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Diversity of the dinoflagellate genus *Tripos* Bory 1823 (Dinophyceae) in the Western coastal waters of Tonkin Gulf, Vietnam

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ABSTRACT

Tripos is a genus of marine dinoflagellates in the family Ceratiaceae, the order Gonyaulacales (Dinophyceae). The genus *Tripos* has been studied in the western coastal waters of Tonkin Gulf, including 6 coastal sampling stations (Tra Co, Cua Luc, Do Son, Ba Lat, Sam Son, Cua Lo) and 2 offshore stations (Co To, Bach Long Vi) in four surveys conducted in March, June, August and November 2022. A total of 21 species belonging to the genus *Tripos* were initially identified in the study area. The number of species of this genus is lower in the estuarine area and higher in the offshore. The species occurred at all sampling stations, including *Tripos furca, T. fusus, T. muelleri, T. trichoceros* and *T. breve*, in which *T. furca* and *T. fusus* were the most common species. Species were common including *Tripos candelabrus, T. ehrenbergii, T. gravidus, T. minutus, T. extensus* and *T. longipes*. The species diversity in the dry season (March and November) was higher than in the rainy season (June and August). Cell density of the *Tripos* species varies spatially and temporally in the study area. The variation in *Tripos* density was related to the sampling stations, tide, sampling time, and water environment factors such as temperature, salinity, DO and pH.

Keywords: Tonkin Gulf, coastal waters, diversity, Ceratium, *Tripos*.

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INTRODUCTION

Tripos Bory 1823 was synonymized with the genus *Ceratium* Schrank 1793 in 1827. The *Ceratium* genus belonging to the family Ceratiaceae, the order Gonyaulacales, the class Dinophyceae (dinoflagellates), includes single-cell and planktonic algae that live in both seawater and freshwater, high and low latitudes, common at sea during the summer months. Most species of the genus *Ceratium* are marine species, but the type species is freshwater. Therefore, Gómez et al., (2010) split the genus *Ceratium* into two separate genera, in which marine species are classified into the new genus *Neoceratium*, while the genus *Ceratium* includes only freshwater species [1]. However, the name genus *Neoceratium* has caused much controversy and is not legal because the type species *Neoceratium furca* has the same basionym as the species *Biceratium furca* Vanhöffen. Hence, the genus *Tripos* was previously used to replace *Neoceratium,* and the new combination names were proposed by Gómez (2013) [2]. The genus *Tripos* includes four subgenera: *Archaeceratium*, *Amphiceratium, Biceratium*, and *Tripos* [3]. The species of genus *Tripos* do not produce toxins. However their blooms can cause water oxygen depletion, affecting aquatic animal life, aquaculture, and coastal ecosystems.

Hoang Quoc Truong (1963) identified 39 species and 6 forms of the genus *Tripos* (*Ceratium*), in Nha Trang Bay, Vietnam [4]. Shirota (1966) found 30 species of this genus in water bodies in South Vietnam (from Hue to Kien Giang) [5]. From investigations across the coastal waters of Vietnam, Nguyen-Ngoc et al., (2012) recorded 52 species of *Ceratium* genus [6]. During the survey of boat M. V. SEAFDEC in 58 stations (of which 7 belonging to Tonkin Gulf) in the Vietnamese waters from April to May 1999, a total of 73 species of Ceratium have been recorded in the Vietnamese waters [7]. A total of 25 species of this genus were recorded in the western waters of Tonkin Gulf [8]. In the narrower spaces such as the marine area of Cat Ba National Park and Ha Long Bay, 24 species of the genus were found [9], or 11 species found in the Ha Long - Ba Tu Long area [10]. In the offshore waters of Nam Yet Island (Spratly Islands), 25 species of the genus have been recorded [11]. Regarding the ecological characteristics of the genus *Tripos* in marine waters, Huynh Thi Ngoc Duyen et al., (2022) published the study results on the influence of environmental factors on two species *Tripos furca* and *T. fusus* in the South-Central coast of Vietnam [12]. The study on the *Tripos* genus in the northern coastal areas of Vietnam is still quite limited, especially in offshore areas such as the Co To and Bach Long Vi Islands and the areas affected by river water flows seasonally as the Red or Ka Long River mouths.

MATERIALS AND METHODS

Study area

Phytoplankton samples were collected at 8 stations in the western coastal waters of Tonkin Gulf, in which there were six coastal stations, including Tra Co, Cua Luc, Do Son, Ba Lat, Sam Son, Cua Lo, and 2 offshore stations, Co To and Bach Long Vi (detail location and coordinates of sampling stations in Table 1 and Fig. 1). The study was carried out in 4 surveys in March, June, August and November 2022.

Sampling and sample analysis

The comparative morphological method has been used to classify and identify species of the genus *Tripos* based on the following documents: Graham & Bronikovsky (1944) [13], Hoang Quoc Truong (1963) [4], Dowidar (1983) [14], Tomas (1997) [15], Dodge & Marshall (2004) [16], McDermott & Raine (2006) [17], Omura et al., (2012) [18], Nguyen-Ngoc et al., (2012) [6].

In 2022, 63 qualitative samples (one sample was not collected during low tide in August at Co To station due to bad weather) and 128 quantitative samples were collected in 4 surveys.

Table 1. Coordinates of sampling stations in the study area

No.	Name of station	Latitude	Longitude
1	Tra Co (Quang Ninh Province)	21°25′50″N	108°01′58″E
2	Cua Luc (Quang Ninh Province)	20°57′00″N	107°03′30″E
3	Do Son (Hai Phong Province)	20°43′00″N	106°50′00″E
4	Ba Lat (Thai Binh Province)	20°15′00″N	106°36′00″E
5	Sam Son (Thanh Hoa Province)	19°43′42″N	103°53′57″E
6	Cua Lo (Nghe An Province)	18°49′36″N	105°43′00″E
7	Co To (Quang Ninh Province)	20°58′21,3″N	107°43′0,9″E
8	Bach Long Vi (Hai Phong Province)	20°08′22,9″N	107°42′1,1″E

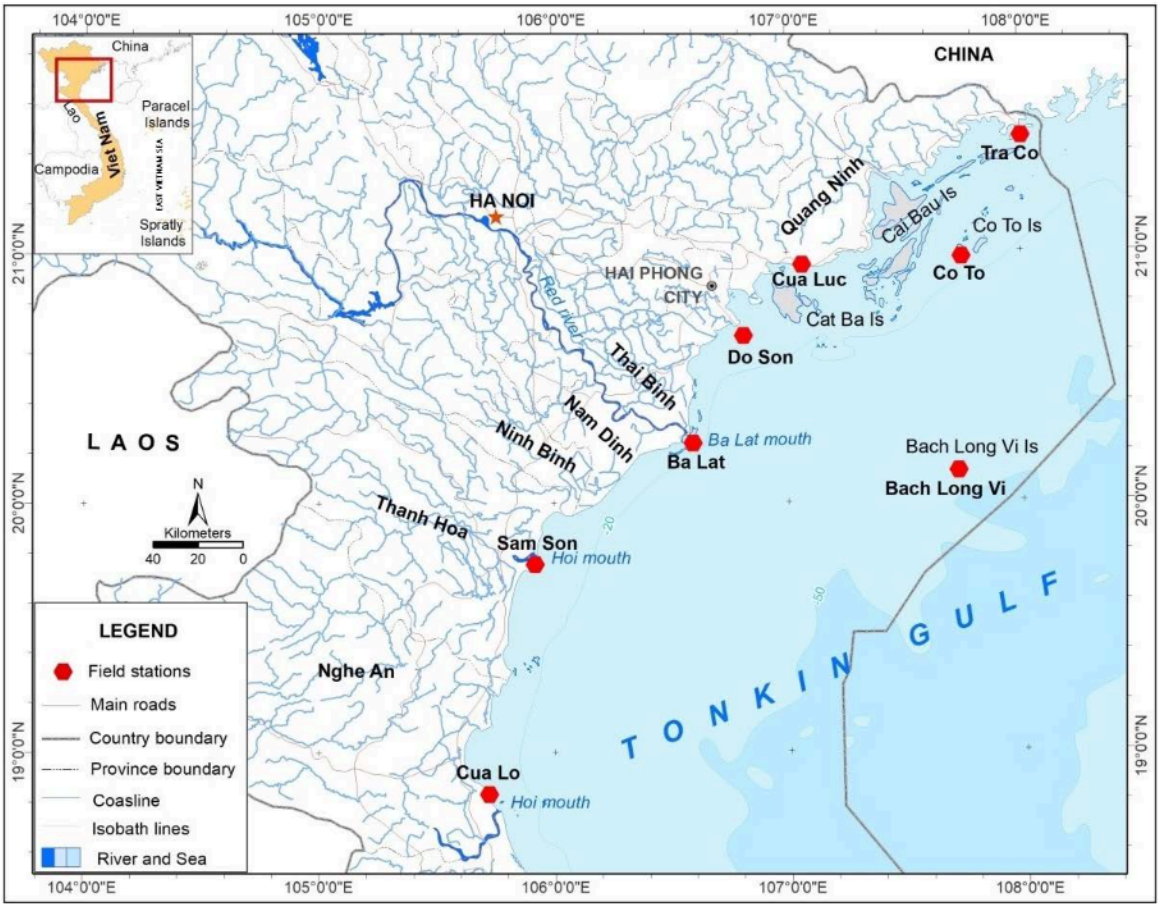

Figure 1. The location of sampling stations

The sampling of phytoplankton samples in the field followed protocols of the Institute of Marine Environment and Resources (2014) [19]. Qualitative samples were collected using a cone-shaped phytoplankton net with a mesh size of 20 µm, pulled vertically from near the bottom to the surface several times. Two qualitative samples at high tide and low tide were collected at each station. Quantitative samples were collected using a Niskin water sampler with a 1–2 liter volume. Four quantitative samples in the surface and bottom layers at high tide and low tide were collected at each station. The samples were fixed immediately with Lugol solution (approx. 4 mL Lugol/L or more, depending on the sample concentration).

Water environment parameters, including salinity, temperature, pH, and dissolved oxygen (DO) were measured *in situ* with portable measurement meters such as Atago S/Mill-E refractometer (Japan), Exrech pH meter (USA), and YSI-55 Pro DO meter (USA) at the same time of collecting phytoplankton samples.

Sample analysis: The qualitative samples were returned to the laboratory for sedimentation. Then, a pipette was used to take a small amount of sample in the vial, put it on a slide, and observed under an OLYMPUS BX51 microscope (100 - 1000x magnifications). Identifying species was based on morphological classification standards under an optical microscope. The quantitative samples were settled for at least 24 hours in the 1-liter cylinders, then siphoned the samples down to 10–20 mL. Sample vials were gently shaken, and then a pipette was used to take 1 mL of sample and put it into the Sedgewick-Rafter counting chamber (volume 1 mL), waiting about 15 minutes for sedimentation. The cell number of each species was counted under a LEICA DMIL inverted microscope (40 - 400x magnifications). The sample analysis was performed at the Institute of Marine Environment and Resources, Hai Phong.

Canonical Correspondence Analysis - CCA (in Excel using the XLSTAT statistical software) and ANOVA test (in Excel) have been used to evaluate the possibility of relationship between the density of *Tripos* genus and water environmental parameters such as salinity, temperature, pH, and DO, and other factors including sampling area, water layer, time, and tide. Tables and graphs were produced using Excel.

RESULTS AND DISCUSSIONS

Species composition of genus *Tripos*

Results of analysis of phytoplankton samples collected at six coastal stations (Tra Co, Cua Luc, Do Son, Ba Lat, Sam Son and Cua Lo) and 2 offshore stations (Co To and Bach Long Vi) in the western coastal area of the Tonkin Gulf in 2022 showed that a total of 21 species belonging to 4 subgenera of the genus *Tripos* have initially been identified (Table 2). Comparing with the central and southern coastal areas of Vietnam [6] and other studies [7, 20], it indicated that the species composition of genus *Tripos* in the western coastal area of the Tonkin Gulf was less diverse than that in the central Vietnam but more diverse than the southern Vietnam. In the same Tonkin Gulf area, the number of *Tripos* species recorded in this study was less than in the previous studies with 25 species found [7, 8]. The reason may be that the number of samples collected in this study was smaller (63). In comparison, the previous studies collected more samples collected, with 401 samples [8] and 500 samples [6], respectively.

A total of 54 species of the genus Tripos (Ceratium is used in the previous studies) have been found in Tonkin Gulf based on the synthesis of the studies of Canh and Hao (2001) [7] and Nguyen-Ngoc et al., (2012)[6] and this study, in which the number of species recorded in Tonkin Gulf are 33, 24 and 21 species, respectively. A few synonyms are used, such as *Ceratium macroceros* and *C. contrarium* [6] will be changed and combined to *Tripos macroceros; Ceratium breve* and *C. breve* var. *parallelum* [7] changed to *Tripos brevis*; *Ceratium extensum* and *C. strictum* [7] changed to *Tripos extensus*; *Ceratium fusus* and *C. fusus* var. *seta* [7] changed to *Tripos fusus*; *Ceratium pennatum* forma *propria and C. pennatum* var. *scapiforme* [7] changed to *Tripos longirostrum* and two freshwater species *Ceratium hirundinella* and *Ceratium lunula* forma *megaceros* [7] will belong to Ceratium genus. Notably, this study added two species (*Tripos ehrenbergii* and *T. gravidus*) to the northern coastal area of Vietnam. However, the species *T. gravidus* was recorded in the central coastal waters [6] but not in the northern coastal area of Vietnam.

Table 2. Species composition of genus *Tripos* in the western coastal area of Tonkin Gulf (followed Guiry and Guiry, 2024) [21]

No.	Scientific name	Notes
	I. Subgenus *Biceratium*	
1	*Tripos setaceus* (Jørgesen) Gómez 2013	Common
2	*Triopos furca* (Ehrenberg) Gómez 2013	Very common
3	*Tripos* sp.1	Rather common
4	*Tripos ehrenbergii* (Kofoid) Gómez 2013	Rare
5	*Tripos minutus* (Jørgensen) Gómez 2013	Rare
6	*Tripos candelabrum* (Ehrenberg) Gómez, 2013	Rare
	II. Subgenus *Archaeceratium*	
7	*Tripos gravidus* (Gourret) Gómez 2013	Rare
	III. Subgenus *Amphiceratium*	
8	*Tripos extensus* (Gourret) Gómez 2013	Rare
9	*Tripos eugrammus* (Ehrenberg) Gómez 2021	Rather common
10	*Tripos inflatus* (Kofoid) Gómez 2013	Common
11	*Tripos longirostrum* (Gourret) Hallegraeff & Huisman 2020	Common
12	*Tripos falcatus* (Kofoid) Gómez 2013	Rather common
13	*Tripos fusus* (Ehrenberg) Gómez 2013	Very common
	IV. Subgenus *Tripos*	
14	*Tripos muelerri* Bory 1826	Very common
15	*Tripos brevis* (Ostenfeld & Schmidt) Gómez 2013	Very common
16	*Tripos trichoceros* (Ehrenberg) Gómez 2013	Very common
17	*Tripos longipes (Bailey)* Gómez 2021	Rare
18	*Tripos vultur* (Cleve) Hallegraeff & Huisman 2020	Rather common
19	*Tripos macroceros* (Ehrenberg) Hallegraeff & Huisman 2020	Rare
20	*Tripos gallicus* (Kofoid) Gómez 2021	Rather common
21	*Tripos massiliensis* (Gourret) Gómez 2013	Common

Notes: Rare: present in < 5 samples; Rather common: present in ≥ 5–10 samples; Common: present in ≥ 10–20 samples; Very common: present in ≥ 20 samples.

The distribution characteristics of the genus *Tripos* in the study area

Species distribution

Spatial distribution: The encounter frequency of genus *Tripos* on the western coast of Tonkin Gulf is presented in Table 3 and Fig. 2. It can be seen that five common species presented at all sampling stations in the study area included *Tripos furca, T. fusus, T. muelleri, T. trichoceros*, and *T. breve*, in which *T. furca* and *T. fusus* were the two most common species, found in over 70% of samples with 52/63 and 48/63 samples, respectively. Common species in the area were *Tripos candelabrus, T. ehrenbergii, T. gravidus, T. minutus, T. extensus*, and *T. longipes*. Among eight survey stations, Ba Lat station had the lowest number of *Tripos* species, with 6 species found in 21 species identified (1.75 species/sample on average).

The species composition of genus *Tripos* tended to increase in areas far from large estuaries, *i.e.*, the farther away from shore, the more abundant the species of genus *Tripos*. Accordingly, the lowest number of species was found at Ba Lat station, gradually increasing from the stations of Cua Lo, Sam Son, Do Son, Tra Co, and Cua Luc to the Co To station where the highest species diversity was found with 18 species (7.9 species/sample on average). In the offshore area (Bach Long Vi station), the diversity of the genus *Tripos* decreased, but here, the species that were not common in the nearshore area were found, such as *Tripos gravidus* and *T. setaceus*. Two groups of species are temporarily divided based on the distribution of species in the study area as follows:

Common species groups in nearshore areas included *Tripos furca*, *T. fusus*, *T. muelleri*, *T. trichoceros* and *T. breve*.

Common species groups in offshore areas included *Tripos setaceus*, *T. gravidus*, *T. inflatus*, *T. falcatus*, *T. longirostrum*, *T.* sp.1, *T. massiliense*, *T. eugrammus*, *T. macroceros*, *T. gallicus*, *T. vultur*, *T. minutus*, *T. extensus*, and *T. longipes*.

Table 3. Encounter frequency of the species of genus *Tripos* by sampling sites

No.	Species	Tra Co	Cua Luc	Do Son	Ba Lat	Sam Son	Cua Lo	Co To	Bach Long Vi
1	*Tripos furca*	8	8	8	5	7	6	7	3
2	*Tripos fusus*	8	7	6	2	5	5	8	6
3	*Tripos muelleri*	6	4	5	2	4	1	5	7
4	*Tripos trichoceros*	8	8	7	3	7	5	8	4
5	*Tripos breve*	5	3	4	1	2	2	5	2
6	*Tripos ehrenbergii*	-	1	-	-	-	-	-	-
7	*Tripos candelabrus*	-	-	-	-	-	1	-	-
8	*Tripos setaceus*	5	3	3	-	2	-	6	8
9	*Tripos gravidus*	-	-	-	-	-	-	-	1
10	*Tripos inflatus*	1	2	3	-	2	1	5	-
11	*Tripos falcatus*	-	-	1	-	1	-	3	1
12	*Tripos longirostrum*	2	3	2	1	2	-	3	1
13	*Tripos* sp.1	1	2	1	-	-	-	1	-
14	*Tripos massiliense*	1	1	3	-	1	1	2	4
15	*Tripos eugrammus*	-	-	-	-	-	-	2	2
16	*Tripos macroceros*	-	-	-	-	-	1	1	1
17	*Tripos gallicus*	1	-	1	-	-	-	2	1
18	*Tripos vultur*	1	-	-	-	1	-	2	-
19	*Tripos minutus*	-	-	-	-	-	-	1	-
20	*Tripos extensus*	-	-	-	-	-	-	1	-
21	*Tripos longipes*	-	-	-	-	-	-	1	-
	Total of species	12	11	12	6	11	9	18	13

Temporal distribution: Results of analysis of qualitative samples collected in March (dry season), June, August (rainy season), and November (dry season) are presented in Table 4.

The data in Table 4 indicated that the average number of species tended to increase from June to November, in which the highest number of species was found in November, with 18 species in a total of 21 species recorded (12 species/sample on average). In the remaining sampling time (March, June and August) the number of species recorded 11 species (7.9 species/sample). Six species presented at all sampling times in the year, including *Tripos furca, T. fusus, T. muelleri, T. trichoceros, T. falcatus*, and *T. setaceus*. The presence of species varied depending on the sampling time, in which species appeared most frequently in March, including *Tripos furca, T. fusus, T. muelleri, T. longirostrum, T. brevis, T. ehrenbergii, T. candelabrus*, and *T. gravidus*; in June (*Tripos* sp.1); in August (*Tripos setaceus*) and in November (*Tripos vultur, T. setaceus, T. eugrammus, T. macroceros, T. massiliense, T. gallicus, T. minutus, T. extensus, T. longipes, T. inflatus* and *T. falcatus*). In general, the species composition in dry season (March and November) was more diversely than those in rainy season (June & August) in the study area.

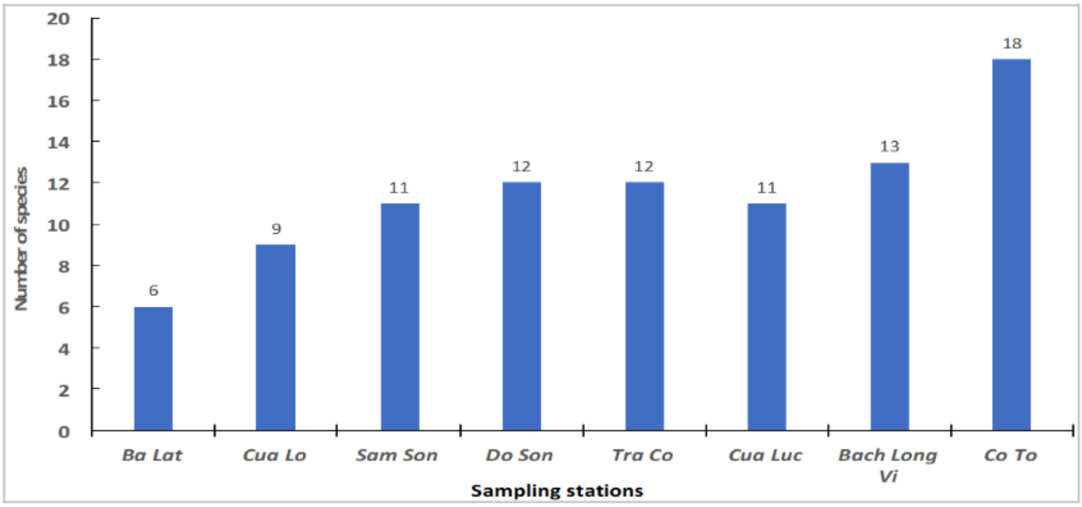

Figure 2. The number of *Tripos* species recorded at the sampling stations

Table 4. Encounter frequency of the species of genus *Tripos* by time in 2022
(The data is the corresponding species was detected in a total of 16 samples collected in each survey)

No.	Species	March	June	August	November
1	*Tripos furca*	15	11	13	14
2	*Tripos fusus*	15	10	13	10
3	*Tripos muelleri*	13	9	1	10
4	*Tripos trichoceros*	13	10	13	12
5	*Tripos breve*	12	5	-	6
6	*Tripos ehrenbergii*	1	-	-	-
7	*Tripos candelabrus*	1	-	-	-
8	*Tripos setaceus*	5	6	8	8
9	*Tripos gravidus*	1	-	-	-
10	*Tripos inflatus*	-	4	4	6
11	*Tripos falcatus*	1	1	2	2
12	*Tripos longirostrum*	8		3	3
13	*Tripos* sp.1	-	3	1	1
14	*Tripos massiliense*	-	3	2	8
15	*Tripos eugrammus*	-	-	1	3
16	*Tripos macroceros*	-	1	-	2
17	*Tripos gallicus*	-	-	-	5
18	*Tripos vultur*	-	-	-	4
19	*Tripos minutus*	-	-	-	1
20	*Tripos extensus*	-	-	-	1
21	*Tripos longipes*	-	-	-	1
	Total of species number	11	11	11	18
	Average/Sample	10.88	7.88	8.57	12.13

Variation in cell density of genus Tripos

Spatial variation: Average cell density of six *Tripos* common species (*Tripos brevis, T. furca, T. fusus, T. macroceros, T. trichoceros*, and *T. muelleri*) at sampling stations in the Western coastal waters of Tonkin Gulf is shown in Figure 3.

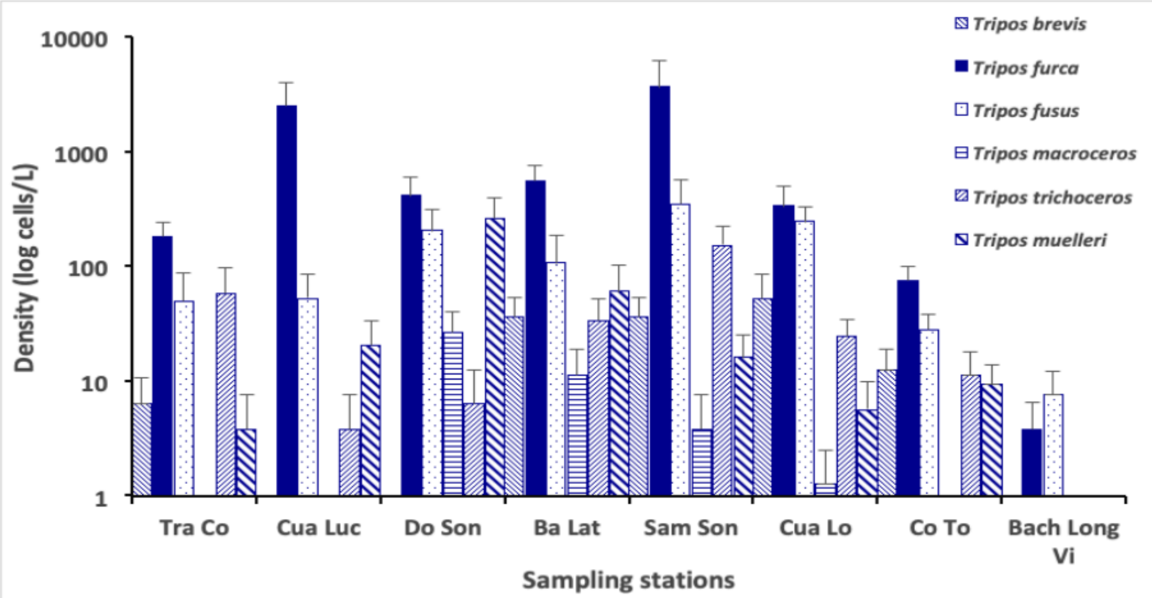

Figure 3. Density of some species of *Tripos* at the sampling stations

It can be seen that Sam Son station had the highest cell density, averaging 705 cells per 1 liter of water (cells/L), followed by Cua Luc station with an average density of 426 cell/L, Bach Long Vi station had the lowest density in the area with an average of only 2 cells/L. Among the six popular species, *Tripos furca* was the most abundant species in terms of overall density of this genus at all sampling stations, followed by *T. fusus*. The remaining species often have lower densities significantly.

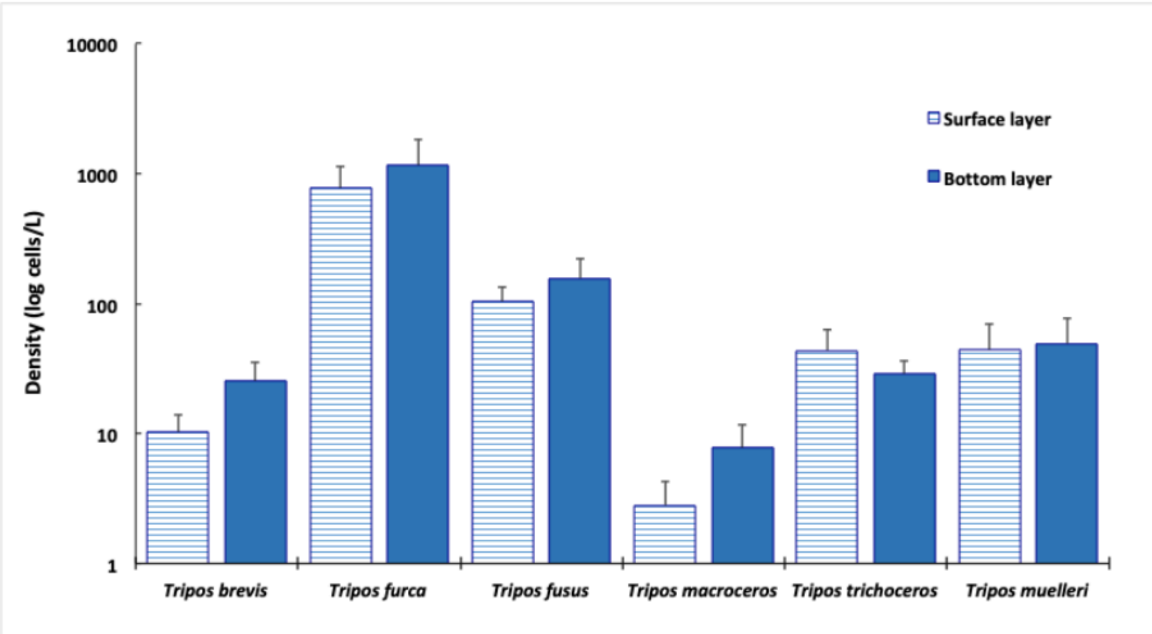

Figure 4. Average density of common species of *Tripos* genus by water layers

Figure 4 shows the variation in the cell density of six common species of genus *Tripos* by the water layer. The average density in the bottom layers was higher than that in the surface layers in the study area, with 233 and 163 cells/L, respectively.

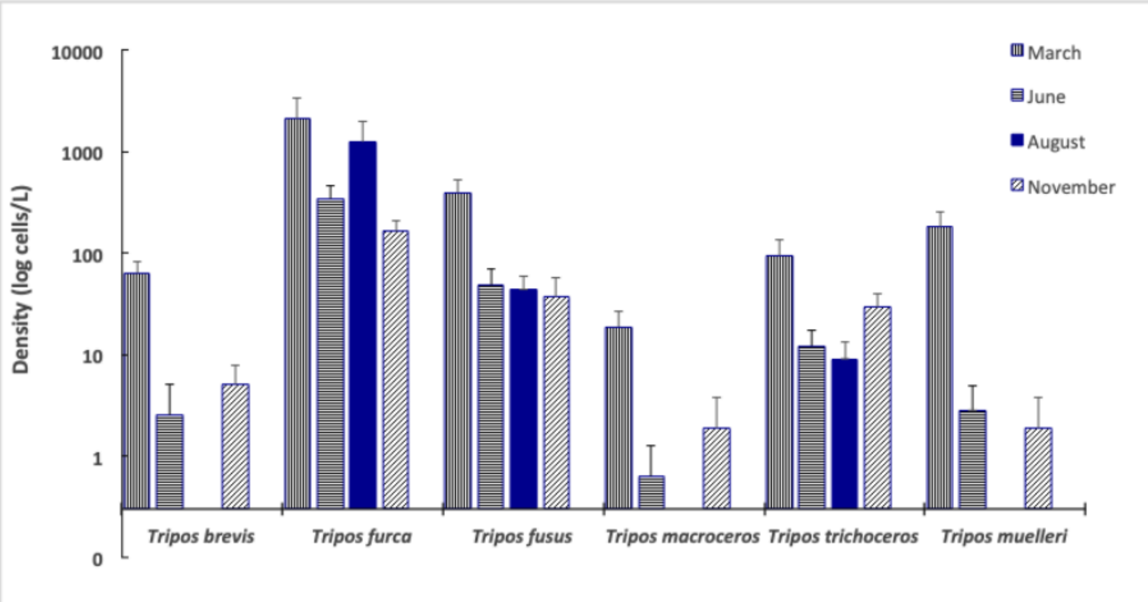

Figure 5. Cell density of *Tripos* genus by sampling time

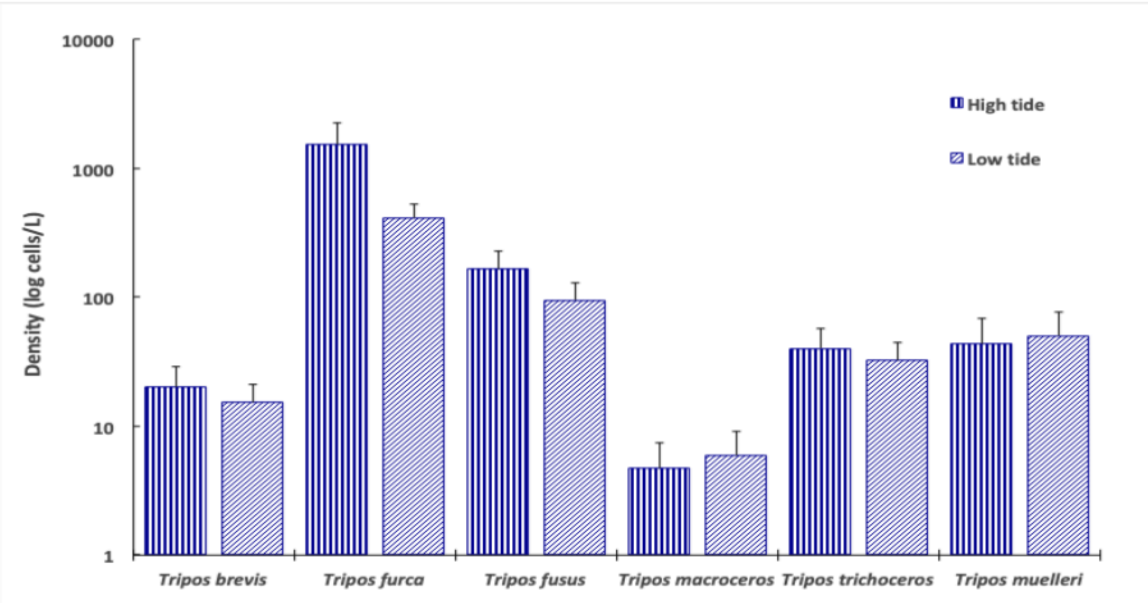

Figure 6. Average cell density of *Tripos* genus by tide

Temporal variation: The cell density of six species of the *Tripos* genus by sampling time is shown in Figure 5. It showed that the highest density was found in March, with an average of 477 cells/L, followed by August with an average density of 216 cells/L and the significant lower densities were found in June and November, with average densities of 68 and 40 cells/L, respectively.

Figure 6 shows the variations in the cell density of the *Tripos* genus by the tide. The density of *Tripos* genus at high tide was higher than that at low tide with average densities of 298 cells/L and 102 cells/L, respectively.

The results of the ANOVA test were presented in Table 5. It indicated that the variations in cell density of genus *Tripos* in the study area were not random and depended on the interaction between species and sampling location (repeatability of 16), sampling time (repeatability of 16) and tide (repeatability of 64), but not depend on sampling layers (repeatability of 64). However, the average algal density of the entire area was higher in the bottom layer than in the surface layer.

Table 5. The result of ANOVA test about density of cell and related factors

Factors	f_A	$f_{A\_standard}$	f_B	$f_{B\_standard}$	f_{AB}	$f_{AB\_standard}$
Sampling station	7.24	2.22	2.21	2.21	1.53	1.41
Water layer	5.98	2.22	2.03	3.0	1.68	1.83
Sampling time	6.1	2.22	2.96	2.38	1.61	1.58
Tide	6.1	2.22	3.65	3.01	3.1	1.83

Notes: f is the ratio of variation between the sample mean squares (mean square between group) and the variation within the mean squares within the samples); $f_{\_standard}$ is the given value of f at the ∝ probability threshold of erroneously rejecting a negative hypothesis (H_0); f_A represents the ability to reject or accept the hypothesis $H_0(A)$ - algal density does not depend on different algal species; f_B represents the ability to reject or accept the hypothesis $H_0(B)$ - algal density does not depend on the related factors; f_{AB} represents the ability to reject or accept the hypothesis $H_0(AB)$ - algal density does not depend on species interaction and related factors.

The correlation of algae density with some water environment factors

Canonical Correspondence Analysis (CCA) is used to evaluate the possible relationship between the water environment factors such as temperature, salinity, pH, and dissolved oxygen (DO) with the density of ten *Tripos* species, which had the most sufficient quantitative data in the study area (Fig. 7). The environmental factors were linear lines going in different directions. However, the species' interaction with temperature and salinity was the same so two lines were almost overlapping. Temperature, salinity, and pH had a strong correlation with *Tripos furca*. A recent study reported that salinity was one of two factors impacted strongly to the abundance of *Tripos furca* and *T. fusus* in the south-central coastal waters of Vietnam [11]. We can see that most species had the same trends in environmental conditions, but the suitable conditions of different species were not the same. Therefore, the samples with high density are often due to the presence of suitable conditions for certain species, leading to the development of their density.

Surface water often has high oxygen concentrations, but the average density of the *Tripos* genus at the bottom layer was higher than at the surface one. During ebb tide, much water flows from the river to the sea, and a decrease of DO and salinity in the surface layer occurs, leading to decrease the cell density in the surface layer. Most sampling stations were located in the northern coastal area close to river mouths, so overall, the density in the surface water layer was lower than that in the bottom layer. However, at the stations far from river mouths, such as Co To and Bach Long Vi, where the effect of river flows was weak, and salinity and DO factors were relatively stable, so the density in the surface water layer was higher than that in the bottom one (Fig. 8).

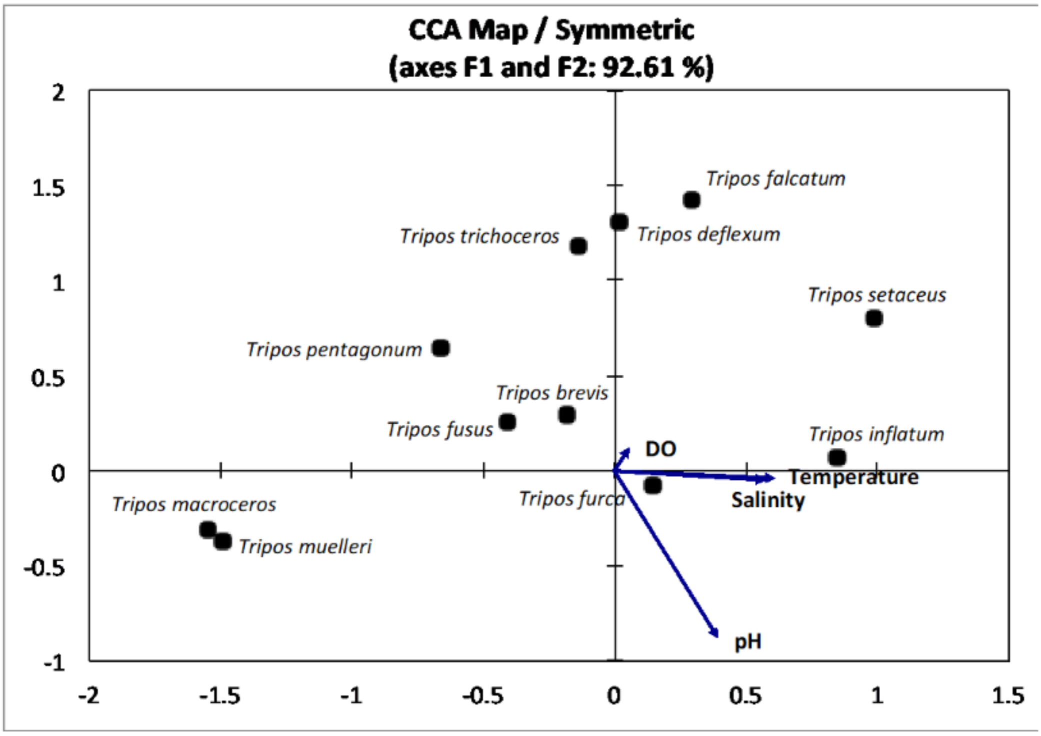

Figure 7. CCA between cell density of *Tripos* species and environmental factors

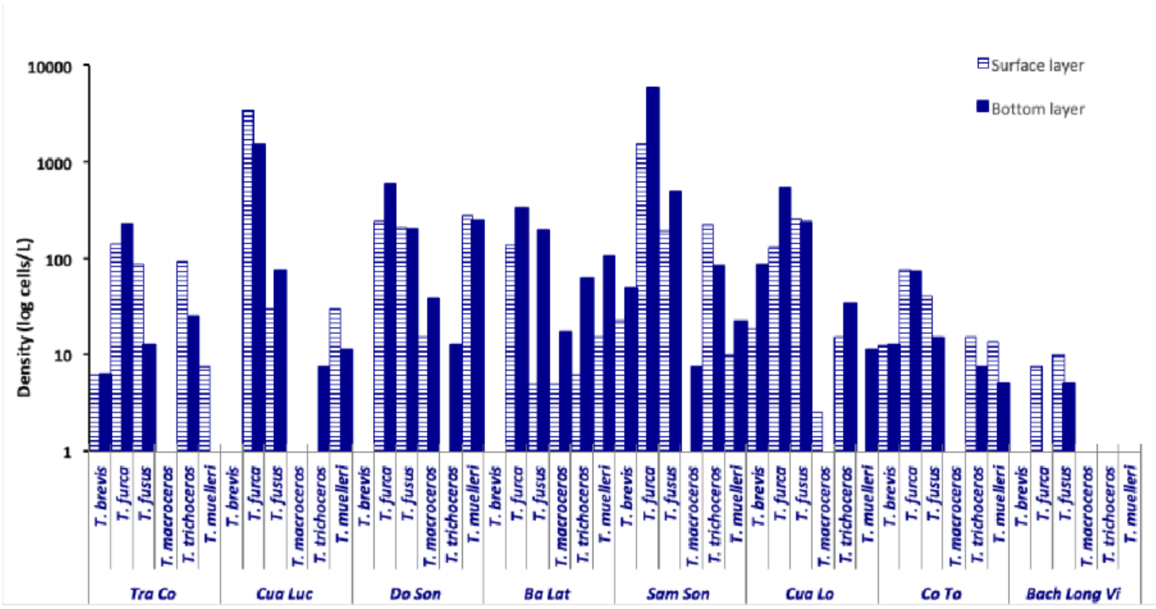

Figure 8. Cell density of common *Tripos* species by sampling stations and water layers

CONCLUSIONS

A total of 21 species belonging to four subgenera of genus *Tripos* was initially identified in the western coastal area of Tonkin Gulf. The number of species of the genus tended to increase from estuarine area to offshore ones. Species presented at all sampling stations in the study area were *Tripos furca, T. fusus, T. muelleri, T. trichoceros,* and *T. breve*, in which *T. furca* and *T. fusus* were the two most common species. Uncommon species were *Tripos candelabrus, T. ehrenbergii, T. gravidus, T. minutus, T. extensus*, and *T. longipes*. The species composition of the *Tripos* genus in the dry season (March and November) was more diverse than in the rainy season (June and August). Six species found at all sampling times during the year: *Tripos furca, T. fusus, T. muelleri, T. trichoceros, T. falcatus*, and *T. setaceus.*

The cell density of the *Tripos* genus varied spatially (by sampling stations, tides, water layer) and temporally (months) in the study area. The variation in *Tripos* cell density by sampling site, tide and time was not random (ANOVA test) and was affected by environmental factors such as temperature, salinity, DO and pH (CCA test). That explained the fluctuations in algae density when environmental factors changed over space and time.

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