Micromonas pusilla (Prasinophyceae) as part of pico- and nanoplankton communities of the Barents Sea

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Micromonas pusilla (Butcher) Manton & Parke appears to be a prominent member of the Barents Sea picoplankton community as revealed by the serial dilution culture method. Cell numbers frequently exceeded 10^7 cells l^{-1}, though they usually varied between 10^3 and 10^6 cells l^{-1}. A number of other identified and unidentified taxa were recorded and quantified. Distribution relative to the marginal ice zone is reported.

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Introduction

Investigations on flagellates in the Barents Sea began with the work of Wulff (1916), who reported the presence of many species previously described from temperate waters. Meunier (1910), working in the neighbouring area, the Kara Sea, likewise added to the knowledge of Arctic flagellate plankton and described new species from the area. Earlier investigations in polar oceans were restricted either to laboratory work on preserved material or to shipboard observations of living material from net hauls.

Recently, quantitative information on the smaller plankton of the Barents Sea was obtained by Reynolds (1973), who made direct counts, and Throndsen (1970), who used a dilution culture technique. Species information from Arctic areas has on the whole been obtained by electron microscopy studies on dried preparations of cell material fixed with osmic acid (e.g. Thomsen 1982, Manton et al. 1975, 1976). This method, however adequate for revealing submicroscopical details necessary for identification, gives very poor indications of cell numbers in the sea. The present survey elucidates the importance of eukaryotic pico- and nanoplankton in the marginal ice zone in Arctic marine waters. The serial dilution culture method was applied in order to give quantitative as well as qualitative information.

When first used in the Arctic in 1969 (Throndsen 1970), the dilution culture technique revealed the presence of a number of genera and species new to the waters off Spitsbergen and Bjørnøya.

Materials and methods

Water samples were collected from stations at different distances from the ice edge (Fig. 1, Table 1) during Pro Mare cruises in the Barents Sea in the spring (April) of 1986 and during the summers (June) of 1984 and 1987.

The sampling device was assumed to be non-toxic; the effect of possible undetected toxicity was minimised by the relatively short handling time and the dilution of the samples into growth medium with chelating agents included. Serial dilution cultures (Throndsen 1978) were set up in Erd-Schreiber medium (S = 32.5) 1–2 hours after sampling. The cultures were kept in dim light at 2–4 °C during the cruise and transportation and in the laboratory culture room at the Department of Biology, University of Oslo.

The cultures were examined after 6–8 weeks by bright field and anoptral contrast microscopy, with additional observations by transmission electron microscopy.

The presence and absence of each species throughout the dilution culture series made it possible to estimate the cell numbers present in the original sample (MPN = Most Probable Number).

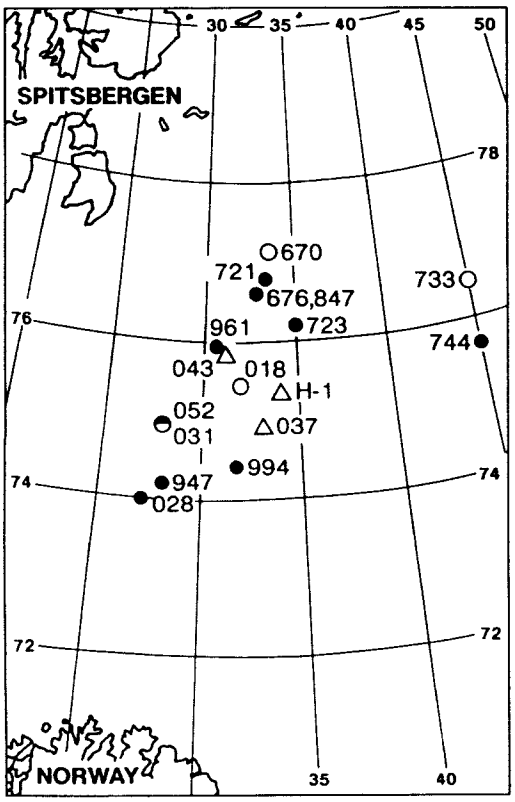

Fig. 1. Map of the Barents Sea showing the location of the stations sampled for serial dilution cultures: solid circles = no ice; open circles = open pack ice; triangles = close pack ice.

Results

Picoplankton (1–2.5 μm)

Micromonas pusilla (Butcher) Manton & Parke appears to be a prominent member of the picoplankton community as revealed by the methods applied. Cell numbers frequently exceeded 10^7 cells l^{-1}, though they usually varied between 10^3 and 10^6 cells l^{-1} (Tables 2, 3). Out of 17 stations sampled, *M. pusilla* was detected at all but three.

Other eukaryotic picoplankton comprised green coccoids 1–1.5 μm, 2 μm, and 2.5–3 μm in size. These were not studied in further detail but probably included non-motile *M. pusilla*, the newly described *Bathycoccus prasinos* (Eikrem & Throndsen 1990), and possibly also undescribed coccoid eukaryotic picoplankton species.

Prokaryotic picoplankton were not prominent in the dilution cultures and are thus ignored in this paper.

Nanoplankton (2.5–20 μm)

Among the smaller nanoplankton (< 5 μm, Tables 2, 3) identified, the five most abundant species were the prasinophycean *Mantoniella squamata*, the prymnesiophytes *Dicrateria inornata* and *Imantonia rotunda*, and the pedinelloid chrysophyte *Pseudopedinella tricostata*.

Table 1. Stations sampled for serial dilution cultures in the Barents Sea in 1984, 1986, and 1987

Year	Date	Ice condition	Station	Position	Depth
1984	4 June	open pack	670	77°10′N, 33°28′E	5 m
	4 June	no ice	676	76°40′N, 33°04′E	25 m
	8 June	no ice	721	76°48′N, 33°07′E	0,5,10,20,30,50,75 m
	9 June	no ice	723	76°10′N, 35°00′E	10 m
	10 June	open pack	733	76°32′N, 45·00′E	5,15,75 m
	11 June	no ice	744	75°45′N, 45°00′E	10 m
	13 Aug.	no ice	847	76°40′N, 33°04′E	10 m
1986	10 April	open pack	018	75°29′N, 32°06′E	10 m
	15 April	no ice	028	74°00′N, 27°11′E	10 m
	16 April	open pack	031	74°58′N, 27°46′E	10 m
	17 April	close pack	037	74°54′N, 32°59′E	ice
	19 April	close pack	043	75°54′N, 30°45′E	ice
	20 April	close pack	H-1	75°26′N, 34°15′E	10,30 m under ice
	21 April	no ice	052	74°58′N, 27°50′E	10 m, bloom
1987	2 June	no ice	945	74°11′N, 28°00′E	10 m
	4 June	no ice	961	75°55′N, 30°30′E	10,30 m
	8 June	no ice	994	74°30′N, 31°31′E	10,60 m

Table 2. Occurrence of *Micromonas pusilla* and selected groups of plankton on stations sampled in the Barents Sea in June 1986, MPN in 10^3 cells l^{-1}, — no cells recorded.

		STATIONS					
Species/groups		670	733	676	721	723	744
	Ice condition	open pack ice			no ice		
	Sampling depth	5 m	5 m	25 m	0 m	10 m	10 m
Micromonas pusilla		>24 000	78	—	68	170	270
Other eukaryotic picoplankton		—	18	—	—	37	48
Nanoplankton <5 μm		0.4	94	120.2	73.2	20	56
Nanoplankton >5 μm		0.8	40	19	72.2	0.2	20
Diatoms		240	920	260	260	35	—
Heterotrophic flagellates		—	—	0.4	6	—	—

Table 3. Occurrence of *Micromonas pusilla* and selected groups of plankton in the Barents Sea in April 1986, MPN in 10^3 cells l^{-1}, — no cells recorded.

	STATIONS						
	037	043	H-1	018	031	028	052
Ice condition	close pack ice			open pack ice		no ice	
Micromonas pusilla	2	—	0.2	40	13 000	—	1.8
Other eukar. picoplkt.	—	—	110	210	—	60	6.8
Nanoplankton <5 μm	—	2.2	22	40	—	55	120
Nanoplankton >5 μm	—	—	0.2	0.2	20	—	0.8
Diatoms	19 500	24 300	84	100	1 830	142	254
Heterotroph. flags	176	22	—	—	—	—	—

Mantoniella squamata (Manton & Parke) Desikachary (3–5 μm) belongs to the smaller nanoplankton. It was recorded on three occasions only. Other prasinophycean flagellate species, e.g. of *Pyramimonas*, were even more scarce in the dilution cultures. Non-motile stages of *Pterosperma*, *Halosphaera* and *Pachysphaera* were encountered in small numbers only.

Dicrateria inornata Parke (3–5.5 μm) and *Imantonia rotunda* Reynolds (2–4 μm) are almost indistinguishable in the light microscope, and for this reason they were referred to as *Dicrateria inornata/Imantonia rotunda*. Most often samples studied in the electron microscope appeared to contain *Imantonia rotunda* which is characterised by fine oval-circular, cartwheel-patterned organic scales covering the cell surface. *Dicrateria inornata* is naked and information from direct EM preparations was rather limited.

Larger nanoplankton (> 5 μm) (Tables 2, 3) was usually dominated by chryso- or cryptophytes. *Pseudopedinella pyriformis* N. Carter (5–8 μm) was the most common chrysophyte identified, the smaller *P. tricostata* (Rouchijajnen) Thomsen (4–5.5 μm) was less common, but recorded both in 1984 and 1987.

Motile cells of the genus *Phaeocystis* (4.5–8 μm) were also occasionally significant.

A new species "Arctic flag sp.#" was probably the most common taxon among the larger nanoplankton. The species has flattened cells 9–12 μm long, with two ventrally inserted flagella, one pointing forwards, the other directed posteriorly. The single chloroplast is golden brown. A more extensive description of the species will be published elsewhere.

Cryptophycean species in the size range 10–20 μm were frequently present. Species of the genera *Isoselmis*, *Plagioselmis* and *Chroomonas* covered the whole nanoplankton size spectrum, whereas specimens which were tentatively identified as *Cryptomonas prora* Conrad & Kufferath were in the upper size range (18–21 μm). This species has been reported to bloom during winter

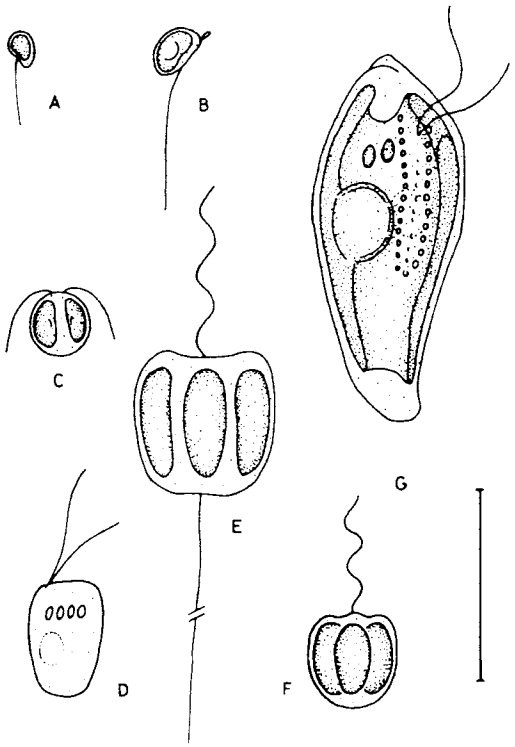

Fig. 2. Selected pico- and nanoplankton flagellates recorded in dilution cultures from the Barents Sea: A. *Micromonas;* B. *Mantoniella* (short flagellum usually not seen in the light microscope); C. *Dicrateria/Imantonia;* D. *Goniomonas;* E *Pseudopedinella pyriformis;* F. *P. tricostata;* G. *Cryptomonas* cf. *prora.* Scale bar 10 µm.

and even to have been found under the ice (−0.5°C) in brackish waters in Belgium (Conrad & Kufferath 1954). Of the *Hemiselmis* species only *H. brunnescens* Butcher was identified.

Heterotrophic flagellates and diatoms were usually only referred to as groups and separated from other nanoplankton e.g. in Tables 2 and 3.

Table 4. Occurrence of *Micromonas pusilla* in the Barents Sea in 1984, 1986, 1987.

Station	Ice condition	Depth	Date	MPN $10^3 l^{-1}$
994	no ice	10 m	87.06.08	>24 000
670	open pack	5 m	84.06.04	>24 000
031	open pack	0 m	86.04.16	13 000
945	no ice	10 m	87.06.02	3 500
994	no ice	60 m	87.06.08	1 700
961	no ice	10 m	87.06.04	1 300
961	no ice	30 m	87.06.04	790
744	no ice	10 m	84.06.11	270
723	no ice	10 m	84.06.09	170
733	open pack	5 m	84.06.10	78
721	no ice	0 m	84.06.08	68
733	open pack	15 m	84.06.10	42
721	no ice	50 m	84.06.08	40
018	open pack	0 m	86.04.10	40
721	no ice	5 m	84.06.08	18
721	no ice	30 m	84.06.08	18
037	close pack	0 m	86.04.18	2
733	open pack	15 m	84.06.10	2
052	no ice	0 m	86.04.21	1.8
721	no ice	20 m	84.06.08	0.2
H-1	close pack	30 m	86.04.20	0.18

The flagellates including *Goniomonas, Pseudobodo* and *Anisonema* were of varying importance, while the diatoms were consistently very abundant in the samples.

Flagellate cell numbers relative to the ice

Micromonas pusilla

Disregarding the different years of sampling (Table 4), the highest cell numbers of *Micromonas pusilla* were found in free water masses well off the ice edge in early June in 1986 (Station 994) or in open pack ice in 1984 (Station 670). The MPN values given in Table 4, >24 million cells l^{-1}, reflects that more dilution steps should

Table 5. Vertical distribution of *Micromonas pusilla* and groups of plankton at an open water station in the Barents Sea. MPN in 10^3 cells l^{-1}, — no cells recorded.

	STATION 721 — 76°48'N, 33°07'E						
Species/groups	Depth	0 m	5 m	10 m	20 m	30 m	50 m	75 m
Micromonas pusilla		68	18	—	0.2	18	40	—
Other euk. picoplkt.		—	18	—	18	—	0.4	2.4
Nanoplankton <5 µm		73.2	54	36	0.4	18	0.2	2.2
Nanoplankton >5 µm		54.4	12.9	121.8	157.2	222.2	18.4	0.6
Diatoms		260	220	120	470	120	60	70

have been used to allow for a better estimate of these high *M. pusilla* populations.

Close to the ice edge (Station 031), MPN up to 13 million cells l^{-1} was recorded in open pack ice in April 1986 (Table 3).

For the stations in oceanic ice-free waters, the *Micromonas* numbers varied between 10^6 and 10^3 cells l^{-1}, except for Station 028 where it was not recorded.

The lowest recorded concentrations of *M. pusilla* were near the limit of detection (180 cells l^{-1}) and were found at 20 m depth at Station 721, 15–20 nm south of the ice edge in 1984 and at 30 m under the ice at Station H-1 in 1986 (Table 4).

Depth distribution (Station 721)

Information on depth distribution is sparse. The MPN for *Micromonas pusilla* and four groups of pico- and nanoplankton from surface to 75 m depth at Station 721 (1984, Table 5) may, however, reflect a common situation; the diatoms had their highest numbers in the surface layer and at 20 m. *M. pusilla* was likewise numerous at the surface, but its deeper maximum was at 50 metres. At Station 733 three depths were sampled showing the highest concentrations of *Micromonas* at 5 m, slightly lower at 15, and lacking at 75 m.

At Station 721 dilution cultures from 5 depths (Table 5) showed that the dominating populations of viable pico- and nanoplankton were confined to the upper 30 m. The population at 75 m consisted of non-motile picoplankton and nanoplankton flagellates: *Mantoniella squamata, Pseudopedinella pyriformis* and the heterotrophic *Pseudobodo minimus* Ruinen, as well as an unidentified heterotrophic *Gymnodinium* species. *M. pusilla* was most numerous at the surface, but the concentration was only 7×10^4 cells l^{-1}.

The concentration of *Pseudopedinella pyriformis* varied from $6 \cdot 10^3$ cells l^{-1} at the surface to $4 \cdot 10^2$ cells l^{-1} at 75 m, with a maximum of $45 \cdot 10^3$ at 30 m.

Occurrence of other flagellates

Pseudopedinella species were most common in summer samples, the highest MPN were $45 \cdot 10^3$ cells l^{-1} (Station 721, 30 m depth) for *P. pyriformis* whereas *P. tricostata* never exceeded $3.7 \cdot 10^3$ cells l^{-1}. *P. pyriformis* was, however, found from the surface to 75 m depth.

Heterotrophic flagellates of nanoplankton size were present at most stations. MPN varied from the detection limit 180 cells l^{-1} to $2.1 \cdot 10^5$ cells l^{-1}. The highest cell number recorded was for *Paraphysomonas* at Station 994, 60 m in June 1987, whereas $1.7 \cdot 10^5$ cells l^{-1} of an unknown species was estimated from an under-ice sample at Station 037.

Discussion

The SDC method

The serial dilution culture method is known to be selective (Throndsen 1978), and other species than those recorded may certainly also have been present in the samples. Lack of viability of the specimens introduced to the dilution tubes, and competitive relations in the single tube, may have reduced the number of tubes with detectable growth of an otherwise culturable species. The culture conditions and the medium used will further influence growth, and hence the MPN estimates (see e.g. Furuya & Marumo 1983, Jochem 1990). The presence of the species recorded, however, cannot be disputed, and they certainly belong to the viable part of the plankton community. Heterotrophic species will only grow provided adequate food organisms or compounds are present, and there is certainly a lack in registration especially of heterotrophic dinoflagellates.

Technically, correct MPN estimates for culturable species will be impossible if their original concentration is too high compared with the dilutions used. The five step dilution used ended with an inoculum of 0.1 µl of the original sample. During the work in the Barents Sea this caused limitations in some of the estimates of the *Micromonas pusilla* population; MPN estimates had to be given as > 24 million cells l^{-1}. For larger species such high numbers of cells would have caused discoloration of the water and hence given visual advice regarding the number of dilution steps.

The MPN estimates could be compared with direct counts at Station 994 (T. F. Thingstad pers. comm.). The MPN for *Micromonas* at 10 m depth was higher than $2.4 \cdot 10^7$ cells l^{-1}, whereas direct counts for picoplankton (not identified to species) gave $1.5 \cdot 10^7$ cells l^{-1}. This indicates that *Micromonas* probably was the major if not the only species in the group. The difference in cell number

estimates is an acceptable discrepancy considering the methods used. At 60 m the difference was significant, MPN estimate for *Micromonas* was $1.7 \cdot 10^6$ cells l^{-1} whereas the counts gave negligible numbers only. *Phaeocystis* MPN estimates were related to colonies (which could contain a variable number of cells) and hence gave lower numbers than the direct cell counts.

Occurrence related to the ice

Micromonas pusilla was apparently not particularly favoured by the conditions in or very close to the ice, though fairly high cell numbers were recorded also at the ice edge (Fig. 1, Table 4), the latter being in accordance with the tolerance of this species for low salinities as well as low temperatures (Throndsen 1976; Throndsen & Heimdal 1976; Jochem 1990). The low surface to volume relation is likely to give the picoplankton species their best competitive advantage under oligotrophic conditions, and it is therefore not surprising to see that diatoms (mostly nanoplanktonic species were recorded by the method applied) rather than picoplankton were dominating in the ice underside samples. The opportunistic *M. pusilla*, however, was very abundant in the surface water of the marginal ice zone.

Information on *M. pusilla* from other polar oceans is mostly restricted to identification in direct EM preparations, and with no real evidence for cell concentrations. Also for eukaryotic picoplankton in general the quantitative information from these areas is very sparse. From the Tromsø area, which is north of the Arctic circle and hence is exposed to "polar" light conditions, *M. pusilla* proved to be present in the sea during all the seasons, but with its highest numbers in summer (Throndsen & Heimdal 1976).

Depth distribution

Micromonas pusilla is known to have a low light saturation level for growth (Throndsen 1976), and its depth distribution in subtropical areas is extensive; viable cells are found down to 500 m in the Bay of Biscay (Manton & Parke 1960) and 600 m off the coast of Japan (Kuroshio area, Throndsen 1983). Hence the presence of *M. pusilla* at 75 m in the Barents Sea is to be expected. Its distribution in the deeper water masses, including productive areas at high latitudes, further confirms its ability for survival and growth.

Studying the subsurface chlorophyll maximum in the western North Pacific Ocean, Furuya & Marumo (1983) found *Micromonas* (probably *M. pusilla*) to be the most abundant genus in this layer. In the Gulf of Panama *M. pusilla* appeared to follow the general distribution of chlorophyll from 10 to 100 m depth; the deeper maximum here was at 75 m (Throndsen 1976).

Picoplankton importance in polar waters

Though *Micromonas pusilla* and other eukaryotic picoplankton species have been reported from both Arctic (Throndsen 1970; Thomsen 1982) and Antarctic waters (H. A. Thomsen pers. comm.), little is known about their importance relative to the prokaryotic picoplankton in these areas. Cyanobacteria are frequently recorded by the dilution culture method e.g. in coastal areas of the temperate zone, but they were not common in the present samples.

Based on the methods known today, future efforts in this field should include an integrated use of serial dilution cultures, direct EM preparations, epifluorescence counts, size fractionated carbon uptake measurements, and laboratory growth and pigment studies.

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References

Conrad, W. & Kufferath, H. 1954: Recherches sur les eaux saumatres des environs de Lilloo. 2. Partie descriptive. *Mém. Inst. R. Sci. Nat. Belg. 127*, 1–346.

Eikrem, W. & Throndsen, J. 1990: The ultrastructure of *Bathycoccus* gen. nov. and *B. prasinos* sp. nov., a non-motile picoplanktonic alga (Chlorophyta, Prasinophyceae) from the Mediterranean and Atlantic. *Phycologia 29*, 344–350.

Furuya, K. & Marumo, R. 1983: The structure of the phytoplankton community in the subsurface chlorophyll maxima in the western North Pacific Ocean. *J. Plankton Res. 5*, 393–406.

Jochem, F. J. 1990: On the seasonal occurrence of autotrophic naked nanoflagellates in Kiel Bight, Western Baltic. *Estuar. Coast. Shelf Sci. 31*, 189–202.

Manton, I., & Parke, M. 1960: Further observations on small green flagellates with special reference to possible relatives of *Chromulina pusilla* Butcher. *J. Mar. Biol. Ass. U.K. 39*, 275–298.

Manton, I., Sutherland, J. & Leadbeater, B. S. C. 1975: Four new species of choanoflagellates from Arctic Canada. *Proc. R. Soc. London, Ser. B 189*, 15–27.

Manton, I., Sutherland, J. & Leadbeater, B. S. C. 1976: Further observations on the fine structure of collared flagellates (Choanoflagellata) from Arctic Canada and West Greenland. *Can. J. Bot. 54*, 1932–1955.

Meunier, A. 1910. Microplankton des mers de Barents et de Kara. *Duc d'Orléans Campagne Arctique de 1907, Bruxelles*. 355 pp.

Reynolds, N. 1973: The estimation of the abundance of ultraplankton. *Br. Phycol. J. 8*, 135–146.

Thomsen, H. A. 1982: Planktonic choanoflagellates from Disko Bugt, West Greenland, with a survey of the marine nanoplankton of the area. *Meddr. Grønland, Biosci. 8*, 1–36.

Throndsen, J. 1970: Flagellates from Arctic waters. *Nytt Mag. Bot. 17*, 49–57.

Throndsen, J. 1976. Occurrence and productivity of small marine flagellates. *Norw. J. Bot. 23*, 269–293.

Throndsen, J. & Heimdal, B. R. 1976: Primary production, phytoplankton and light in Straumsbukta near Tromsø. *Astarte 9*, 51–60.

Throndsen, J. 1978: The dilution culture method. Pp. 218–224 in Sournia, A. (ed.): *Phytoplankton Manual, UNESCO Monographs on Oceanographic Methodology, No. 6*. Paris.

Throndsen, J. 1983: Ultra- and nanoplankton flagellates from coastal waters of southern Honshu and Kyushu, Japan (including some results from the western part of the Kuroshio off Honshu). *Working party on taxonomy in the Akashiwo Mondai Kenkyukai fishing ground preservation division, Research department, Fisheries Agency, Japan*. 62 pp.

Wulff, A. 1916: Ueber das Kleinplankton der Barentssee. *Arb. Dt. Wiss. Kommn Int. Meeresforsch. A. Aus Dem Laboratorium Für Internationale Meeresforschung in Kiel. Biol. Abt. No. 28*, 95–125.