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Gonyaulax tamarensis - the causative organism of mussel toxicity in Trondheimsfjord

by

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INTRODUCTION

All outbreaks of shellfish toxicity reported in Norway have been due to consumption of toxic mussels (*Mytilus edulis* L.) and have occurred exclusively in Oslofjord. Investigations carried out in that area suggested that a marine dinoflagellate, *Gonyaulax tamarensis* Lebour which had been implicated with shellfish toxicity in other parts of the world (Prakash 1963, Ingham *et al.* 1968) may have been the source of toxic mussels in Oslofjord (Oftebro 1965).

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In 1964, toxic mussels were reported for the first time in Trondheimsfjord, central Norway (Oftebro 1965). This finding resulted in intensified investigations on the association of mussel toxicity with the occurrence of *G. tamarensis* in Trondheimsfjord.

Most of the results of investigations during 1963 - 1969 have been submitted for publication (Sakshaug and Jensen, 1971). The present report summarizes hitherto unpublished results and deals particularly with the relationship between *G. tamarensis* abundance and accumulation of poison in mussels.

We gratefully acknowledge professor Steinar Hauge at the Veterinary College of Norway, where the mouse assays were carried out.

MATERIAL AND METHODS

The sampling sites for phytoplankton and mussels are shown in Fig. 1.

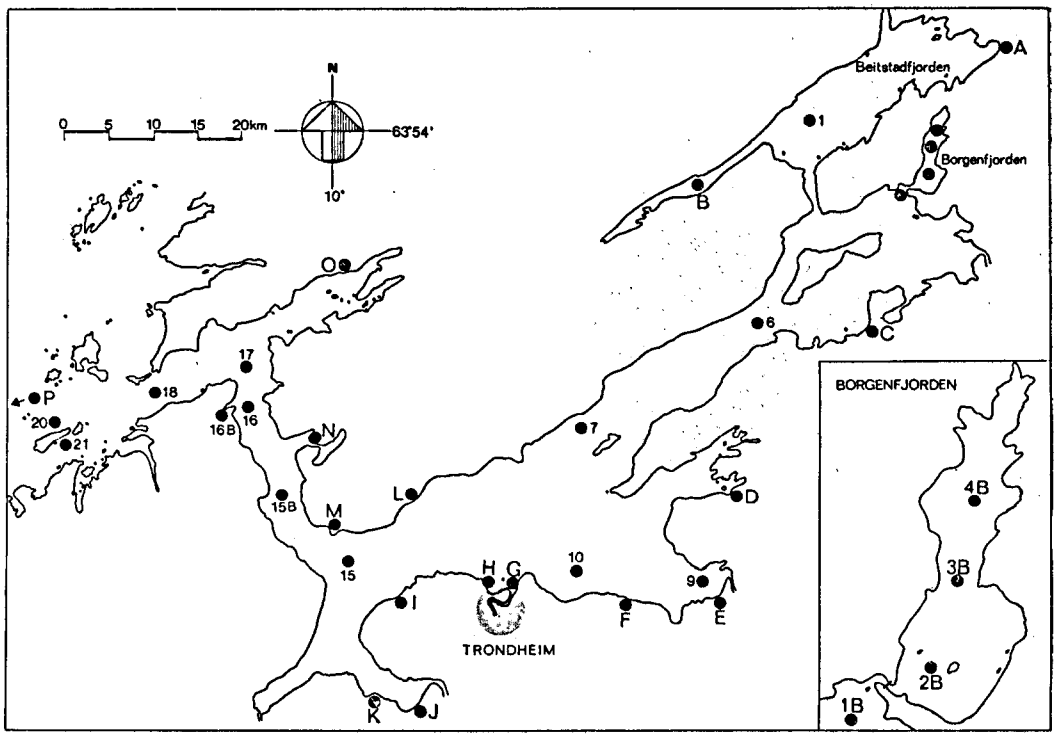

Fig. 1. Map showing the location of the sampling sites.

Phytoplankton was collected during weekly or monthly cruises in 1963 - 1971. The stations selected for investigation varied from year to year. Detailed investigations of the phytoplankton abundance in waters surrounding selected mussel beds were carried out from 1968 to 1970. Estimates of phytoplankton abundance are based on counts of preserved samples using an inversed microscope technique (Utermöhl 1931).

Mussels for toxicity analyses were colledted from the subtidal zone and were shipped to the Institutt for Næringsmiddelhygiene in Oslo where they were assayed for toxicity within 36 hours after collection. The toxicity bioassays were carried out according to methods given by Oftebro (1965).

RESULTS AND DISCUSSION

Seasonal abundance and year to year fluctuations of G. tamarensis

Observations carried out during 1963 - 1971 have shown that *G. tamarensis* is a normal component of the phytoplankton in Trondheimsfjord. It has been observed every year, and concentrations as high as 31 500 cells per litre have been recorded. The organism is most common during April - June each year, reaching its peak abundance usually in May. The only exceptions have been 1963 and 1966 when no increases in the dinoflagellate population took place in the late spring (Sakshaug 1971). In some years *G. tamarensis* has been observed in small numbers as early as March and as late as September in Trondheimsfjord.

Considerable fluctuations in year-to-year abundance of *G. tamarensis* occur in Trondheimsfjord as indicated by their maximum counts recorded during 1963 - 1971 (Table 1). In view of somewhat long intervals between the cruises, the coarse spacing of the stations and eventual patchiness of the populations, the figures serve only as rough estimates of the general abundance during the periods investigated.

Table 1.

Maximum abundance of *G. tamarensis* (cells per litre) and mussel toxicity (M.U. per 100g mussel meat) at neighbouring stations in Trondheimsfjord, 1963 - 1971. Sampling sites are given in parentheses.

Year	*G. tamarensis*	Date	Toxicity	Date
1963	160 (1)	July 8	?	
1964	31 500 (15)	May 20	2 400 (E,6)	June 3-10
1965	20 500 (6)	April 12	2 077 (E)	
			2 833 (C)	May 21-26
1966	20 (H)	April 7	0 [1]	May
1967	22 500 (E)	June 12	13 400 (E)	June 7
1968	524 (E)	May 13	768 (E)	June 4
	2 300 (16 B)		1 120 (16 B)	
1969	1 280 (E)	May 22-27	486 (E)	May 28
	21 000 (4 B)			
1970	1 970 (15)	May 7-8	532	May 27
	300 (Borgenfj.)		(Borgenfj.)	
1971	1 240 (15)	June 6	< 200 (E,I,J)	June 15
	10 480 (4B)			

1) two samples from unknown locality

Relationship between G. tamarensis and mussel toxicity

The maximum numbers of *G. tamarensis* recorded each year and the highest toxicities of mussels found at neighbouring stations are given i Table 1. It is seen that mussels can be quite toxic in Trondheimsfjord, but the peak toxicities show wide fluctuations. Nevertheless, the years with the highest *G. tamarensis* counts also showed the highest toxicity levels.

The timing of the rise in mussel toxiciy and abundance of *G. tamarensis* in the area generally coincided, except in 1965, when the peak of *G. tamarensis* abundance occurred in the first half of April but the maximum toxicity occurred during the last half of May as shown below:

Date	Maximum of *G. tamarensis* (St. 6, 15), cells/l	Maximum toxicity (St. E, G) M.U./100g meat
March 31	80	
April 13	20 500	
" 27		230
May 4		720
" 6	5 000	
" 14		1 537
" 25	280	2 077
June 1		1 188
" 9		200

This disparity between high *Gonyaulax* counts in April and high toxicities in May appears to be linked with temperature characteristies in the two months. During the first half of April mean surface temperature was about 4.0 C. In May it increased to about 16 C. It is possible that the low temperatures in April slowed down the pumping rate and consequently the feeding activity of the mussels, whereas in subsequent months, elevated temperatures speeded up the feeding activity resulting in accumulation of high amounts of toxin (Prakash *et al.* 1971).

In order to get a more precise picture of the relationship between *G. tamarensis* abundance and the corresponding toxicity level, special investigations in the near-shore water surrounding the mussel beds were undertaken in 1968, 1969 and 1970. In 1968, water overlying mussel beds along an approximately 100 m long stretch of the shore was sampled for *G. tamarensis* near St. E. Sampling was done every second day at 20 m intervals at a depth of 1 m, thus giving 5 samples in one horizontal sweep. Some of the typical results (cells/l) are given below (data from Sakshaug 1970):

Sample	I	II	III	IV	V	Mean
May 7	200	560	20	60	120	192
" 13	0	260	1920	380	60	524
" 27	520	140	180	60	500	280

It is seen that the horizontal variations at 1 m depth are large. It may be mentioned that *Skeletonema costatum* (Grev.) Cleve, which by far dominated these samples (10 - 20 mill.

cells per litre) was far more uniformly distributed horizontally. It is probable that not only small-scale hydrographic irregularities, but also the motility of *Gonyaulax* may have contributed to its patchy distribution. Day-to-day variations in *Gonyaulax* abundance were also noticed but these were even larger than the spatial variation as shown in Fig. 2.

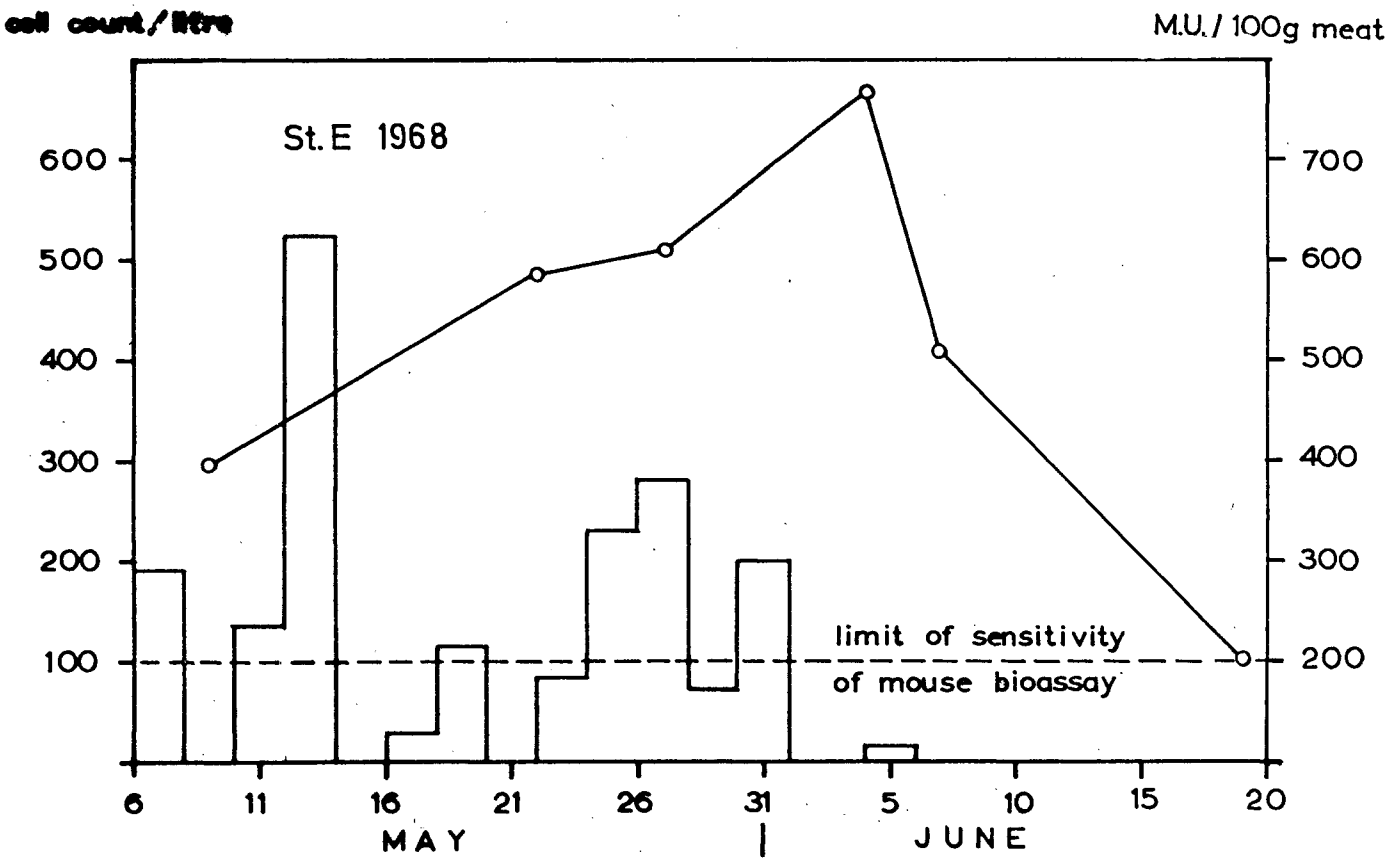

Fig. 2. Variations in *G. tamarensis* abundance (histogram) and mussel toxicity (curve) at St. E, 1968 (*G. tamarensis* given as mean count of 5 samples every second day).

These results made it clear that if an adequate estimate of the amount of *G. tamarensis* available to the mussels

was to be obtained, one had to use a device which could sample near the mussel population so that all variations set up by hydrographic irregularities, tidal fluctuations and patchiness in the algal population could be integrated. Such an automatic pump sampler was constructed and put into work in 1969 at St. E and in 1970 in Borgenfjord (for description of the sampler, see Jensen and Sakshaug 1970). The sampler was preset to obtain samples at intervals of one hour, and it worked continuously from March to November both years. In 1969, the intake of the sampler was attached near the mussel beds about 1 m below the low tide level. In 1970, the sampler was operated very close to the mussel population (*Mytilus edulis* L. and *Modiolus modiolus* (L)) which was hanging from a floating buoy at 1 m depth.

From the analysis of phytoplankton counts using the sampler, it became obvious that the amount of phytoplankton available to the mussels undergoes very rapid changes. It was therefore decided to pool the 24 hourly samples obtained each day, and estimates of total phytoplankton present were made. The amounts of *G. tamarensis* recorded by this method are given in Fig. 3. They confirm the huge daily variations noticed earlier.

When comparing the amounts of *G. tamarensis* and the corresponding toxicities given in Fig. 2 - 3, it is seen that numbers of *G. tamarensis* usually regarded as negligible (< 1 000 cells/l) were sufficient to give measurable toxicity in mussels. On the basis of field data avaiable, it is not possible to elaborate on the relationship between toxin accumulation in mussels and *G. tamarensis* abundance, since this would require adequate knowledge of pumping rates and detoxification rates in the mussels.

Toxicity of G. tamarensis cultures.

Gonyaulax tamarensis was isolated from a net haul taken in Trondheimsfjord (St. 15) on May 8, 1970 and has been successfully cultured in our laboratory in an enriched sea water medium (modified F/2, Guillard and Ryther, 1962).

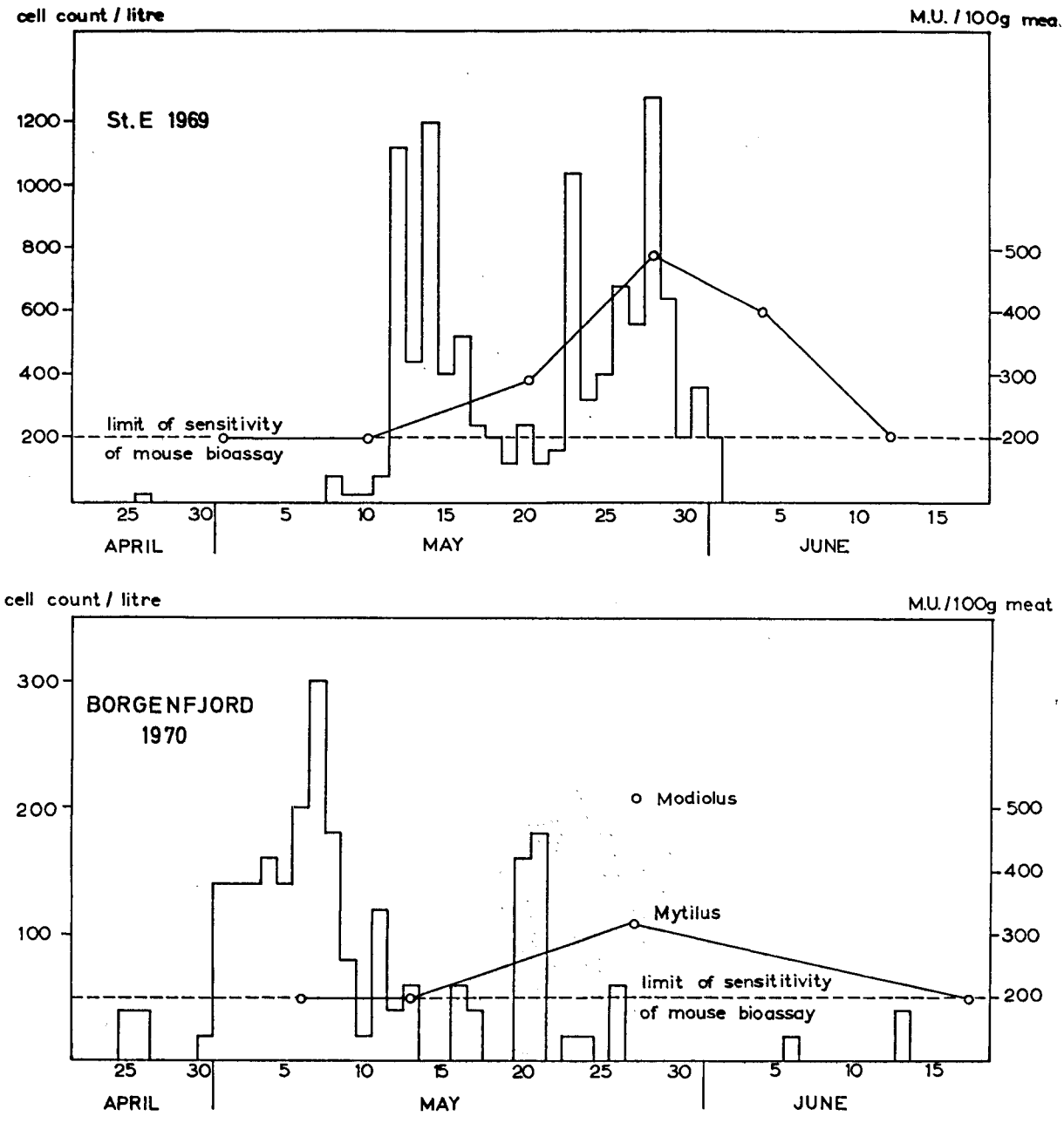

Fig. 3. Variations in *G. tamarensis* abundance (histogram) and mussel toxicity (curve) at St. E, 1969 and Borgenfjord, 1970 (*G. tamarensis* sampled by the automatic pump sampler, see text).

The establishment of this unialgal culture provided an opportunity to test it for toxicity. The toxic principle was extracted from filtered cells according to method

developed by Prakash (1963) and the bioassays on *G. tamarensis* extracts were carried out at Institutt for Næringsmiddelhygiene in Oslo. Results of these bioassays have revealed the toxic nature of *G. tamarensis* of Trondheimsfjord. Present estimates based on very few samples indicate that ca. 20 000 cells are required to yield one Mouse Unit of shellfish toxin. This yield seems lower than that estimated from field data on *Gonyaulax* abundance and mussel toxicity scores in Trondheimsfjord. Further experiments are in progress to establish precisely the cell density-toxin yield relationship.

In view of the association between mussel toxicity and abundance of *G. tamarensis* in the fjord discussed earlier and the results of toxin bioassays on culture extracts, there remains little doubt that *G. tamarensis* is the primary source of toxin in mussels in Trondheimsfjord.

Factors affecting the abundance of G. tamarensis.

G. tamarensis is a minor component of the phytoplankton in Trondheimsfjord. Even during its period of abundance it is outnumbered by the diatoms (Sakshaug 1971).

As mentioned earlier, the period of abundance of *G. tamarensis* in Trondheimsfjord is April - June. The growth conditions for phytoplankton are generally good during this period.

The most characteristic feature of this season is the enormous freshwater discharge into the fjord (over 5×10^9 m^3 in the fjord area of 1420 km^2 in the month of May). This of course affects the hydrography, chemistry and the biology of the upper layers profoundly.

River water collected during April - June 1971 contained 3-22,5 and 0,20-0,88 µgatoms of nitrate - N and phosphate - P respectively. These figures are comparable to the nutrient concentrations in the upper layers of the fjord before the early spring bloom starts. The large amounts of nutrients, particularly nitrate - N supplied this way are significiant, since nitrate - N has proved to be a limiting factor after the early spring bloom (Sakshaug 1971, unpubl.). The freshwater therefore contributes significantly to the nutrient supply of the fjord at

this time, since the supply through vertical mixing tends to be impeded because of the increased stability of the upper layers.

In late spring the surface salinities are lower than the rest of the year, mostly in the range of 16 - 29 ‰. Optimum salinity for the Bay of Fundy *G. tamarensis* was found to be about 20 ‰ (Prakash 1967), this is also true for our local strain (Sakshaug 1970, unpubl.). It is probable that the salinity conditions in late spring stimulate growth of *G. tamarensis* in the same manner as described by Prakash (1967) for the Bay of Fundy strain.

Another aspect of the freshwater discharge in Trondheimsfjord is the large amount of yellow-coloured humic matter carried to the sea by the runoff. In Trondheimsfjord the maximum input of humic matter occurs in late spring (Sieburth and Jensen 1968), and this yellowish brown water can be seen in the upper 2 m during most of May - June. Humic compounds are known to stimulate growth of *G. tamarensi* and other dinoflagellates (Prakash and Rashid 1968).

During May - June, the surface temperature increases rapidly and is generally over 10 C. Temperatures above 10 C have been found well suited for growth of *G. tamarensis* cultures (Sakshaug 1971, unpubl.).

In view of what has been said above, we believe that the growth conditions for *G. tamarensis* are most favourable in late spring, and this is the season when a maximum is to be expected in Trondheimsfjord.

After reaching its peak in May, the decline in *G. tamarensis* abundance is quite rapid in June. After May, the *G. tamarensis* population has a high incidence of large cells and resting spores, an indication of suboptimal growth conditions.

Another factor which may influence abundance of *G. tamarensis* in Trondheimsfjord is grazing by zooplankton. In two of the years, viz. 1963 and 1966, *G. tamarensis* showed no maximum at all. During May and June in these years huge stocks of *Calanus finmarchicus* were found in Trondheimsfjord. Horizontal as well as seasonal distribution of *Calanus* and phytoplankton in these years indicated that *Calanus* not only grazed diatoms down to a minimum, but

G. tamarensis as well (Sakshaug 1971).

In 1971, such large *Calanus* stocks appeared in May, and the *G. tamarensis* maximum was delayed until June. This is illustrated in Fig. 4 which also includes a "normal" cycle represented by the cycle of spring 1970. Bioassays on sea water from May 1971 showed good growth capacity for *G. tamarensis* without any nutrient enrichment. It seems therefore that the grazing by *Calanus* may be an important factor in limiting the abundance of *G. tamarensis* in some years.

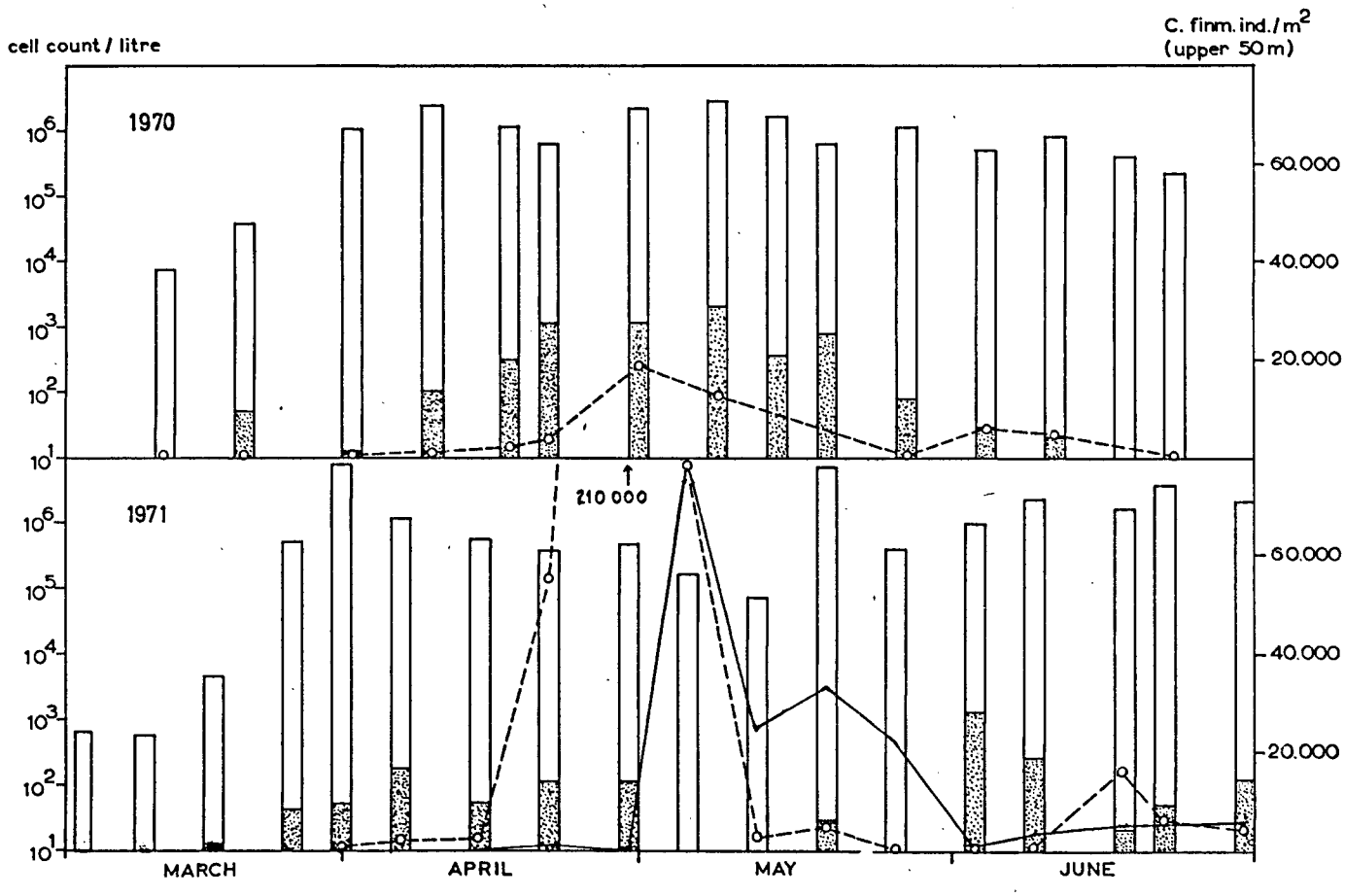

Fig. 4. Seasonal variations in total diatoms (open columns), *G. tamarensis* (filled columns), *Calanus finmarchicus* copepodite stages IV-V (fully drawn curve) and copepodite stages II-III (broken curve) at St. 15 in March - June, 1970 and 1971. Zooplankton data from Strømgren (unpubl.).

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