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Growth performances and natural diet of European eel (*Anguilla anguilla* L.) reared in muddy and sandy ponds

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Abstract – This study evaluated the growth performance and survival rate of *Anguilla anguilla* glass eels reared in 4750 m^2 earthen ponds and dependent on trophic resources provided by natural macrobenthic populations. Two ponds (M ponds) had mostly muddy-soil bottom whereas the other two (S ponds) had mostly sandy-soil bottom. A total of 4795 pigmented glass eels (0.73 ± 0.26 g) were stocked, at a density of 1.6 fish/m^2. After 360 days of growth, in S ponds eels exhibited a final mean body weight (15.57 ± 5.36 g) significantly higher than those seeded in M ponds (9.85 ± 4.11 g). Mean final length of S eels (21.55 ± 3.33 cm) was statistically higher than that of M eels (17.85 ± 2.42 cm). In M ponds, relatively higher abundances of *Crustacea* appeared to promote higher survival of glass eels in the post-seeding phase. Consequently, the relatively higher densities of eels in M ponds could have resulted in slower growth compared to eels in S ponds; however, eels grown in M ponds exhibited a significantly higher conditioning factor at the end of the growth period. We conclude that M ponds host highly viable macrobenthic prey populations that better sustain the early growth phases of *A. anguilla*. Furthermore, this study demonstrated that artificial feed supplements are not necessary for the rearing of *A. anguilla* juveniles in earthen ponds.

Keywords: European eel (*Anguilla anguilla*) / glass eel / pond culture / macrobenthic communities / growth performance

1 Introduction

Extensive fish culturing utilizes natural trophic sources to generate energy flow and enhance animal production. Differently, intensive culturing relies exclusively on supplementary energy delivered by artificial feed (Rossi et al. 1988; Bosma and Verdegem 2011). In Italy, extensive cultures are managed in coastal lagoons, and North Adriatic lagoons constitute nearly half of the total exploited Italian brackish lagoon environments. In this area, eels (*Anguilla anguilla* L.) are reared extensively via the traditional *Vallicoltura* (Mordenti et al. 2013), carried out in the *Valli*, a sector of a lagoon or enclosed earthen pond (Ciccotti 1997).

In the pond environment, there are close trophic interactions between the fish and their prey (Ingram and de Silva 2007); therefore, identifying the preferred prey of juvenile fish reared in earthen ponds is important for improving fish production. Managing ponds to increase the abundance of preferred prey could enhance growth and survival of cultured fish stocks. This approach, often referred to as green-water culturing, has been adopted worldwide for the rearing of numerous species of juvenile fish in earthen ponds (Egna and Boyd 1997; Castelo Branco et al. 2006; Young-Sulem et al. 2006; Ingram and de Silva 2007; Bosma and Verdegem 2011; Biswas et al. 2012), but only a few studies have investigated alimentary shifts of juvenile eels and these have been largely limited to coarse descriptions of diet (zooplankton, macroinvertebrates, and fish) (Breteler et al. 1990). Macrobenthic populations in coastal lagoons normally exhibit high spatial variability as their distribution can be influenced by various environmental parameters including salinity, water exchange ratio, and bottom characteristics, among others (Guelorget and Perthuisot 1992; Ponti et al. 2005). Identifying the ideal habitat for macrobenthic populations that yield higher survival and growth of glass eels would in turn lead to enhanced production of juvenile eels that can be reared for silver eel production in *Valli* (Parisi et al. 2014). The objectives of the present study were to evaluate the productive performances (final mean weight, condition factor, specific growth rate and final density) and survival rate of the European eel, starting from the glass eel phase, reared in earthen ponds with different bottom characteristics.

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Table 1. Types of substrate in ponds and farming parameters of *A. anguilla* during growth phase.

		Growth
Pond	*n*	4
Sandy substrate size	μm	125–500
–incidence	%	70 ± 5
Muddy substrate size	μm	4–63
–incidence	%	65 ± 7
Area	m^2	750
Volume	m^3	900
Stocking density	*n* total	1200
density	n/m^2	1.6
Days	*n*	360
Initial mean weight	g	0.73 ± 0.26
Initial mean length	mm	8.89 ± 0.95
Food type		Natural
Water origin		Lagoon water
Type of water circulating system		Closed
Flow rate	L/s	–

2 Materials and methods

In May 2013, glass eels were captured from two different river mouths, (Tevere River: 41° 44'25.97"N – 12° 14'00.2"E; Marta River: 42° 14'06.75"N – 11° 41'44.12"E), in central Italy. They were transferred to an experimental station of the *Bonello Valle*, a small coastal lagoon (50 ha) located in north-eastern Italy. For the trial, 3.5 kg of pigmented glass eels (n = 4,795 fish; 0.73 ± 0.26 g body weight) (BW) were distributed at random into 4750 m^2 ponds. The ponds differed in sediment type in relation to the prevalence of sandy (S) or muddy (M) substrate (S:M ratio) (Table 1): two ponds (S ponds) were characterized by a mostly sandy-soil bottom (3:1); the other two (M ponds) had a mostly muddy-soil bottom (1:3). The water depth in each pond was 1.2 m (Table 1) and glass eels were stocked at the initial density of 1.6 fish/m^2.

One month before the beginning of the growth phase, all the ponds were drained and allowed to dry for 7 days. The bottoms were levelled and then filled with brackish water coming from the *Bonello Valle*. The water was filtered with a 5 mm mesh net to prevent the introduction of large organisms into the ponds, especially predatory fish. There was no flow to or from the ponds, nor was supplementary aeration provided during the growth experiment. To prevent bird predation, the ponds were protected with a 12 cm side mesh net. The growing phase lasted 360 days, from July 2013 (T0, Start of the experiment) to June 2014 (T4, Day 360). Samples were carried out at different times: September 2013 (T1, Day 95), November 2013 (T2, Day 155), March 2014 (T3, Day 272). Water temperature (°C) and dissolved oxygen (mg L) were measured daily (9–11 a.m.) in all ponds using portable electronic devices (Mod Hanna Instr. mod HI9146), and water salinity was monitored weekly using a salinometer (Milwaukee mod MR100ATC).

At the same times, in order to evaluate the composition and abundance of macrobenthic communities, six substrate samples (surface: 115 cm^{-2}; thickness: 12 cm) were collected from the bottom of each S and M ponds, at the same distance among them, using a coring device. Macrobenthic organisms were separated by repeatedly suspending each sediment sample in water and decanting the supernatant into a 250 μm test sieve. The macrobenthic community was described following Borja et al. (2000) and Ponti et al. (2005) by computing the mean abundance of each taxon at each sample site. The species richness was determined as relative distribution (rate of macrobenthic species) and density (n.100 cm^{-2}). Taxon identification was performed in the laboratory by means of a binocular microscope (4–40X).

Fish sampling in M and S ponds was performed using traps and fishing nets (30 eels per pond) and coincided with the sampling of the macrobenthic community. All the fish samplings were carried out at the same time of the day (6:00 a.m.). A winter sampling was not performed in order to prevent capture-induced mortality and, because fish are more active during late spring and summer, the environmental effects on growth are more evident (Carvalho et al. 2007). The fish captured from each pond were individually weighed to the nearest 0.01 g with electronic balance scales (Bel Engineering mod. Mark K12); total body length (BL; measured from the most anterior extremity to the caudal fin, squeezed to give the maximum length measurement) was determined to the nearest millimeter using an ictiometer. Condition factor (K) was calculated according to the formula $K = (BW \times BL^{-3}) \times 10^3$ where BW: body weight (g), BL: body length (cm) (Mordenti et al. 2013). At T2 and T4, 20 eels per pond were randomly selected and immediately sacrificed with an overdose of anaesthetic (2-phenoxyethanol) in order to excise their stomach and determine the gut fullness index (GFI) according to the procedure indicated by Ingram and de Silva (2007). GFI values were determined as one of three categories: GFI 0 (zero) = empty stomach; GFI 1/2 = half full stomach; GFI 1 = full stomach.

At the end of the growth phase, the all ponds were drained and all fish were harvested. All eels were counted and 100 specimens per pond were individually weighed and measured in order to evaluate growth performances (BW, BL and K) of fish reared in ponds with different bottom types. Survival rate was recorded as percentage of fish harvested in relation to the number of fish stocked at the beginning of growth phase. The specific growth rate (SGR), was expressed as the percentage increase in BW per day (% day^{-1}) using the following formula:

$$SGR = 100 \times (\ln BW_t - \ln BW_0)/t;$$

where t is time in days; $\ln W_0$ is the natural logarithm of the average body weight at time zero; $\ln W_t$ is the natural logarithm of the average body weight at time t (Ingram et al. 2001).

Zootechnical performances of eels reared in M and S ponds were compared using a one-way analysis of variance (ANOVA; SSP software, Smith's Statistical Package). The means were separated by a Student Newmann Keuls test. Differences were considered statistically significant at $p \leqslant 0.01$.

All the fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC). Approval for this study was obtained by Ethics Committee of Bologna University.

3 Results

Several growth parameters varied significantly between eels reared in M and S ponds at T4. Eels in S ponds exhibited a

Table 2. Growth performances of eels in the two different ponds.

		S ponds	M ponds
Initial mean weight	g	0.73 ± 0.26	0.73 ± 0.26
Initial mean length	mm	8.89 ± 0.95	8.89 ± 0.95
Initial condition factor (*K*)		1.04 ± 0.31	1.04 ± 0.31
Final mean weight	g	15.57 ± 5.36[a]	9.85 ± 4.11[b]
Final mean length	mm	21.55 ± 3.33[a]	17.85 ± 2.42[b]
Final condition factor (*K*)		1.43 ± 0.14[b]	1.63 ± 0.14[a]
SGR	%/d	0.850[a]	0.723[b]
Final specimen density	kg/pond	12.74 ± 0.8	9.96 ± 0.60
	n/pond	818.4 ± 51.6	1011.6 ± 61.2
Survival rate	%	68.2 ± 4.3[b]	84.3 ± 5.1[a]

Different letters (a, b) on the same line indicate significant differences, with a > b ($p < 0.01$).

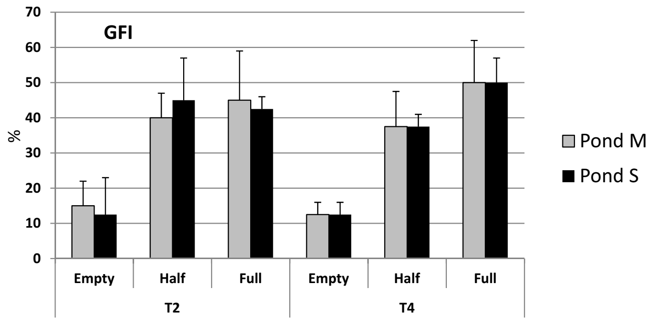

Fig. 1. Gut Fullness Index (GFI) registered at the end of growth phases T2 and T4.

final mean body weight (15.57 ± 5.36 g) that was significantly higher than those eels seeded in M ponds (9.85 ± 4.11 g); the mean final length of S eels (21.55 ± 3.33 cm) was also statistically higher than those of M eels (17.85 ± 2.42 cm; Table 2). SGR was also significantly higher for eels reared in S ponds (Table 2). Condition factor (*K*) steadily increased during the growth period in both pond types; however, in contrast to the differences in body weight and length at T4, the *K* value of eels reared in M ponds (1.63 ± 0.14) was significantly higher those reared in S ponds (1.43 ± 0.14; Table 2). Eel survival rate at the end of the trial was also significantly higher in M ponds (Table 2). The GFI of eels reared in M and S ponds was not significantly different (Fig. 1). Empty stomach percentage was very low at both T2 and T4, and was never higher than 15%.

Macrobenthos analysis identified 12 taxa, 7 of which were classified at the species level. The most abundant species were *Cerastoderma glaucum* and *Abra alba* for Bivalves, *Hydrobia ventrosa* for Gastropoda, *Corophium* spp. for Crustaceans, and Polichaeta were represented exclusively by *Neanthes succinea*. At the beginning of the growth phase (T0), M and S ponds exhibited a macrobenthic faunal community that contained the same four taxa (Fig. 2). However, the relative distribution of species in the two pond types was significantly different; in M ponds it was mainly composed of Crustaceans (58.2%, 93% of which were *Corophium* spp.), followed by Polichaeta, Bivalves, and Gastropoda. S ponds were mainly inhabited by Polichaeta (60.5%, all of which were *Neanthes succinea*), followed by Bivalves, Crustaceans and Gastropoda (Fig. 2). The total macrobenthic population showed a more significant density in M ponds than that observed in S ponds (12.7 animals.100 cm^{-2} vs. 4.3 animals.100 cm^{-2} respectively; Fig. 2). As concerns the different taxa, only *Crustacea* exhibited significant difference in favour of M ponds compared to S ponds (7.4 animals.100 cm^{-2} vs. 0.5 animals.100 cm^{-2} respectively; Fig. 2).

Similar trends were observed for the macrobenthic community composition in both pond types during the eel growth period (Fig. 3). In all the ponds of both the types, the relative abundances of Polichaeta and Crustaceans significantly decreased over time, and consequently, Bivalves and Gastropoda notably increased during the growth period. The largest reduction of Crustacea (67.2% in S ponds and 45.7% in M ponds) was observed during the first growth time (from T0 to T2), and a dramatic reduction in the Polichaeta population (94.1% and 87.8% in S and M ponds, respectively) occurred during the second growth time (from T2 to T4; Fig. 3). Over the entire growth period, the macrobenthic population density was reduced by 73.4% in M ponds (from 12.7 to 3.3 animals.100 cm^{-2}) and 85.1% in S ponds (from 4.3 to 0.6 animals.100 cm^{-2}).

Water analyses and sediment samples showed high uniformity in chemical composition between the two pond types, with oxygen concentrations ranging from 5.16 mg L to 8.74 mg/l and salinity ranging from 35–40 in summer and 20–25 in winter (Fig. 4). The maximum and minimum temperatures were 30 °C and 3.5 °C, respectively (Fig. 4).

4 Discussion

The present study investigated the effects of benthic communities in earthen valley ponds on feeding behavior, growth, survival, and rearing of the European glass eel. The macrobenthic invertebrate compositions observed in the two pond treatments were significantly different but qualitatively corresponded to that of a littoral ecosystem (i.e., a transitional ecosystem). The introduction of glass eels into soft-bottom earthen ponds has also been shown to impact the structure of the macrobenthic community (Carvalho et al. 2007). At seeding, all ponds exhibited high taxonomic diversity, including high abundances of *Polichaeta* in S ponds and *Crustacea* in M ponds. M ponds were richer in terms of macrobenthic density (12.7 animals.100 cm^{-2}), compared to S

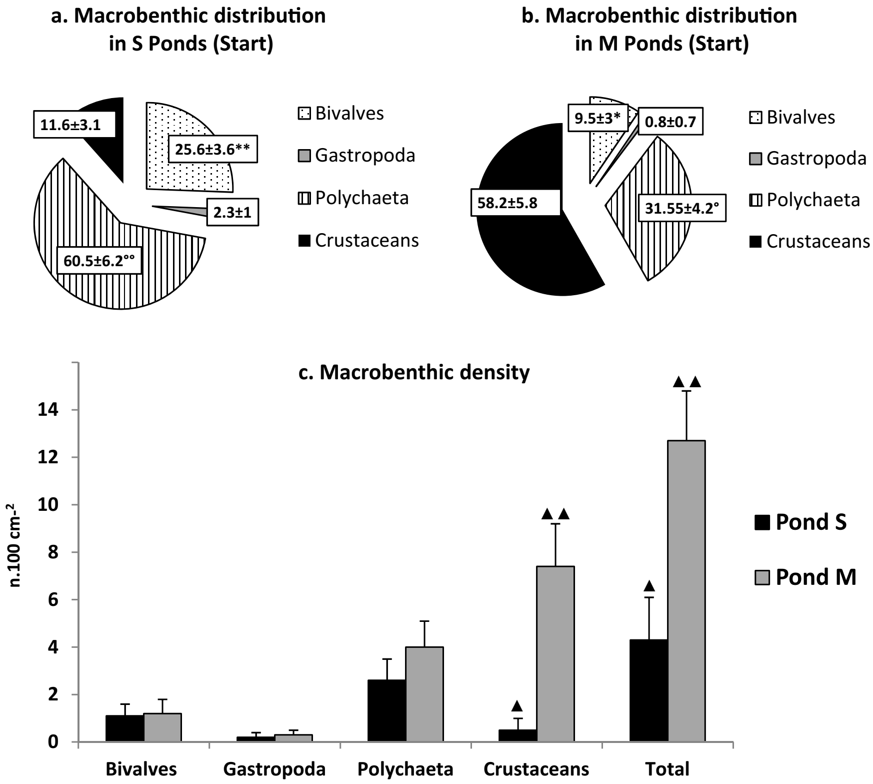

Fig. 2. Relative distribution (%) of macrobenthic species (a, b) and macrobenthic density (c) in S and M ponds at the beginning of the growth phase. Different symbols of the same taxa show significant differences ($p < 0.01$).

ponds (4.3 animals.100 cm^{-2}). During the growth phase, organisms belonging to *Crustacea* and *Polichaeta* were reduced to 13.2% and 4.0% of the total macrobenthic population in M and S ponds, respectively. As reported by Ingram and de Silva (2007), the demand for food resources increases fish biomass.

Glass eel feeding habits shifted from an initial preference for *Crustacea*, which were largely reduced in both M and S ponds from T0 to T2, to *Polichaeta*, which almost completely disappeared from T2 to T4. This size selective feeding behavior is expected by predacious fish, as juveniles typically consume the most abundant prey within the limitations imposed by mouth gape (Fox 1989; Hapher et al. 1989; Schael et al. 1991; Christofferson et al. 1993; Ingram and De Silva 2007). Finally, the relative increases in Bivalves and *Gastropoda* abundance throughout the growth period could be due to their reproductive activity and relatively reduced predatory pressure, as eels seem to have avoided these species. Gut content analysis revealed that eels also rarely consumed zooplankton (i.e. rotifers and copepod nauplii), which are common and abundant in these lagoon habitats. Although it was not possible to verify if prey size increased with eel growth during the current study, juveniles fishes are expected to feed on small plankton only when prey choice is limited (Rowland 1992; Ingram and de Silva 2007).

In M ponds, eels showed lower weight, length and SGR but higher survival rate in comparison with juveniles in S ponds. In M ponds, eels had an initial feeding availability more favourable probably due to the high content of *Crustacea*; this taxa represented almost the 60% of the benthic community and could have enhanced the survival rate in this environment. This result is in agreement with the findings of Ravagnan (1978) which showed that eels consumed *Corophium* spp. as the most available benthic prey in lagoon ponds. The higher stocking density partially affected the growth rate although the condition factor was favourable in all the types of pond.

5 Conclusion

In the M ponds, the high *Crustacea* content appeared to promote a higher survival of glass eels during the initial growth phase. Subsequently, the higher eel population density in M ponds could have negatively impacted eel growth (i.e., increases in biomass and body length), which could explain the lower condition factor (K) observed upon harvest (T4) in comparison to S ponds. It seems that muddy ponds were more suitable for rearing eels, as they provide habitat for a macrobenthic population that is highly viable for glass eels during the initial growth phases. This is supported by the fact that eel sampling in S ponds was always more time consuming and labor intensive compared to M ponds, likely due to reduced eel abundance.

The current study demonstrated that juvenile *A. anguilla* can be reared in earthen ponds without supplementary artificial feeding. These results were satisfactory, although the initial stocking density used in this study was relatively low (i.e., glass eel 1.6 m^{-2}) compared to those used in tank cultures (Heinsbroek and Kreuger 1992; Roncarati et al. 1997)

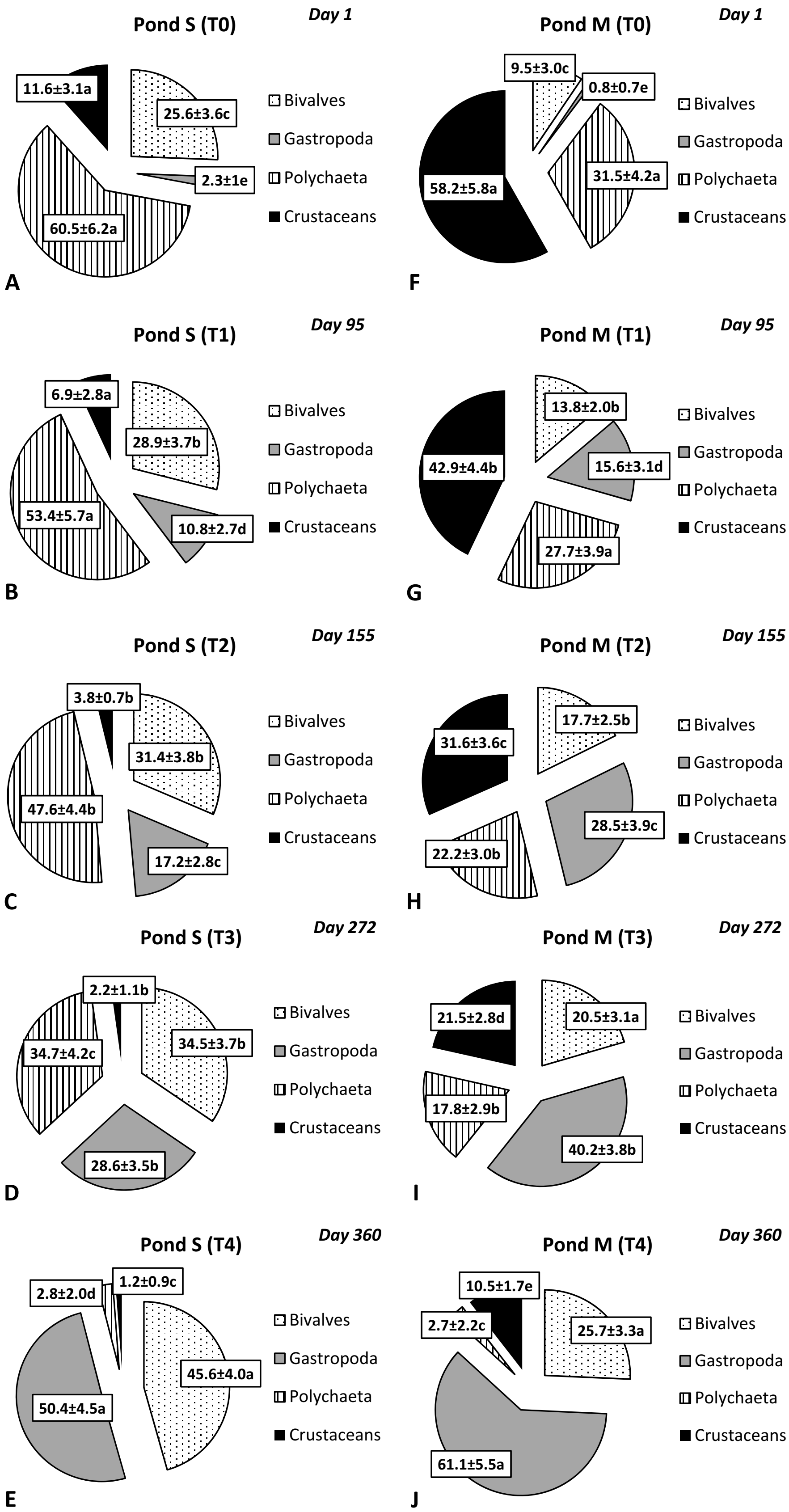

Fig. 3. Changes in the relative abundance of the macrobenthic community over the sampling period. Different letters of the same taxa, belonging to the same type of Pond, show significant differences ($p < 0.01$).

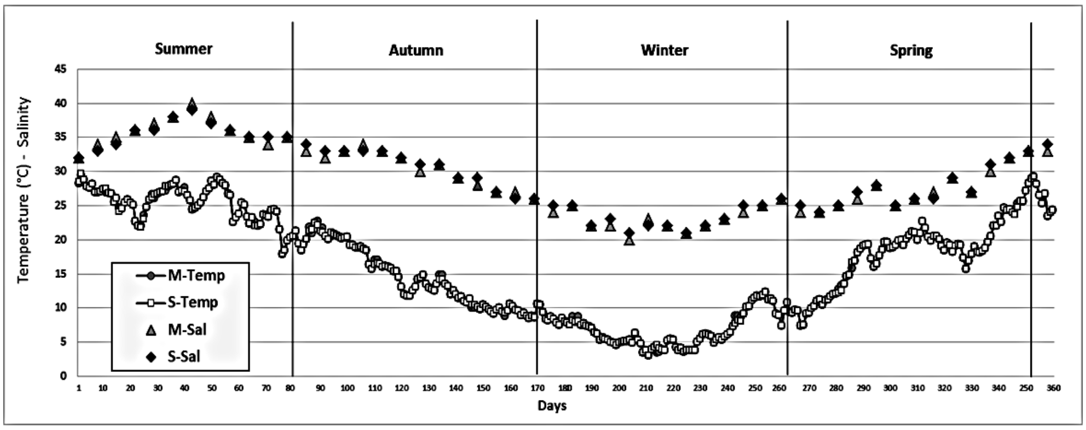

Fig. 4. Water temperature and salinity in M ponds and S ponds through the growth period.

or for *A. australis* in a fertilized pond (Ingram et al. 2001). Belpaire et al. (1990), after 190 days of stocking glass eels at higher initial densities in fertilized earthen ponds without supplemental feeding, obtained lower BW (1–2 g) compared to those reached in the present paper. The stocking of juvenile *A. anguilla* at high densities resulted in reduced growth in earthen ponds (Breteler et al. 1990) and tank cultures (Degani and Levanon 1983; Roncarati et al. 1997). High seeding densities, which are normally employed for intensive rearing, can lead increased feeding competition and larger variability in fish body weight. A greater size range in eels induces an increase in aggression between fish, and may result in cannibalism of smaller fish (Sadler 1979). It is possible that low seeding densities in ponds can lead to higher female eel production. Even though sexual dimorphism was not yet evident in the current study due to the small size of the fish, Tesch (1991) observed that, given equal space in nature or farming, an increase in the number of individuals corresponded to a modified sex ratio in favor of male eels, which are generally believed to grow slower than females. Ravagnan (1978) and Roncarati et al. (1997) observed that the growth of eels reared in ponds at high stocking densities tended plateau at 150 g, due to the high number of males or specimens with male gonads.

Interestingly, the biometric parameters of glass eels observed in the current study were comparable with those of a European eel culture that was reared in captivity, using a traditional intensive technique relying on artificial food (Gousset 1990). In rearing tanks, Roncarati et al. (1997) obtained 7.5 g juveniles after 120–150 days, while in the current study, glass eels reared in earthen ponds increased to 10–15 g in 170 days. It should be taken into account that eels feed mainly when water temperature is between 18 and 30 °C (Altun et al. 2005), reducing the optimal growth period to 170 days out of the 360 day trial length. The water temperature remained below 30 °C in summer, while temperatures were lower than 5 C for 22 days during winter. According to Sadler (1979) and Walsh et al. (1983), eels enter a state of numbness at temperatures varying from 1 to 5 °C, while the critical thermal maximum varies from 33 to 39 °C. Increased basin depth helped to buffer temperatures and reduced eel mortality and other problems that could result from excessive cooling in winter or overheating in summer.

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