Glomerular disease associated with *Polysporoplasma sparis* (Myxozoa) infections in cultured gilthead sea bream, *Sparus aurata* L. (Pisces: Teleostei)

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SUMMARY

Polysporoplasma sparis infection was studied in gilthead sea bream from different mariculture systems of the Spanish coasts. Culture conditions influenced the infection dynamics, as the parasite appeared only in semi-intensive cultures and was not found in intensive closed systems nor in open ones. No clear seasonal pattern was observed. No fish weighing less than 51 g was found parasitized in any group. A statistically significant dependence between infection prevalence and host weight was observed in some growing stocks. Light and transmission electron microscope observations revealed serious damage in the trunk kidney. Glomerular disease was provoked by the progressive occupation of the glomerular capillaries by *P. sparis* spores. Tubular epithelial cells were also affected. Inflammatory responses appeared towards the end of the infection, and consisted mainly of melanomacrophages and eosinophils. Rodlet cells were common close to infected capillaries and debris of rodlet sacs formed a belt encircling capillary vessels. Cytochemistry demonstrated the lipidic nature of these sacs and the glycogen and glycoprotein composition of the cytoplasmic granules of rodlet cells.

Key words: *Polysporoplasma sparis*, Myxozoa, gilthead sea bream, electron microscopy, histopathology, epidemiology.

INTRODUCTION

The gilthead sea bream, *Sparus aurata*, is a marine teleost successfully cultured in the Mediterranean area. Nevertheless, increasing numbers of pathological problems associated with its culture have been reported. Parasitic diseases are responsible for important economical losses in aquaculture (Bauer, Egusa & Hoffman, 1981; Klesius, 1994). Among these, Myxosporea play a significant role as pathogens for both marine (Álvarez-Pellitero & Sitjà-Bobadilla, 1993*a*) and freshwater fish (El-Matbouli, Fischer-Scherl & Hoffman, 1992). These include the recently described genus *Polysporoplasma* and the new species *P. sparis* from the gilthead sea bream (Sitjà-Bobadilla & Álvarez-Pellitero, 1995). This species had been previously referred to as a *Sphaerospora* sp. (Sitjà-Bobadilla, Franco-Sierra & Álvarez-Pellitero, 1992).

In the present work we analyse *P. sparis* infections in cultured gilthead sea bream. Data on the prevalence and intensity of the infections in different culture systems are presented. The pathology caused by the parasite is studied at light and electron microscope levels, and histochemical studies are included. The significance of this myxosporidiosis for gilthead sea bream culture is also discussed.

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MATERIALS AND METHODS

Fish and sampling procedure

A total of 851 gilthead sea bream from several culture systems in the Mediterranean and South Atlantic coasts of Spain were sampled from January 1990 to May 1997. Details of the fish groups and culture facilities are summarized in Table 1. Information on the sampling periods and frequency is also included. Groups 1 (G–1) and 2 (G–2) were followed periodically through random samplings. Group 3 (G–3) was sampled periodically in only some periods. The remaining groups include fish from occasional samplings.

Fish were killed by overexposure to the anaesthetic MS-222 (Sigma, St Louis, MO, USA), weighed, measured, necropsied and their organs excised for fresh and histological examination for parasites. The presence of *P. sparis* was registered, and the prevalence of infection was calculated as the percentage of infected fish in each sampled group. The intensity of infection was semi-quantitatively evaluated according to a scale ranging from 1+ to 6+. This scale was based on the number of parasitic stages per microscope field at 300×, with the scaling range being 1+ = 1–5; 2+ = 6–10; 3+ = 11–25; 4+ = 26–50; 5+ = 51–100; 6+ > 100.

Histological processing

For light microscope (LM) studies, thin sections (1–3 μm) were obtained from material fixed in 10%

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Table 1. *Sparus aurata* groups sampled and the culture systems surveyed

Group	Location	Total no. of fish	Culture conditions	Age/weight	Temperature (°C)*	Salinity (‰)	Sampling periods and frequency
G–1	Southern Atlantic Coast	99	Intensive open hatchery	< 45 days	18	21	Apr. 90–Aug. 94: every 2–3 months until 9 months old; once or twice a year later on
		48	Intensive open nursery	45–100 days	20	20	
		151	Semi-intensive and extensive growing stocks	7·8–419 g	10–28·5	25–40	
G–2	Western Mediterranean Coast (Valencia)	200	Intensive closed system	0·65–450 g	16–27	16	Jan. 90–May 91: every 2 months
G–3	Western Mediterranean Coast (River Ebro Delta)	198	Semi-intensive growing system	6·3–431 g	8·1–29·4	28–36	1993–1995: every 2–3 months in spring and summer; occasional samplings out of these seasons
G–4	Western Mediterranean Coast (IATS)†	100	Intensive open system	4·8–1000 g	9–27	37·8	Mar. 93–Jun. 94: every 1–2 months
G–5	Western Mediterranean Coast (Alcanar)	55	Sea cages	220–378 g	9–27	37·8	May 96; Jun. 96; May 97

* Range of monthly averages, with minimum values from December to February, and maximum values from July to September. For the nursery and hatchery systems, well water temperature was constant. † Instituto de Acuicultura Torre de la Sal facilities (Castellón).

buffered formalin and embedded in Technovit resin (Kulzer, Heraeus, Germany). They were stained with toluidine blue (TB) or the periodic acid-Schiff (PAS) method followed by counterstaining with haematoxylin. For transmission electron microscope (TEM) examination, small pieces of the organs were fixed in 2·5 % (v/v) glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2), for 1 h at 4 °C. Samples were washed several times with the same buffer, post-fixed in 1 % (w/v) cacodylic OsO_4, dehydrated through a graded ethanol series, and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were double-stained with uranyl acetate and lead citrate (Reynolds, 1963). Some sections mounted on golden grids were stained by the Thiéry reaction for polysaccharides (Thiéry, 1967), the OTO technique for lipids (Seligman, Wasserkrug & Hanker, 1966), or the method of hydrochloric phosphotungstic acid stain (acid PTA, Courtens, 1978) for glycoproteins. The sections were studied in Philips CM-200 and Hitachi-800 TEM, operating at 80–100 kV.

Statistics

The influence of the host weight on the prevalence of infection was statistically analysed in G–1, using a chi-square test of independence ($P < 0{\cdot}01$) (Sokal & Rohlf, 1981).

RESULTS

Location in the host

Polysporoplasma sparis was found in the trunk kidney. Disporoblasts (DB) and spores (SP) were located mainly in the glomerulus, but some spores were occasionally observed in the Bowman's space, the lumen of renal tubuli or the interstitial tissue. Pre-sporogonic stages were not detected in the trunk kidney, though abundant macrophages engulfing possible residual stages and possible proliferative stages were observed in the anterior kidney of some infected fish.

Prevalence and intensity of infection

Data on total prevalence and mean intensity of infection in the different fish groups are presented in Table 2. The parasite was only found in fish from G–1 and G–3, with a relatively low prevalence of infection. Nevertheless, in G–1 prevalence reached 28·47 % in fish from growing ponds, whereas it was zero in fish from the hatchery or the nursery. No fish from the other systems, regardless of its age or size, was found to be parasitized.

Seasonal variations

Table 3 summarizes the infection levels along the 4-year study in the growing facilities of G–1. No clear

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Table 2. Total prevalence and intensity found for infections with *Polysporoplasma sparis* in the different *Sparus aurata* groups, along the surveyed years

Group	Number of fish examined	Prevalence (%)	Intensity (mean)
G–1	298	14·4	+ + +
G–2	200	0	–
G–3	198	12·1	+ +
G–4	100	0	–
G–5	55	0	–

Table 3. Seasonal prevalence and intensity found for *Polysporoplasma sparis* infections in *Sparus aurata* growing stocks of G–1 group, along the surveyed years

Sampling period	Number of fish examined	Prevalence (%)	Intensity (mean)
Spring	70	32·85	+ + +
Summer*	13	53·84	+ +
Autumn	39	15·38	+ + + +
Winter	29	24·13	+ + +

* Only 1994.

seasonal pattern was found for the prevalence of infection, as the parasite was regularly found in every season. Nevertheless, prevalence of infection was generally higher in spring and summer, though data for the latter comes only from 13 fish sampled in 1994. The parasitic stages found in autumn and winter consisted mainly of degenerated spores in old granulomas. In G–3, seasonal variations were not analysed due to the lack of data in winter. However, infection levels were irregular in the remaining seasons along the surveyed years (data not shown).

Influence of the host weight on the infections

In the growing stocks of G–1, an increase of prevalence of infection with host weight was observed, which was statistically significant at $P < 0{\cdot}01$ (Table 4). The prevalence of infection decreased moderately in fish in the 2 highest weight classes, but it remained higher than that in young fish or than the total prevalence in the growing stocks (28·47 %). No fish weighing less than 51 g was found to be infected, which was also the case in G–3 (data not shown).

Pathology

Most infected fish showed no external signs of disease, but the histopathological study at light microscope (LM) level revealed the presence of the parasite in the trunk kidney, which could be invaded by the parasite (Fig. 1 A), with numerous DB and SP in the glomeruli. Occasionally, some stages were detected in renal tubuli (in the epithelium or the lumen) (Fig. 1 B), producing vacuolation of epithelial cells, in the interstitial tissue or, more rarely, in the lumen of blood vessels.

Different degrees of histopathological damage were observed. The most outstanding feature was the gradual hypertrophy and eventual destruction of infected renal corpuscles in a process apparently linked to spore maturation. In the first steps in which the myxosporea was clearly identified, the capillary vessels of the glomerular tuft were occupied by DB, few mesangial cells were still present, the Bowman's space was narrow, and the Bowman's capsule started to thicken. In a following step, glomerular capillaries were completely devoid of blood cells and occupied by mature SP, showing thickening of the capillary walls and dilated lumens. Groups of two SP were located following the original architecture of the segments of the capillary tuft, in a rosette-like manner (Fig. 3 C). The extensive reduction of Bowman's space and the thickening of Bowman's capsule, resulted in a close contact between its visceral and parietal layers. In cases of low intensity

Table 4. Relationship between host weight and the infection level of *Polysporoplasma sparis* in *Sparus aurata* growing stocks of G–1 group, along the surveyed years

Weight class	Number of fish examined	Prevalence (%)*	Intensity (mean)
3·6–10 g	11	0	–
11–50 g	23	0	–
51–100 g	30	6·6	+ + + +
101–200 g	40	52·5	+ + +
201–300 g	29	41·4	+ + +
> 300 g	18	44·4	+ + +

* Statistical dependence at $P < 0{\cdot}01$.

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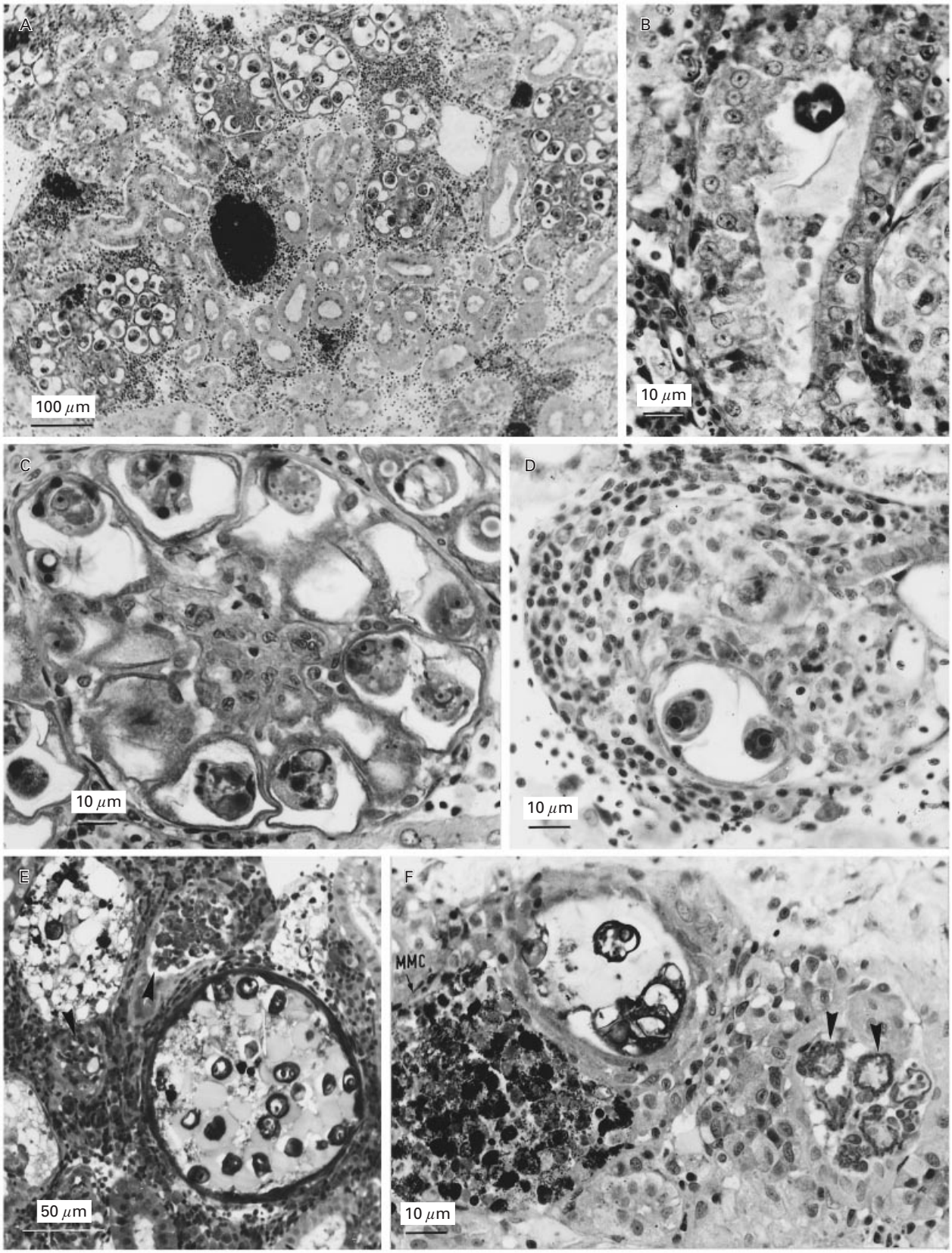

Fig. 1. For legend see p. 252.

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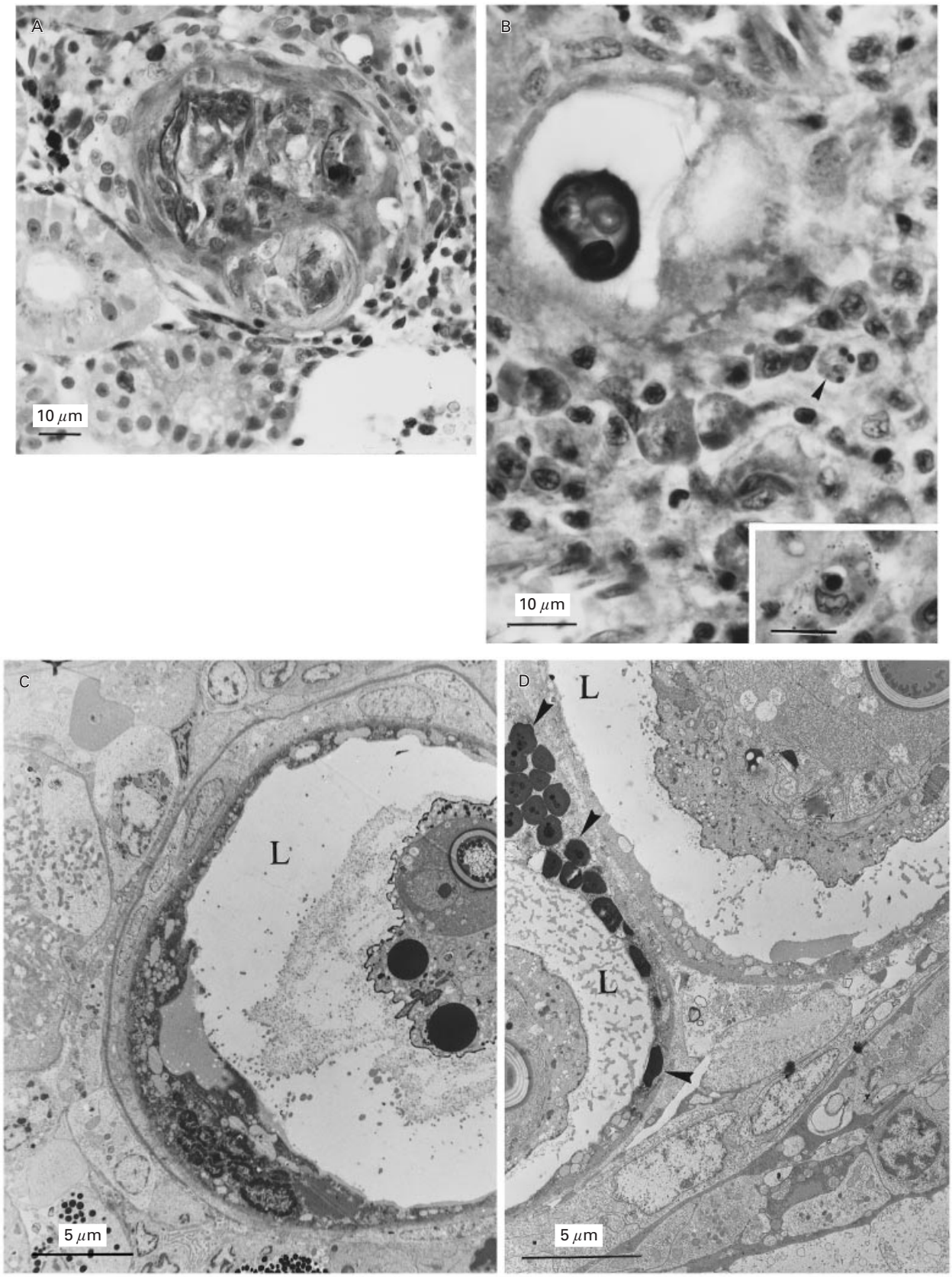

Fig. 2. For legend see p. 252.

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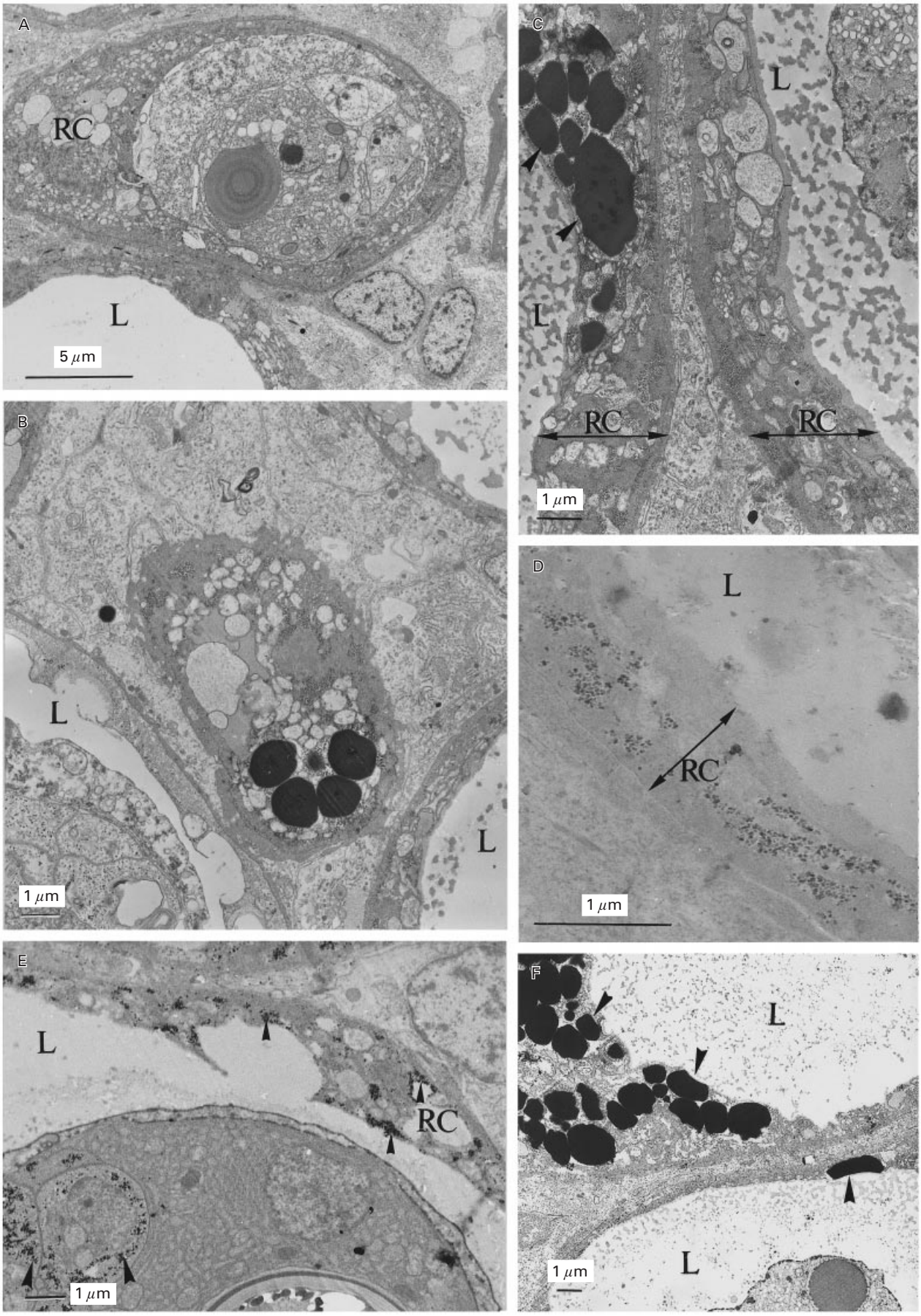

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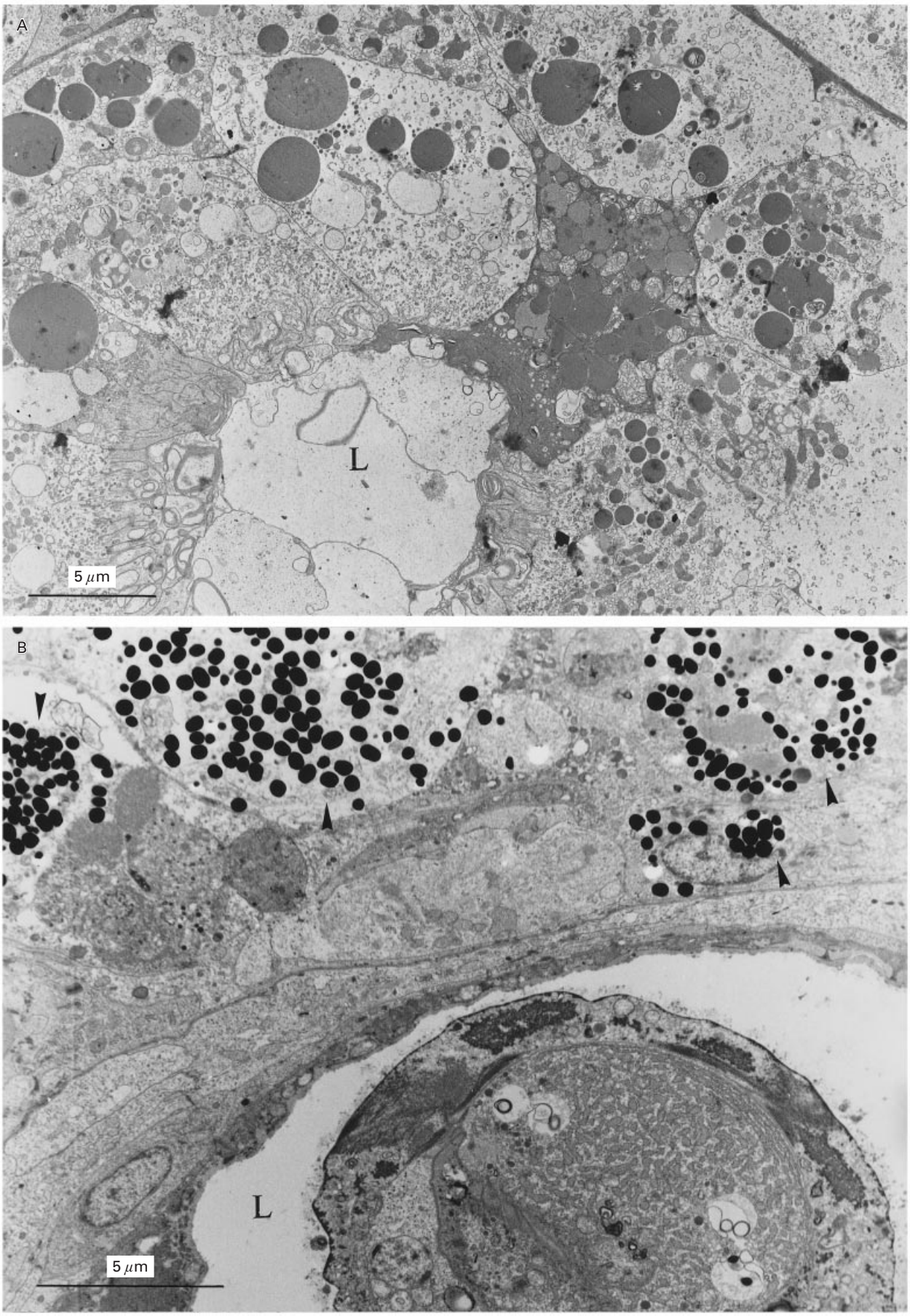

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of infection, only few glomerular capillaries exhibited mature SP, but in the remaining glomerular tuft, mesangial cells proliferated (Fig. 1 D). In a more advanced stage, nothing of the glomerular tissue was left; instead darkened spores embedded in an amorphous, hyaline material spread beyond the capillaries. The thickening of the basal membrane and the inconspicuousness of Bowman's capsule against the surrounding tissue layers were outstanding (Fig. 1 E). Sometimes, SP reached and enlarged Bowman's space, which appeared also filled with cellular debris. Initially, a slight fibrotic reaction surrounded parasitized renal corpuscles, which gradually thickened by the juxtaposition of epitheliod-like cells, resulting in necrotic granulomas, with cellular and parasitic debris (spore valves were sometimes distinguishable) (Fig. 1 F). Frequently, darkly pigmented melanomacrophage centres were found associated to these granulomas (Fig. 1 F), and eventually only debris was left within them (Fig. 2 A). Some glomeruli without detectable myxosporean stages were visibly altered, with enlarged Bowman's space and prominent podocytes or thickened capillaries filled with granulated material (Fig. 1 F).

In severely infected kidneys, the renal parenchyma atrophied, and haemorrhagic areas close to infected renal corpuscles were very infrequent. Inflammatory reaction to the parasite was obvious. It consisted mainly of leucocyte infiltration, involving lymphocytes (Fig. 1 E), monocytes (Fig. 2 B), macrophages – which could appear engulfing myxosporean stages (Fig. 2 B inset)–, eosinophils, and other granular cells. Some degenerated spores could be seen trapped within melanomacrophages (Fig. 1 F).

The ultrastructural study confirmed and extended the LM observations. Most of the TEM samples corresponded to advanced *P. sparis* infections. Frequently, mature SP occupied and distended glomerular capillaries, and a finely granulated material appeared in the capillary lumen (Fig. 2 C). The presence of the parasite provoked the compression of the trilaminate structure typical of a vertebrate glomerulus, that is to say, podocytes, the basement membrane and endothelial cells were pushed outside towards the neighbouring vessels (Fig. 2 C). Mesangial cells among capillaries appeared disorganized, with fibrous material (Fig. 2 D).

Bowman's space disappeared as a result of the capillary hypertrophy, and Bowman's capsule made

Fig. 1. (A–F) Toluidine blue-stained sections of *Sparus aurata* trunk kidneys parasitized by *Polysporoplasma sparis*. (A) Massive infection and hypertrophied glomeruli invaded with spores. Note the leucocyte infiltration and the atrophy of the intertubular tissue. (B) Detail of a SP in the lumen of a renal tubule. Note the extensive vacuolation of epithelial cells. (C) An advanced stage of parasite invasion. Note the dilated capillaries, with thickened walls, harbouring mature spores. The thickening of the basement membrane is evident. (D) Hypertrophied mesangial cells and leucocyte infiltration in a slightly infected gomeruli. (E) Darkened spores embedded in a hypertrophied renal corpuscle. Compare its size with that of apparently not infected ones (arrowheads). Note peri-glomerular fibrosis, surrounded by haemorrhage, and lymphocyte infiltration. (F) Necrotic granuloma with some spore valves inside. A strongly melanized MMC is attached. Note the neighbouring glomerulus with thickened capillary walls (arrowheads).

Fig. 2. (A and B) Toluidine blue-stained sections of *Sparus aurata* trunk kidneys parasitized by *Polysporoplasma sparis*. (A) Granuloma at a final stage of degradation, some debris is left inside and several layers of epitheliod-like cells are juxtaposed. (B) Detail of monocytes infiltrated in the interstitial tissue close to a spore, and a probable initial stage (arrowhead). Inset. Detail of a macrophage engulfing a parasite stage. (C and D) Transmission electron microscope images of *S. aurata* trunk kidneys infected by *P. sparis*. Spores located in the capillary lumen (L) have destroyed the podocytes and the endothelial cells, pushing the visceral and parietal layers of the Bowman's capsule towards the interstitial cells. Note the deposition of electron-dense material at the periphery of the capillary lumen, as well as finely flocculated material in (C) and (D). (D) Rod sacs of disintegrated rodlet cells (arrowheads) encircle a capillary vessel, and mesangial cells are conspicuously disorganized.

Fig. 3. (A–F) Transmission electron microscope images of *Sparus aurata* trunk kidneys infected by *Polysporoplasma sparis*. (A) Detail of an immature spore close to a rodlet cell (RC), between 2 capillaries. (B) Rodlet cell in the mesangial matrix of the capillary tuft, close to an infected glomerular capillary. (C) Detail of the cytoplasmic residues of rodlet cells (RC) at the periphery of 2 contiguous infected capillaries. Note the thick wall, the rod sacs (arrowheads), and the abundant granules and vacuoles in the cytoplasm. (D) Acid-PTA-positive staining of some glycoprotein granules of the rodlet cell cytoplasm. (E) Thiéry-positive staining of some granules of the rodlet cell cytoplasm (small arrowheads). Note the positively stained granules in the sporoplasms of a spore (arrowheads). (F) OTO-positive staining of the rod sacs of rodlet cells at the periphery of glomerular capillaries (arrowheads).

Fig. 4. (A and B) Transmission electron microscope images of *Sparus aurata* trunk kidneys infected by *Polysporoplasma sparis*. (A) Renal tubule, adjacent to an infected glomerulus, with vacuolized epithelial cells. Note the abundance of membranous debris in the lumen (L), the disorganized brush border, and the abundance of lysosomes and mielinic figures in epithelial cells. (B) Numerous melanomacrophages (arrowheads) with engulfed residual material close to an infected glomerular capillary.

a continuum with interstitial cells. In a final stage of degradation, the old trilayered structure was not distinguishable and appeared to be full with heterogeneous debris. Among it, some highly electron-dense structures were identified as disintegrated rodlet sacs, the elongated sac-like structures with a central core of dense material typical of rodlet cells (Fig. 2C). They frequently appeared surrounding the capillaries (Fig. 2D), in close contact with myxosporean stages (Fig. 3A), or embedded in the mesangial matrix (Fig. 3B). Rodlet cells contained numerous vacuoles of different size, lysosomes with membranous material, and scattered electron-dense granules (Fig. 3B). These structures were more densely packed in advanced stages of capillary destruction, and myelinic figures were frequent (Fig. 3C). Cytochemistry demonstrated glycoproteins (Fig. 3D) and glycogen (Fig. 3E) as part of the composition of these granules. The Thiéry-positive staining of these cells coincided with the PAS-positive reaction of the glomerular capillaries at LM. The lipidic nature of the rodlet sacs was demonstrated by the OTO staining (Fig. 3F).

The damage to the tissue was evident even outside the renal corpuscles. Adjacent renal tubules appeared clearly affected. Their lumen was frequently filled with membranous debris, and epithelial cells showed marked vacuolation, with increased amounts of lysosomes and myelinic figures (Fig. 4A). Melano-macrophages were common in the interstitial tissue close to infected glomeruli (Fig. 4B), some of them showing abundant secondary lysosomes, with engulfed material.

DISCUSSION

The results obtained in this study demonstrate the influence of culture conditions on the infection dynamics of *Polysporoplasma sparis*. The parasite appeared only in semi-intensive cultures (G–1 and G–3), whereas it was not found in intensive closed systems (G–2), open systems (G–4), nor in sea cages (G–5). The introduction of infected juveniles in semi-intensive growing systems should be disregarded, as the parasite was only detected in fish larger than 50 g (G–1 and G–3). Moreover, fish coming from the same hatchery and nursery, introduced later in the different growing systems studied, developed the infection (G–1 and G–3) or no infection at all (G–2). These data strongly suggest an introduction of the infective stages with the water supply. The absence of infection in fish from cage cultures is also remarkable, although only occasionally sampled. We have also diagnosed the parasite in fish from cage cultures in the Adriatic Sea (samples sent by Dr Ceschia). The involvement of an intermediate host, demonstrated for several freshwater myxosporean species (El-Matbouli *et al*. 1992; Lom & Dyková, 1995), including *C. shasta* (Bartholomew *et al*. 1997) is also probable in the life-cycle of *P. sparis*. If this is the case, the semi-intensive culture conditions found in G–1 and G–3 growing stocks, in earth ponds with a rich organic sediment, would be adequate for the hypothetical intermediate host. In the intensive open system (G–4), sediments which support a rich invertebrate fauna are also found in the sea-water income pools. However, the use of sand filtration of the water, or even a restricted ecogeographical distribution of the putative intermediate host, could account for the absence of *P. sparis* infections in this sytem, in which other myxosporoses can be highly prevalent (Palenzuela, Sitjà-Bobadilla & Álvarez-Pellitero, 1997).

Host age and size are known to influence the infection rates of fish parasites, including myxosporean species. Prevalence of *Ceratomyxa labracis* in cultured Mediterranean sea bass (*Dicentrarchus labrax*) rose with age in fish aged up to 1 year, but decreased in older animals (Álvarez-Pellitero & Sitjà-Bobadilla, 1993*b*). Su & White (1996) described a very similar pattern in atherinid fish infected by *Zschokkella leptatherinae*, with prevalence of infection increasing with host length and declining in the largest animals. In gilthead sea bream, prevalence of infection by *C. sparusaurati* increased with host weight in fish from groups similar to G–3 and G–4 of the present work, although a decrease in the oldest animals was not so clear (Palenzuela *et al*. 1997). In the current study, we also observed an initial rise in the infection prevalence with host weight in G–1, with a moderate decrease in the oldest animals.

Seasonal fluctuations of myxosporean infections have frequently been recorded. In general, temperature has been considered the major factor involved in these variations (McGeorge, Sommerville & Wootten, 1996). The prevalence of other myxosporean infections tends to increase with temperature, as in *Sphaerospora dicentrarchi* infections in Mediterranean sea bass (Sitjà-Bobadilla & Álvarez-Pellitero, 1993), or *C. shasta* in salmonid fish (Ching & Munday, 1984; Hendrickson, Carleton & Manzer, 1989). In the current study, no clear seasonal pattern of *P. sparis* infections was found either. Nevertheless, the observation of advanced granulomas in winter-examined fish and the evolution of infection levels in G–1 growing stocks, could indicate an initial infection in spring.

LM and TEM studies revealed the serious injury produced by *P. sparis* in the trunk kidney of gilthead sea bream. This organ is a very common target site for myxosporea, which cause different degrees of damage and disorders according to the intensity of infection and the location within the renal tissue (see Lom & Dyková, 1995; Feist, 1997). Those myxosporea located in the tubular lumina and renal corpuscles are considered celozoic, thus *P. sparis*

should be considered celozoic, as most stages were found in renal corpuscles, mainly in glomerular capillaries. There are many myxosporean species which, at least in some stage of development, are found in renal corpuscles. Among them, only *Sphaerospora epinepheli* (Supamattaya *et al*. 1991), *Chloromyxum inexpectatum* (Baska, 1990) and *Myxidium lieberkuehni* (Lom, Dyková & Feist, 1989) have been found in the capillary network.

The histopathological damage of glomeruli prompts us to describe the disease as a glomerulonephritis. The hypertrophy of the renal corpuscle and the atrophy of the renal parenchyma are similar to those described for *Myxidium* sp. (Atsuta, Sakai & Kobayashi, 1989), *M. lieberkuehni* (Lom *et al*. 1989), *M. rhodei* (Dyková, Lom & Grupcheva, 1987), *S. epinepheli* (Supamattaya, Fischer-Scherl & Hoffman, 1993), or *S. truttae* (Fischer-Scherl, El-Matbouli & Hoffman, 1986). The development of some myxosporeans in Bowman's space pushes the capillary network of the glomerulus towards the periphery, atrophying and eventually destroying it, as with *M. rhodei* (Dyková *et al*. 1987), *M. giardi* (Ventura & Paperna, 1984), *Ceratomyxa hungarica* (Molnár, 1992), or *Sphaerospora colomani* (Baska, 1993). In some other myxosporoses, glomerular capillary loops appear dilated, but the particular occupation and eventual destruction of the glomerular capillaries by *P. sparis* has not been described before. In just two cases, *C. inexpectatum* (Baska, 1993) and *M. lieberkuehni* (Lom *et al*. 1989), the parasites invade and hypertrophy the podocytes or the endothelial cells of the glomerular capillaries, respectively. In *P. sparis* infection, the lumen of the glomerular capillaries is occupied by the parasite, and consequently the renal corpuscle is replaced by masses of spores. The possible intracellular location of *P. sparis* in capillary cells could not be determined.

Host response to renal myxosporea seems to depend on the exact location and stage of development of the parasite (Feist, 1997). The most representative case of a dramatic host response, is the massive inflammatory reaction elicited by the extrasporogonic stages of the myxosporean organism responsible for proliferative kidney disease (PKD) in salmonids (MacConnell *et al*. 1989). On the other hand, *M. rhodei* plasmodia provoke a tissue response in *Rutilus rutilus* kidney interstitium, but not in renal corpuscles (Dyková *et al*. 1987). Probably, the presporogonic stages of *P. sparis*, not found in trunk kidney, are capable of producing a more important host reaction. The granulomatous changes evoked by *P. sparis* in the renal interstitium appeared at the end of the infection process, when spores appeared partially degenerated.

The different cellular effectors observed in the granulomatous area close to parasitized renal corpuscles might have contributed to the destruction of the parasite. The frequently observed old granulomas with residual spores in the largest animals, suggest that the inflammatory reaction could prevent the parasite from spreading in the fish body. Among these cells, melanomacrophages are thought to play a pivotal role in the control of myxosporean infections (Dyková, 1984; Supamattaya *et al*. 1993), and probably they took part in *P. sparis* destruction. Eosinophils, are the most abundant granulocyte cell type in gilthead sea bream head kidney (Meseguer, López-Ruíz & Esteban, 1994; Calduch-Giner *et al*. 1995), and have been reported to engulf bacteria (Noya, Magariños & Lamas, 1995). Eosinophilic granulocytes were also involved in the gilthead sea bream reaction to *C. sparusaurati* (Palenzuela *et al*. 1997). The morphological and staining features of the other granular cell type observed close to infected areas prompt us to consider them mast-like cells.

The possible role of rodlet cells in *P. sparis*-infected kidneys deserves special attention, and adds more questions to the old controversy on the nature and function of this cell type in fish. Our observations seem to support the view of some authors about their possible contribution to the host response (Smith *et al*. 1995; Leino, 1996; Reite, 1997). We have also observed the presence and discharge of rodlet cells in other myxosporean infections (Palenzuela *et al*. 1997; Álvarez-Pellitero & Sitjà-Bobadilla, 1993*b*). Nevertheless, this is the first time that rod sacs have been shown to be clearly associated to a parasite. Rodlet cells, frequently reported in fish epithelia, could proliferate or migrate massively to the endothelium of infected capillaries, and discharge their contents into the capillary lumen contributing to stopping myxosporean invasion. This hypothetical process could explain the presence of rodlet cell-belts, or their residues, in the periphery of glomerular capillaries harbouring the parasite. Rapid proliferation of rodlet cells in fish epithelia at sites of tissue injury, has also been suggested to participate in tissue repairing (see Leino, 1996).

Although the ultrastructure of the renal corpuscle of the gilthead sea bream reflects a low glomerular filtration rate (Zuasti, Agulleiro & Hernández, 1983), the infection by *P. sparis* must have produced a loss of functional glomeruli, resulting in a reduction of the filtration rate capacity. According to Feist (1997), it is often surprising how well the host is able to adapt physiologically when a large proportion of the renal tissues is destroyed or rendered non-functional by disease. In contrast to mammals, fish heavy nephropathies are not always correlated to clinical symptoms or mortality, because renal excretion can be taken over by the gills. In our case, the weakened condition of *P. sparis*-infected gilthead sea bream could imply an increase of mortality under stress-inducing situations (Sitjà-Bobadilla *et al*. 1992) which have a diuretic effect, or could render the host

susceptible to other secondary pathogens. Thus, in those types of facilities in which the infective stage can enter, and fish are systematically stressed, we consider this infection an indicator of population risk.

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