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An integrated fish–plankton aquaculture system in brackish water

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Abstract:

Integrated Multi-Trophic Aquaculture takes advantage of the mutualism between some detritivorous fish and phytoplankton. The fish recycle nutrients by consuming live (and dead) algae and provide the inorganic carbon to fuel the growth of live algae. In the meanwhile, algae purify the water and generate the oxygen required by fishes. Such mechanism stabilizes the functioning of an artificially recycling ecosystem, as exemplified by combining the euryhaline tilapia *Sarotherodon melanotheron heudelotii* and the unicellular alga *Chlorella* sp. Feed addition in this ecosystem results in faster fish growth but also in an increase in phytoplankton biomass, which must be limited. In the prototype described here, the algal population control is exerted by herbivorous zooplankton growing in a separate pond connected in parallel to the fish–algae ecosystem. The zooplankton production is then consumed by tilapia, particularly by the fry and juveniles, when water is returned to the main circuit. *Chlorella sp.* and *Brachionus plicatilis* are two planktonic species that have spontaneously colonized the brackish water of the prototype, which was set-up in Senegal along the Atlantic Ocean shoreline. In our system, water was entirely recycled and only evaporation was compensated (1.5% volume/day). Sediment, which accumulated in the zooplankton pond, was the only trophic *cul-de-sac*. The system was temporarily destabilized following an accidental rotifer invasion in the main circuit. This caused *Chlorella* disappearance and replacement by opportunist algae, not consumed by *Brachionus*. Following the entire consumption of the *Brachionus* population by tilapias, *Chlorella* predominated again. Our artificial ecosystem combining *S. m. heudelotii*, *Chlorella* and *B. plicatilis* thus appeared to be resilient. This farming system was operated over one year with a fish productivity of 1.85 kg/m^2 per year during the cold season (January to April).

Keywords: IMTA ; Tilapia ; *Chlorella* ; *Brachionus plicatilis* ; photosynthetic recycling aquaculture system

Implications

Aquaculture production systems all face the same problems, i.e., maximising production or feed efficiency while minimizing water input, and wastes that must be removed (faeces) or transformed into non-toxic compounds (e.g. from ammonia to nitrates). Recirculating water systems, which operate a combination of mechanical and biological filters recycle water but necessitate periodical waste removal. In an integrated rearing system, wastes are recycled and contribute to enhancing production. Our integrated recirculating system uses no filter but a simple artificial ecosystem (phytoplankton, zooplankton and fish) and enables fish (tilapia) production while recycling almost all organic wastes, without water exchange.

Introduction

Among Integrated Multi-Trophic Aquaculture (IMTA), closed systems combining intensive and extensive rearing have been tested in Taiwan and Singapore (Liao and Chen, 1983; Chin *et al.*, 1993), and notably in Israel (Mires *et al.*, 1990; Diab *et al.*, 1992; van Rijn, 1996). The identification of autochthonous detritivorous fish species adapted to such technology (mutualism between these fishes and phytoplankton) is a key to its implementation (Gilles *et al.*, 2008). Fish recycle nutrients 74 by consuming alive and dead micro-algae, and provide remaining live algae with the inorganic carbon needed to their growth. In the meanwhile, algae purify the water and generate the oxygen required by the fish (Hargreaves, 2001 and 2006; Neori *et al.*, 2004).

Generally, fish grazing is unable to control phytoplankton growth (Turker *et al.*, 2003) resulting from the input of nutrients from fish feed, even when the reared species are phytoplanktivorous. Furthermore, it has often been reported that fish such as tilapia, by recycling nutrients through excretion, eventually promote the production of phytoplankton in ponds and lakes (McQueen *et al.*, 1986; Drenner *et al.*, 1987; Lazzaro, 1987; Northcote, 1988; Elser *et al.*, 1990). Without algal biomass control, an excessive algal bloom may occur, followed by a collapse of the algal population, an increase in ammonia concentration, and an oxygen depletion (Rimon and Shilo, 1982).

Two main types of Photosynthetic Suspended-Growth (PSG) systems (Hargreaves, 2006) have been developed, both relying on a periodic or continuous removal of phytoplankton. In the Dekel Aquaculture system (Mires *et al.*, 1990; Mires and Amit, 1992), the rearing water is discarded at the end of the production cycle, and must be fully renewed for the next cycle, which might be an issue wherever water resources are limited. In the Partitioned Aquaculture System (PAS), phytoplankton must be continuously collected in the sewage-treatment channel by a rolling filter and removed from the system (Drapcho and Brune, 2000; Brune *et al.*, 2001 and 2003).

We developed a prototype of an alternative system, which does not require periodic or continuous removal of phytoplankton, installed at the IRD centre in Mbour, on the Atlantic shore of Senegal. It mimics a brackish water natural ecosystem where phytoplankton is largely grazed by zooplankton. It comprises tanks for intensive fish culture, linked with sewage ponds, two for phytoplankton (almost exclusively *Chlorella*

sp., naturally seeded), and one for zooplankton (rotifer, *Brachionus plicatilis,* naturally seeded) as an additional volume to the one occupied by the fish-alga ecosystem. Rotifers contribute to regulate the growth of phytoplankton, and are distributed as additional food to the fry and juveniles of tilapia. Water is slowly fully recycled with only freshwater being periodically added to compensate for evaporation. Only sediment has to be removed from the zooplankton pond. This system is partly similar to those developed by Shnel *et al.* (2002) for tilapia, and Burford *et al.* (2003) for shrimp.

Our artificial ecosystem has originally been developed for rearing the euryhaline tilapia, *Sarotherodon melanotheron heudelotii* (Trewavas, 1983; Falk *et al.*, 2000), which is endemic to the coastal regions of Senegal and Guinea. The fry and fingerlings are zooplanktivorous, whereas juveniles are omnivores, and adults are essentially detritivorous (Pauly, 1976). This robust fish can adapt to integrated aquaculture because it mainly consumes sediment (i.e. dead algae, uneaten food, and its own faeces) when fed restricted food rations, therefore contributing to clean up its own farming environment.

Recently we provided information on how *Chlorella* biomass, in the presence of *S. m. heudelotii,* varied with fish biomass and feeding level, depending on its nutrients uptake (Gilles *et al.*, 2008). In this paper, we focus on the system productivity during a routine operation period over several months. We also describe the system resilience, i.e., how it responds to a sudden drop in phytoplankton concentration following an invasion of the main circuit by rotifers from the zooplankton pond.

Materials and Methods

123 *Description of the prototype*

124 The prototype was developed at IRD (Institut de Recherche pour le Développement)
125 Centre in Mbour, Senegal (14° 23' 30.89" N, 16° 57' 26.80" W), from June 2003 to
126 August 2009. It operated as a recycling system including an intensive fish rearing unit
127 and a depuration unit.

128 The intensive farming unit was composed of ten cylindrical 30-L tanks (useful
129 volume), stocked with fry (one progeny per tank, i.e. on average 300-500 fry; Fig. 1 [5]),
130 and nine cylindrical 1300-L (useful volume, black polyethylene;) growth tanks (Fig. 1
131 [4]). The overall volume of these tanks was 12 m^3. Aeration columns, for exchange of
132 oxygen and carbon dioxide, were placed on the water feeding line.

133 The wastewater treatment included three lined ponds (13-m long x 4-m wide x
134 0.50-m deep each; Fig. 1 [1, 2, 3]), covered with translucent greenhouses in order to
135 maintain water temperature above 25°C during the cold season (December-March).
136 Their total useful volume was 78 m^3, i.e., about 6.5 times the volume of the intensive
137 rearing section (Fig. 1 [4]). Ponds 1 and 2, interconnected with a pipe (Ø 140 mm),
138 received wastewater from the intensive rearing tanks and were stocked with *S. m.*
139 *heudelotii*. The intensive farming tanks plus sewage ponds 1 and 2 (Fig. 1) constituted
140 the main rearing circuit. Pond 3, dedicated to zooplankton rearing, was filled with algae-
141 rich water from the main circuit and sown with rotifers (Table 1). When phytoplankton
142 was depleted by zooplankton, water of pond 3 was periodically drained into the main
143 circuit using a set of valves and the main recirculation pump (Fig. 1 [7]). In contrast to
144 ponds 1 and 2, pond 3 was devoid of fish, and continuously mixed by air bubbling (using
145 a 12 m^3 h^{-1} blower) in order to reduce sedimentation. During the cleaning procedure,
146 sediment was either returned to the circuit or thrown out of the system, when *Chlorella*

147 density was over 35 10^6 cells mL^{-1} in the main circuit, while keeping some rotifer stock
148 in order to reinitiate the food chain for subsequent farming cycles.

149 Tilapias *S. m. heudelotii* were obtained from neo-males (XX females treated with
150 a masculinising hormone, 17 α methyltestosterone), and their male:female sex ratio was
151 about 1:9 (i.e. the mean phenotypic sex ratio in the progenies of neo-males in this
152 subspecies). This enabled to slow down the proliferation of fry, which is systematic with
153 balanced sex ratios. Two circulating pumps (24 m^3 h^{-1}, Fig. 1 [7]) used alternatively
154 enabled an overall renewal rate of 200 % h^{-1} in the intensive farming volumes, and 46 %
155 h^{-1} in the sewage volume (ponds 1 and 2).

156 Within the whole system, water salinity was 15-, a suitable level for the phyto-
157 (*Chlorella sp*.) and zooplankton species (*Brachionus plicatilis)* that naturally colonized
158 this artificial environment. A well, located 50 m from oceanside, supplied brackish water
159 (15) used for filling the circuit. Evaporation was compensated for (on average 1.5 % of
160 overall volume per day; i.e., about 1 m^3 day^{-1}) with fresh tap water from the public water
161 system.

162 Fig. 1

163

164 *Study periods*

165 Fish productivity was calculated from early January to end of April 2009, after about 2
166 years of operation so as to refine the production parameters. Rotifers invasion and the
167 description of the system resilience occurred in March 2007. Nitrogen absorption within
168 the system was calculated during a 7-day period, in February 2007.

169

170 *Monitoring of physico-chemical parameters*

171 Water temperature and dissolved oxygen concentration (mg O_2 L^{-1}) were measured in
172 all rearing tanks and ponds twice a day, at 7:00 (before sunrise) and 15:00, using a
173 CyberScan DO 300/310 dissolved oxygen meter (Eutech Instruments, Singapore).
174 Salinity was measured every day at 15:00 using an ATAGO S-10e refractometer
175 (Tokyo, Japan).

176 Parameters monitored after pond samplings were: pH measured using an Hanna
177 HI 9025 pH meter (Hanna Instruments Inc., RI, USA), dissolved nitrogen ($N\text{-}NH_4^+$ + $N\text{-}NH_3$ and $N\text{-}NO_2^-$ + $N\text{-}NO_3^-$) and phosphorus (PO_4^{3-}) in ponds 2 and 3 assessed by
178
179 colorimetric methods after vacuum filtration using Whatman GF/F membrane filters (0.7-
180 µm pore size, Ø 47 mm; Florham Park, NJ, USA) and preservation with chloroform.
181 Concentrations of total ammonia nitrogen (TAN = $N\text{-}NH_4^+$ + $N\text{-}NH_3$) (Koroleff, 1969) and
182 (ortho)phosphate (PO_4^{3-}) (Murphy and Riley, 1962) were measured in the ponds with a
183 Helios UV-visible spectrophotometer (Thermo Electron Corp., Winsford, Cheshire, UK).
184 Concentrations of nitric nitrogen ($N\text{-}NO_3^-$) (Grasshoff, 1976) and nitrous nitrogen ($N\text{-}NO_2^-$) (Bendschneider and Robinson, 1952) were measured using a TECNICON II auto-
185
186 analyzer (Bran and Luebbe Analyzing Technologies, Inc., Elmsford, NY, USA). Water
187 alkalinity was measured using a Varian model Spectra AA220 flame absorption
188 spectrophotometer (Victoria, Australia).

189

190 *Biological parameters*

191 Fish were collected and weighed individually using a 0.5-g precision scale balance.
192 *Chlorella* density was determined by colorimetry using a Hanna C203 photometer
193 (Hanna Instruments Inc., RI, USA), with the ammonia medium range program at 420-
194 nm wavelength and a tungsten lamp source. Prior to this study, a calibration curve was

established by comparing the optical density (OD) measured with the photometer and the actual algal density (AD), which was assessed from counts using a Burker cell and an Olympus CX41 microscope (x20 magnification). Each sample count was achieved by computing the mean density of 12 optical fields, of which the highest and lowest algal densities were removed. The observed relationship between optical density (OD) and algal density (AD) was:

$$AD \text{ (cells } mL^{-1}) = (6.62 \times 10^{6} \text{ OD}) + 127.90,\ r^2 = 0.9995,\ df = 3$$

Rotifer densities (ind. mL^{-1}) in ponds 2 and 3 were determined from counts under an OLYMPUS SZX9/12 stereomicroscope (after adding 5% formaldehyde). As for algae, 12 counts were performed on 50-µL samples, of which the highest and lowest values were removed. Specific compositions of phytoplankton in ponds 2 and 3 were determined using an OLYMPUS CX41 microscope (x100 magnification, x0.65 eyepiece).

Fish feeding and growth

Total nitrogen concentrations of fish feed were determined using a CHN Thermo Finnigan Flash Series EA1112 analyzer (Milan, Italy). Fish feed used during the nitrogen absorption study contained 4.5% nitrogen, (28.1% as protein), and 38.7% carbon. Floating feed used during the fish productivity study had a 32 % protein content, and was specifically formulated for tilapia. Floating feed allowed to control the effective food intake by fish, and thus to adjust subsequent food distribution.

In this article, we focus on fish production rather than growth. Nevertheless, to facilitate comparisons between studies, the Specific Growth Rate (SGR, % ww day^{-1}) has been calculated as:

$$SGR = (\ln ww_2 - \ln ww_1) * (t_2-t_1)^{-1} * 100$$

Where ww_2 and ww_1 are the mean individual wet weights (g) of fish at times t_2 and t_1 (days), respectively, corresponding here to the end and the start of the operation period.

Nitrogen balance during the productivity period

To assess the nitrogen balance in the system the following equations were used:

$$N\ (period\ end) = N\ (period\ start) + N\ (inputs) - N\ (outputs)$$

Where, in the overall circuit:

$$N\ (period\ start) = N\ (initial\ algae) + N\ (initial\ dissolved\ in\ water) + N\ (initial\ rotifers) + N\ (initial\ fish)$$

$$N\ (inputs) = N\ (feed)$$

$$N\ (period\ end) = N\ (final\ algae) + N\ (final\ dissolved\ in\ water) + N\ (final\ rotifers) + N\ (final\ fish)$$

$$N\ (outputs) = N\ (rejected\ sediment)$$

Results

During the early working period of the prototype, we had no specific idea of the best planktonic species, and of the optimal salinity, in relation to temperature and dissolved

241 oxygen. Tests with *Dunaliella* and copepods as grazers were unsuccessful. Thereafter,
242 salinity was stabilized at 15 g L^{-1} in the system, leading to spontaneous propagation of
243 algae *Chlorella* sp. (formerly *Nannochloris*, 4 – 5-µm diameter) and rotifer *Brachionus*
244 *plicatilis*. Rotifers were probably introduced through faeces of wild fishes that had been
245 caught and farmed. *Chlorella* blooms occur when fish biomass is high, in relation with
246 CO_2 concentration (Turker *et al.*, 2003).

247 The main objective achieved was the total recycling of water, with only
248 compensation for evaporation. Recycling of sediment from the zooplankton pond was
249 partial, and mud was eventually returned to the main circuit, according to the
250 concentration in algae. When fish biomass reached about 1 kg m^{-2} in the sewage
251 volumes no sediment was found in ponds 1 and 2.

252

253 *Physico-chemical parameters*

254 Table 2

255 The minimal average temperatures were observed in January, and the maximum in
256 October. Daily variations of DO in the intensive tanks were low because of the
257 mechanical action of the aeration columns. Greater variations of DO occurred in pond 2
258 because photosynthetic oxygen was not expelled by mechanical action. Water salinity
259 buffered the variations of pH, especially high values due to the uptake of inorganic
260 carbon by photosynthesis. As the evaporated water from the circuit was replaced by
261 alkaline tap water (about 1.5 % day^{-1}), a heavy precipitate of calcium carbonate was
262 observed in all tanks, and alkalinity remained constant. During the rare cloudy (or sand
263 winds) days oxygen in sewage ponds dropped by about 5 mg L^{-1} at 15:00 because light

intensity fell approximately to 500 W h m^{-2} day^{-1}. Routine variations of values of TAN, N-NO_2^-/NO_3^- and PO_4^{3-} are indicated in Table 2.

Fish productivity

Results of fish production were obtained at the end of the test, when the prototype system was run with a correct balance of algae, rotifers, and fish biomasses. This period occurred between two drainages of sewage ponds, allowing control of total fish biomass in ponds 1 and 2, as well as in the intensive rearing tanks (Table 3). The feed distributed during this period was 149 kg, equivalent to 8.6 g m^{-2} day^{-1} at the beginning and 13 g m^{-2} day^{-1} at the end, for the whole prototype (including the zooplankton pond). Global density of fish was 1.2 kg m^{-2} or 3.1 kg m^{-3}, as 8.1 kg m^{-3} in the intensive rearing tanks, and 1.1 kg m^{-2} in the sewage part (ponds 1 and 2). Fish productivity in the sewage part was 1.1 kg m^{-2} $year^{-1}$ in pond 1 and 0.6 kg m^{-2} $year^{-1}$ in pond 2.

Nitrogen balance during the productivity period

The ratio between the N quantity included in feed and fish (i.e. by reference to the gain of biomass) was 4.8.

During the entire test period the algal density remained stable, between 30 and 45 10^6 cells mL^{-1}. Likewise, TAN and N-NO_2^-/NO_3^- concentrations were stable during the whole period (minima in table 2). In the main circuit the rotifers were completely consumed by fish during the 117-day period. Henceforth, it can be assumed that N was not lost from plankton and dissolved elements. This interpretation is largely supported by the observation that the combined N amount included in sediment, 6,284 g (outputs), and

287 the increase of N in fish biomass, 1,315 g, almost perfectly matched with the N amount
288 included in feed, 7,596 g (inputs).

289 Cycles of the zooplankton pond occurred three times per week. Over the 117 days of
290 the test period, sediments from this pond were either returned to the circuit (15 times) or
291 removed from the system (30 times). Rejected sediment (total dry weight of 127,323 g),
292 had 47.6 % water content, thereby meaning that a total of 61 litres had been removed,
293 which represents a negligible water loss (i.e. on average 0.5 L day^{-1}).

294

295 *Rotifer invasion and the ecosystem resilience*

296 At one moment during the test, when algal density had considerably declined to 16.10^6
297 cells mL^{-1}, and when a large amount of fish (fry and fingerlings, which actively consume
298 rotifers) had been removed from sewage ponds 1 and 2, draining of pond 3 induced an
299 invasion of rotifers into the circuit (from 4 to 41 ind. mL^{-1}), which could not be regulated
300 by fish predation. This resulted in a near-complete disappearance of *Chlorella* sp from
301 the circuit, and blooms of centric diatoms *Thalassiosira* sp. and *Tetraselmis* sp.
302 (Prasinophycae) (Fig. 2, B). Twenty-nine days later, after newly hatched fry in the circuit
303 had largely consumed rotifers, these opportunistic algae disappeared. Concomitantly,
304 *Chlorella* progressively started propagating again in the circuit. During the period of
305 rotifer invasion, tilapias in the rearing tanks were not fed so as to avoid eutrophication.
306 At the beginning, rotifer invasion was accompanied by a progressive increase in TAN
307 (from 1.7 to 3.1 mg L^{-1}), $N\text{-}NO_2^-/NO_3^-$ (from 2.3 to 12.2 mg L^{-1}), and PO_4^{3-} concentrations
308 (from 5.2 to 7.7 mg L^{-1}) (Fig. 2, A). Nine days later, TAN and PO_4^{3-} concentrations
309 began to decrease, and returned to their original levels until the end of the period.
310 Conversely, $N\text{-}NO_2^-/NO_3^-$ concentrations continued to increase, and peaked at 32.2 mg

311 L^{-1} on day 33, before declining progressively down to 0.12 mg L^{-1} on day 37. The high
312 capacity of *Chlorella* to absorb nitrates, which had been demonstrated in a previous
313 experiment (Gilles *et al.*, 2008), was confirmed here with this rotifer invasion. In pond 2,
314 which was not mechanically aerated, DO concentration at 15:00 h decreased on
315 average from 12 mg O_2 L^{-1} on day 1, to 9 mg O_2 L^{-1} on day 21, when the opportunistic
316 algae were abundant in the circuit (Fig 2, C). By day 26, when almost all algae had
317 disappeared, the DO concentration (at 15:00 h) drastically dropped down to 1.68 mg O_2
318 L^{-1}. Eight days later, during the new *Chlorella* bloom, DO rose up to 16 mg O_2 L^{-1}, and
319 then remained stable. No fish died during the rotifer invasion, even when DO dropped
320 drastically. Such resilience of this artificial ecosystem was not exceptional, as it had
321 been observed on several other occasions before and after this observation.

322 Fig. 2

323

324 *Uptake of total nitrogen*

325 During the nitrogen absorption study the biomass of *S. m. heudelotii* was 8.1 kg m^{-3} in
326 the intensive part and 0.6 kg m^{-2} in the sewage part, for an overall fish biomass of 1.4
327 kg m^{-2} in the circuit, except the zooplankton pond. Initial TAN concentration was 0.12
328 mg L^{-1}, and concentration of $N\text{-}NO_2^-/NO_3^-$ was null, due to an almost total absorption of
329 nitrates by *Chlorella* (Gilles *et al.*, 2008). Fish were fed with a 4.99 % N feed, at a daily
330 rate of 1.6 % body weight, equivalent to 15.2 g m^{-2} day^{-1}. Considering that all feed was
331 consumed, and 53 % of absorbed N was excreted (Beveridge *et al.*, 1991), the
332 expected final N concentration was 13.7 mg L^{-1}, taking into account the initial N
333 concentration dissolved in the water. The final N concentration was 0.22 mg L^{-1}.
334 Nitrogen absorption was thus 1.9 mg L^{-1} day^{-1} with a mean *Chlorella* concentration of

335 35.10^6 cells mL^{-1}. The PO_4^{3-} concentration showed little variation, from 2.5 mg L^{-1} to 4.4
336 mg L^{-1}, due to phosphorus excretion by rotifers.
337
338
339 **Discussion**
340
341 *Control of algal biomass*
342 The inclusion of a rotifer pond (pond 3) and the way it was operated (i.e., draining at
343 regular intervals) proved it is a good tool for controlling algal blooms in the circuit
344 although rotifers brought in extra TAN and phosphates. The periodic drainage of pond
345 3, after algae had been depleted, resulted in dilution events of phytoplankton
346 concentration in the rest of the circuit. Pond 3 water, rich in rotifers, lead to an additional
347 reduction in algal concentration in the main circuit immediately after draining.
348 Consequently such control of algal biomass was at least as effective as the periodic or
349 continuous removal of algae in the Dekel Aquaculture System (Mires and Amit, 1992) or
350 in the Partitioned Aquaculture System (Drapcho and Brune, 2000). The efficient control
351 of algal biomass in the present study was also facilitated by the fact that *Brachionus*
352 *plicatilis* did not suffer from the concurrence or predation by other zooplanktonic
353 species.
354
355 *Fish productivity*
356 The euryhaline tilapia *S. m. heudelotii* is not domesticated and no dedicated selection
357 program has been undertaken until now. It is likely that its growth performances can be
358 improved. In the system, each intensive rearing tank received one batch constituted

359 with a single progeny, and we observed substantial between-tank (and thus between-
360 progeny) variations in growth, although the very same amounts of food were distributed.
361 The use of fry obtained from neo-males did not completely prevent reproduction, and
362 this certainly affected fish growth. In the present study, the Specific Growth Rate (SGR)
363 was 0.51 % ww day^{-1} whereas in another test made with all mono-sex male groups
364 using fish obtained through hormonal treatment, the SGR was 1.96 % ww day^{-1}.

365 During the production trials in the present study, the daily feeding rate was 13 g
366 m^{-2} for an instantaneous biomass of 1.8 kg m^{-2}. Yet, maximum productivity of this
367 system still remains to be determined.

368 A test with Nile tilapia *Oreochromis niloticus* was also implemented in the
369 prototype (S. Gilles, unpubl. data) and showed that *O. niloticus* is able to adapt to this
370 planktonic artificial ecosystem running in brackish water, at least regarding the intensive
371 rearing unit. This is of particular interest since *O. niloticus* grows faster than *S. m.*
372 *heudelotii* and is currently found in most tropical countries around the world, as a result
373 of introductions. Nevertheless, an associated detritivorous species is needed for the
374 sewage ponds, as *O. niloticus* is not efficient in recycling sediments.

375 It could probably be possible to achieve a more efficient recycling of the sediment
376 in pond 3, and thus to avoid N loss, by increasing the rotifer number seeded at the start
377 of each cycle, thereby increasing grazing and limiting the amount of dead algae sinking
378 to the bottom of the pond. To this respect, the subdivision of pond 3 into two separate
379 volumes would allow reciprocal seeding, as well as an increase of productivity and
380 global conversion efficiency in the system.

381

382 *The rotifer invasion and the ecosystem resilience*

383 Algae belonging to the genera *Thalassiosira* and *Tetraselmis* are reputedly not grazed
384 by rotifers (Lavens and Sorgeloos, 1996). Hence, these algae proliferated in the main
385 circuit while *Chlorella* almost disappeared, and rotifers remained abundant for a while.
386 The later disappearance of these algae along with the return of *Chlorella* is more difficult
387 to explain. Drapcho and Brune (2000) pointed out that the addition of inorganic carbon
388 to PAS resulted in a predominance of green algae, to the detriment of cyanobacteria.
389 King (1970) also reported that green algae benefited from high dissolved CO_2
390 concentrations. Dissolved CO_2 tends to increase concomitantly with increasing fish
391 biomass and feeding rate. Indeed, fish metabolism, and thus CO_2 production are
392 proportional to food intake. Witt *et al.* (1981) showed that optimum salinity for
393 *Nannochloris* sp. (now renamed within the *Chlorella* genus) growth ranged from 10 to
394 20. The conjunction of these two factors (i.e. inorganic carbon load and salinity) is then
395 likely to account for the predominance of green algae in this prototype.

396 After the sixth day of rotifer invasion, TAN elimination from the circuit could have
397 resulted from nitrifying bacteria, which developed particularly in the circuit pipes (Dvir *et*
398 *al.*, 1999), as well as from opportunistic algae. We must emphasize, however, that these
399 algae do not regulate nitrites or nitrates, the levels of which only began to decrease
400 after *Chlorella* returned. This significant control of $N\text{-}NO_2^-/NO_3^-$ by *Chlorella* was
401 illustrated in a previous experiment conducted *ex situ* but with biological material (algae,
402 fish) from the prototype (Gilles *et al.*, 2008).

403

404 *Uptake of nitrogen*

405 During this experimental period with a feeding rate of 15.2 g m^{-2} day^{-1}, the N
406 assimilation in the prototype was 1.90 mg L^{-1} day^{-1}, which fits with the range given by

Hargreaves (2006) in his review of photosynthetic suspended-growth systems in aquaculture. Yet, the depuration capacity of the prototype can be higher, as demonstrated during previous experiments with the prototype, when the uptake of N from fish excretion was as high as 4.4 mg $N\text{-}NH_4$ L^{-1} day^{-1} (for feed with a 8.32 % nitrogen content; Gilles *et al.*, 2008). The prototype was evaluated in Senegal (14°N) where solar radiation is effective for photosynthesis all year around and cloudy days are rare. Conversely, other systems studied at higher latitudes experienced instantaneous TAN peaks of up to 17 mg L^{-1} during protracted cloudy periods. This was observed for a marine recycling system using macro-algae in southern France (44°N; Blancheton, 2000; Pagand *et al.*, 2000; Deviller *et al.*, 2004; Metaxa, 2006) or for PAS in South Carolina (35°N; Brune *et al.*, 2003).

Conclusion

This study by focusing on resilience and purification capacities of a prototype of recycling aquaculture system, provided further evidence of its relevance in comparison to conventional systems in clear water with mechanical and biological filters, especially wherever water resources are limited. It is possible to implement a stable outdoor artificial planktonic ecosystem, in tropical conditions, thanks to constant or at least sufficiently stable light and temperature conditions all year long, and to a brackish water environment. By contrast, it would probably be very difficult to have such a system stabilized outdoor in temperate climatic conditions. With their fast growth potential, *Chlorella* and rotifers seem to be the ideal candidates for planktonic artificial ecosystems in brackish water, especially with their spontaneous development.

In view of the increasing constraints on water supplies, planktonic recirculating aquaculture systems (PRAS) may certainly have a bright future. In particular, efforts should be deployed to develop similar approaches in marine and fresh waters, while using local resources. To this respect, new research efforts are currently undertaken by the authors to develop a similar system adapted to the Amazonian waters and fish fauna. The second step in the effective development of PRAS will be their transfer from the research sector to the industry, which is not always familiar with the way of operating these new systems. The recent transfer to a private tilapia farm in Senegal of the prototype originally developed in Mbour indicates that this is feasible, at least after proper information exchange and staff training.

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551 Table 1
552 Characteristics of the various compartments of the system
553

Compartment	Volume (m^3)	Flow rate ($m^3\ h^{-1}$)	Retention time (h)
Fry tanks	0.3	0.6	0.5

Grow out tanks	11.7	23.4	0.5
Sewage pond 1	24.4	24.0	1.0
Sewage pond 2	24.4	24.0	1.0
Zooplankton pond	24.4	0.5	48.0

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556 Table 2
557 Physico-chemical parameters
558

Mean daily water temperature °C	From 26 to 37
Mean Dissolved Oxygen (DO) mg L^{-1} at 7:00 and 15:00	
in intensive tanks	4 and 8
in sewage pond 1	3 and 12
in sewage pond 2	1 and 15
Mean pH at 7:00 and 15:00	7.9 ± 0.5 and 9.2 ± 0.8
Total alkalinity meq L^{-1}	3.47
Mean light intensity W h m^{-2} day^{-1}	4000
$N\text{-}NH_4^+/NH_3$ (TAN) mg L^{-1}	From 0.2 to 1.7
$N\text{-}NO_2^-/NO_3^-$ mg L^{-1}	From 0.0 to 2.3
PO_4^{3-} mg L^{-1}	From 2.3 to 4.1

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561 Table 3
562 Performances parameters of the system (2008)
563

Growth period (days)	117
Initial tilapia biomass weight in the prototype (kg)	99.7
Final tilapia biomass weight in the prototype (kg)	187.1
Total biomass produced (kg)	87.4
Global productivity (kg m^{-2} $year^{-1}$)	1.85
Global Specific Growth Rate (SGR) in grow out tanks (%)	0.51
Global FCR of the system	1.69
Survival in grow out tanks (%)	98.3
Average daily water evaporation (% total water volume)	1.5
Specific water consumption (liters kg^{-1} fish produced)	799

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568 **Figure captions**
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570 Figure 1: Schematic view of the prototype: (1) sewage pond 1, (2) sewage pond 2, (3)
571 sewage pond 3 as zooplankton producer, (4) intensive rearing tanks, (5) fry tanks, (6)
572 connection between ponds 1 and 2, (7) alternative operating pumps, and (8) fish filters.
573
574 Figure 2: Temporal evolutions in the main circuit of: (A) Rotifer density, $N\text{-}NO_2^-/NO_3^-$,
575 TAN and PO_4^{3-} concentrations, (B) Algal densities as *Chlorella*, *Tetraselmis*, and
576 *Thalassiosira*, and (C) DO in pond 2 at 7:00 and 15:00, during the rotifer invasion.
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Figure
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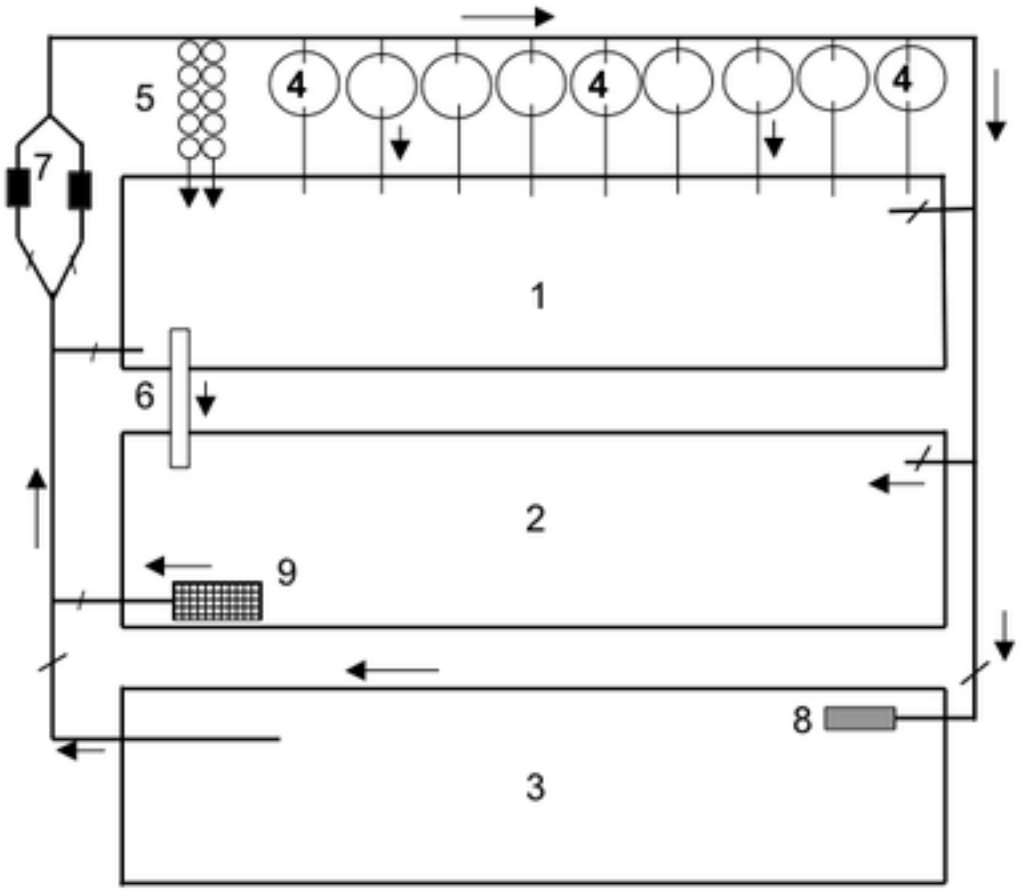

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