International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 18-06-2020; Accepted: 04-07-2020; ; Published: 19-07-2020
Volume 2; Issue 3; 2020; Page No. 59-66

A systematic account of freshwater diatoms of Kerala, India: Potential source as live feeds in larviculture and biodiesel

Aswathy Krishnan[1], Aneykutty Joseph[2*]
[1-2] Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Fine Arts Avenue, Cochin University of Science and Technology, Cochin, Kerala, India

Abstract
In the present study, diatom samples were collected from the tropical river systems in Kerala, India. The investigation was done for a period of two years from January 2017 to December 2018. Thirty four species of diatoms belonging to fifteen genera *viz. Nitzschia*, *Navicula*, *Diadesmis*, *Achnanthidium*, *Synedra*, *Encyonema*, *Sellaphora*, *Lemnicola*, *Eunotia*, *Pinnularia*, *Aulacoseira*, *Surirella*, *Stauroneis*, *Gomphonema* and *Cymbella* were isolated using methods *viz.* serial dilution, agar plating and single cell isolation. Out of the 34 species isolated and identified, 16 species could be maintained as pure cultures in f/2 medium under laboratory conditions for further analysis *viz.* standardization of culture conditions and nutrient analysis. Species identification was done with the help of light microscope and scanning electron microscope images. The diatom cells were subjected to acid treatment for analyzing the structure and morphology. The study was aimed at screening, isolation and identification of the diatoms from freshwater sources and to find out its potential as live feeds in the larviculture of fin fish and shell fish species of aquaculture importance and also as a source for biodiesel production.

Keywords: diatoms, isolation, live feed, biodiesel, screening

Introduction
Diatoms belong to the class Bacillariophyceae, which is the major group of photosynthetic microalgae found in almost all water sources. Over 8000 different species are described growing worldwide in lakes and sea, but according to different authors [1], extant species are estimated to range between 20000 and 200000 species. These microscopic autotrophic microalgae possess highly ornamented cell wall composed of glass silica called frustules which provide a variety of shapes from nano- to micro-scale structures. The size, shape and sculpturing of diatom cell walls are taxonomically diagnostic [2].

Microalgae are utilized diversely in aquaculture, but their main applications are related to nutrition. They are used as a sole component or as a food additive to supply basic nutrients, color the flesh of salmonids or for other biological activities [3]. Microalgae must possess a number of key attributes to be useful to aquaculture species. They must be of an appropriate size for ingestion and readily digested, they must have rapid growth rates, be amenable to mass culture, and they must have a good nutrient composition, including an absence of toxins that might be transferred up the food chain [4]. With the development of the mariculture in the last two decades, microalgal cultivation is increasing its importance due the fundamental use of microalgae for feeding many animal species in aquaculture [5]. Microalgae contain essential nutrients which determine the quality, survival, growth and resistance to disease of cultured animal species [6].

Diatoms are widely used as larval feeds. The small diatoms *Chaetoceros muelleri*, *Chaetoceros calcitrans* and *Thalassiosira pseudonana* are used for mollusc culture [7]; the chain forming diatom *Skeletonema costatum* for larval prawn culture [8]; and benthic mat forming pennate species (e.g. *Nitzschia* sp.) for juvenile abalone culture. PUFAs derived from microalgae, i.e. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are known to be essential for various larvae [9]. These are important essential fatty acids required for gametogenesis [10]. When compared to other groups of microalgae, diatoms are the only algal group containing a high level of cholesterol, the sterol essential for mollusc growth [11]. Diatoms are fed to different fresh water aquaculture animals which include fishes such as milk fish, gold fish, etc. and shell fishes such as *Lamellidens* sp.

The imminent depletion of world oil reserves and the global environmental deterioration associated with the exhaustive consumption of fossil fuel have generated renewed interest in exploring microalgae as alternative and viable resources of renewable biofuels [12]. The promise of diatoms as potential candidates for biodiesel arose from the fact that most species synthesize considerable amounts of glycerides (monoglycerides, diglycerides and triglycerides) which are the key lipid feedstock of biodiesel [13].The present study was aimed at the identification of diatoms from the microalgal sample from fresh water sources which can be used as potential live feeds in the field of larviculture. Number of studies has been done on the marine diatoms for their nutritional value. But fresh water diatoms undergo very few studies on their distribution, biochemical composition and nutritional importance.

2. Materials and methods
2.1 Collection of diatom sample
The microalgal samples were obtained from the rocks and macrophytes collected from the river. Diatoms were removed by

vigorously scrubbing the upper surface of the rocks with a small brush to dislodge the diatom community. For separating diatoms from macrophytes the plant parts were placed in a plastic bag together with enough stream water. The plants were shaken vigorously and squeezed in the plastic bag and the resulting brown suspension was poured into a sample bottle. Plankton net (40 microns) was also used to get the free floating diatoms from the upper surface of the stream. The collected samples were brought to laboratory in containers along with water taken from the sample site for further analysis i.e. isolation and identification [2].

2.2 Isolation

For isolating diatom species three methods were adopted such as, agar plating, serial dilution and single cell isolation. Serial dilution involved five steps. First, 9 ml f/2 medium was taken in 5 test tubes each and 1ml diatom sample was added to the first test tube. The tube was shaken well and from this tube 1ml sample was transferred to the second tube. The procedure was continued up to the fifth test tube. Thus the dilution series is 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The diluted sample was then kept for growth [14].

For preparing agar plates, 1% agar was prepared by adding 1gm of agar to 100 ml of filtered and sterilized freshwater. The agar solution was then autoclaved and f/2 medium was added to it for enriching algal growth. Then it was poured in to sterilized Petri dishes and kept for 24 hours. One drop microalgal sample was then spread on the agar medium present in each Petri plate. After spreading, the Petri dishes were kept for 4-6 days, providing 1500 lux light with a photoperiod of 12 hours light: 12 hours dark and 25ºC temperature. Within this time the microalgal groups present in the sample form different colonies. Later each colony was separated and spread on another plate to obtain monoculture.

In single cell isolation Pasteur pipette was used. The desired organism was sucked in through the pipette and was transferred to a test tube containing f/2 medium. Thus the organism was allowed to grow.

2.3 Identification

For identifying the diatoms, the frustules were cleaned and subjected to acid treatment [2]. The treated samples were studied under light microscope for identification. Scanning electron microscope analysis was also done to confirm the identification of the diatoms. The diatoms obtained through laboratory pure cultures were identified by consulting various literatures [15, 16, 17, 18], monograph [19] and books [2, 20, 21, 22].

3. Results

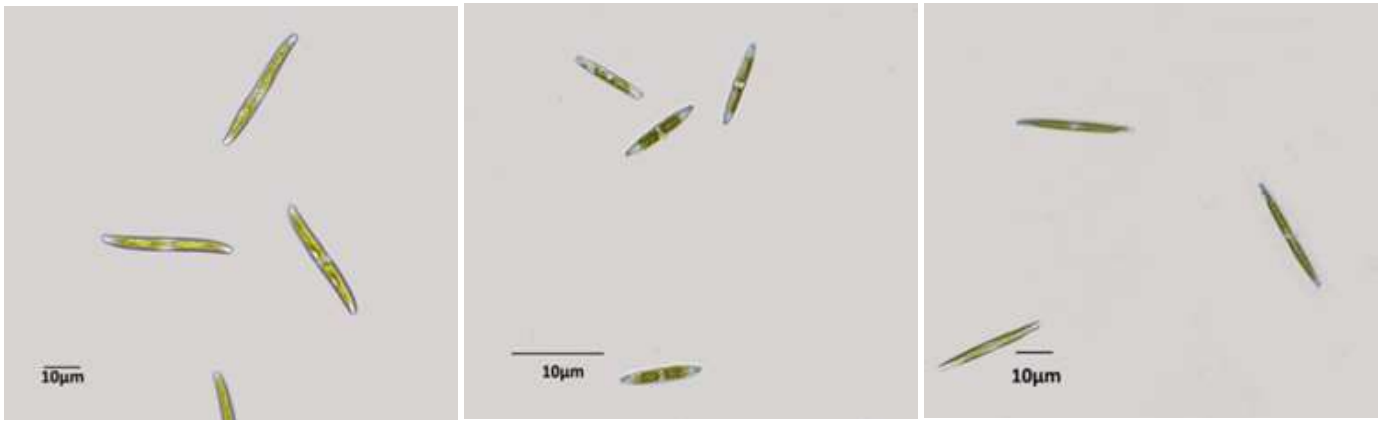
From the microalgal samples collected, thirty four species of diatoms were identified (Table 1 and 2).

Table 1: Size, shape and number of striae of the diatoms identified

Sl. No.	Species	Shape	Size		Number of striae in 10µm
			Length	Width	
1	*Nitzschia scalpelliformis*	Sigmoid	60- 80	4-7	27- 34
2	*Nitzschia amphibia*	Lanceolate	14- 37	5-6	16- 18
3	*Nitzschia angustata*	Lanceolate	45- 50	4-5	42- 45
4	*Nitzschia minuta*	Lanceolate	35- 40	4-5	36- 38
5	*Nitzschia perminuta*	Lanceolate	15- 20	3- 4	88- 90
6	*Nitzschia balcania*	Lanceolate	46- 51	4-5	27- 30
7	*Nitzschia inconspicua*	Elliptical	13- 17	2-5	18- 25
8	*Nitzschia palea*	Linear lanceolate	19- 28	2-4	30- 36
9	*Nitzschia linearis*	Linear- lanceolate	80- 110	3-6	30- 36
10	*Navicula anglica*	Naviculoid	15- 21	7-9	12- 20
11	*Navicula ingenua*	Naviculoid	15-18	3-4	-
12	*Navicula radiosa*	Lanceolate	45-52	5- 10	9-11
13	*Navicula tripunctata*	Linear- lanceolate	20-45	5-10	9-12
14	*Navicula kotschyi*	Naviculoid	18-19	4-5	20-24
15	*Navicula rostellata*	Linear- lanceolate	24- 32	6-8	12-14
16	*Sellaphora seminulum*	Linear- elliptic	10- 15	4-5	20-22
17	*Achnanthidium exiguum*	Elliptical- lanceolate	15-17	4-6	28-34
18	*Synedra acus*	Linear	120-130	4-5	13-15
19	*Lemnicola hungarica*	Linear- elliptical	20- 30	4-8	18-24
20	*Diadesmis confervacea*	Elliptical	14- 18	6-7	18-25
21	*Diadesmis gallica*	Linear- elliptical	7-15	6-8	10-15
22	*Encyonema nicafei*	Half elliptic	40-70	12-19	9-10
23	*Surirella robusta*	Cuneate	95-110	8-16	-
24	*Pinnularia brevicostata*	Rectangular	80-106	9-12	8-10
25	*Pinnularia gibba*	Linear- lanceolate	60-90	11-14	10-12
26	*Pinnularia nodosa*	Linear	45-80	7-9	9-11
27	*Gomphonema tumens*	Linear	23-34	3-4	13-14
28	*Stauroneis lauenburgiana*	Linear- elliptical	25-37	6-10	16-20
29	*Stauroneis anceps*	Linear- lanceolate	28-56	10-12	18-20
30	*Eunotia zygodon*	Linear with undulate dorsal margin	18-34	5-7	14-16
31	*Eunotia clavata*	Linear with undulate dorsal margin	26-52	5-9	14-18
32	*Eunotia incisa*	Linear with convex dorsal margin	12-32	3-5	16-21
33	*Aulacoseira granulata*	Cylindrical	2-4	-	-
34	*Cymbella cymbiformis*	Lanceolate	32-54	7-9	8-10

Table 2: Systematic position of identified diatoms

Sl. No.	Class	Order	Family	Genus	Species
1	Bacillariophyceae	Bacillariales	Bacillariaceae	*Nitzschia*	*Nitzschia scalpelliformis*
2					*Nitzschia amphibia*
3					*Nitzschia angustata*
4					*Nitzschia minuta*
5					*Nitzschia perminuta*
6					*Nitzschia balcanica*
7					*Nitzschia inconspicua*
8					*Nitzschia palea*
9					*Nitzschia linearis*
10		Naviculales	Naviculaceae	*Navicula*	*Navicula anglica*
11					*Navicula ingenua*
12					*Navicula radiosa*
13					*Navicula tripunctata*
14					*Navicula kotschyi*
15					*Navicula rostellata*
16			Sellaphoraceae	*Sellaphora*	*Sellaphora seminulum*
17			Diadesmidaceae	*Diadesmis*	*Diadesmis confervacea*
18					*Diadesmis gallica*
19			Pinnulariaceae	*Pinnularia*	*Pinnularia brevicostata*
20					*Pinnularia gibba*
21					*Pinnularia nodosa*
22			Stauroneidaceae	*Stauroneis*	*Stauroneis lauenburgiana*
23					*Stauroneis anceps*
24		Achnanthales	Achnanthaceae	*Achnanthidium*	*Achnanthidium exiguum*
25				*Lemnicola*	*Lemnicola hungarica*
26		Cymbellales	Cymbellaceae	*Encyonema*	*Encyonema nicafei*
27				*Cymbella*	*Cymbella cymbiformis*
28			Gomphonemataceae	*Gomphonema*	*Gomphonema tumens*
29				*Eunotia*	*Eunotia zygodon*
30					*Eunotia clavata*
31					*Eunotia incisa*
32		Surirellales	Surirellaceae	*Surirella*	*Surirella robusta*
33	Fragilariophyceae	Fragilariales	Fragilariaceae	*Synedra*	*Synedra acus*
34	Coscinodiscophyceae	Aulacoseirales	Aulacoseiraceae	*Aulacoseira*	*Aulacoseira granulata*



A **B** **C**

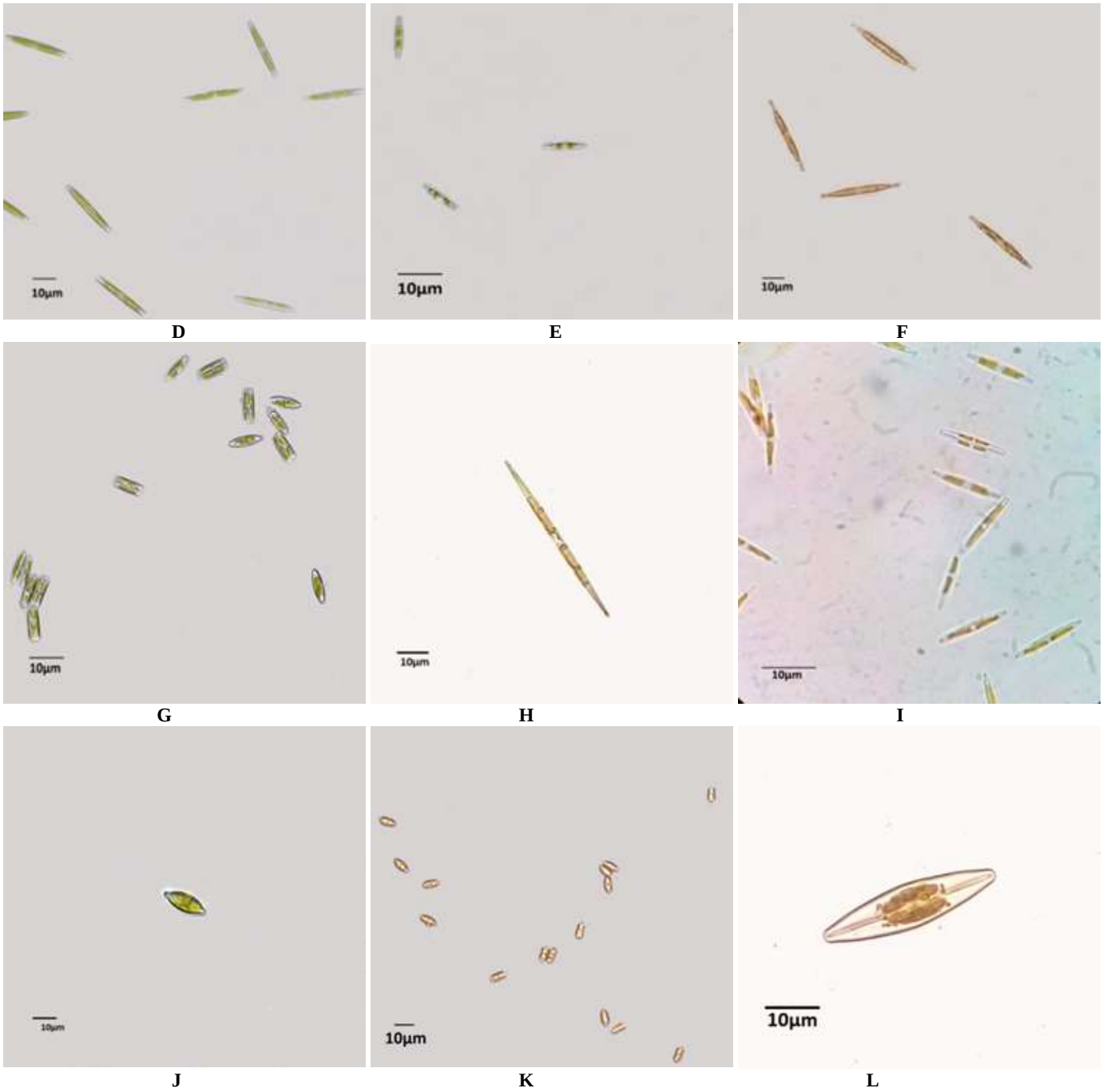


Fig 1: A- *Nitzschia scalpelliformis*; B- *Nitzschia amphibia*; C- *Nitzschia angustata*; D- *Nitzschia minuta*; E- *Nitzschia perminuta*; F- *Nitzschia balcanica*; G- *Nitzschia inconspicua*; H- *Nitzschia linearis*; I- *Nitzschia palea*; J- *Navicula anglica*; K- *Navicula ingenua*; L- *Navicula radiosa*

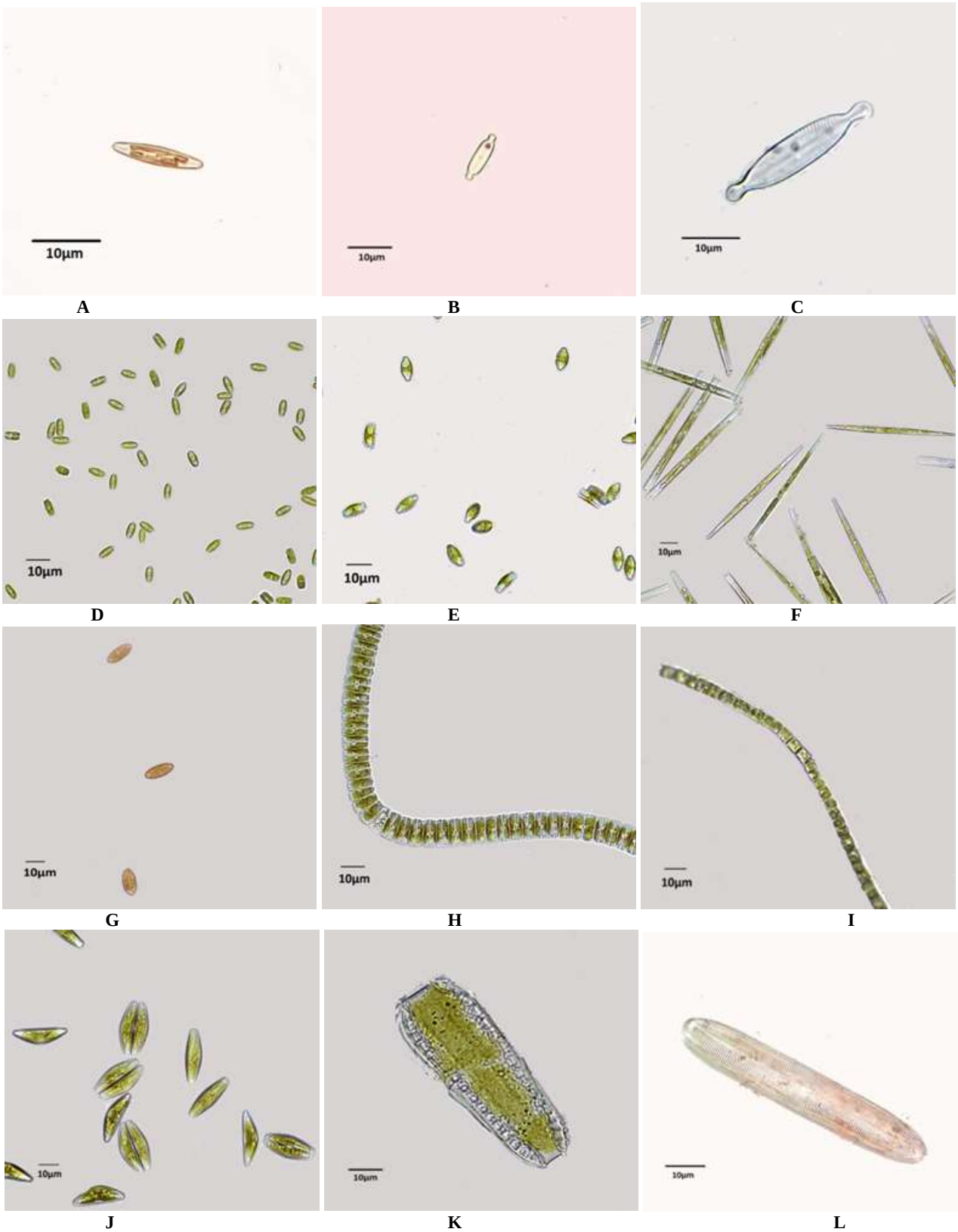


Fig 2: A- *Navicula tripunctata*; B- *Navicula kotschyi*; C- *Navicula rostellata;* D- *Sellaphora seminulum*; E- *Achnanthidium exiguum*; F- *Synedra acus*; G- *Lemnicola hungarica*; H- *Diadesmis confervacea*; I- *Diadesmis gallica*; J- *Encyonema nicafei*; K- *Surirella robusta*; L- *Pinnularia brevicostata*

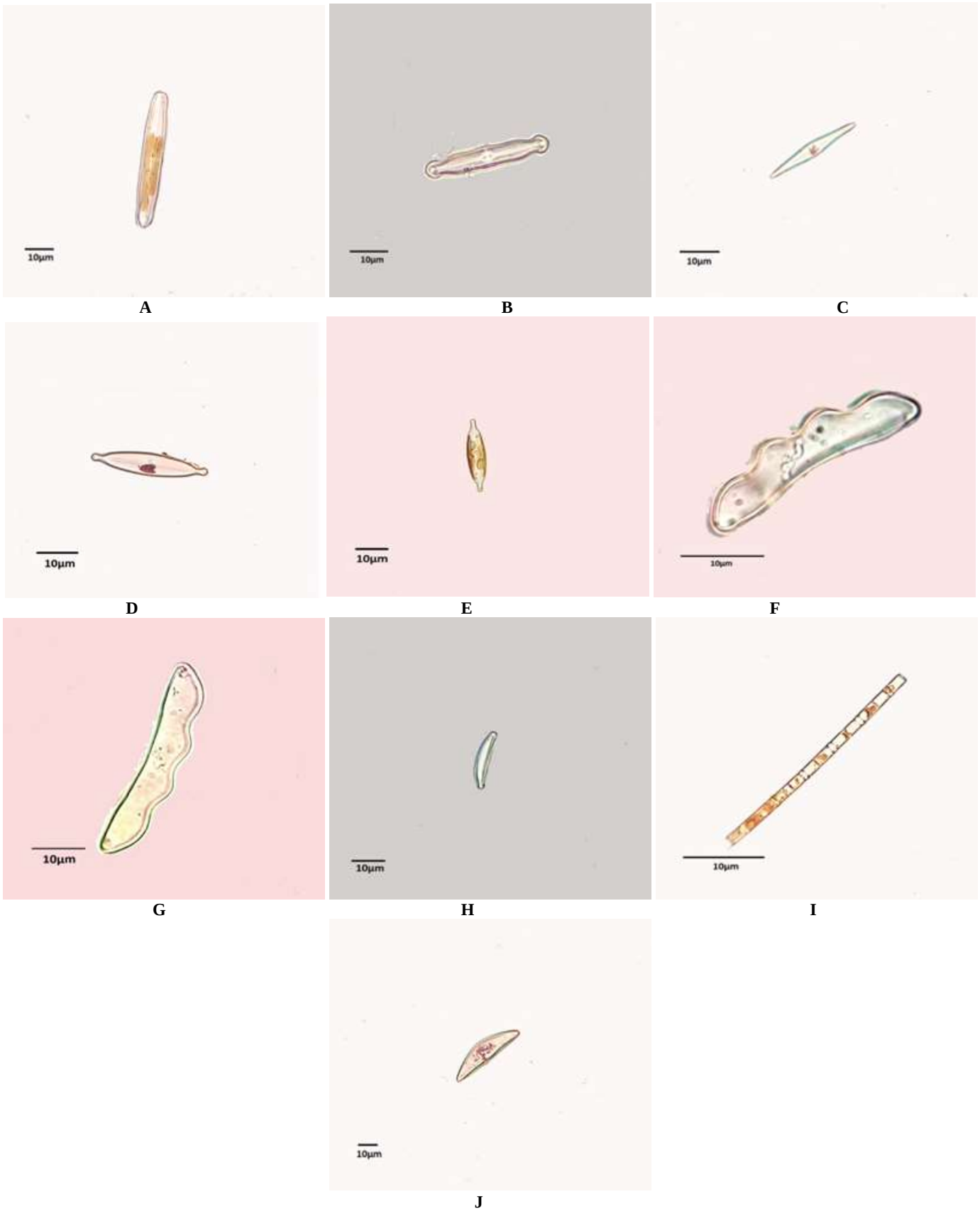


Fig 3: A- *Pinnularia gibba*; B- *Pinnularia nodosa*; C- *Gomphonema tumens*; D- *Stauroneis lauenburgiana*; E- *Stauroneis anceps*; F- *Eunotia zygodon*; G- *Eunotia clavata*; H- *Eunotia incisa*; I- *Aulacoseira granulate*; J- *Cymbella cymbiformis*

4. Discussion

Among the phytoplankton, diatoms are one of the dominating group of organisms. Diatoms are among the most productive and environmentally flexible eukaryotic microalgae on the planet [23]. Notably, United States Department of Energy (USDOE) had collected over 3,000 strains of oil-producing organisms, which after screening, isolation and characterization efforts, the collection was narrowed down to 300 species, mostly green algae and diatoms. 60% of these were diatoms, which were chosen based on criteria such as high growth rates and lipid yields, tolerance of harsh environmental conditions, and performance in large-scale cultures [12].

Microalgae composed of one diatom and one or more flagellates, assure a better balance in essential nutrients [24]. Microalgae feeds are currently used mainly for the culture of larvae and juvenile shell and finfish, as well as for raising the zooplankton required for feeding of juvenile animals [25]. They are required for larval nutrition during a brief period, either for direct consumption in the case of molluscs and peneid shrimp or indirectly as food for the live prey, mainly rotifers, copepods and *Artemia* nauplii, which in turn are used for crustaceans and fish larvae feeding [3, 7]. Several diatoms routinely cultured in mollusc hatcheries lead to good performances in the aspects of larval survival and metamorphosis (*Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *Chaetoceros calcitrans*, *Chaetoceros calcitrans* forma *pumilum*, *Chaetoceros tenuissimus*) [26].

Diatoms are excellent lipid accumulators with a substantial portion of the cells' volume is occupied by lipid droplets that accumulated rapidly, especially under Si limitation [27]. A wide variety of TAG composition has been identified in diatoms, with fatty acid (FA) side-chains ranging from 14:0, 16:0, 16:1 to 16:1, 20:5, 22:6 [28]. Also diatoms are unusually high in EPA which is not prevalent in chlorophytes [29, 30, 31]. EPA performs many vital functions in biological membranes and serves as a precursor of a variety of lipid regulators in cellular metabolism [32]. Extracellular polysaccharides (EPS) produced by diatoms are responsible for the attachment of the cells and that well attached *Navicula* sp. produced the largest number of post-larvae with the highest survival rate [33].

Using even very small amounts of microalgal biomass can positively affect the physiology of animals by improved immune response, resulting in growth promotion, disease resistance, antiviral and antibacterial action, improved gut function, probiotic colonization stimulation, as well as by improved feed conversion, reproductive performance [34]. Algal diets rich in polyunsaturated fatty acids and with high levels of carbohydrate are reported to produce the best growth for juvenile oysters *(Ostrea edulis)* [35]. and larval scallops *(Putinopecten yessoensis)* [36], while high dietary protein provides best growth for juvenile mussels *(Myths trossulus)* [37].

In the present study 34 species of diatoms were identified, out of which 16 species could be maintained in the laboratory. *Nitzschia* sp. dominated the diatom sample collected. *Nitzschia* sp. can be used as live feed for abalone *Haliotis iris* which produced rapid growth rate of post larvae of these organisms and showed high digestion efficiency too [38]. *Nitzschia* sp. is determined to be good producer of EPA [30]. According to the results of various studies *Navicula* sp. can be used as food for sea scallop larvae [39]. *Nitzschia* sp. is capable of accumulating substantial amounts of fat in the biomass [40]. The fresh water diatom *Nitzschia palea* is reported to have 20- 25% lipid in its total proximate composition [41] *Navicula* cf. *lenzii* has reported to support high post-larval growth and a high survival rate of *Haliotis discus* [42]. *Nitzschia* sp., *Navicula britannica*, *N. ramosissima*, *Navicula* sp. and *Nitzschia ovalis* were used to culture *Haliotis iris* post-larvae [38]. The properties of algae lipids-based biodiesel is largely determined by the structure of fatty acids feedstock [43]. Studies indicate that the long chain fatty acids with carbon atoms number between C12 and C24 represent suitable feedstock for biodiesel [44]. The main raw material for diatom-based biodiesel is the enormous range of glycerides (monoglycerides, diglycerides and triglycerides) which are hybrid compounds of fatty acids and glycerol [45].

5. Acknowledgement

The first author acknowledges the financial assistance as JRF, Cochin University of Science and Technology. The authors are thankful to the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology for providing the necessary research facilities.

6. References

1. Mann DG, Droop SJM. Biodiversity, biogeography and conservation of diatoms. In Biogeography of freshwater algae. Springer Netherlands, 1996, 19.
2. Taylor J C, Harding W R, Archibald C G M. A methods manual for the collection, preparation and analysis of diatom samples. WRC Report No TT 281/07. Water Research Commission, Pretoria, 2007, 11-23.
3. Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. J. Appl. Phycol. 2000; 12(3-5):527-534.
4. Brown MR. Nutritional value and use of microalgae in aquaculture. In: Cruz- Suarez, L.E., Ricque- Marie, D., Tapia- Salazar, M., Gaxiola- Cortes, M.G., Simoes, N. (Eds.). Avances en Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3 al 6 de Septiembre del. Cancun, Quintana Roo, Mexico, 2002.
5. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J. Biosci. Bioeng. 2006; 101:87-96.
6. Gouveia L, Batista AP, Sousa I, Raymundo A, Bandarra NM. Microalgae in novel foods products. In KN Papadopoulos (Ed.) Food Chemistry Research Developments (ed.). Nova Science Publishers, 2008, 75-111.
7. Brown MR, Jeffrey SW, Volkman JK, Dunstan GA. Nutritional properties of microalgae for mariculture. Aquaculture. 1997; 151:315-331.
8. Wilkenfeld JS, Lawrence AL, Kuban FD. Survival, metamorphosis and growth of penaeid shrimp larvae reared on a variety of algal and animal foods. Journal of World Mariculture Society. 1984; 15:31-49.
9. Langdon CJ, Waldock MJ. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. JMBA. 1981; 61(2):431-448.
10. Ehteshami F, Christianus A, Rameshi H, Harmin SA, Saad CR. Proximate and fatty acid composition of the gonads of wild versus hatchery-conditioned *Pinctada margaritifera* broodstock. Aquac. Nutr. 2011; 17:675-682.

11. Trider DJ, Castell JD. Effect of dietary lipids on growth, tissue composition and metabolism of the oyster (*Grassosirea virginica*). J. Nutr. 1980; 110(7):1303-1309.
12. Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US Department of Energy's aquatic species program: biodiesel from algae. National Renewable Energy Laboratory, 1998, 328.
13. Lombardi A, Wangersky PJ. Particulate lipid class composition of three marine phytoplankters *Chaetoceros gracilis*, *Isochrysis galbana* (Tahiti) and *Dunaliella tertiolecta* grown in batch culture. Hydrobiologia. 1995; 306:1-6.
14. Andersen RA. Algal culturing techniques. Elsevier Academic press, London, 2005, 578.
15. Gandhi HP. A contribution to ourknowledge of the freshwater diatoms of Partabgarh Rajasthan. J. Indian Botanical Society. 1955; 34(4):307-338.
16. Pareek R, Singh R. Some fresh water diatoms of Galta kund, Jaipur, India. J. Soil Sci. Environ. Manage. 2011; 2(4):110-116.
17. Borgohain D, Tanti B. Diversity of freshwater diatoms from few silica rich habitats of Assam, India. J. Research in Biology. 2014; 4(1):1162-1173.
18. Narayan P, Barupal GK. A Systematic Account of the Fresh Water Diatom from Kaylana Lake of Jodhpur District, Rajasthan (India). Int. J. Recent Sci. Res. 2015; 6(4):3435-3439.
19. Joseph KJ, Saramma AV. Marine benthic microalgae of India- A monograph, OASTC Marine Benthos- 03. Ocean and Atmosheric Science and Technology Cell, Cochin University of Science and Technology (CUSAT), Kochi, India, 2011, 292.
20. Heurck HF. A Treatise on the Diatomaceae. William Wesley and Son, London, 1896, 380-408.
21. Desikachary TV. Atlas of Diatoms-Marine diatoms of the Indian Ocean region, Vol. VI. Madras Science Foundation, Madras, 1989, 1809.
22. Wehr JD, Sheath RG. (Eds.). Freshwater algae of North America: ecology and classification. Elsevier, 2003.
23. Merz CR, Main KL. Microalgae (diatom) production- The aquaculture and biofuel nexus. In Oceans-St. John's, 2014, 1.
24. Coutteau P, Sorgeloos P. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve mollusks: an international survey. J. Shellfish Research. 1992; 11(2):467- 476.
25. Benemann JR. Microalgae aquaculture feeds. J. Appl. Phycol. 1992; 4:233-245.
26. Ponis E, Probert I, Veron B, Mathieu M, Robert R. New microalgae for the Pacific oyster *Crassostrea gigas* larvae. Aquaculture. 2006; 253(1-4):618-627.
27. Hildebrand M, Davis AK, Smith SR, Traller JC, Abbriano R. The place of diatoms in the biofuels industry. Biofuels. 2012; 3(2):221-240.
28. Yu ET, Zendejas FJ, Lane PD, Gaucher S, Simmons BA, Lane TW, *et al*. Triacylglycerol accumulation and profiling in the model diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* (Baccilariophyceae) during starvation. J. Appl. Phycol. 2009; 21(6):669.
29. Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, *et al*. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The Plant Journal. 2008; 54(4):621-639.
30. Chen GQ, Jiang Y, Chen F. Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change. Food Chem. 2008; 109(1):88-94.
31. Mansour MP, Frampton DM, Nichols PD, Volkman JK, Blackburn SI. Lipid and fatty acid yield of nine stationary-phase microalgae: applications and unusual C24–C28 polyunsaturated fatty acids. J. Appl. Phycol. 2005; 17(4):287-300.
32. Gill I, Valivety R. Polyunsaturated Fatty Acids, Part 1: Occurrence, Biological Activities and Applications. Trends in Biotechnology. 1997; 15:401-409.
33. Daume S, Krsinich A, Farrell S, Gervis M. Settlement, early growth and survival of *Haliotis rubra* in response to different algal species. J. Appl. Phycol. 2000; 12:479- 488.
34. Harel M, Clayton D. Feed formulation for terrestrial and aquatic animals. US Patent 20070082008 (WO/2004/080196), 2004.
35. Enright CT, Newkirk GF, Craigie JS, Castell JD. Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros calcitrans* Schutt of varied chemical composition. J. Exp. Mar. Biol. Ecol. 1986; 96:15-26.
36. Whyte JNC, Boume N, Hodgson CA. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. Aquaculture. 1989; 78:333-347.
37. Kreeger DA, Langdon CJ. Effect of dietary protein content on growth of juvenile mussels, *Mytilus trossulus* (Gould 1850). Biological Bulletin. 1993; 185:123-139.
38. Kawamura T, Roberts RD, Nicholson CM. Factors affecting the food value of diatom strains for post-larval abalone *Haliotis iris*. Aquaculture. 1998; 160(1-2):81-88.
39. Gouda R, Kenchington E, Hatcher B, Vercaemer B. Effects of locally-isolated micro-phytoplankton diets on growth and survival of sea scallop (*Placopecten magellanicus*) larvae. Aquaculture. 2006; 259:169-180.
40. Gladue RM, Maxey JE. Microalgal feeds for aquaculture. J. Appl. Phycol. 1994; 6(2):131-141.
41. Krishnan A, Joseph A, Ganga RS. The diatom *Nitzschia palea* as a potential live feed for ornamental fish and molluscan hatchery. In: Marine Biodiversity and Bioprospecting for Sustainable Livelihood (Joseph, A. & Philip, R., Eds.) The Directorate of Public Relations and Publications, Cochin University of Science and Technology, Cochin, 2016, 286-301.
42. Gordon N, Neori A, Shpigel M, Lee J, Harpaz S. Effect of diatom diets on growth and survival of the abalone Haliotis discus hannai postlarvae. Aquaculture. 2006; 252(2-4):225-233.
43. Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Processing Technol. 2005; 86:1059-1070.
44. Basha SA, Jebaraj KRGS. A review on biodiesel production, combustion, emissions and performance. Renew. Sustain. Energy Rev. 2009; 13:1628-1634.
45. Ramachandra TV, Mahapatra DM, Karthick B. Milking Diatoms for Sustainable Energy: Biochemical Engineering versus Gasoline-Secreting Diatom Solar Panels. Ind. Eng. Chem. Res. 2009; 48:8769-8788.