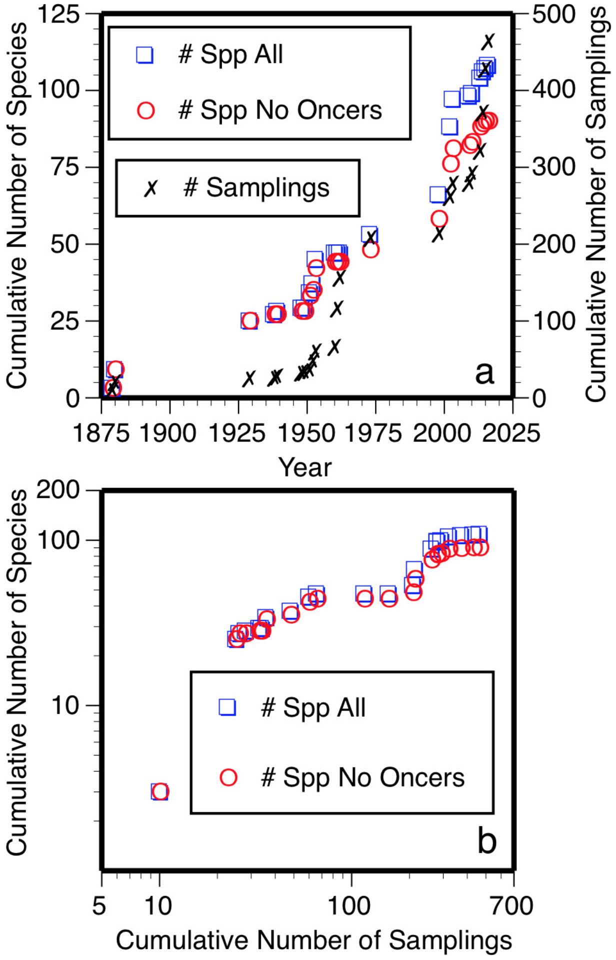

Fig. 1. (a) Temporal changes in the cumulative number of species recorded from the Bay of Villefranche and the cumulative number of samplings. Cumulative number of species (pooling probable synonymous records, see Table 2) are shown both with (# Spp All) and without species recorded but once (# Spp No Oncers). (b) Cumulative number of species recorded from the Bay of Villefranche as a function of sampling effort.

Temporal growth in the species inventory, both with and without 'oncers', along with sampling effort is shown in Fig. 1a. Growth in the number of species appears to increase markedly in recent years. However, a different pattern is evident from the plot of changes in the species inventory as a function of sampling effort (Fig. 1b). The log–log plot, similar to a Preston Species–Area curve relating sampling effort to species discovery (Preston 1962), shows a linear increase (log–log) up to about 100 samplings (approx. 1960) followed by moderate increases in species from 100 to 300 samplings (early 2000s). Notably only minor increases in the species inventories resulted from recent intensive sampling from 300 to 461 samples (2013–2016).

Comparing the species list from recent (2013–2016) sampling to the previous inventory revealed some differences. Although a large number of previously recorded species have been found in recent sampling, 39 previously recorded species are absent and 9 new species have been found. Most species recorded previously but missing from recent sampling were either oncers or apparently temporary residents (found only during one year) representing 14 and 12 species, respectively. However, 12 species were recorded from multiple years in previous sampling suggesting a relatively common occurrence in the past (Table 4). Likewise since 2013, among the species recorded as species new to the bay, 6 have been recorded from multiple years and can be considered as current resident species. In terms of gross morphology, there are no obvious characteristics distinguishing or uniting the apparently new nor absent species (Table 4). Both groups of species range widely in lorica dimensions. The majority of both groups of species are not unusual for Mediterranean waters as they are known from other coastal Mediterranean sites (Table 4).

Discussion

The possibility of a systematic shift in the composition of microplankton in a given system due to environmental change is possible (e.g. Whyte et al. 2016). However, it is also evident that species inventories can only increase, as it is impossible to prove that a species, once recorded, is subsequently completely absent. Consequently, historical growth in a species inventory is to be expected. The questions addressed here focus on how complete a listing does the cumulative historical record appear to provide compared to recent sampling and is there any evidence of large changes in the composition of the tintinnid assemblage?

As noted previously, sampling methods and frequencies varied considerably over the past 137 years. Plankton net mesh size, bottle vs net sampling, and season of sampling varied. Thus, one might expect a very weak relationship between cumulative sampling effort and increases in the numbers of species encountered. Interestingly, there is a strong relationship between cumulative sampling effort, varied as it was, and increases in the species inventory (Fig. 1a). Furthermore, the increases in the species inventory of Villefranche corresponding with increases in cumulative sampling effort (Fig. 1b) suggest that substantial sampling effort (hundreds of dates) are needed to provide a near complete species list (as indicated by a plateau). However, as the historical series includes any changes in the species composition it may be useful to compare it with a similar sampling effort curve derived from only the recent intensive sampling (2013–2016). Fig. 2 shows cumulative number of species encountered as a function of the cumulative number of samplings for both the periods 1879–2010 and 2013–2016. The two curves reach a similar value at about 100 samples, corresponding to multiple year coverage for the recent sampling. Subsequent increases in both series may then be attributable to year to year variability. As nicely remarked by Haeckel (1891), time-series data are needed to capture changes in composition and inter-annual variability can be considerable:

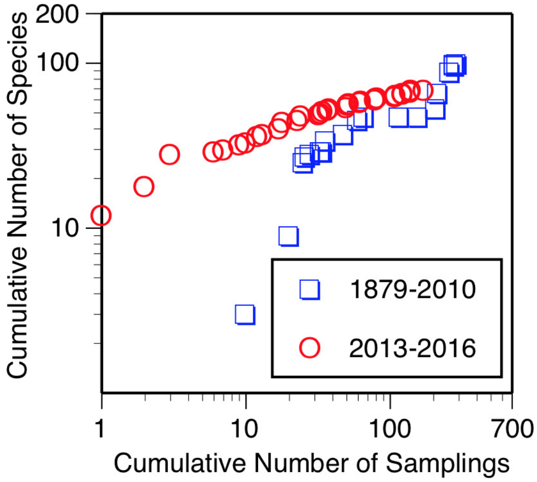

Fig. 2. Comparison of cumulative number of species recorded from the Bay of Villefranche as a function of sampling effort for recent intensive sampling (2013–2013) and the preceeding historical sampling (1879–2010).

> "*To obtain a complete and more certain survey of the temporary variations of plankton composition requires an unbroken series of observations, carried on at one and the same place at least for the space of a full year—still better for several successive years—to obtain from the yearly and monthly oscillations a general average. . . . . . As there are good and bad wine and fruit years, so there are rich and barren plankton years*"

Comparing the species list from recent sampling (2012–2016) to the historical list (1879–2010) some differences are evident. Of the species previously found, but not noted in recent years, none were described as abundant and likewise among species first noted in recent years, none were abundant. There are no obvious morphological characteristics distinguishing either group of recently absent or recently new. Most of the species of both groups are distributed widely in the Mediterranean (Table 4). The differences in the species lists of recent versus previous years may represent a change in the species composition of the tintinnid assemblage. However, the more likely explanation is that the differences are due to chance detections of species intermittently present in low concentrations.

Previous studies of seasonal changes in the assemblage of tintinnid species in Villefranche have yielded distinct descriptions. Rassoulzadegan (1975) noted that in his 1973–1974 series common was *Steenstrupiella steenstrupii* and rare

Table 4. Characteristics of “new species” and “species absent in recent years” (2013–2016). The list excludes ‘oncers’ and species recorded from a single year. Morphological characteristics given are lorica oral opening diameter (LOD), overall length lorica length (LL). Occurrences in other coastal Mediterranean sites are (arranged in order of increasing distance from Villefranche): SR = San Remo, Italy (Rampi 1948), M = Marseille, France (Travers and Travers 1971), CS = Catalan Sea, Spain (Dolan and Marrase 1995), C = Castellon, Spain (Duran 1951 1953), N = Naples, Italy (Modigh and Castalado 2002), T = Trieste, Italy (Monti et al. 2012), JB = Jounieh Bay, Lebanon (Abboud-Abi Saab 1989), PI = Palm Island, Lebanon (Abboud-Abi Saab 2002).

	LOD (μm)	LL (μm)	Last year	Mediterranean sites
New species				
Amphorellopsis tetragona	45	110	2015	C, T, JB
Eutintinnus permintus	34	162	2016	SR
Eutintinnus stramentus	28	145	2016	M, C, T, JB
Proplectella amphora	29	59	2015	
Xystonella lanceolata	55	245	2016	
Codonella amphorella	44	89	2016	JB, PI
Missing species				
Acanthostomella minutissma	24	33	2010	N
Ascampbelliella oxyura	28	40	2002	
Codonella aspera	50	75	2003	M, T, JB
Dictyocysta entzi	38	48	2009	N
Eutintinnus macilentus	48	233	2010	SR, M, C, JB, PI
Favella serrata	116	264	2002	M, N, T
Metacylis jörgensenii	47	55	2002	SR, N, T, JB, PI
Proplectella claparedei	35	85	1998	M, C, N, T, PI
Salpingella minutissma	34	106	2003	CS
Salpingella rotundata	43	334	1953	M, N, T
Steenstrupiella intumescens	36	205	2003	JB, PI
Tintinnopsis compresa	56	69	2002	M, T, PI

Table 5. Presence (as % dates sampled) of the most common species in 2014 and 2015. The species listed were found on at least 25% of the dates sampled in either the 50 dates sampled in 2014, or the 57 dates sampled in 2015. Note the high variability in detection of some species such as *Stenosomella ventricosa* and *Tintinnopsis campanula*.

Species	2014	2015
Amphorella quadrilineata	54	48
Codonaria cistellula	34	18
Codonella elongata	58	44
Codonella nationalis	40	23
Codonellopsis morchella	72	67
Dictyocysta lepida	18	25
Eutintinnus apertus	30	42
Proplectella fastigata	50	40
Rhabdonella spiralis	34	37
Salpingella accuminata	54	42
Salpingella attenuata	28	32
Steenstrupiella steenstrupii	18	37
Stenosemella nivalis	54	16
Stenosomella ventricosa	60	16
Tintinnopsis campanula	26	4

was *Proplectella claparedei*, in contrast with the data of Posta who found found *P. claparedei* abundant and found no *S. steenstrupii* (1972) from her sampling in 1960–1962. Rassoulzadegan (1975) attributed the difference to sampling methods. However, a comparison of the presence of the most common species in 2014 and 2015, sampled using the same methods, shows that a species found often one year may be relatively rare another year, i.e. the conspicuous species *Stenosomella ventricosa* and *Tintinnopsis campanula* (Table 5). While we have no data on historical concentrations, the recent temporal variabilities in the presence of *Stenosomella ventricosa* or *Tintinnopsis campanula* (2014 vs. 2015) are in regard to detection limits in range of 1 cell in 20 l. Thus species inventories may differ due not only to sampling methods but also large inter-annual variability in relatively abundant and common species, as pointed out long ago by Haeckel (1891). It should be noted that data on possible historical changes in the physical and chemical characteristics is lacking. However data from the late 1960s to the present suggests that periodic oscillations of sea surface temperature exists on a 10–15 year scale and may influence zooplankton abundances (Howes et al. 2015).

Conclusions

Data presented here show historical growth in the species inventory of tintinnid ciliate species in the Bay of Villefranche. Temporal increases in species inventories are to be expected in any open or semi-open system, as with time inter-annual variability will be captured and occasional species are more likely to be found. Remarkable for Villefranche is the magnitude of diversity recorded, over 100 species after accounting for likely synonymous records. The comparison of recent and historical sampling in Villefranche suggests a very large sampling effort, weekly sampling over multiple years, is required to provide an inventory representing the majority of species currently found in the Bay. Based upon data from Villefranche, a species checklist of tintinnids is at best an approximation, an incomplete list of species possibly to be found. Consequently, claims of a tintinnid species as 'new' to a locality should be made with caution and caveats.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejop.2016.10.004.

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