Macroalgae in marine fish farms at Egypt (Ismailia province)

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Abstract: The aim of this study is assessing the possibility of detection of the most predominant agents which causes high losses in some marine fishes. Identification the most appropriate algae to feed the marine fishes. Many species of the Seaweeds of the Suez Canal are important and can be used as a marine resource of the area and being used as human food, fish feed and raw material for many industries.

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1. Introduction

Seaweeds are rich sources of structurally diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that seaweeds originated compounds exhibit various biological activities. During the last years, many studies have been made on biological activities of the seaweed and could be potential rich sources of natural antioxidants (Pulz 2004**).**

The seaweeds are a diverse group of large marine macroalgae that are as important to our nearshore coastal marine world as land plants. Seaweeds were the evolutionary precursors to land plants, and like land plants they are critical primary producers, forming living links between the inorganic and the organic world, using photosynthesis to convert CO2 and nutrients into living biomass. These primary producers support other marine life through the production of oxygen, their contribution to marine food webs, and by providing structure and habitat for fish and invertebrates (Dawes, 1981 and Smit, 2004).

Seaweeds can be cultivated in the sea on suspended lines, rafts, or nets, or on land in tank--based culture systems. A sustainable, low impact process, seaweed culture can provide much-needed employment and independence to rural coastal communities. The development of a seaweed aquaculture industry can also encourage development of other aquacultured species that are higher up in the food chain (Hoek,. *et al.,* 1995 and Dillehy*et al*., 2008).

Global seaweed aquaculture production occupies approximately 20% of the total world marine aquaculture production by weight, with an annual value of US $6.7 billion in 2013 (Bjerregaard *et al*. 2016, Cottier-Cook *et al.* 2016 and FAO, 2017).

In fish farming, wet feed usually consists of meat waste and fish waste mixed with dry additives containing extra nutrients, all formed together in a doughy mass. When thrown into the fish ponds or cages it must hold together and not disintegrate or dissolve in the water. A binder is needed, sometimes a technical grade of alginate is used. It has also been used to bind formulated feeds for shrimp and abalone. However, cheaper still is the use of finely ground seaweed meal made from brown seaweeds; the alginate in the seaweed acts as the binder. The binder may be a significant proportion of the price of the feed so seaweed meal is a much better choice. However, since the trend is to move to dry feed rather than wet, this market is not expected to expand. Nakagawa *et al.* (1997) indicated that dietary algal meal improved the physiological condition of fish and the muscle protein increased in response to the algal level. Feeding represents over 73 % of the operational costs of aquaculture (Hanan A. Abo-State and Noor El-Deen (2017). Therefore, fish nutritionists have made several attempts to replace fish meal with less expensive, locally available protein source (Nakagawa *et al*., 1997, Attia, 2001 and Hegazi, *et al*., 2003).

The present study was planned to investigate the most prevailing Seaweeds in marine fish culture in third stage of project.

2. Material and Methods

Seaweeds Collections

Private fish farms area in Ismailia governorate was studied during a number of reconnaissance field trips, intensive collections of seaweeds were done from different depths and habitats. The seaweeds collected by different methods in order to secure complete alga. The best time for collecting the

seaweeds was during the hours time level of water as low as possible.

This is allow to observe the algae in their natural habitat, to record such observations and to collect the specimens.

Field containers:

Plastic containers were used, but for intertidal collection, buckets and bags for various sizes were used, and for the collection by SCUBA (self – contained –underwater breathing apparatus) diving perforated plastic bags were used.

Algal identification:

Species of common Seaweeds collected from private fish farms, all collected species were isolated and identified macroscopically. That had been identified and compared with materials in the Marine Botany Laboratory, Marine Science Department, Suez Canal University according to the methods described by Hegazi (2006) and Abdel-Rahman & Hegazi (2006). Seaweeds were collected and transferred directly to the laboratory aquarium in ice box.

Cultivation Methods

The methods used for the cultivation of seaweeds in the present study were two dominant worldwide cultured species of algae *Gracilaria*, *Sargassum* and *Ulvaas* a model and ideal specimen for cultivation in our private fish farms area in these period of the year. A unialgal culture was established by either spore or tip isolation. Clean, healthy, actively growing, reproductive 'parent' fronds exhibiting desirable characteristics were selected. Fronds were chosen from existing cultures. When making a wild collection, fronds were held and transported in an environment cooler than the one it was collected in to minimize stress. Clean plastic bags, plastic containers, or buckets can be used for collection and transport. Gracilaria was transported in moist paper towels and gauze to avoid drying and exposure.

The area of new and active growth was located at the tips of every branch on a *Gracilaria* frond, the apical tissue. New 'starter plants' were obtained to initiate a culture, tips was cut from the parent frond, cleaned thoroughly, and placed in favorable growing conditions. Each tip was then grow, elongate, and branched into a new frond. To prepare a tip for isolation, a clean working area was set up in a clean room with a draft-free, clean working space and a flame for sterilization of metal instruments to avoid any contamination of cultures. A small section of the frond was removed for tip isolation, and placed in a small dish with sterilized seawater and moved to the clean working space. The free contaminants tip was placed in sterilized seawater prepared with Von Stosch's Enrichment medium (VSE) and germanium dioxide (GeO2). Multiple tips were isolated from each frond in order to increase chances of obtaining a clean culture.

Individual tips were isolated and cultured separately in small Petri dishes and flasks. They should be placed in VSE seawater with GeO2 under low light (10-20 µmol photons m -2 s -1), with a 12:12, L:D photoperiod, at 20°C. Tips began to elongate in about a week or two. Once tips had begun to grow and appear clean, light aeration was applied to cultures to increase growth rates. Cultures were changed once every two weeks initially, then once per week as growth rates increase. Once tips began to elongate and branch into larger fronds, they were transferred to larger and larger containers to encourage growth. To preserve or maintain original culture strains over long periods of time in the laboratory, growing environments were minimized, reducing light, reducing temperature, space, and frequency of media changes (FAO,2017).

Spore Isolation:

Clean cultures were initiated from either carpospores or tetraspores. Both types of spores were released and isolated in the same way, though each will give rise to a different phase in the life cycle. Carpospores were obtained from mature cystocarps, which were apparent as bumps on the female thallus. These were easily identifiable, being obvious without the aid of a microscope. A microscope, was only needed to observe the presence of the tetrasporangium on the thallus of the tetrasporophyte. These appeared as small reddish spots scattered throughout the cortex of the thallus. To release spores, short sections of the fronds were placed in sterilized seawater in a Petri dish over glass slideskept under low light at 20°C. Spore release was checked under a dissecting microscope. Once there are spores released in the water, a small sample can be removed from the Petri dish and placed in a fresh dish with new medium. Individual spores were selected with a very fine-tipped Pasteur pipette under a microscope. Selected spores were placed on cut glass slides (25mm x 25mm) or on cover slips in small Petri dishes with sterilized VSE seawater. Dishes can be kept undisturbed at 20°C under 30 µmol photons m -2 s -1 light, with a 12:12, L: D photoperiod. Spores were settled within 12-24 hours after release, adhere to the glass slide, and began to divide. After the initial division, the diameter began to increase as a multi-cellular disc was formed, a few days after settlement. The culture was easily expanded by breaking apart, or fragmenting, the fronds, that continued to branch and grow into new fronds.

Nutrient Medium

(von stosch's enriched seawater medium (ott, 1965)

The seawater was filtered (What man's No.1) to remove large organic particles and sand. Then

sterilized by autoclaving (time: 100 ml required 10 minutes; 2 litters required 40 minutes; 3 litters required 50 minutes; and 5 liters required 70 minutes). A stock solution of each salt in distilled water was prepared; of such concentration that 1 ml of the stock solution gave the required concentration of each ingredient. The three vitamins were incorporated in the same refrigerated stock solution. Stock solutions use deionized distilled water and clean volumetric flasks. The salts and vitamins after preparation into stock solutions were filter sterilized. Each stock solution was filtered through separate 0.22 um Millipore filters. Each solution will have to be sterilized separately.

Table (1): Showing ingredient of stock solution.

Salts	**1 liter stock solution (g)**	**2 liter stock solution (g)**
Na N03	42.500	85.000
Na2HP04 12H2O	10.750	21.500
FeSO4 7H2O	0.278	0.556
MnCl2 4H2O	0.0198	0.039
Na2EDTA 2H2O	3.720	7.440
Vitamins		
Thiamine-HCl	0.200	0.400
Biotin	0.001	0.002
B12	0.001	0.002

All solutions prepared separately using clean sterilized volumetric flasks, clean pipettes, digital balance, and mixed with magnetic stirring bars. Filter sterilize was used in each solution using a 0.2 µm filter and a vacuum pump assembly. Vitamins should not be heat sterilized. Media solutions were stored in the refrigerator, while vitamins stored in the freezer and thawed before use. All chemicals were dated when received and when opened. All solutions were clearly labeled at every step, and aseptic technique was used in preparation.

Nutrient Media Supplies List:

Volumetric flask (1000mL; measuring), Erlenmeyer flasks (3000mL; mixing and storing), Graduated cylinders (500 or 1000 mL), Storage flasks and bottles.

Digital scale (0.0001 g; 3 decimal places), Weigh paper/boats, Metal chemical spoon or spatula (measuring), Safety goggles and gloves, 0.2 micron filter for media sterilization (Corning Disposable Sterile Bottle Top Filter, 150mL Funnel, #25965-45), Pyrex screw-cap media storage bottles (media storage), Flame (for sterile technique; Bunsen burner, etc), Sterile pipettes, 10mL (measuring; disposable plastic or glass autoclavable), Pipette bulb or motor, Potable pH meter and associated buffers, Stirring plate and magnetic stir bars and Parafilm.

Growth rate:

Individual test seedlings were weighted before test planting. Actual planting was numbered tag sandals for identification purpose. The test plant was cleared daily. Test plant was tested individually with avoidance of direct exposure to sunlight or rain. Plants were weighted to get growth rate from the test plant with aduration of 6 weeks. Test plants were weighted weekly or every 15 days to avoid stress on the plants. Test plants were checked and missing plants should be replaced immediately. The seedlings growing points was indicator of good growth. Triple beam balance/string balance or actual calculation was used in determining the incremental weight.

Formula for getting growth rate:

$$\frac{\square\square - \square\square}{\square\square.\square\square\ \square\square\square\square\square\square\square\ \square\square\square\square} = \%\ \text{daily growth rate}$$

(FAO,.2017)

Where: TW = Total weight of plant after test planting, OW = Original weight of the plant before test planting and No. of culture days = Period of test planting.

Microscopical examination:

According the morphological characteries, the isolation and identification of macroalge as recorded by Dawes.1981and Hoek *et al*.,1995.

3. Results and Discussion

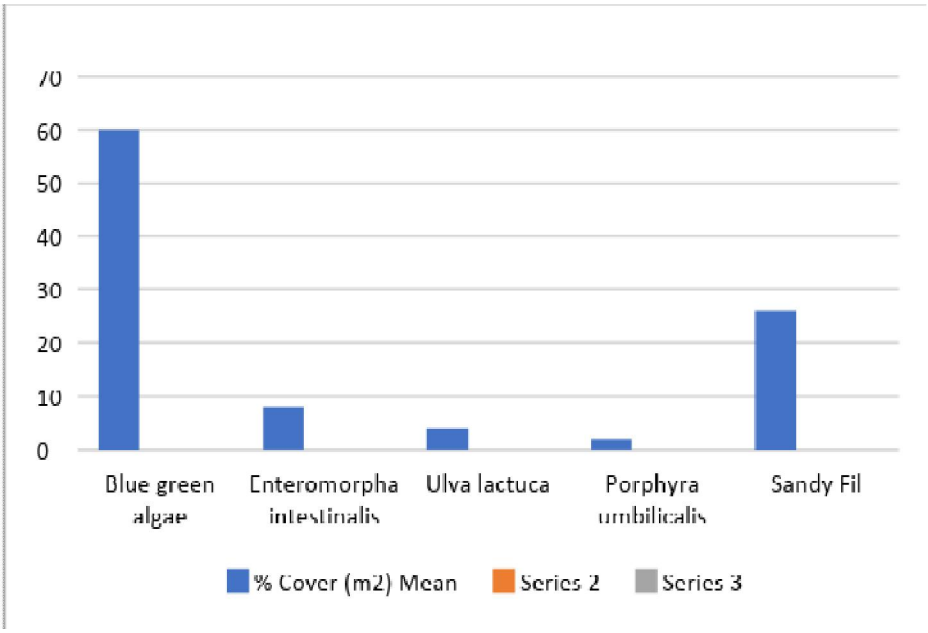
The aim of the Ismailia project is not an environmental study of seaweeds, which requires monthly or seasonal samples from both Suez Canal and fish farms to conduct statistical analysis of seaweeds distribution.

Field trips that were carried out from the Suez Canal areas in the Ismailia are intensive collection according to the geographical and distribution of the seaweeds in this area and selection of some species suitable for aquaculture. The 50 x 50 cm square method was used to obtain the percentage covered of the seaweeds; the area was divided into the following zones:

In which species of blue-green algae and percentage covered of green algae represented by Enteromorpha intestinalis and Ulva lactuca respectively, with a small percentage of red algae represented by Porphyraumbilicalis. (Table 2)

A- Supra Tidal zone

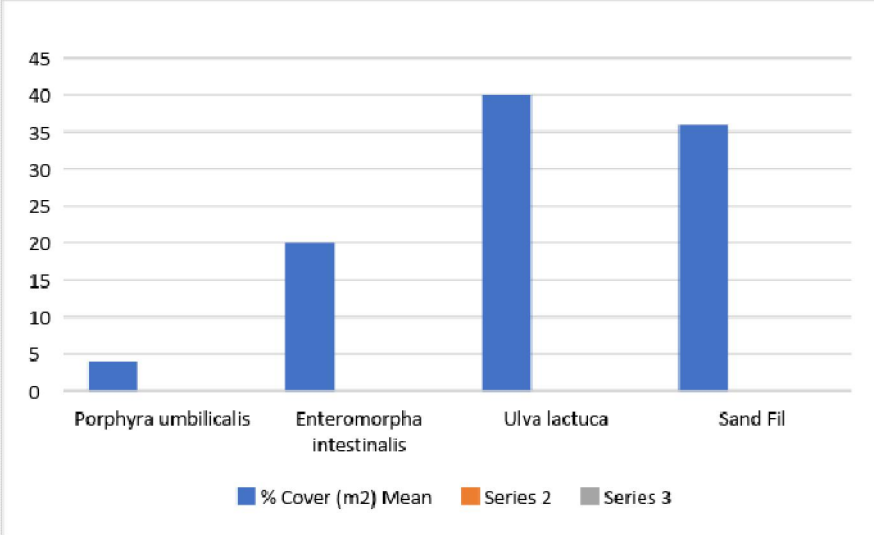
Species	means
Blue green algae	60
Enteromorpha intestinalis	8
Ulva lactuca	4
Porphyraumbilicalis	2
Sandy Fil	26



B- Intertidal Zone

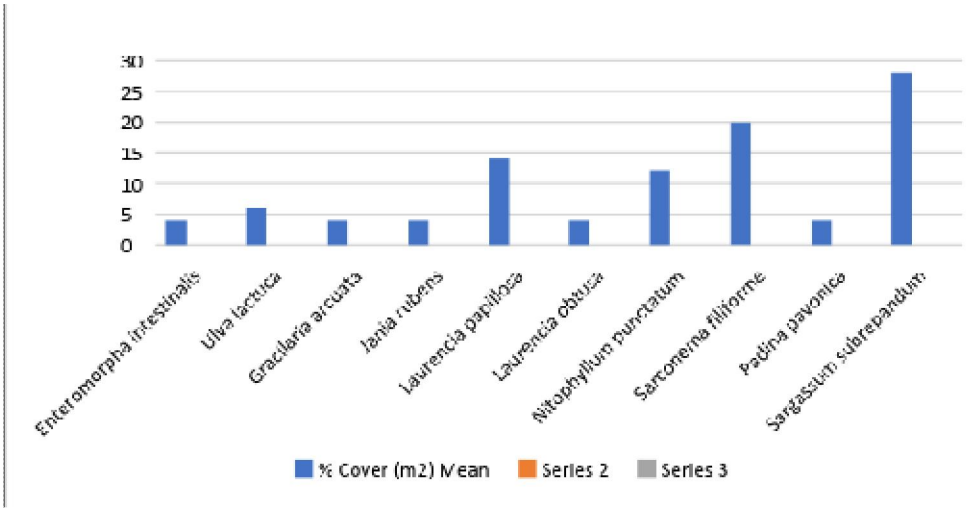
Which represented by Porphyraumbilicalis, Enteromorpha intestinalis and Ulva lactuca. (Table 3)

Species	% Cover (m2) Mean
Porphyraumbilicalis	4
Enteromorpha intestinalis	20
Ulva lactuca	40
Sand Fil	36



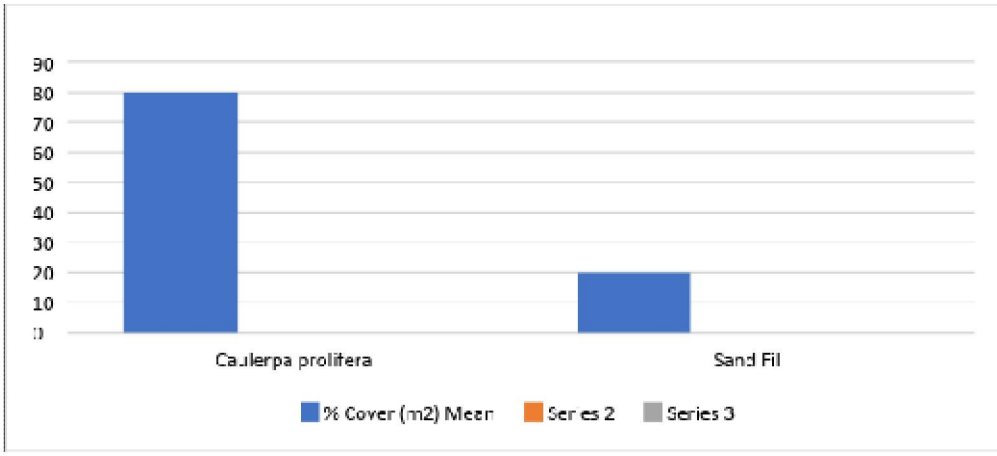
C- Sub-tidal Zone up to 1m depth. (Table 4)

Species	% Cover (m2) Mean
Enteromorpha intestinalis	4
Ulva lactuca	6
Gracilariaarcuate	4
Janiarubens	4
Laurenciapapillosa	14
Laurencia obtuse	4
Nitophyllumpunctatum	12
Sarconemafiliforme	20
Padinapavonica	4
Sargassumsubrepandum	28



D- Sub-tidal Zone from 1-3 m depth. (Table 5)

Species	% Cover (m2) Mean
Caulerpaprolifera	80
Sand Fill	20



3- There is no intensive density of seaweed within Ismailia marine Fish ponds, because there is no suitable hard substrate for attachment, and the presence of some drifted species were due to the water current and waves on the surface of water or the presence of a few rocks substrate within the pond bottoms. So according to the project target, different type of nets and ropes will be placed in the ponds to increase the seaweeds presence and distribution to improve water quality and increase the efficiency of food chains inside Ismailia marine fish ponds.

The common and abundant species of selected Seaweeds from both littoral and sub-littoral of the Suez Canal pathway and fish ponds were (*Caulerpaprolifera, Enteromorpha intestinalis, Ulva lactuca, Gracilariaarcuata, Janiarubens, Laurenciapapillosa, Laurenciaobtusa, Nitophyllumpunctatum, Porphyraumbilicalis, Sarconemafiliforme, Padinapavonica* and *Sargassumsubrepandum*) had been identified and compared with materials in the Marine Botany Laboratory, Marine Science Department, Suez Canal University.

Some worldwide cultured species of green seaweeds were isolated and identificated in private fish farms area such as Ulvalactuca, Enteromorphaintestinalis and Caulerpaprolifera from temsah lake.

A species of genus Ulvalactuca (Sea lettuce) it was cultured in our laboratory as an important source of food. Both in respect of the region where it grows and its ecological characteristics, this is seaweed that shows striking resemblance with 'Enteromorpha'. The methods of culturing either this species or Enteromorpha are also very similar. It is also used as the principal ingredient for soups in many countries.

The culture medium in such cultures was replenished once in every week. The more preminant Ismailia Seaweeds species valid for cultivations:

Chlorophyta: Ulvalactuca, Enteromorphaintestinalis and Caulerpa sp. Simmelar to that recorded by Buschmann, *et al*., 2008.

Techniques of growing cultured Ulva have yet to be developed to the level of 'Enteromorpha'. One has so far depended on nature for seeding Ulva, though more recent studies have led to the development of artificial methods that have since been applied widely. Artificial seeding of cultured Ulva consists of culturing in tanks zygotes of the summer generation and artificially inducing adhesion of zoospores emerging in early autumn on to seeding nets. The technique also facilitates easy retention of the seeding nets. On the other hand, increase in the number of culture nets of late has tended to overcrowd the culture grounds. At times this causes diseases of the leaf fronds and, accordingly, affects both the yield and quality of cultured Ulva. Studies must now be taken up to stabilize production by installing the exact number of culture nets in the culture grounds according to their expected production. Research is also needed in the field of manufacturing technology for food products using Ulva to help increase demand for this seaweed.

Influence of environmental factors on spore release of Gracilaria culture in lab. have shown that the highest number of spores were released at 20–25 °C Table 2. According to field surveys, the reproductive season of Gracilaria was between March to May. During these months, the natural seawater temperature ranged from 20–25 °C. The experimental results coincided with natural reproductive seasons as reported by Zheng*et al*. (1987).

Table,6: The relationship between released quantity and seawater temperature.

Temperature	8-10	12-15	20-22	25
Tetraspore	100	478	2876	1389
Carpospore	610	1057	1436	746

The impact of aquaculture on the environment and effects of environment on aquaculture production have become important issues in recent years. There is evidence from many countries that environmental deterioration is a major threat to aquaculture production and product quality. So, the mean object of our research is to minimize the environmental side effects and improved the water quality through the use of macroalgae (Seaweeds) in marine fish farms.

The present work phase was focused in the massive production of nutrients within seaweeds which in turn became more effective in bio-form in adult fish nutrition. This includes the proper indoor macroalgale new species growth associated with both growth curve and determination of nutrients accumulation rate.

Also, the current studies concluded that in these studies we expected to introduce and improve seaweeds new species adapted with mariculture.

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References

1. Abdel-Rahman, I. F. and M. M. Hegazi (2006). Depositional Environments, Facies Pattern and Marine Plants Distribution in Ras Muhammad Area, Sinai, Egypt. CATRINA, 1 (2): 81-89.
2. Amar, E.C., V. Kiron, S. Satoh and T. Watanabe, (2004). Enhancement of innate immunity in rainbow trout (Oncorhynchusmykiss) associated with dietary intake of carotenoids from natural products. Tokyo University of Marine Science and Technology, Minato, Japan. pp: 527-537.
3. Attia, Z. I. (2001). Effect of diet composition on kinetic properties of acetyle cholinesterase in thermally acclimated Oreochromisniloticus. J. Egypt. Ger. Soc. Zool., 36 A: 61-70.
4. Barman, N., G.G. Satpati, S. Sen Roy, N. Khatoon, R. Sen, S. Kanjilal, R.B.N. Prasad, and R. Pal. (2012). Mapping algae of sundarban origin as lipid feedstock for potential biodiesel application. Journal of Algal Biomass Utilisaton 3(2): 42–49.
5. Becker, E.W., and L.V. Venkataraman. (1984). Production and utilization of the blue green alga Spirulina in India. Biomass 4:105.
6. Ben-Amotz, A. & Avron, M. (1989). The wavelength dependence of massive carotene synthesis in Dunaliellabardawil (Chlorophyceae). Journal of Phycology 25: 175-178.
7. Berker, E, W, (2004). Microalgae as a source of protein. Biotectology advances.25:207-2010.
8. Bjerregaard, R., Valderrama, D., Radulovich, R., Diana, J., Capron, M., Mckinnie, CA., Cedric, M., Hopkins, K., Yarish, C., Goudey, C. & Forster, J. (2016). Seaweed aquaculture for food security, income generation and environmental health in Tropical Developing Countries. Report #107147.
9. Borowitzka, M.A. (1998). Algae as food. In Microbiology of fermented foods, 2nd ed, ed. B.J.B. Wood, 585–602. London: Blackie Academic and Professional.
10. Brown, M.R., S.W. Jeffrey, J.K. Volkman, and G.A. Dunstan. (1997). Nutritional properties of microalgae for mariculture. Aquaculture 151: 315–331.
11. Buschmann, A. H., Varela, D. A., Hernández-González, M. C. & Huovinen, P. (2008). Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of Macrocystis and Gracilaria as biofilters. J Appl Phycol. 20:571–577.
12. Chapman, H.D. and Pratt, P.F. (1978) Methods of Analysis for Soils, Plants and Waters. Univ. California Div. Agric. Sci. Priced Publication, Oakland.
13. Choi, K.J., V. Nakhost, J. Krukonis, and M. Karel. 1987. Supercritical fluid extraction and characterization of lipids from algae Scenedesmus obliquus. Food Biotechnology 1(2): 263–281.
14. Cottier-Cook, EJ., Nagabhatla, N., Badis, Y., Campbell, ML., Chopin, T., Dai, W., Fang, J., He, P., Hewitt, CL., Kim, GH., Huo, Y., Jiang, Z., Kema, G., Li, X., Liu, F., Liu, H., Liu, Y., Lu, Q., Luo, Q., Mao, Y., Msuya, FE., Rebours, C., Shen, H., Stentiford, GD., Yarish, C., Wu, H., Yang, X., Zhang, J., Zhou, Y. & Gachon, CMM. 2016. Safeguarding the future of the global seaweed aquaculture industry. United Nation University (INWEH) and Scottish Association for Marine Science Policy Brief, South Hamilton, ON, 12 pp.
15. Dawes, C. J. 1981. Marine Botany. 2nd Edition, John Wiley & Sons, P. 496.

16. Dillehay, TD.,Ramírez, C., Pino, M., Collins, MB., Rossen, J. & Pino-Navarro, JD. 2008. Monte Verde: seaweed, food, medicine, and the peopling of South America. Science. 320:784–786.
17. Edge, R., D.J. McGarvey, T.G. Truscott, 1997. The carotenoid as antioxidants a Review. Journal of Photochemistry and Photobiology: Biology, 189-200.
18. El-Sayed, A. B. and Abdel-Maguid, A.A. (a) (2010). Immobilized-microalga Scenedesmus sp. for biological desalination of Red Sea Water: II. Effect on macronutrients removal. Journal of American Science, 6(9):637-643.
19. El-Sayed, A. B., El-Fouly, M.M. and Abou El-Nour, E. A. (b) (2010). Immobilized-microalga Scenedesmus sp. for biological desalination of Red Sea water: I. Effect on Growth Metabolites. Nature and Science, 8(9):69-76.
20. El-Sayed, AB; EMHoballah, MA Khalafallah 2012. Utilization of citrate wastes by Scenedesmus sp. I-Enhancement of vegetative growth. 8(2):739-745.Journal of Applied Sciences Research.
21. El-Shafey Y.H., M.M. El-Fouly, M.M. Khalil, A.B. Abdalla, F.E. and El-Sayed 1999. Secondary carotenoid accumulation in some green algae species. The First Congress on the Recent Technologies in Agriculture. 27-29 Nov., 1999, Fac. of Agric., Cairo Univ. Cairo, Egypt.
22. FAO (Food and Agriculture Organization of the United Nations). (2017). The state of world fisheries and aquaculture.
23. Gasse, F. (1986) East African diatoms: Taxonomy, ecological distribution. Biblioteca Diatomologica 11, Crammer, Berlin/Stuttgart, 201 pp.
24. Guillard, R.R.L. and Ryther, J.H. 1962. Studies of marine planktonic diatoms. I. Cyclotella *nana* Hustedt and *Detonulaconfervacea* Cleve. *Can. J. Microbiol.* **8:** 229-239.
25. Hamed A.F. (2008). Biodiversity and Distribution of Blue-Green Algae/Cyanobacteria and Diatoms in Some of the Egyptian Water Habitats in Relation to Conductivity. Australian Journal of Basic and Applied Sciences, 2(1): 1-21.
26. Hanan A. Abo-State and A.I. Noor El-Deen (2017). Practical aspects of phytobiotic (Veto-Acid®) supplemented to Nile tilapia (Oreochromisniloticus) diets and its susceptibility to Aeromonashydrophila challenge. International Journal of ChemTech Research, 2017,10(2): 265-273.
27. Hassan, A.M., S.H. Abdel-Aziem and M.A. Abdel-Wahhab, (2012). Modulation of DNA damage and alteration of gene expression during aflatoxicosis via dietary supplementation of Spirulina (Arthrospira) and whey protein concentrate. Ecotoxicol. Environ. Safety, 79: 294-300.
28. Hegazi, M. M. (2006). Distribution of the invasive species Caulerpaprolifera along the coasts of the Suez Canal, Egypt. CATRINA, 1 (2): 31-35.
29. Hegazi, M., Sharaf, M. and El-Shenawy, N. (2003). Growth response of nile Tilapia Oreochromisniloticus (L.) fed on artificial food mixed with different species of seaweeds. Egypt. J. Zool., 40: 47-61.
30. Hoek, C. van den, Mann, D.G. and Jahns, H.M., 1995. Algae An introduction to phycology. Cambridge University Press, Cambridge.
31. Huervana FH, De La Cruz JJY, Caipang CMA. Inhibition of luminous Vibrio harveyi by “green water” obtained from tank culture of Tilapia, Oreochromismossambicus. Acta Ichthyol Piscat. 2006;36:17–23.
32. Mark L. Wells; Philippe Potin; James S. Craigie; John A. Raven; Sabeeha S. Merchant; Katherine E. Helliwell; Alison G. Smith; Mary Ellen Camire and Susan H. Brawley 2017. Algae as nutritional and functional food sources: revisiting our understanding. Journal of Applied Phycology, 29(2):949–982.
33. Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. J ApplPhycol. 2000;12:527–534.
34. Nakagawa, H., Umino, T. and Tasaka, Y. 1997. Usefulness of Aschophyllum meal as a feed additive of red sea bream, Pagrus major. Aquaculture, 151 (1-4): 275-281.
35. Nichols, B.W. 1970. Comparative lipid biochemistry of photosynthetic organisms. In Phytochemical phylogeny, ed. J.B. Harbone,105–118. London: Academic Press New York.
36. Ott, F.D., 1965. Synthetic media and techniques for the xenic cultivation of marine algae and flagellata. Virg. J. Sci. 16:205-218.
37. Palmer PJ, Burke MJ, Palmer CJ, Burke JB. Developments in controlled green-water larval culture technologies for estuarine fishes in Queensland, Australia and elsewhere. Aquaculture. 2007;272:1–21.
38. Percival, E.E., and J.R. Turvey. 1974. Polysaccharides of algae. In CRC, handbook of microbiology, condensed edition, 532nd ed, ed. A.L. Laskin, and H.A. Lechevalier. Cleveland: CRC Press Inc.
39. Qie G, Makridis P, Reitan KI, Olsen Y. Protein and carbon utilization of rotifers

(Brachionusplicatilis) in first feeding of turbot larvae (Scophthalmus maximus L.). Aquaculture. 1997;153:103–122.
40. Renaud, S.M., D.L. Parry, and L.V. Thinh. 1994. Microalgae for use in tropical aquaculture I: Gross chemical composition and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. Journal of Applied Phycology 6:337–345.
41. Round, F.E. (1971). "The taxonomy of the Chlorophyta, 2". Brit. Phycol. J. 6 (2): 235–264.
42. Sen Roy S., Ruma Pal Microalgae in Aquaculture: A Review with Special References to Nutritional Value and Fish Dietetics Proceedings of the Zoological Society, June 2015, Volume 68, Issue 1, pp 1–8.
43. Sen Roy, S., N. Barman, and R. Pal. 2009. Stess Induced changes in total lipid and fatty acid composition of Navicula minima Grun. Journal of Botanical Society of Bengal 63(1): 47–51.
44. Smit, A.J., 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. Journal of Applied Phycology, 16: 245-262.
45. SAS (2002) Statistical Analysis System, version 9.1. SAS Institute Inc., Cary.
46. Tachibana, K., M. Yagi, K. Hara, T. Mishima and M. Suchimote, 1997. Effects of feeding of β-Carotensupplemented rotifers on survival and lymphocyte proliferation reaction of fish larvae Japanese parrotfish (Oplegnathusfasciotus) and spotted parrotfish (Oplegnathupunctatus): preliminary trials. Hydrobiologia, 358: 313-316.
47. Tendencia EA, de la Peňa MR, Fermin AC, Lio-Po G, Choresca CH, Jr, et al. Antibacterial activity of Tilapia hornorum against Vibrio harveyi. Aquaculture. 2004;232:145–152.
48. Tim, J., T.J. Bowden, K.D. Thompson, A.L. Morgan and S. Nikoskelainen, 2007. Seasonal variation and immune response: A fish perspective. Department of Zoology, University of Aberdeen, Scotland, UK., pp:695-70.
49. Tonon T, Harvey D, Larson TR, Graham IA. Long chain polyunsaturated fatty acid production and partitioning to triacylglycerols in four microalgae. Phytochemistry. 2002;61:15–24.
50. Tornabene, T.G., G. Holzer, S. Lien, and N. Burris. 1983. Lipid composition of the nitrogen starved green alga Neochlorisoleoabundans. Enzyme and Microbial Technology 5(6): 435–440.
51. Venkataraman, L.V., and E.W. Becker. 1985. Biotechnology and utilization of algae—The Indian experience. Mysore: Central Food Technological Research Institute. 257.
52. Wang, Y.J., Y. Huchien and Ch. Hugpan, 2006. Effects of dietary supplementation of carotenoids on survival, growth, pigmentation and antioxidant capacity of characins, (Hyphessobrycallistus). Department of Aquaculture, National Taiwan Ocean University Keelung, Taiwan, 202.
53. Webb, K. L., F E Chu. 1983. Phytoplankton as a food source for bivalve larvae. In Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. eds.G. D. Pruder, C.J. Langdon and D. E. Conklin 272–291. Louisiana State University, Baton Rouge, LA.
54. Zenetos A, Gofas S, Verlaque M, Çinar M, García Raso E, Bianchi CN, Morri C, Azzurro E, Bilecenoglu M, Froglia C, Siokou I, Violanti D, Sfriso A, San Martín G, Giangrande A, Katağan T, Ballesteros E, Ramos Esplá A, Mastrototaro F, Ocaña O, Zingone A, Gambi MC, Streftaris N (2010) Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. Mediterranean Marine Science 11: 381–493,.
55. Zheng C. K., Wang, S. J. and S. J. Liu, 1987. Phycoculture. Shanghai House of Science and Technology Publication. pp. 225–254.

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