Effect of Location Transfer on the Purification of the Carpet Shell Clam, *Ruditapes decussatus* in Southern Tunisia

Leila Hmida *[1,2] , Fedia Nasri [2] , Driss Ben Naila[4], Sana Lengliz [4,5] and Mohamed Salah Romdhane [2,3]

1 Institut Supérieur de Biotechnologie Monastir. 5000 Monastir/ Tunisie
Tel:+216 99 98 14 21- Fax: +216 73 90 79 27. E-mail: hmidaleila@yahoo.fr
2 Institut National Agronomique de Tunisie. UR03AGRO1 Écosystèmes & Ressources Aquatiques. 43 av. Charles Nicolle 1082 Tunis .Tel : +216 71 28 71 10 - Fax : +216 71 79 93 91
E-mail: n.fedia@hotmail.com , E-mail: ramadhanms@gmail.com
3 Institut de Recherche Vétérinaire de Tunis. R Djebel El Akhdhar Errabta 1006, Tunis Tunisie.
E-mail: bennailaidriss@yahoo.fr , E-mail: lengliz_sana@yahoo.fr
(*) corresponding author e mail: hmidaleila@yahoo.fr

1 ABSTRACT

The aim of the present study was to evaluate possible purification of the carpet shell clam *Ruditapes decussatus* of toxic dinoflagellates, by the transfer from areas of high toxic algae concentrations to coastal areas with low toxic algae concentrations. The experiment concerns contaminated clams from Boughrara lagoon (high contamination) transplanted to the estuarine area of Oued Maltine (low contamination) in southern Tunisia. A positive relationship was observed between abiotic (physico-chemical), trophic (chlorophyll a and phytoplanctonic biomass) and physiological parameters (condition index and mortality rate). Condition Index showed Boughrara clams transported to Oued Maltine acclimatized well. Mortality rate very low from November 2011 until April 2012, but increased to 85% at the end of June 2012, when temperatures were highest. Toxicity test by qualitative method showed that transplanted clams were not toxic to mice five months after transplantation to Oued Maltine.
Conclusion was??

Keywords: Clam, *Ruditapes decussatus,* Purification, Phytoplankton, Condition Index. Southern Tunisia.

2 INTRODUCTION

~~During the previous decade,~~ Tunisia has shown a renewed interest in aquaculture in general and shellfish in particular by developing a plan for the development and management of this important economy throughout the national seashore. The target species under this plan is the carpet shell, *Ruditapes decussatus*, commonly called "clam," which occupies a place of importance in the shellfish industry in Tunisia (Hmida ~~et al.,~~ *et al.,* 2004).

In Tunisia, the species *R. decussatus* occurs along almost the entire coast (Lubet, 1976. Medhioub, 1983), and is especially abundant in the Gulf of Gabes. Indeed, it is an important economic product, both in terms of employment and at the level of the trade balance: 8,000 fishers are directly tied to the *R. decussatus* fishery and 699 ~~tonestonnes~~tonnes/years are exported to Europe (GIPP, 2012). However, the indiscriminate and unregulated collection outside the opening fishing periods affects the availability of this resource due to overexploitation and contributes to the depletion of the stock. (Ibn Chbili 2010; Ibn Chbili ~~et al.,~~ *et al.,* 2011).

Toxic dinoflagellates also affect~~s~~ the availability of this resource, it is a major problem in shellfish. Diarrheic (DSP) and Paralytic (PSP) shellfish poisons is usually a consequence of eating toxic bivalves that have ingested, by filter feeding, a large quantities of toxic dinoflagellates in ~~plankto~~plankton. To overcome this problem, The National Phytoplankton and Phycotoxins monitoring network for Tunisia coastal waters was created in 1998 "REPHY" to observe the development of phytoplankton species along Tunisia coasts, monitor exceptional perturbations like "colored water" or "blooms" and ensure the protection of both public health and livestock health by detecting toxic phytoplankton species. Water samples and analyses are run at least once every two weeks. When toxic blooms occur, samples are taken once a week, the number of sampling points is increased and shellfish samples are taken to measure the toxicity. The results are sent to Authorities ~~which~~that can decide to prohibit the marketing and harvesting of shellfish.

Two approaches have been proposed to promote sustainable harvest of this species: the first is the preservation of this natural resource through the establishment of appropriate management of fishing activities and collection systems for this species, and the second is the repopulation of the stock from ~~hatchery produced~~hatchery-produced spat. The latter requires optimal development of hatchery production techniques and control of livestock and agricultural runoff from land to minimize toxic algae blooms (Loosanoff and Davis, 1963. Le Pennec, 1987).

The objective of the present study is to evaluate methods for purification of the carpet shell clam, *R. decussatus* against toxic dinoflagellates by the transfer from risky "unsafe" to healthy coastal areas.

3 MATERIALS AND METHODS

The ~~experiment monitored~~experiment-monitored clams *R.decussatus* collected in the contaminated Boughrara lagoon (southern Tunisia) before and after they were transplanted the estuarine "healthy" area of Oued Maltine (southern Tunisia). The methods used to determine contaminated and health areas is "REPHY"monitoring network. Physicochemical (Water temperature, salinity and pH), physiological parameters (Condition Index, mortality rate) and presence of phytoplanctonic species (*Alexandrium sp, Dinophysis sp, Gymnodinium sp, Prorocentrum sp, and Peridinium sp*) were measured every two weeks in water for a period of 6 months. Relative toxicity of the clams ~~were~~was measured during the ~~autum~~autumn and early summer in 2011 and 2012. Three campaigns of relaying clams outcome from Boughrara lagoon. Two reference campaigns from Oued Maltine are performed in parallel throughout the experiment to monitor the possibility of contamination. Meanwhile, identification of phytoplankton is carried out in Oued Maltine and Boughrara site. Oued Maltine has been also monitored physical, chemical and tropic parameters.

Clams were transported from Boughrara Lagoon (contaminated) to Oued Maltine (healthy) on three different occasions. Water quality parameters of Oued Maltine were measured at two sites on a weekly basis as during the sampling period to test for contamination of the water. Water samples were kept for further phytoplankton identification and quantification during this period from both locations.

3.1 Parameters Studied

3.1.1 Physical and chemical parameters

Water temperature (C), salinity (ppt) and pH at Oued Maltine were measured with a multiparameter unit (WTW MULTI 3430 SET G). The determination of dissolved oxygen in ~~sea water~~seawater is carried out by the chemical method called also Winkler method was used to measure dissolved oxygen (mg/L) (Montgomery ~~et al.,~~ *et al.,* 1964). The nutrients nitrate, ammonium and phosphate were measured by testing kits (VISOCOLOR MN). Chlorophyll a was measured using fluometric method in order to quantitatively determine the total biomass of phytoplankton (mg chla/l). The identification of phytoplankton was made by the decanting method, phytoplankton samples should only be decanted after they have been allowed to settle for a minimum of 24 hours. (Paxinos and Mitchell, 2000).

3.1.2 Physiological parameters

The survival rate of transplanted clams was estimated bimonthly. Dead clams were removed from the cage and enumerated to estimate the percentage of mortality ((# dead clams/# live clams)*100)) per sampling period.

To measure the relative health of the clams, a condition index (CI) was calculated as:
CI=Dried weight of clam flesh/dry weight of clam shell
The dry weight of the flesh of clams (+/-0.001g) was obtained after drying in an oven for 48 hours at a temperature of 80 ° C. Shells were dried by precision scale The CI was measured for 30 individual on a random sampling basis.
A total of 4 kg of dried clam was used for analysis of toxicity in order to search for DSP (Diarrheal Toxicity) by qualitative method (mouse test) (Stabell ~~et al.,~~ *et al.,* 1991). DSP is a gastrointestinal illness without neurologic manifestations reported worldwide ~~It~~it is caused by the consumption of contaminated shellfish. More ~~then~~than 50% of dead mouse bioassay is considered toxic for human health. (Haystead ~~et al.,~~ *et al.,* 1989, .Aune and Yndstad 1993).

Results and Discussion

Physical and chemical parameters

The temperature at Oued Maltine declined from November until the beginning of February, then increased until through the end of the sampling period in April (Fig. 1). A minimum of 11 ° C was recorded during February and a maximum of 23.9 ° C during the month of May. Salinity values at Oued Maltine fluctuated monthly, with a minimum of 38 psu during the month of February and a maximum of 42.3 psu recorded during December (Fig. 1). The high levels of salinity may be due to the presence of the River close to this site. The pH of Oued Maltine ranged between 8 and 8, 5. The average value of dissolved throughout the experiment oxygen was 8.86 ± 0.91mg/l (Fig. 1). This value decreased from the month of May to the lowest level in June (6.67 mg/l). This is likely related to the seasonal increase in water temperature.

Nutrients

The nitrite concentration remained almost unchanged throughout the experiment and did not exceed 0.03 mg/l (Fig. 2). According to the European standard, the concentration of toxic nitrite is about 0.2 mg/l, which shows that the relaying environment is healthy. The ammonuim maximum level was 0.7mg/l recorded in November (Fig. 2). However, during the time of this experiment the level did not exceed 0.4mg/l and remained stable around 0.3mg/l during the spring period. The high level of ammonium observed at the beginning of sampling imentmay ~~be~~is due to ammonium ~~fertilizers.The~~fertilizers. The phosphate content in the site was between 0 and 0.25 mg/l (Fig. 2). During the winter period, the content of phosphate ranged from 0 to 0.2mg/l. During the spring season, the phosphate is stable at 0.1 mg/l. It is important to note that the level of 0.1 mg/l corresponds to the limit of normal concentration of phosphate in water according to the European standard.

Trophic parameter

Chlorophyll a

Chlorophyll a concentrations showed a maximum of 1.4 µg/l during the month of ~~January which corresponds to the maximum value of the dissolved oxygen content~~January, which corresponds to the maximum value of the dissolved oxygen content, is of the order of 10 mg/l (Fig. 3).

Phytoplankton results

Identification and quantitative estimation of the phytoplankton confirmed the presence of toxic phytoplankton ~~both in risky coastal areas (Bougrara lagoon) and healthy~~in both risky coastal areas (Bougrara lagoon) and healthy coastal areas (Oued Maltine) (Fig.4).
The species of phytoplankton suspected to be toxic are: *Alexandrium sp, Dinophysis sp, Gymnodinium sp, Prorocentrum sp, and Peridinium sp.*
Chaetoceros and *Tetraselmis* were present in the first season (November-December 2011) than the second (January-June 2012). This diversity of phytoplankton species demonstrates nutrient availability at the site of clam relocation. This promotes healthy growth of individuals by providing them the essential nutritional reserves, ensuring proper functioning of metabolism, such as respiration, filtration and

physiological essentially reproductive mechanisms. During the second season, there is a more pronounced in phytoplankton species belonging to the families of diatoms and Dinophyceae. In addition, ~~we note~~ the appearance of phytoplankton species indicator of pollution *Euglena sp*. was noted.

Physiological parameters

Survival rate

During the first period "November-December 2011", the survival rate was high 99% during the 60 days of the experiment. In addition, no signs of physiological weakness were observed. This demonstrates that during the winter period, clams were able to acclimate with the relaying site environment.

During the second period "January-June 2012", the survival rate was high and constant for the first three months, but the end of the campaign it fell from 60% to only 15% in June (Fig.5). Higher mortality observed during the last two months of the experiment may be due to:

- The increase in temperature and the decrease in dissolved oxygen in the water.
- The appearance of phytoplankton species such as *Euglena sp*, causing a degradation of water quality

Condition index

The Condition index for Boughrara samples and Oued Maltine showed a decrease followed by a slight stabilization, then an increase towards the end of the experiment, however, it is still lower than the initial values. The clams from Boughrara lagoon have a lower growth than native clams site. The clam batches B2 and B3 had similar IC at the end of the experiment. This may be because contaminated clams were well adapted to the medium, and their CI increased from April simultaneously with the increase in temperature (seasonal variation), this influencing the filtration and purification of the speed of the animal. (Fig.5)

Toxicity monitoring

We note that at the end of the second campaign "January-June 2012", contaminated clams were purified. During the two campaigns, there is a positive result: 2 to 3 mouse died after injection this is confirm the presence of biotoxin. The ~~result which deserves attention~~result, which deserves attention, is of April 2012 where the test is ~~negative~~negative, i.e. 3 surviving mouse this mean that biotoxin has disappeared and clams are purified. However, Maltine reference campaign, the test is negative until February, and then from the month of March it became positive. Results are conformed to those of the monitoring network.

Conclusion

This work has the objective of contributing to the exploitation of clams from areas at risk: the Boughrara lagoon. In fact, this study was conducted in Oued Maltine site (Mahes-Gulf of Gabes, south Tunisia).

The physical, chemical and trophic parameters contributed to the suitability of the site clams relaying. It is to retain an increased index condition after an adjustment period (2-4 weeks). For ~~mortality~~mortality, it did not exceed 1% in the first campaign, then, it increases substantially at the end of the second campaign. This result shows that relaying is not feasible during the summer, where the species has a physiological weakness. This condition can be further accentuated by possible bacterial or viral infections.

Identification of phytoplankton species and estimating their biomass, the results obtained during the first campaign can meet the dominance of Chlorophycea in the relaying site and the absence of toxic phytoplankton species, except the genus *Alexandrium*. It is important to note that Oued Maltine site has considerable phytoplankton diversity: Diatoms, Dinophyceae, Chlorophycea, with a dominance of the family Dinophyceae ~~which~~that some species may be toxic.

Purification of contaminated clams, a result of monitoring toxicological test deserves attention. During ~~April~~April, the mouse test is negative that mean that *Ruditapes decussatus* has been purified which confirm the possibility of purification clams in natural site. This requires an operation results in a natural environment in order to achieve a follow-up purification relaying, ~~and also~~and the realization of a health monitoring via a parasitic and viral diagnosis.

Acknowledgements

The Authors would wish to thank the teams from the laboratory of Research Unit Ecosystems and Aquatic Resources (INAT/Carthage University) and the laboratory of National Veterinary Research Institute (IRVT/Sfax) for their valuable support.

References

Aune T and Yndstad M: 1993. Diarrhetic shellfish poisoning. in: IR Falconer (Ed), Algal Toxins in Seafood and Drinking Water. *London: Academic Press: 87-104.*

GIPP : 2012. Groupement Interprofessionnel des Produits de Pêche. Résultats de la production annuelle des coquillages en 2012. *Bulletin No 22.*

Haystead T.A.J, Sim A.T.R, Carling D., Honnor R.C., Tsukitan Y., Cohen P. and. Hardy D.G:1989. Effects of the tumor promoter okadaic acid on intracellular protein phophorylation and metabolism. *Nature 337: 78-81*

.Hmida L, Medhioub M.N, Cochard JC. and Le Pennec M: 2004. Evaluation of the effects of serotonin (5-HT) on oocyte competence in Ruditapes decussatus (Bivalvia, Veneridae). *Aquaculture*, 239: 413 - 420

Ibn Chbili A : 2010. Analyse de la filière palourde dans la zone de Zaboussa (Délégation de Graiba, Gouvernerat de Sfax)". *Mémoire de mastère de recherche, Institut National Agronomique de Tunisie. Tunis.*

Ibn Chbili A, Hmida L, Lovatelli A, Belkahia R. and Diei-Ouadi Y :2011. Amélioration des revenus des femmes dans la filière palourde: Approche chaine des valeurs et exploitation venericole dans le golfe de Gabès, FAO~~. –~~. *Rapport sur les pêches et l'aquaculture no 990.*

Le Pennec M : 1987. Genèse de la coquille larvaire et post larvaire chez divers bivalves marins. *Thèse d'état. Université de Bretagne Occidentale. France.*

Loosanoff V. and Davis HC: 1963. Rearing of bivalve molluscs~~. –~~. *Advances in Marine Biology*. 1: 1 - 136.

Lubet, P : 1976. Écophysiologie de la reproduction chez les mollusques lamellibranches. *Haliotis*. 7 : 49 - 55.

Medhioub M.N : 1983. La conchyliculture en Tunisie. *Projet Tunis/92/002. Direction Générale de la Pêche et de l'Aquaculture. PNUD/FAO.* 83pp.

Montgomery H.A.C, Thom N. S. and Cockburn A:1964. Determination of dissolved oxygen by the winkler method and the solubility of oxygen in pure water and ~~sea water~~seawater. *Journal of Applied Chemistry. 14, (7): 280–296*

PaxinosR. and Mitchell J.G: 2000. A rapid Uthermohl method for estimationg algal numbers. Journal of plankton research. 22 (12): 2255-2262.

Stabell O.B. HormazabalI V. Steffenak I and Pedersen K: 1991. Diarrhetic shellfish toxins: Improvement of sample ~~clean-up~~clean up for HPLC determination. *Toxicon. 29 (1): 21-29.*

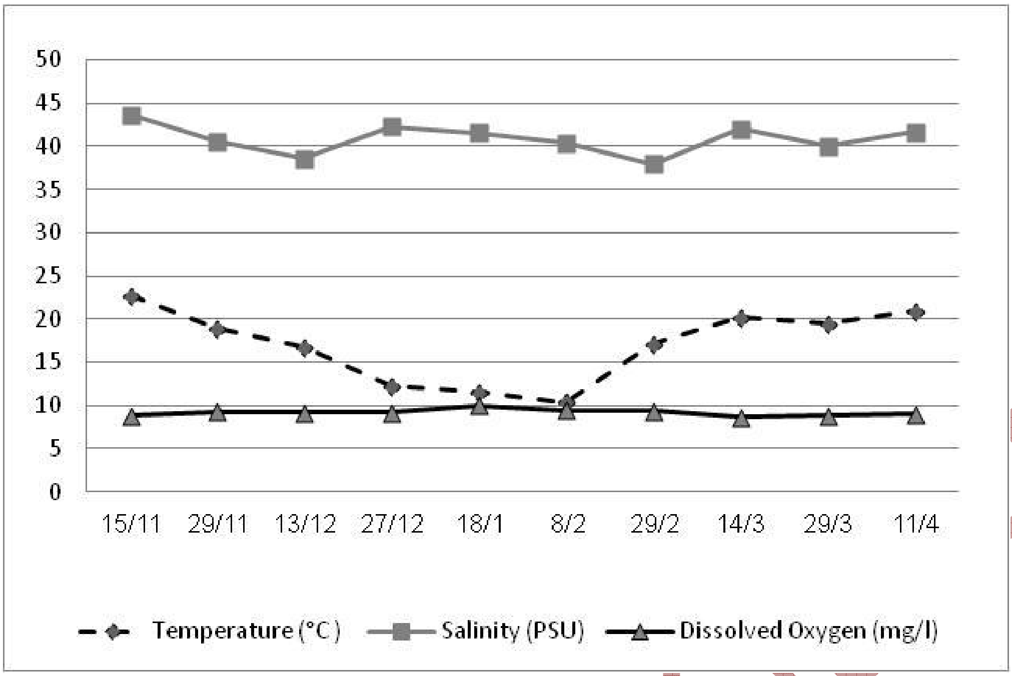

Fig. 1: Variation of physical parameters measured at healthy coastal areas (Oued Maltine, Gulf of Gabes, south Tunisia)

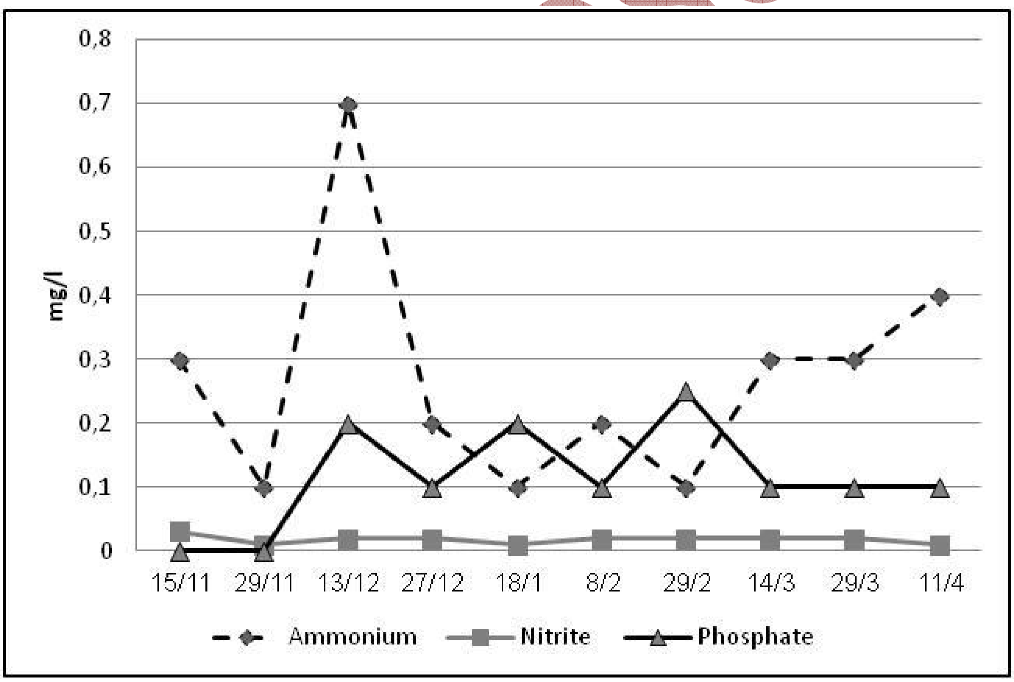

Fig. 2: Variation of nutriments at healthy coastal areas (Oued Maltine)

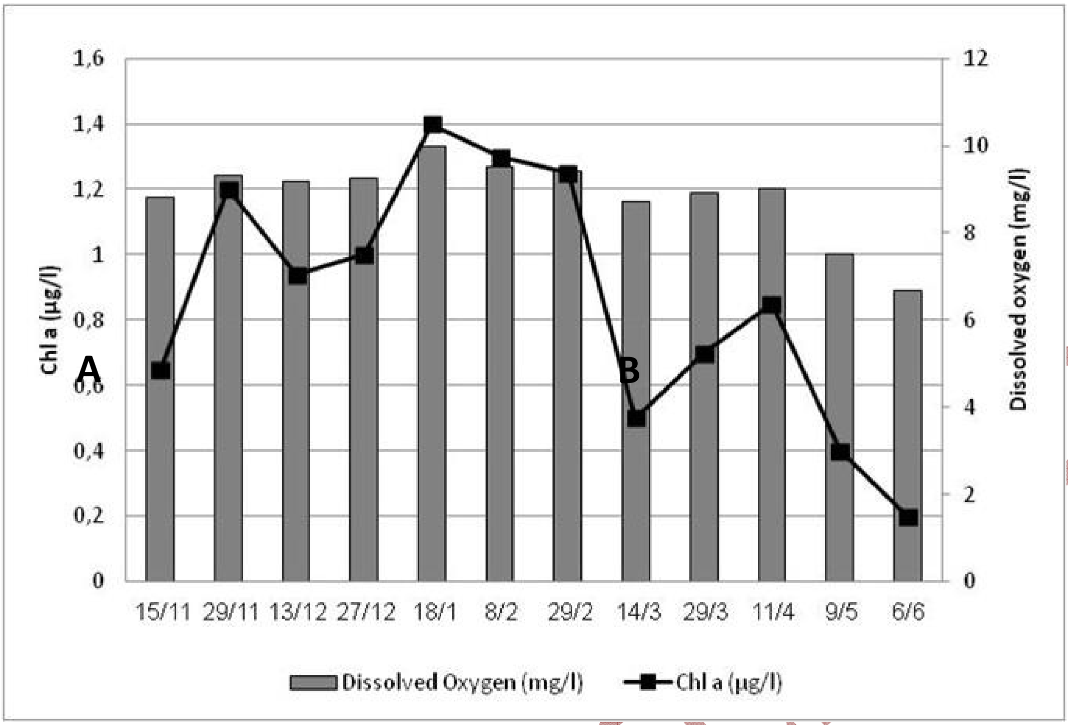

Fig. 3: Dissolved oxygen and chlorophyll a concentrations during the experiment at Oued Maltine.

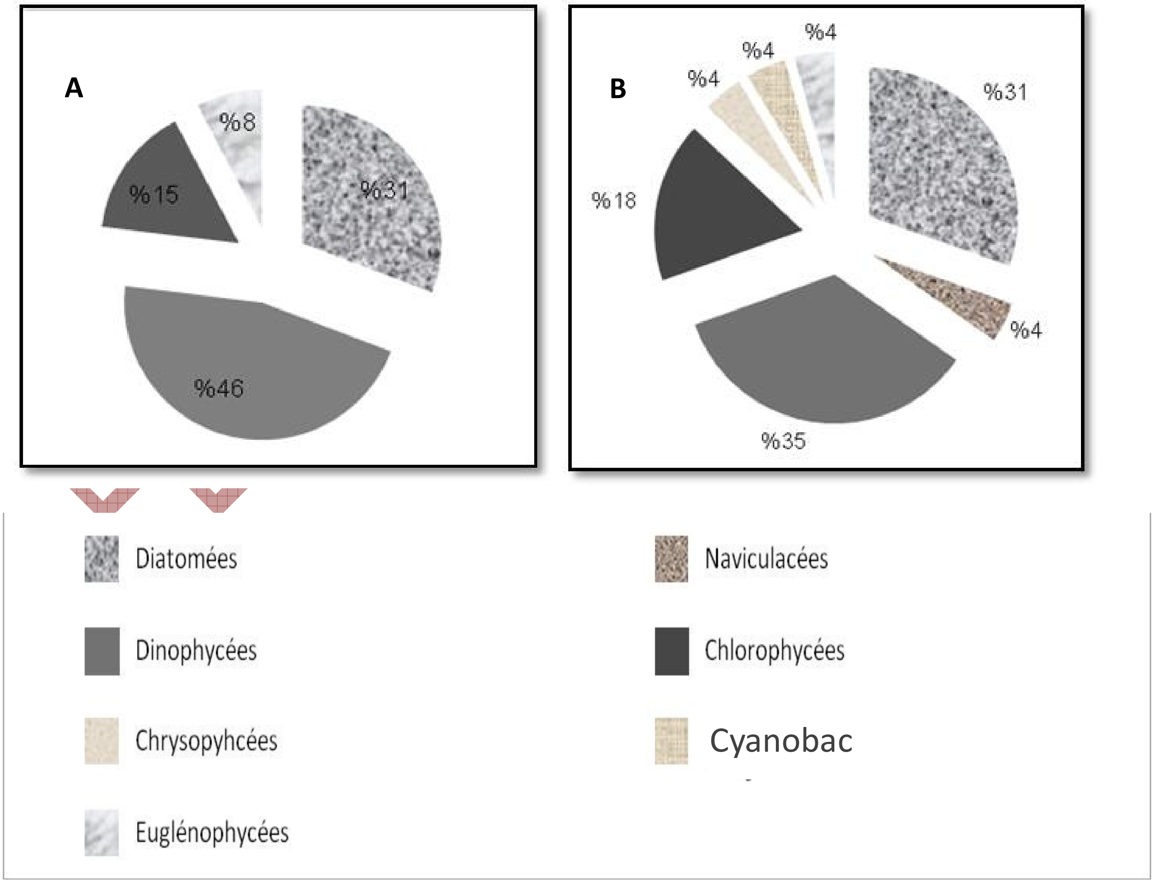

Fig
4:

Principals phytoplankton species observed in risky coastal areas (A. Bourgaga lagoon) and healthy coastal areas (B. Oued Maltine)

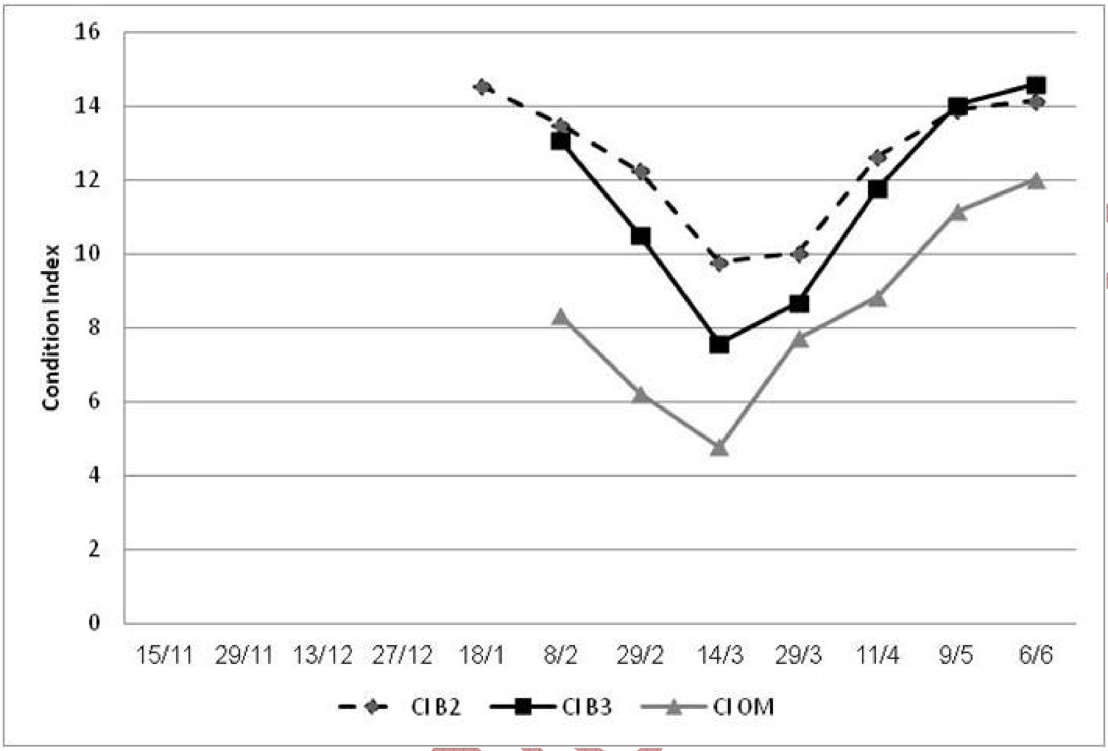

Fig. 5: Variation of condition index measured at healthy coastal areas (Oued Maltine, Gulf of Gabes, South Tunisia)