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Periphyton-based Apparatus For Improving Aquaculture Production and Method
Preparing Thereof

Field of Invention

5 The present invention relates to production of a portable and recyclable apparatus which is used to improve aquaculture production by utilizing specific or mixture of periphyton species in the system. The present invention particular includes a method to culture purified periphyton.

10 **Background of Invention**

Current survival rate of shrimp larvae in shrimp hatcheries nationwide ranged from 30-60%. Higher survival rate can only be obtained if water is exchanged 90-100% every day. However, with limited availability of clean sea-water and widespread disease causing agents, changing water in culture tanks poses high risk to the larvae. Thus
15 method to maintain high water quality that does not involve frequent water exchange is needed in order to improve larviculture technology

Another cause for low survival in larval rearing sector is the high larval mortality due to cannibalism. Some aquatic animals such as shrimps are cannibalistic. Cannibalism can
20 be high when the larval sizes are not uniform. Thus, in a hatchery tank with clear water,

cannibalism can run high due to absence of refuge for the weak/smaller shrimps/fish to escape. Availability of hiding places would offer shelter for the larvae.

In addition, the growth of the larvae can also be increased with additional supply of
5 natural feed such as microalgae, either in planktonic or attached form, the later is referred to as periphyton. Researches have shown that shrimp/fish larvae supplemented with microalgae showed significantly higher growth and survival since microalgae have high content of essential fatty acid eicosapentaenoic acid (EPA-20: 5n-3) and docosahexaenoic acid (DHA- 22 : 6 n-3).

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According to prior art, US patent 5476787, planktonic microalgae is utilized for removing inorganic nitrogen-based impurities from water. Said invention is useful in cleaning water effluent from sewage treatment facilities or plant. Patent US5985147 disclosed an integrated system to purify water, produce pulp and improve soil quality by
15 using microalgae. Patent US2006024404 utilizes microalgae culture as high nutrient-contained feed for aquatic animals. While patent WO2004032610 relates to method using culture of microrustacea, *Parvocalanus* sp. nauplii, as the feed for the larviculture to enhance the aquaculture production. However, so far, no invention being disclosed as a portable and integral microalgae culture system or apparatus for improving
20 larviculture or aquaculture by not only providing high nutrient-contained feed but also functions as refuge and a water treatment system.

Summary of the Invention

The present invention is directed to a method to manufacture periphyton based apparatus for improvement of aquaculture therefore enhance the harvest obtained.

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The present invention also related to an apparatus which can be applied in aquaculture to maintain water quality by removing pollutants in the water thus greatly reduce the rate of water changes and limit the contact of aquatic animals with disease causing agents.

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The present invention further provides an apparatus that functions as refuge for small or weak aquatic animals by avoiding the cannibalism therefore elevate the survival rate of these animals and increase the harvest.

15 Another objective of the present invention is to disclose an apparatus that provides high nutrient natural feed to aquatic animals constituted from specific periphyton species or mixture of periphyton species.

Brief Description of the Figure

20 Figure 1 is the graph shows the percent of reduction of ammonia in water by different periphyton species.

Figure 2 is the graph shows the percent of reduction of nitrite in water by different periphyton species.

5 Figure 3 is the graph shows the percent of reduction of soluble reactive phosphorus in water by different periphyton species.

Figure 4 is the graph shows percentage of survival of shrimp larvae in rearing tank with the presence of the apparatus without water change for
10 duration of 17 days.

Figure 5 is the graph shows the percentage of survival of shrimp larvae in rearing tank under the reverse salinity stress test.

15 **Detail Description of the Invention**

The term microalgae used in the specification thereafter is a general term to denote minute algal species. Microalgae can be planktonic or attached, known as periphyton.

The periphyton types of microalgae attach to the surface of suitable material in the aquatic environment for growth. Periphytons have different names depending on the
20 kind of substrates on which they are attached such as plants (epiphytic), sediments (epidemic), rocks (epilithic), sand (episammic) or animals (epizootic).

Isolation, Purification and Culture of Periphyton

The present invention includes a method to culture purified periphyton before utilizing the obtained purified periphyton for manufacturing of the microalgae-based apparatus to improve the quality of aquaculture. The abovementioned method comprising the steps of collecting and cleaning of periphyton mixed samples; concentrating said periphyton mixed samples; washing said concentrated periphyton; culturing said washed periphyton under suitable condition to obtain first culture; obtaining purified periphyton species by inoculating the interested species from said first culture; culturing said inoculated periphyton species under suitable condition to obtain the purified second culture.

In one of the embodiments of the present invention, samples of periphyton are collected from the natural environment such as brackish-waters. The periphyton samples collected are normally mixed samples constituted from different species of periphyton. Therefore cleaning and purification steps are required to get a purified species from the mixed sample collected before utilizing the periphyton species for manufacturing of said apparatus. The collected mixed samples are at first re-suspended in sterilized filtered seawater and centrifuged between 1000-3000 rpm for 5 to 20 minutes. The sterilized filtered water is prepared by autoclaving seawater that being filtered through 0.2 μ m Milipore filter. The re-suspended periphyton samples are then centrifuged at

1000-3000 rpm for 5 to 20 minutes and the aqueous phase are disposed. This is followed by washing the periphyton samples with autoclaved filtered seawater. These centrifugation and washing steps are repeated 3 times to reduce the unwanted materials. The washed periphyton samples are re-suspended in autoclaved filtered waters before

5 culturing the mixed samples. The washed periphyton are streaked onto solid microalgae medium for 4 to 10 days under temperature of 20°C to 35°C to obtain the first culture includes all the periphyton species available in the mixed samples used. After the incubation period, each cell colony of the periphyton species in first culture is inoculated respectively from the first culture to separate culture medium by selecting

10 single cell colonies in the first culture. The inoculated cell colonies are further cultured to obtain the second culture which composes of specific purified periphyton in each separate culture medium. The optimum condition for culturing the second culture is 4 to 10 days under temperature of 20°C to 35°C. During the culturing process of the second culture, the growth of each specific periphyton species are monitored closely to ensure

15 the culture is axenic and pure without contamination of other species. If the culture was impure, the process is repeated until pure cultures are obtained. To ascertain the purity and species of the periphyton in each of the second culture, identification of the species is done by preparing either fresh or permanent slides and checked under high resolution microscope. The whole isolation and purification process are conducted under sterile

20 condition. To avoid cross-contamination, in the preferred embodiment of the present invention, two different sets of stocks, the undisturbed stock and the starter stock, are

maintained for each species of periphyton. The starter stock is frequently used to initiate mass propagation of the interest species and said mass propagation may involve more than one type of species in the culture tank depends against the requirement. Therefore the starter stock is subjected to cross-contamination of other species. Instead the
5 undisturbed stock is used to maintain pure lines of said species.

To be noted that the above mentioned periphyton culture is preferably used on the genus *Oscillatoria* sp., *Navicula* sp., *Cymbella* sp., and *Amphora* sp. Nonetheless said method of the present invention is described in its preferred embodiments, it is to be understood
10 that the present invention may be embodied in other specific forms and is not limited to the sole embodiment described above.

Manufacturing of the Aquaculture Improving Apparatus

Another embodiment of the present invention involves a method to manufacture an
15 apparatus for aquaculture improvement. Following the preferred procedures, the method includes the steps of providing at least one suitable construct a purified periphyton culture container; growing said purified periphyton on the surface of said suitable construct until its surface coated with said periphyton; and removing said coated construct from said culture container for use.

In accordance to the embodiment of the present invention, the construct are moulded or prepared in a preferred shape before providing into the culture container. In order to have the apparatus perform in optimum condition, shape of the construct is important as regards the surface area available for the purified periphyton attached to. In the most preferred embodiment, said construct is prepared in the form of hollow tube or pipe as to larger surface area provided and convenience to be placed into tanks or ponds where the aquaculture activities carried out.

Further in accordance to the preferred embodiment of the apparatus, material for preparing or moulding the construct is very important as regards to survivability of the periphyton species attach on the construct. Exemplary materials suitable for preparing the construct are plastic, wood, bamboo, scrubs or tiles. It is most preferable that the construct is made of plastic especially polyvinylchloride which is capable to resist erodent aquatic environment and being recycled for re-utilization once the periphyton species depleted on the construct.

Of importance to propagate or culture the interest periphyton species, the physical parameters of the culture tank or container have to be maintained in certain narrow range for the cultured periphyton grow exuberantly. For the purpose of obtaining the optimum condition in the culture tank, the ratio of the purified stock culture to culture medium is 1:20 to 1:100. The other physical parameters to achieve the optimum

condition include adequate light intensity, aeration, salinity of 15 to 35 ppt, pH of 5.5 to 8.5 and temperature of 20°C to 35°C. Furthermore, the constructs have to be soaked in the culture tank or container for duration of 5 to 8 days for the interest purified periphyton species fully coat or attach onto the surface of the construct.

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In accordance with one of the main features of the present invention, different periphyton species are used respectively or in combination to produce synergistic effect. In the most preferred embodiment, the periphyton species is selected from a group of periphyton species comprising genera *Oscillatoria* sp., *Navicula* sp., *Cymbella* sp., and

10 *Amphora* sp. For example, periphyton species derive from genus *Oscillatoria* sp. is of importance in reducing those toxicant substances in the aquatic environment such as ammonia, nitrite, reactive phosphorous and the same thus providing an appropriate aquatic environment for its own survival as well as for aquatic animals. Another example is regarding to the use of diatoms type periphyton such as *Navicula* sp.,
 15 *Cymbella* sp., and *Amphora* sp. in catering nutrients to the aquatic animals to enhance their growth and boost up their health status. It is found in practice that the diatoms types periphyton are cultured in the same tank or container so that the construct can be coated with different types of diatoms periphyton at the same time which not only ease the process of manufacturing but also enable different types of diatoms periphyton
 20 complement each other in providing nutrients to the aquatic animals once the apparatus being used for improving the aquaculture. In like manner, the constructs can be soaked

into culture tank or container with mixed culture of genus *Oscillatoria* sp. and diatoms periphyton. Similarly, the constructs can be prepared in culture tank or container with each periphyton species respectively.

5 In another embodiment of the present invention, different constructs with different types of periphyton species are joined together to form an integral system that provides different desired improvement for the aquaculture activities. In this embodiment, each single construct acts as subassembly for said integral system.

10 As hereinbefore set forth, the apparatus coated with different types of periphyton species are transferred to the aquaculture farm such as larval rearing tank and placed inside the tank or pond. The apparatus improves water quality in the aquaculture tank or pond by maintaining low level of ammonia and nitrite levels. In addition, it also provides natural food supplement consisting of beneficial diatoms that enhances growth
15 and health of the aquatic animals. The apparatus also give refuge to the aquatic animals to minimize cannibalism especially among shrimp and fish larvae. Therefore, the apparatus can greatly enhance quality and quantity of the output of the aquaculture industry.

20 It is to be understood that the present invention may be embodied in other specific forms and is not limited to the sole embodiment described above. However

modification and equivalents of the disclosed concepts such as those which readily occur to one skilled in the art are intended to be included within the scope of the claims which are appended thereto.

5 Example 1

Selected periphyton as natural foods (the diatoms) has good nutritional values with protein ranging from 30% - 38%, lipid from 26 – 33% and carbohydrate from 31 to 46% (Table 1). In addition, the diatoms also have adequate essential fatty acids for high quality growth of shrimp/fish larvae. Table 1 shows the proximate composition (% dry weight) of different periphyton (diatom) species. Table 2 shows the fatty acid (% total fatty acid) composition of periphyton species compared to commonly used shrimp larval feed.

Diatoms	Parameters				
	Protein	Lipid	Carbohydrate	Moisture	Ash
<i>Cymbella</i>	38.25±1.02	30.50±1.77	30.77±0.75	0.38±0.009	0.08±0.002
<i>Navicula</i>	29.60±0.12	25.46±0.47	44.59±0.42	0.28±0.002	0.086±0.0006
<i>Amphora</i>	30.89±0.70	33.05±2.10	35.38±1.78	0.54±0.01	0.136±0.003

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Table 1

Parameters	Periphyton			Normal larval feed	
	<i>Navicula</i>	<i>Cymbella</i>	<i>Amphora</i>	<i>Artemia</i>	Artificial diet
SFA	57.41	55.89	30.01	13.52	16.40
MUFA	27.72	25.16	30.77	45.80	20.30
PUFA	4.50	4.40	7.44	20.80	58.43
DHA	2.00	3.00	2.00	Nd	8.07
EPA	8.00	5.01	15.09	Nd	0.64
Total n-3	10.07	8.19	20.35	13.17	33.70
Total n-6	2.85	2.84	10.24	7.64	2.27

Table 2

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SFA = saturated fatty acids

MUFA = monounsaturated fatty acids

PUFA = polyunsaturated fatty acids

DHA = docosahexaenoic acid (22 : 6 n-3).

10 EPA = eicosapentaenoic acid (20: 5n-3)

Example 2

In terms of water quality improvements, *Oscillatoria* significantly ($p < 0.05$) reduced total ammonia nitrogen by approximately 90%, nitrite by 91% and soluble reactive phosphorus by 83%. Similarly other types of periphyton also significantly reduced the toxic compounds such as ammonia (Figure 1), and nitrite (Figure 2), and improved the water quality in the larval rearing tanks. In addition, the periphyton species also reduced the concentration of soluble reactive phosphorus (Figure 3) and decrease the likelihood of water turning into green soup, which is undesirable for larval rearing activities.

Example 3

The aquaculture improving apparatus shows significantly higher survival compared to those without rings (Figure 4). In fact, larvae reared with said apparatus show significantly higher growth rate and are more tolerant to stress. When exposed to stress
5 test, those larvae cultured in tanks with the aquaculture improving apparatus show significantly higher survival rate (Figure 5), indicating the booster effects of the aquaculture improving apparatus on the health and robustness of the larvae produced. In conclusion, the larvae live under the effect of the aquaculture improving apparatus are healthier and have high resistance to stress.

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The present disclosure includes as contained in the appended claims, as well as that of the foregoing description. Although this invention has been described in its preferred form with a degree of particularity, it is understood that the present disclosure of the preferred form has been made only by way of example and that numerous changes in
15 the details of construction and the combination and arrangements of parts may be resorted to without departing from the spirit of the invention.

Claims:

1. A method to culture purified periphyton comprising the steps of:
 - (a) collecting and cleaning of periphyton mixed samples;
 - 5 (b) concentrating said periphyton mixed samples;
 - (c) washing said concentrated periphyton;
 - (d) culturing said washed periphyton under suitable condition to obtain first culture;
 - 10 (e) obtaining purified periphyton species by inoculating the species from said first culture;
 - (f) culturing said inoculated periphyton species under suitable condition to obtain the purified second culture; and
 - (g) identifying said purified species of the second culture to determine their use.
- 15 2. A method as claimed in claim 1, wherein said concentrating said periphyton mixed samples is done by using centrifugation.
3. A method as claimed in claim 1, wherein said suitable condition is at temperature of 20°C to 35°C for 4 to 10 days.
- 20 4. A method as claimed in claim 1, wherein said periphyton species are genus *Oscillatoria* sp., *Navicula* sp., *Cymbella* sp., and *Amphora* sp.
5. A method as claimed in any of the preceding claim is conducted under sterilized condition.
- 25 6. A method to manufacture an apparatus for aquaculture improvement, comprising the steps of:

- (a) providing at least one suitable construct into purified periphytons culture container;
 - (b) growing said purified periphyton on the surface of said suitable construct until its surface coated with said periphyton; and
 - 5 (c) removing said coated construct from said culture container for use.
7. A method as claimed in claim 5, wherein said culture container comprises at least one type of said purified periphyton.
- 10 8. A method as claimed in claims 5 and 6, wherein said periphyton is selected from a group consisting of genus *Oscillatoria* sp., *Navicula* sp., *Cymbella* sp., and *Amphora* sp.
- 15 9. A method as claimed in claim 5, wherein said construct is plastic, wood, bamboo, scrubs or tiles.
- 20 10. An apparatus for improving aquaculture comprising a hollow construct with surfaces pre-coated with purified periphyton, wherein the periphyton is selected from a group consisting of genus *Oscillatoria* sp., *Navicula* sp., *Cymbella* sp., and *Amphora* sp..
11. An apparatus according to claim 10, wherein the hollow construct is made of plastic, wood, bamboo, scrubs or tiles.