Abstract:

Periphyton is an additional food source in African and Asian brackish and freshwater fish ponds. The present study was a preliminary assessment of periphyton development on artificial substrates in temperate marine ponds. The effects of submersion time, substrate type, water depth, and total or partial sampling methods on the quantity and quality of periphyton collected, were evaluated. Four types of substrate (W: wooden poles, S: smooth fiber-glass strips, m: mosquito screen (1 mm-mesh) and M: garden netting (5 mm-mesh)) were deployed in a marine pond, and periphyton was collected 15 and 30 days later. The total amount of periphyton per substrate unit was collected as one sample or as 5 sub-samples. Results showed that (i) periphyton biomass in a marine pond increased between day 15 and day 30, (ii) more periphyton was collected on mosquito screen than on wooden poles, fiberglass strips and garden netting, (iii) periphyton biomass increased with submersion depth, (iv) sub-sampling leads to an underestimate compared to whole unit sampling, and (v) a correction of periphyton weight must be carried out considering the dissolved inorganic salts present in periphyton samples from marine and brackish ponds. Whole substrate unit sampling using a tube and stopper is recommended to avoid underestimation of periphyton development. Finally, the autotrophic fraction in the periphyton communities was very low compared to periphyton developed on biodegradable substrates in fertilized tropical ponds. Studies on fertilization and use of biodegraded substrates (i.e. long-time submerged wood) are recommended to further optimize periphyton development in temperate marine ponds.

Keywords: Periphyton; Fouling; Artificial substrate; Marine pond; Aquaculture
1. Introduction

Periphyton refers to the entire complex of attached aquatic biota on submerged substrates, including associated non-attached organisms and detritus (van Dam et al., 2002). This assemblage comprises bacteria, fungi, protozoa, phyto and zoo-plankton, benthic organisms and detritus (Azim et al., 2005). It can be used as additional food in aquatic production systems. Aquaculture based on periphyton was originally derived from traditional fishing methods known in Africa as Acadja (Welcomme, 1972) and in Asia as Kathas and Samarahs (Van Dam et al., 2002). Artificial substrates are added into aquatic system to enhance the food availability. This semi-extensive aquaculture system is well known to increase the production of fish (Ramesh et al., 1999; Umesh et al., 1999; Azim et al. 2001a). Although widely tested in freshwater fish culture (Azim et al., 2005), the use of periphyton in brackish or marine waters (van Dam et al., 2002; Huchette and Beveridge, 2005; Khatoon et al., 2007) is limited to shrimp (Bratvold and Browdy, 2001; Moss and Moss, 2004; Arnold et al., 2006) and abalone cultures (Kawamura et al., 2005).

Variation of periphyton quantity and quality depends on a range of factors such as (i) submersion time (Azim and Aseada, 2005), (ii) substrate type (Ramesh et al., 1999; Keshavanath et al., 2001; Azim et al., 2002a), and (iii) light intensity and quality (Kirk 1994; Goldsborough et al., 2005). The latter is strongly influenced by the depth of the substrates (Asaeda and Son 2000). Thus, Azim et al. (2001a, 2003b) waited minimum 2 weeks to allow periphyton to develop on the substrates before stocking fishes. Keshavanath et al. (2001) observed that fish production based on periphyton depends on artificial substrate type and preferred to use bamboo rather than PVC pipes or sugarcane bagasse bundles when culturing masheer (Tor khudree) fingerlings. Azim et al. (2001b, 2002a, 2004a) and Keshavanath et al. (2001) pooled several sub-samples of periphyton collected at equally spaced depths along vertical substrates to analyse the composition of periphyton. This pooled sample was
considered by these authors to represent the mean composition of periphyton developed on substrate, going from the photic zone close to the surface to the aphotic zone above the bottom.

The potential contributions of semi-extensive aquaculture to environmental protection and restoration of coastal areas have been clearly recognised within EU policy. The SEACASE program (Sustainable extensive and semi-extensive coastal aquaculture system in Southern Europe) was started in 2007 to develop sustainable extensive and semi-extensive coastal aquaculture systems in Southern Europe (Conceição et al., 2007). The present SEACASE study is a preliminary assessment of the feasibility to grow periphyton on artificial substrates in temperate marine ponds. The effects of submersion time, substrate type, water depth, and total or partial sampling methods, on the quantity and quality of periphyton collected, were evaluated. The goals of this study were to identify (i) the best periphyton substrate type and (ii) a methodology of periphyton sampling for further studies on periphyton-based marine aquaculture.

2. Materials and methods

2.1. Experimental site and design

The experiment was carried out from 9 May till 6 June 2007 in a 200 m² marine pond in the IFREMER-L’Houmeau experimental facilities, located on the Atlantic coast of France, near La Rochelle. Four types of substrates were used for this experiment (Fig. 1): (i) 2.5 cm wide square wooden poles (fir tree: W), and 5 cm wide strips of (ii) smooth fiber-glass (S), mosquito screen (1mm-mesh; m) and (iv) garden netting (5mm-mesh; M). The mean submersion depth of the substrates (± SE) was 76.3 ± 2.7 cm after 15 days of submersion
whereas it was 66.3 ± 4.6 cm after 30 submersion days. The mean submerged surface area (±
S.E.) was 713 ± 9.3 cm² and equal for each substrate type. Eleven poles or strips (called units)
of each substrate type were deployed in the marine pond. The units were put 20 cm apart from
the closest other units in 4 parallel rows with 11 units each within a 1.0 m x 2.4 m plot,
randomly assigning the different unit types to the available locations. The different strip types
were suspended in the water column from iron bars fixed on a horizontal wooden frame
standing slightly above the surface on poles driven in the bottom, while the pole units were
standing in the sediment, under the iron bar.

Total sampling: Influence of substrate type and submersion time

On sampling days, four units of each substrate type were randomly collected. All the
periphyton on each unit was collected. Sampling was done 15 (23 May 2007; T₁₅d) and 30
days after submersion (4-5 June 2007; T₃₀d). Collected units were not placed back. In total, 32
units were collected (4 units/type/date x 4 types x 2 dates).

Sub-sampling: Influence of substrate type and submersion depth

At the end of the experiment (T₃₀d), the remaining 3 units of each substrate type (W, S,
m, M) were sampled in a random order. The submerged area of each unit was divided in five
15-cm-segments starting from the bottom (Fig. 2a: 1: 0-15 cm, 2: 15-30 cm, 3: 30-45 cm, 4:
45-60 cm, 5: 60-75 cm). Each 15-cm sub-sample (i.e. 1 to 5; Fig. 2a) was completely cleaned.
The order of the segment cleaning was randomly assigned for each unit. Each sub-sample was
next separately stored. In total, 60 samples were collected (3 units/type x 4 types x 5 sub-
samples/unit).
Total vs. sub-sampling: comparison of both sampling methods

Each 15-cm sub-sample was analysed separately. The average periphyton composition on each unit was calculated in two ways (Fig. 2b):

1. Per unit, the data of the five 15-cm sub-samples (1 to 5) were added together, to represent the whole surface area (S-5), and

2. Per unit, the top (1: 0-15 cm), middle (3: 30-45 cm) and bottom (5: 60-75 cm) sub-samples were added together, and extrapolated to the total unit area (S-3).

These data were compared with the results of the whole unit samples (T) collected on the same day (30d). 28 data were thus used for each set of comparison ((4 units/types x 4 types) + (S-3 or S-5 sampling method 3 units/type * 4 types)).

Three units (one W, m and M) were incorrectly treated and could not be included in the data set. It explains why the total degree of freedom was lower than expected (Tables 1 through 3).

2.2. Sampling and storage

At T\textsubscript{15d} and T\textsubscript{30d}, water temperature (°C), salinity, pH were measured with a multi-parameter probe (HI9828 HANNA) at the water top 15 cm of three sites in the pond, at 5:00 PM. Mean water temperature, salinity and pH (± SE) were 24.4 ± 0.76 °C, 32.2 ± 0.14 ppt and 8.1 ± 0.07 at T\textsubscript{15d} vs. 26.3 ± 0.93 °C, 32.9 ± 0.14 ppt and 8.2 ± 0.03 at T\textsubscript{30d}. Mean oxygen concentration (± SE) was at 6.4 ± 0.1 mg.L\textsuperscript{-1} (92.8 ± 2.2 %) T\textsubscript{15d} and 6.9 ± 0.1 mg.L\textsuperscript{-1} (102.7 ± 2.7%) at T\textsubscript{30d}. The water samples were collected immediately after the probe recording. Means of suspended matter (± SE) and particulate organic matter were 13.7 ± 1.04 mg.L\textsuperscript{-1} and 1.9 ± 0.2 mg.L\textsuperscript{-1}, respectively, at T\textsubscript{15d}. The suspended matter was composed of 85.9 ± 0.4 % of
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inorganic matter. At $T_{30d}$, mean Chl $a$ was $5.3 \pm 0.3 \, \mu g.L^{-1}$. Chlorophyll pigments included 15.2 ± 1.2 percent of Phaeophytin $a$.

Periphyton

The order and the location of collected units were randomly assigned. Each unit was sampled by putting a PVC tube (diameter of 6 cm x 110 cm of length) over it and closing it with a 100 μm-meshed stopper to avoid periphyton loss. The length of the submerged part of the collected substrate was measured in order to calculate the exact substrate area with periphyton (cm²). Each unit was carefully and completely cleaned with fingers and a toothbrush into a plastic flask with a fixed volume of 0.7 μm-filtered sea-water (200 ml for total unit samples and 40 ml for 15-cm samples). All material from the inner part of the net of meshed substrates was removed. Each sample was next sub-sampled using a Motoda box-splitter (Motoda, 1959): 1/8 part was stored in a dark box at -20°C for Chla analysis, 7/16 parts were stored with 4% formalin for taxonomic analysis, and 7/16 parts were used for periphyton weight analyses, putting it directly in pre-weighted box at 60°C.

2.3. Sample analyses

Dry weight and Ash free dry weight

Periphyton samples were dried at 60°C for 72h, weighed (DW: dry weight), and burned for 4h at 450°C to calculate the ash-free dry weight (AFDW; Byers et al., 1978). DW, AFDW and the weight of ash (ASH) were measured to the nearest $10^{-5}$ g with an AE240 Mettler Toledo Balance. As filtered sea-water was used to clean units, “salt correction” was applied on
periphyton weight. The effects of added filtered seawater (7/16 of 200ml or 40 ml according to the type of sampling) on DW, ASH and AFDW of periphyton were determined considering the salinity of the cleaning water and the corresponding calibration curves (DW (g.L⁻¹) = 1.17 Salinity (R²=0.99), ASH (g.L⁻¹) = 0.94 Salinity (R²=0.99), and AFDW (g.L⁻¹) = 0.23 Salinity (R²=0.96). These equations were established using based on DW, AFDW and ASH content of three replicates of 0.7 µm-filtered water in which the salinity was either 0, 10.7, 20.4, 28, 28.7, 36.37, 36.42, 38.03, 38.12, 40.17 or 40.38 ppt. Sea-water (28 to 40) was collected in marine ponds. Water in which the salinity ranged from 10 to 20 corresponded to diluted sea water by Milli-Q water (0 ppt). Values were reported to the total sample volume (200 or 40 ml) and to the total length of the unit. DW, ASH and AFDW were thus expressed in mg.cm⁻².

Chlorophyll a and Phaeophytin a

Chlorophyll a (Chl a) and phaeophytin a (Phaeo a) observed in periphyton were determined with a Turner TD 700 fluorometer after 12 hours of acetone extraction at 4°C in the dark without and with acidification. Nine ml of 100% acetone were added to 1 ml-periphyton as could performed Azim’s team (M.C.J. Verdegem, Pers. Com.). Chl a and Phaeo a data were reported to the total sample volume (200 or 40 ml) and to the total length of the unit. Values were expressed in µg.cm⁻² for periphyton. The ratio of phaeophytin vs. sum of chlorophyll pigments was also calculated as (Phaeo a)/(Phaeo a + Chl a)⁻¹ and expressed in % (% Phaeo a). The autotrophic index (AI) was calculated as: AFDW (mg.cm⁻²)/ Chl a (µg.cm⁻²)² * 1000 µg/mg (APHA 1992).

2.4. Statistical analyses
The assumptions of normality and homoscedasticity were evaluated using Shapiro-Wilk (Shapiro and Wilk, 1965) and Brown-Forsythe (Brown and Forsythe, 1974) tests, respectively. When required, data were transformed to satisfy both assumptions. ANOVAs were next performed to test the influence of (i) submersion time (TIME), (ii) substrate type (TYPE), (iii) submersion depth (DEPTH), (iv) sampling method (SAMPLING), and (v) their interactions on periphyton DW, AFDW, Chl \(a\), Phaeo \(a\), \%Phaeo \(a\) and AI. Tukey’s HSD (honestly significant differences) pairwise multiple comparison tests were used to identify the differences when a source of variation was significant (P < 0.05).

3. Results

3.1. Total sampling: Influence of submersion time and substrate type

According to the ANOVA results (Table 1), dry weight, ash free dry weight, chlorophyll \(a\) and phaeophytin \(a\) varied significantly among submersion time (TIME; Table 1). Means were greater at T\(_{30d}\) than at T\(_{15d}\). Mean AFDW and Phaeo \(a\) were more than twice higher at T\(_{30d}\) than at T\(_{15d}\) (DW: 6.3 mg.cm\(^{-2}\) vs. 2.3 mg.cm\(^{-2}\) and Phaeo \(a\): 0.2 \(\mu\)g.cm\(^{-2}\) vs. 0.1 \(\mu\)g.cm\(^{-2}\); Fig. 3a, b).

Substrate type (TYPE) significantly affected the amount of periphyton collected in terms of DW, AFDW, Chl \(a\) and Phaeo \(a\) (Table 1). Tukey HSD tests revealed that means of DW, AFDW, Chl \(a\) and Phaeo \(a\) were larger on mosquito screen (m) that on the other substrate types (M, S or W; Fig. 3c, d). Mean periphyton DW and total chlorophyll pigment varied between 3.4 and 6.4 mg.cm\(^{-2}\) (Fig. 3c) and between 0.5 and 1 \(\mu\)g.cm\(^{-2}\), respectively, among substrate types (Fig. 3d).
The interaction of both factors (TYPE x TIME) was a significant variation source of % Phaeo a (Table 1). Relatively more Phaeo a was present on mosquito screen at T\textsubscript{30d} (m-T\textsubscript{30d}: 27.7%) than at T\textsubscript{15d} (m-T\textsubscript{15d}: 16.7%). In contrast, mean % Phaeo a did not significantly differ over time on wooden poles (W), fiber-glass (S) and garden netting (M). Means (± SE) were respectively 30.2 ± 0.9 %; 28.2 ± 1.1 % and 18.7 ± 1.6 %. At T\textsubscript{15d}, a higher % Phaeo a was observed on smooth substrates (W, S) than on meshed substrates (m, M). At T\textsubscript{30d}, the % Phaeo a observed on wooden poles (W) was higher than on garden netting (M).

The autotrophic index was significantly different for the factors TYPE and TIME, and showed a significant interaction (Table 1). The mean AI observed on wooden poles (W) was more than 6 times lower at T\textsubscript{15d} (1554 ± 410) than at T\textsubscript{30d} (9449 ± 1479). In contrast, the mean AI observed on the other substrates (S, m, M) did not vary over time.

3.2. Sub-sampling: Influence of substrate type and submersion depth

Chl \textsubscript{a}, % Phaeo a and the AI were significantly different among substrate type (Table 2). HSD tests showed that at T\textsubscript{30d}, the Chl \textsubscript{a} mean was greater on meshed substrates and fiberglass than on wooden poles (m, M, S: 0.6 ± 0.12 µg.cm\textsuperscript{-2} > W: 0.26 ± 0.08 µg.cm\textsuperscript{-2}). At T\textsubscript{30d}, % Phaeo a varied such as W > S, M ≥ M, m. The AI mean was almost three times higher on wooden poles (W: 2815 ± 816) than on the other substrates (S, M, m: 939 ± 299).

Periphyton DW, Chl \textsubscript{a} and Phaeo a significantly changed with depth (DEPTH; Table 2). More periphyton was collected at 60-75 cm depth than at 0-15 cm depth (Fig. 4). Respectively 2 and 12 times more DW and total chlorophyll \textsubscript{a} was collected in the bottom 15 cm than at the top 15 cm. Mean differences between sampling depths of DW and Phaeo a were not statistically significant whereas means seemed to increase between 15 and 60 cm depth. In contrast, Chl \textsubscript{a} increased gradually with depth (Fig. 4b). The mean AI (± SE) was
more than three times larger in the top 15 cm (3406 ± 1002) than between 15 and 60 cm (900 ± 260). The % Phaeo a did not vary with depth whatever the type substrate (Table 2) and was 25.7 ± 2.05%.

3.3. Total vs. sub-sampling: comparison of both sampling methods

Five sub-sampling

The mean DW, AFDW, Chl a and Phaeo a differed significantly between sampling methods (SAMPLING (S-5 vs. T); Table 3). Means were higher with the total sampling (T) than the S-5 sub-sampling method whatever the substrate type (Fig. 5a, b). It was particularly right for periphyton quantity rather than quality. DW and AFDW determined through S-5 sampling were 2 and 8 times, respectively lower than T means (Fig. 5a) whereas the mean of chlorophyll pigment obtained with S-5 sampling corresponded to 82.6% of means obtained with total sampling T (Fig. 5b).

Three sub-sampling

Sampling was a significant source of variation for DW and AFDW (SAMPLING (S-3 vs. T); Table 3). More DW and AFDW were measured with total sampling (T) than with the S-3 sub-sampling method (Fig. 5a). As S-5 means, S-3 means of DW and AFDW were 2 and 8 times, respectively, lower than the T means (Fig. 5a). In contrast, Chl a and Phaeo a means did not significantly differ between S-3 and T (Fig. 5b).

4. Discussion
4.1. Marine periphyton and its variation sources

Marine periphyton

A thin mat of matter was observed on all the immersed surface of the different types of substrate after 15 days of submersion. The inorganic fraction of periphyton (ASH) could originate from trapping of suspended inorganic particles. The latter would be favoured during resuspension caused by wind driven turbulence or people working around units during sampling. The organic matter (AFDW) fraction originated from the accumulation of detritus, bacteria, fungi, flora and fauna on substrates. The presence of photosynthetic pigments (Chl a and Phaeo a) could indicate flora colonization of artificial substrates. The presence of phaeophytin a indicated that the flora observed was partly degraded (15 to 30 %). The mean autotrophic index ranged between 250 (60-75 cm section of mosquito screen at T30d) and 9450 (wooden poles at T30a). These high values indicate that the periphyton contained mainly heterotrophic organisms and dead organic matter, as specified by Huchette et al. (2000) for an AI above 200. In situ observations showed that periphyton was also composed of detritus and small-sized organisms as harpacticoid copepods (Richard et al., unpublished data).

Submersion time

A significant increase of periphyton DW, AFDW and photosynthetic pigments was shown on all substrate types. According to periphyton colonization models (Hoagland et al., 1982; Steinman, 1996), AFDW and Chl a levels increase exponentially until a biomass peak. Organisms at the base of the biofilm become light and nutrient limited, eventually die and detach from the substrate (Hansson et al., 1992; Asaeda et al., 2000; Keshavanath et al.,
2001a; Azim and Aseada, 2005). In this investigation, periphyton was still in its accretion phase on day 30 on all substrate types. As noted Eding et al. (2006), biofilm establishment seems to be slower in marine than in freshwater. It would be better to wait a minimum of 4 weeks rather than 2 as Azim et al. (2001a, 2003b) did in freshwater, before introducing fish in marine periphyton-based ponds.

Substrate type

DW, AFDW, Chl $a$, Phaeo $a$ and $\%$Phaeo $a$ varied according to substrate type. Keshavanath et al. (2001) showed that biodegradable substrates could be more efficient than synthetic substrates (e.g. Bamboo vs. PVC tubes) because of the nutrient leaching that occurred at the substrate-water interface (van Dam et al., 2002). In the same way, Anderson and Underwood (1994) reported higher recruitment by epifauna on plywood than on fibreglass or aluminium substrates in an estuary. In contrast, periphyton biomass was not larger on natural (i.e. wooden poles) than on fiberglass strips in this study. 30 days-submersion time might have been too short to permit to a significant nutrient leaching at the interface of wooden poles. Nevertheless, the periphyton grown on wooden poles contained relative more phaeophytin (higher $\%$ Phaeo $a$) and non autotrophic matter (higher AI) than the other substrates. The observed increase in AI could have originated from uptake of decomposition products from the wood.

More dry matter and Chl $a$ were found on meshed substrates (mosquito and garden meshes) than on smooth substrates (i.e. wood and fiber-glass). The meshes might favour the trapping of particles, in contrast to smooth surfaces. Moreover, higher circulation of water and nutrients across the meshed substrates could stimulate periphyton growth and explain this result.
The substrate type could also influence the nature of the heterotrophic associated community. Richard et al. (2007) observed that mesh substrates, as aquaculture pens, offered appropriate structures for infauna, as Corophium sp. whereas newly submerged smooth substrate, favoured epifauna recruitment. In this study, some polychaete tubes were observed on mosquito screen, but not on smooth substrates. The results of this study indicate that more and qualitatively better periphyton grew on mosquito screen than on the other substrates.

Submersion depth

The light intensity and its spectral composition change with depth, influencing the quality and type of flora (Boston and Hill, 1991; Hansson, 1992; Kirk, 1994), as periphyton (Goldsborough et al., 2005). In contrast to the observations of Azim et al. (2002a), periphyton DW and chlorophyll pigments increased with depth in this investigation. A decrease of the 10 cm-water level at T_{30d} could explain why less periphyton was collected on the 0-15 cm part of substrates than on the deeper parts. Nevertheless, the lower chlorophyll pigment concentration observed on the 15-60 cm part of substrates compared to the deeper part (60-75 cm) could be due to a photo-inhibition processes, as Hansson (1992) suggested when periphyton Chl a was negatively correlated with light. Unfortunately, light incidence was not measured during this study.

Maximal periphyton biomass could be observed where the combination of light and nutrient are optimal (Hansson et al., 2002). In this way, periphyton observed on the deeper part of substrates could have the advantage over the one observed on the surface part by benefiting from nutrient released at the water-sediment interface. Moreover it could benefit from trapping suspended sediment and microphytobenthos present at the bottom of the pond.
4.2. Comparisons

Sampling method

The sum of 3 samples taken between 0-15 cm, 30-45 cm and 60-75 cm (S-3 method) led to comparable Chl_a and Phaeo_a means with the ones obtained with total sampling (T). That was not the case with S-5 method. Nevertheless, the DW and AFDW of the periphyton collected with both sub-sampling methods (S-5 and S-3) were significantly lower than with total sampling. The sub-sampling, especially the one of mesh substrates, necessitated extra handling for cutting before periphyton collection. Each handling event results in losses, making both the S-5 and S-3 methods less accurate than whole unit sampling. Total sampling was easier and more periphyton was collected. In further studies, the total sampling method will be preferred to sub-sampling one.

In this investigation, the use of 200 ml of filtered salt-water for unit cleaning induced over-estimation of periphyton weight. The DW, the ASH and the AFDW added when cleaning 750 cm²-periphyton substrate with 200 ml of 0.7 µm seawater of 32.55 ppt were respectively 10.1, 8.1 and 2 mg.cm⁻². These values are very important compared to the real periphyton weight (Table 4), especially for DW and ASH. Without the salt correction, ASH would be more than 6 times greater than the real values (with correction) at T₁₅d and 3 times at T₃₀d. Analysis of three blanks of cleaning water should be envisaged at each sampling date in subsequent studies. To avoid the salt correction, the use of milliQ water could be envisaged in case where the determination of periphyton weight would be the only analysis to carry out on the sampled unit. The periphyton fauna and flora could be analysed from other units cleaned with filtered seawater to avoid osmotic shock of the living cells.
Variation sources of marine periphyton

Periphyton in other aquatic systems

Absolute values which described the quantity and the quality of periphyton developed on our substrates deployed in marine water were different with the one observed mainly in freshwater by others authors (Table 4). The mean DW observed on our substrates reached 8.8 mg.cm\(^{-2}\) on mosquito screen m at T\(_{30d}\) (Table 4). This is relatively high since 10 studies out of 13 found a DW < 5mg.cm\(^{-2}\). Maximal mean organic periphyton (AFDW) observed in this study (4.5 mg.cm\(^{-2}\)) was greater than means observed by others authors which generally did not exceed 1 mg.cm\(^{-2}\) with the exception of Azim et al., 2002b (Table 4). In contrast to this investigation, in most of the cited studies, periphyton substrates are simply removed from the water causing probably a lot of loosely attached to be lost and could explain lower mean of AFDW. The use of a tube with a stopper for substrate sampling is recommended to avoid underestimation of periphyton development.

High autotrophic index of this investigation (Table 4) was induced by greater AFDW but also by very low chlorophyll \(a\) concentration observed on substrates (0.4 to 0.6 \(\mu g.cm^{-2}\); Fig. 3b). Numerous studies observed Chl \(a\) levels above 10-15 \(\mu g.cm^{-2}\) (Azim et al., 2001b,c, 2002a 2003a; Keshavanath et al., 2001; Table 4). Low periphyton concentration could originate partly from the use of inert substrate (Huchette et al. 2000; Azim et al. 2003b; Liboriussen and Jeppesen, 2006; This study: Table 4) rather than nutrient-leaching substrate (Azim et al., 2001b, 2002a, 2002b). Nevertheless, others factor could influence the primary productivity, such as temperature, light and nutrient availability (Liboriussen and Jeppesen, 2005; Vermaat et al., 2005). The high densities of periphyton recorded by Azim et al. (2001b, 2002a, 2002b) and Keshavanath et al. (2001) were observed in tropical ponds in Bangladesh and India with more light and higher temperatures than in temperate ponds in France, in the Netherlands (Azim et al., 2003b) or Denmark (Liboriussen and Jeppesen, 2006). It is the same in water
column where mean Chl a was above 200 µg.L\(^{-1}\) in Bengali fresh ponds (Azim et al. 2002b), whereas it was 5 µg.L\(^{-1}\) in our temperate marine pond. Productivity in freshwater is generally higher than in marine water. However, the ponds were fertilized with urea, manure, food in most studies listed in Table 4 whereas our pond was not fertilized. Azim et al. (2001c, 2003a) showed that periphyton biomass increased with increasing fertilization rate up to a maximum. Thus, in future studies, as part of EU policy of environmental protection and restoration of coastal areas, fertilized effluents of intensive farms could be used to maximise periphyton production and the associated production of herbivorous fishes.

The present investigation showed that (i) periphyton biomass in a marine pond increased between day 15 and day 30, (ii) more periphyton was collected on mosquito screen than on wooden poles, fiberglass strips or garden netting, (iii) periphyton biomass increased with water depth submersion, (iv) sub-sampling methods underestimated periphyton development compared to whole unit sampling, and (v) a correction of periphyton biomass must be carried out for the dissolved inorganic salts present in marine or brackish systems using blank weight of cleaning salt filtered water. The use of a tube with stopper for substrate sampling will reduce periphyton sampling losses. Finally, the autotrophic fraction in the periphyton communities was very low compared to periphyton developed on biodegradable substrates used in fish cultures in fertilized tropical ponds. Thus, pond fertilization and use of biodegraded substrates (i.e. long-time submerged wood) should be envisaged in further studies on periphyton-based marine aquaculture in temperate regions.

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Tables

Table 1

Results of analyses of variance (ANOVAs) testing the effect of substrate type (TYPE: W: wooden poles, S: fiber-glass strip, m: mosquito screen, M: garden netting), submersion time (TIME: T\textsubscript{15d}, T\textsubscript{30d}) and their interactions on periphyton dry weight (DW), ash free dry weight (AFDW), Chlorophyll a (Chl a), Phaeophytin (Phaeo a), % Phaeo a (Phaeo a.(Chl a + Phaeo a)\textsuperscript{-1}) and autotrophic index (AI: AFDW.Chl a\textsuperscript{-1}) observed on collected substrates. df: degrees of freedom, MS: mean square, F: Fischer, * P < 0.05, ** P < 0.01, *** P < 0.001

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<th>Variation source</th>
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<td>6.46</td>
<td>0.0025 **</td>
<td>217.81</td>
<td>20.98</td>
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<td>119.05</td>
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<td>&lt;0.0001 ***</td>
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Table 2

Results of ANOVAs testing the effect of substrate type (TYPE: W: wooden poles, S: fiberglass strips, m: mosquito screen, M: garden netting), submersion depth (DEPTH: 1: 0-15 cm; 2: 15-30 cm; 3: 30-45 cm; 4: 45-60 cm; 5: 60-75 cm) and their interactions on Periphyton dry weight (DW), ash free dry weight (AFDW), chlorophyll (Chl \(a\)), phaeophytin (Phaeo \(a\)) and % Phaeo \(a\) (Phaeo \(a\)./(Chl \(a\) + Phaeo \(a\))\(^{-1}\)) and autotrophic index (AI: AFDW.Chl \(a\)\(^{-1}\)) observed on collected substrates. df: degrees of freedom, SS: sum square, MS: mean square, F: Fischer, * P < 0.05, ** P < 0.01, *** P < 0.001

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<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<td>log (AFDW + 1)</td>
<td>log AI</td>
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<tr>
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<td>0.02</td>
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<td>0.2786</td>
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<td>log Chl (a)</td>
<td>log Phaeo (a)</td>
<td>% Phaeo (a)</td>
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<td>DEPTH</td>
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<td>0.19</td>
<td>30.57</td>
<td>1.70</td>
<td>0.1176</td>
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Table 3

Results of ANOVAs testing the effect of periphyton sampling method (SAMPLING: T: total vs. S-5: addition of all five sub-samples and vs. S-3: addition of three sub-samples 0: 0-15 cm, 3: 30-45, 5: 60-75 cm), substrate type (TYPE: W: wooden poles, S: fiber-glass strips, m: mosquito screen, M: garden netting) and their interactions on Periphyton dry weight (DW), ash free dry weight (AFDW), Chlorophyll \( \alpha \) (Chl \( \alpha \)) and phaeophytin (Phaeo \( \alpha \)) observed on collected substrates. df: degrees of freedom, MS: mean square, F: Fischer, * P < 0.05, ** P < 0.01, *** P < 0.001

<table>
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<th>Variation source</th>
<th>df</th>
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<th>F</th>
<th>P</th>
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<th>F</th>
<th>P</th>
<th>MS (Chl ( \alpha ))</th>
<th>F</th>
<th>P</th>
<th>MS (Phaeo ( \alpha ))</th>
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<td>24.383</td>
<td>130.80</td>
<td>&lt;0.0001 ***</td>
<td>0.085</td>
<td>9.30</td>
<td>0.0069 **</td>
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<td>2.30</td>
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<td>0.626</td>
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<td>0.156</td>
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<td>0.008</td>
<td>3.09</td>
<td>0.0532</td>
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<td>0.454</td>
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<td>0.0537</td>
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<td>0.0421 *</td>
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Table 4

Ranges of mean variables characterizing quantity and quality of periphyton developed on different submerged substrates in natural and exploited aquatic systems observed by different authors over the world

<table>
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<tr>
<th>References</th>
<th>Location</th>
<th>Season (date and range of temperature)</th>
<th>Fertilization</th>
<th>Substrate type</th>
<th>Presence (+) and absence (-) of fish</th>
<th>Periphyton quantity (range of mean DW, AFDW mg cm$^{-2}$)</th>
<th>Periphyton quality (range of mean DW, AFDW mg cm$^{-2}$)</th>
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<tr>
<td>Boston and Hill, 1991</td>
<td>American streams</td>
<td>(16°C to 22°C)</td>
<td>NF</td>
<td>Ceramic tiles on natural rocks</td>
<td>natural presence</td>
<td>AFDW: 0.25 to 2.1</td>
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<td>Huchette et al., 2000</td>
<td>Tilapia reared on floating cages in a Bengali fresh farm</td>
<td>March to May (29.5°C)</td>
<td>No fertilization (NF)</td>
<td>Plastic bottle</td>
<td>presence and absence</td>
<td>AFDW: +: 0.5 to 0.9 -: 0.75 to 0.9</td>
<td>Chl a: +1 to 1.5, -: 1.2 to 2.8</td>
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<td>Azim et al., 2001b</td>
<td>Polyculture of carp in Bengali fresh ponds (75 m$^2$)</td>
<td>Sept to December (23.5 to 33.7°C)</td>
<td>Continuous fertilization on (CF)*</td>
<td>Bamboo</td>
<td>presence</td>
<td>DW: 0.7 to 2.5</td>
<td>AFDW: 0.6 to 0.8</td>
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<td>Azim et al., 2001c</td>
<td>Bengali fresh ponds (75 m$^2$)</td>
<td>July to September (27.8 to 33.1°C)</td>
<td>CF: + 4 rates of *</td>
<td>Bamboo</td>
<td>absence</td>
<td>DW: 0.5 to 5</td>
<td>AFDW: 0.5 to 3.3</td>
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<td>Keshavanath et al., 2001</td>
<td>Masur fingerlings rearing in Indian fresh water tanks (25 m$^2$)</td>
<td>Trial 1: May to June (31.6°C)</td>
<td></td>
<td>Bamboo, PVC, sugarcane bagasse</td>
<td>Trial 1: absence</td>
<td>DW: 0.5 to 1.9</td>
<td>AFDW: 0.4 to 1.2</td>
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<tr>
<td>Azim et al., 2002a</td>
<td>Polyculture of carp in Bengali fresh ponds (75 m$^2$)</td>
<td>April to September (26.4 to 31.7°C)</td>
<td>CF: * + Rice bran and mustard oil cake</td>
<td>Bamboo, jute stick, Kanchi Bamboo</td>
<td>Trial 2: absence and presence</td>
<td>DW: 2 to 10</td>
<td>AFDW: 2 to 6</td>
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<tr>
<td>Azim et al., 2002b</td>
<td>Polyculture of carp in Bengali fresh ponds (75 m$^2$)</td>
<td>August to November (27.1 to 32.7°C)</td>
<td>CF: *</td>
<td>Bamboo</td>
<td>Trial 1: absence</td>
<td>DW: 2 to 10</td>
<td>AFDW: 2 to 6</td>
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<tr>
<td>Azim et al., 2003a</td>
<td>Bengali fresh ponds (75 m$^2$)</td>
<td>Trial 2: May to July (29°C)</td>
<td>Trial 2: Aug to September (22.5°C)</td>
<td>CF: Trial 1: *</td>
<td>absence</td>
<td>DW: 1.2 to 5</td>
<td>Trial 2: 0.9 to 2.6</td>
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<td>Azim et al., 2003b</td>
<td>Tilapia rearing in fresh tanks (1 m$^3$) in The Netherlands</td>
<td>Trial 1: May to July (29°C)</td>
<td>Trial 2: Aug to September (22.5°C)</td>
<td>CF: NaN3 + single superphosphate (SSP) each week</td>
<td>absence</td>
<td>DW: +0.2 to +0.4</td>
<td>AFDW: +0.08 to 0.25</td>
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<td>December to April (17°C to 26°C)</td>
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<td>presence</td>
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<tr>
<td>Keshavanath et al., 2004</td>
<td>Tilapia rearing in Indian freshwater tanks (25 m$^2$)</td>
<td>Danish lakes</td>
<td>May to August (25°C to 31°C)</td>
<td>Strips of Tape</td>
<td>natural presence</td>
<td>DW: 0.1 to 0.35</td>
<td>AI: 30 to 60</td>
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<td>Liboissen and Jeppesen, 2006</td>
<td>Tilapia rearing in Danish lakes</td>
<td>May to August (16°C to 21°C)</td>
<td>CF: TSP at start, daily</td>
<td>Strips of Tape</td>
<td>presence</td>
<td>DW: 0.1 to 0.35</td>
<td>AI: 30 to 60</td>
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<tr>
<td>Liboissen and Jeppesen, 2006</td>
<td>Tilapia rearing in Indian freshwater tanks (25 m$^2$)</td>
<td>September</td>
<td>CF: TSP at start, daily</td>
<td>Strips of Tape</td>
<td>presence</td>
<td>DW: 0.1 to 0.35</td>
<td>AI: 30 to 60</td>
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<td>Khubal et al., 2007</td>
<td>Mussel limnabicnhich water shrimp pond</td>
<td>(30°C to 33.3°C)</td>
<td>CF: TSP at start, daily</td>
<td>Shrimp pellets</td>
<td>presence of shrimp</td>
<td>nd, 56 to 168 polychaete tubes cm$^{-2}$</td>
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<tr>
<td>Richard et al. (This study)</td>
<td>French marine pond</td>
<td>May to June (224° to 26.3°C)</td>
<td>NF</td>
<td>Ceramic tiles</td>
<td>absence</td>
<td>DW: 1.5 to 8.8</td>
<td>AFDW: 0.2 to 4.5</td>
</tr>
</tbody>
</table>
Figures captions

Fig. 1: Pictures and schemes of the four types of periphyton substrate deployed in the marine pond: a) wooden poles (W), b) fiber-glass strips (S), b) mosquito screen (m) and d) garden netting (M). 

Fig. 2: a) Scheme of sub-sampling of the submerged substrate surface carried out along the submersion depth gradient, b) scheme of three methods of sampling (Total, 5 sub-samples, 3 sub-samples).

Fig. 3: Mean (± Standard Error) periphyton dry weight (a, c) and chlorophyll pigment (b, d) observed on substrates according to a, b) submersion time (TIME: T\textsubscript{15d}, T\textsubscript{30d}) and c, d) substrate type (TYPE: W: wooden poles, S: fiber-glass strips, m: mosquito screen, M: garden netting). Different letters indicate statistically difference among variation source. Lower cases are linked to means represented by the bars of the bottom (AFDW, Chl a). Capital letters are associated with DW and Phaeo a means.

Fig. 4: Mean (± Standard Error) periphyton dry weight (a) and chlorophyll pigment (b) observed on substrates according to the submersion depth (DEPTH; 1: 0-15 cm; 2: 15-30 cm; 3: 30-45 cm, 4: 45-60 cm, 5: 60-75 cm). Different letters indicate statistically difference among depth. Lower cases are linked to means represented by the bars of the bottom (AFDW, Chl a). Capital letters are associated with DW and Phaeo a means.

Fig. 5: Mean (± Standard Error) periphyton dry weight (a) and chlorophyll pigment (b) observed on substrates according to the sampling method (SAMPLING: S-5: addition of five sub-samples; T: total sample; S-3: addition of three sub-samples 0: 0-15 cm, 3: 30-45, 5: 60-75 cm). Different letters indicate statistically difference among sampling method. Normal letters are used for the T vs. S-5 comparison, whereas italic letters are used for the T vs. S-3 comparison. Lower cases are linked to means represented by the bars of the bottom (AFDW, Chl a). Capital letters are associated with DW and Phaeo a means.
Figures

Fig. 1. Richard et al.
Fig. 2. Richard et al.
Fig. 3. Richard et al.
Fig. 4. Richard et al.
Fig. 5. Richard et al.