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MÉMOIRES ORIGINAUX

Sporozoan infection in cultured *Sparus aurata* L. and wild *Siganus luridus*

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Summary.

White nodules containing intracellular ovoid Sporozoans were observed in internal organs (kidneys, liver and gut wall) of cultured *Sparus aurata* and in the liver of wild *Siganus luridus* in the gulf of Aquaba in the Red Sea. Nodules consisted of parasitised hypertrophic macrophage-like cells enclosed in a well-defined collagenous fibrous capsule. These parasites are evidently the cystozoite stage of a haemogregarine.

Résumé.

Infection à Sporozoaires chez des *Sparus aurata* en culture et des *Siganus luridus* sauvages.

Des nodules blancs renfermant des Sporozoaires intracelllulaires ovoïdes ont été observés dans les organes internes (reins, foie et paroi intestinale) de *Sparus aurata* en culture et dans le foie de *Siganus luridus* sauvage, cela dans le golfe d'Akaba en mer Rouge. Les nodules étaient faits de cellules macrophages hypertrophiques enfermées dans une capsule au collagène filamenteux bien déterminé. A l'évidence, ces parasites sont le stade cytozoïque d'une haemogrégarine.

Ferguson and Roberts (1975) and Kirmse and Ferguson (1976), described a proliferative condition with tumors of the monocyte cell series associated with a spo-

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rozoan infection in cultured Turbot (*Scophthalmus maximus*). Kirmse (1978, 1978 *a*) identified this sporozoan as *Haemogregarina sachai* and experimentally reproduced its developmental cycle through fish and leech. White nodules containing intracellular sporozoans were observed in internal organs of cultured *Sparus aurata* and wild *Siganus luridus*. The *S. aurata*, cultured at the Israel Oceanographic and Limnologic Research Mariculture Laboratory at Elat, on the gulf of Aqaba (Red Sea), were reared in tanks supplied by open sea water circulation pumped to the system from a well dug in the beach rock. The infected specimen of *S. luridus* was caught in the northern gulf of Aqaba. Temperatures of the gulf water range perrenially from 22 to 26 °C and the salinity is constant at 39 to 40 %.

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Materials and methods

Tissues were fixed either in Buffered Neutral Formalin or in Bouin's fluid. Sections of paraffin embedded tissue were stained by Meyer's Hematoxilin eosin, periodic acid-Schiff (PAS), Martius scarlet-blue (MSB), Best's Carmin and Overnight Giemsa; tissue smears on glass slides were fixed in Methyl alcohol absolute and stained in Giemsa. Smears of fresh unfixed tissue were prepared for immediate microscopic examination. Measurements in µm were taken from a sample of 12 parasites and data are presented by calculated mean and standard deviation.

Results

Infection in *S. aurata:* Infection was found in three fish 145-160 mm long, approximately ten months old, coming from a group of fish collected nine months earlier in Bardawil lagoon on the Mediterranean coast of Sinai. Infection was not observed in histological material from any of the other 19 autopsied fish from the same tank, nor in over 200 autopsied fish of the same origin kept in parallel tanks. No external signs of infection were observed. On autopsy, kidneys were mottled grey but not hypertrophic, spleens were slightly enlarged but retained their normal color. In the spleen of one fish a 0.5 mm white nodule was observed. All other internal organs were of a normal appearance. Systemic granuloma was suspected as the parasitic infection was noticed only when processed tissue were histologically examined, thus blood and tissue smears were not prepared.

Clusters of parasitised macrophage-like cells, enclosed in a well-defined, collagen rich, fibroblast capsule (*fig. 1, 2, 3*) were observed upon histological examination, predominantly in the hematopoietic tissue of the head kidney and in the spleen. Encapsulations of parasitised cells were seen in the hematopoietic tissue of the trunk kidney (*fig. 1, 7*), on the surface of the intestine within the tunica serosa (*fig. 5*),

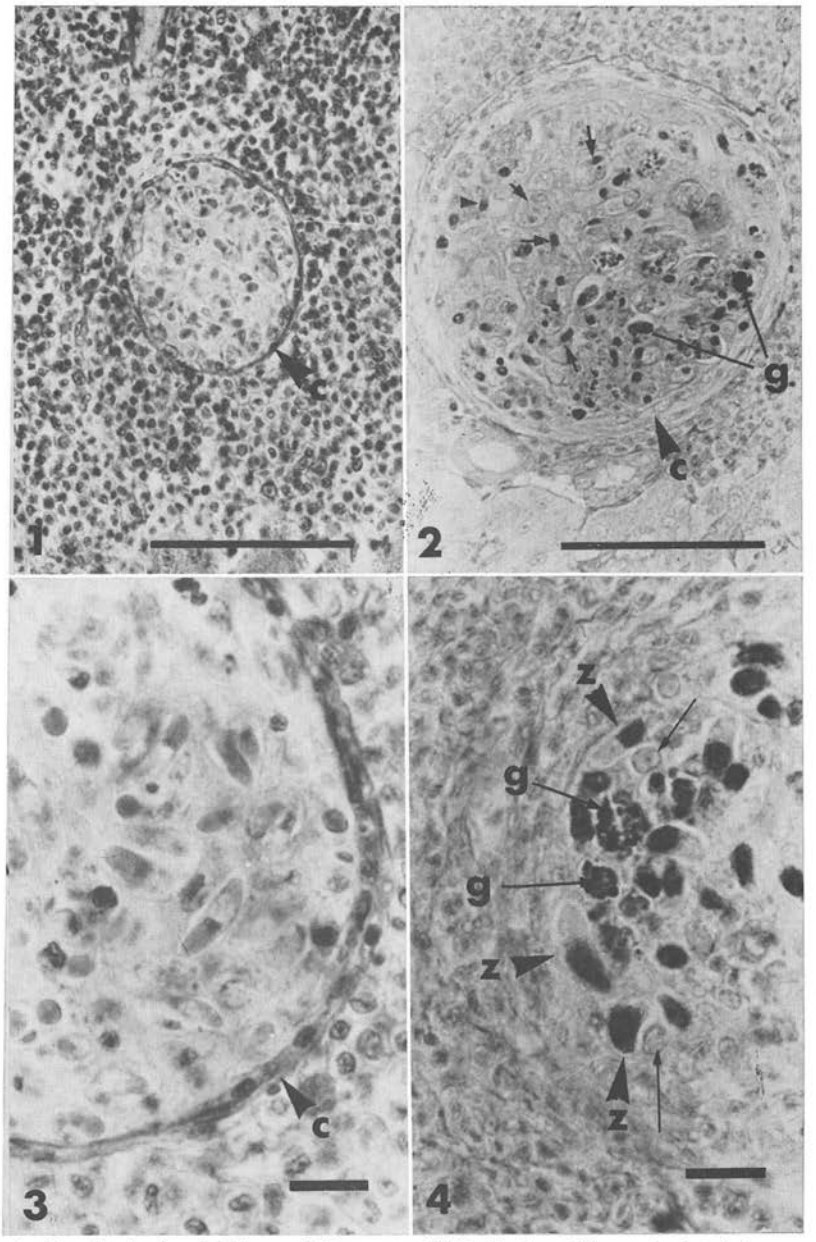

Fig. 1. Cyst in the head kidney of *S. aurata*. MSB, Scale : 100 µm (pointed by arrows)
Fig. 2. Young cyst in the head kidney of *S. aurata* containing predominantly early developmental stages and ovoid elongated forms of the sporozoan. PAS-phloxine tatrazine. Scale : 100 µm
Fig. 3. Cyst in the head kidney of *S. aurata* containing the ovoid elongated stage of the parasite. Hematoxylin-Eosin, scale : 10 µm
Fig. 4. Same as fig. 3, stained PAS-phloxine tatrazine, scale : 10 µm

in the tunica mucosa in the intestinal wall, in the mesentries in vicinity of the pancreatic cells and in the liver. Infected cells were never seen outside the encapsulations or among the blood cells seen in sections. The encapsulations varied in size from less than 0.1 mm in diameter to 0.3-0.5 mm in diameter, and numbered from five to ten infected cells in the smaller capsules to more than 100 in the larger ones. The smaller encapsulations were enclosed in a delicate fibroblast, collagen-containing capsule and were further subdivided internally in a concentric pattern by thin septae of fibroblasts and collagen. The macrophage-like cells in the smaller encapsulations contained parasites apparently of earlier stages of development. Several apparent stages in the development of the parasites within the macrophage-like cell were recognized:

A. Cells containing one eosinophilic inclusion with one or two compact basophilic nuclei.

B. Cells containing two small eosinophil inclusions, each one having a nucleus. Host cells with the above stages of infection also frequently included an additional vacuole containing dark pigment granules, often PAS positive. The encapsulation was frequently heavily loaded with extracellular brownish-black melanin granules (*fig. 2*).

Within the capsules containing cells infected by the earlier parasitic stages, few cells contained parasites apparently in a state of schizogony. Such cells contained two to four eosinophil inclusions, each containing two distinct nuclei.

C. Cells containing two parasites differentiated into an elongated ovoid organism, tapering at one pole (*fig. 3, 6*). The nucleus, consisting of aggregated basophilic granules, was positioned either centrally or acentrally toward the blunt pole. An aggregate of vacuoles containing PAS positive substance (*fig. 4*) occupied the region between the nucleus and the tapering pole. The PAS positivity resulted from the presence of glycogen, which was demonstrated by Best's carmin with diastase control. Both parasites were enclosed within a common membrance limited inclusion. The infected cells were larger than the uninfected macrophages outside the encapsulation and their nuclei were vesiculated and displaced. Two size groups of parasite of this morphological stage were observed: 9.55 ± 0.55 long, 2.07 ± 0.67 wide and 11.41 ± 0.71 long, 3.79 ± 0.39 wide.

D. Cells containing, each, two stout ovoid parasites, 9.08 ± 0.59 long, 3.83 ± 0.72 wide, enclosed in a common membrance or cyst which were PAS negative (*fig. 7, 8, 9*).

Such parasites were densely packed with refractory eosinophil granules, their nucleus was central and was accompanied with a small vacuole or inclusion. The host cell was enlarged and swollen in appearance, its nucleus pyknotic and compressed to the margins of the cell. Encapsulation predominantly containing parasites of this stage were enclosed in a very thick collagenous capsule (*fig. 7*), the inner septae were absent, and the extra-cellular aggregates of melanin pigment were either residual or absent.

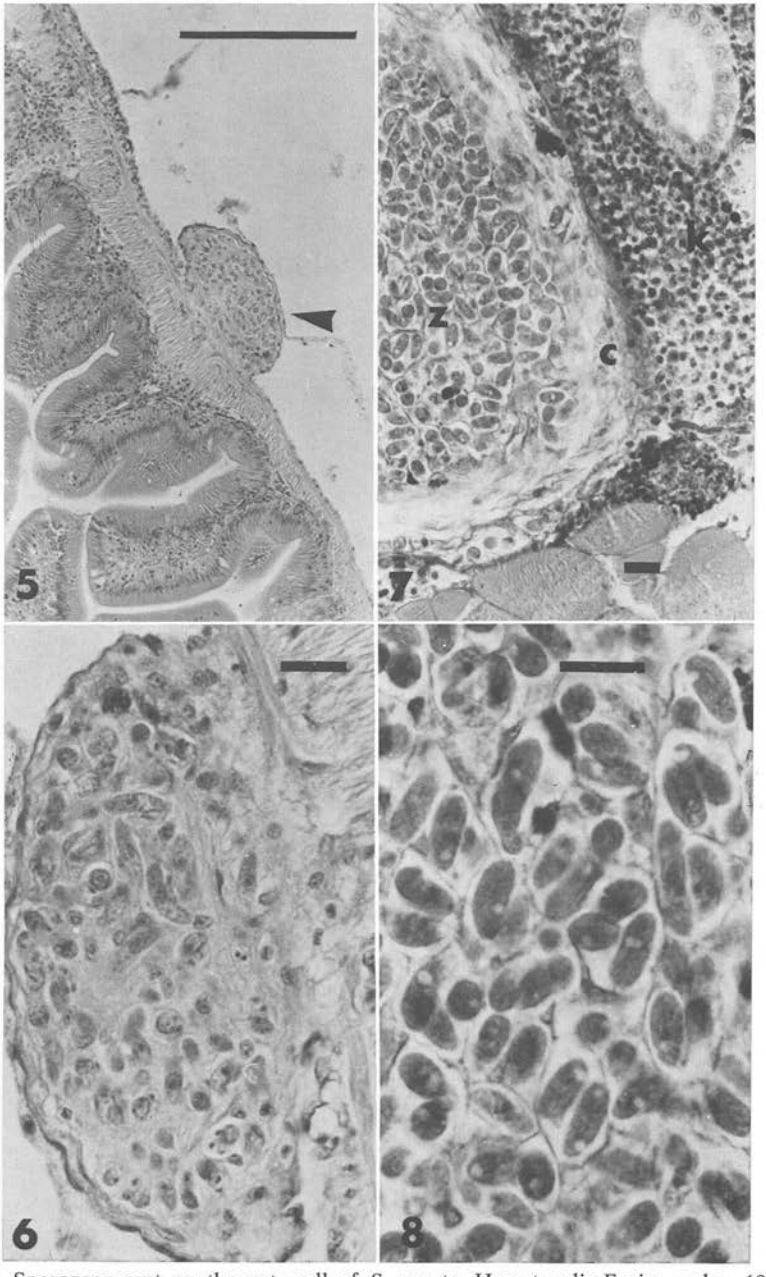

Fig. 5. Sporozoan cyst on the gut wall of *S. aurata*. Hematoxylin-Eosin, scale : 100 μm
Fig. 6. Same as fig. 5, enlarged. Scale : 10 μm
Fig. 7. Large cyst in the head kidney of *S. aurata* containing stout ovoid parasites. MSB, scale : 10 μm
Fig. 8. Enlarged sector of fig. 7, scale : 10 μm

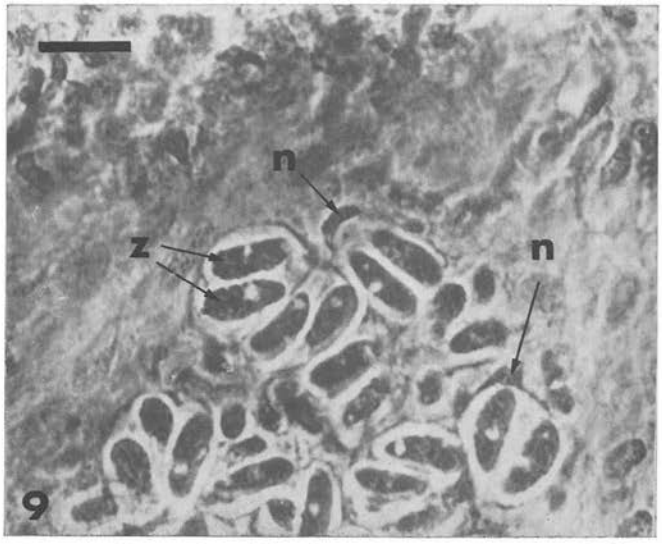

Fig. 9. Same as fig. 8, viewed by phase contrast microscopy. Scale : 10 μm

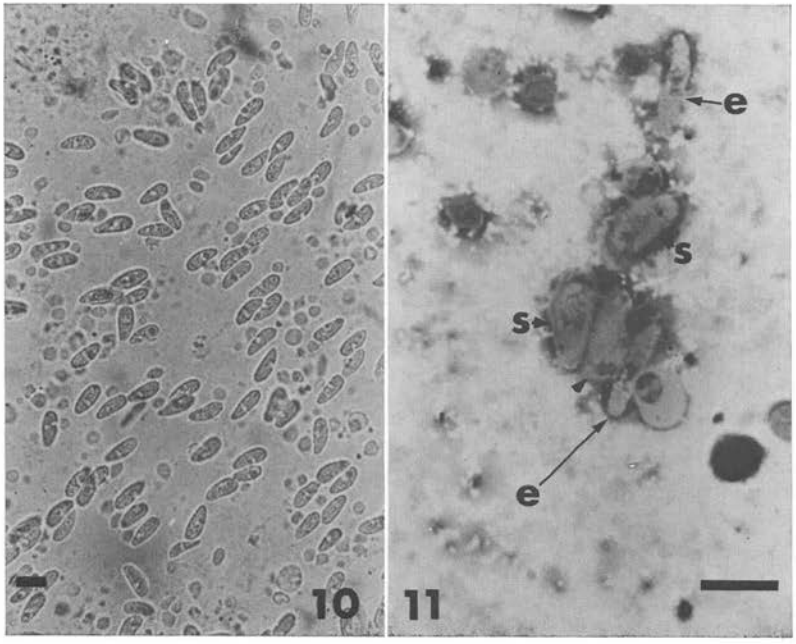

Fig. 10. Sporozoans from *Siganus luridus* (stout ovoid stage), in fresh tissue smear from a liver cyst. Scale : 10 μm

Fig. 11. Sporozoans in a smear from a liver tissue stained by Giemsa, scale : 10 μm

Abbreviations : *c* — fibrous cyst wall; *e* — ovoid elongated form; *g* — pigment granules ; *n* — nuclei of the parasitised cell; *s* — stout ovoid forms; *z* — parasite.

Infection in *Siganus luridus:* Infection was recovered from one specimen, 90 mm long, caught in the north end of the gulf of Aqaba. Autopsy of 200 additional fish from the north of the gulf of Aqaba yielded negative results. Infection was noticed on examination of fresh tissue smear prepared from a white nodule observed in the liver of the autopsied fish.

From this single nodule it was possible to prepare only tissue smears. The Giemsa stained smears contained both elongated ovoid forms, 13.50 ± 1.00 long, 2.66 ± 0.44 wide and stout ovoid form, 11.95 ± 1.21 long and 5.50 ± 0.41 wide (*fig. 10, 11*). In spite of the differences in size between these and the parallel forms seen in infected *S. aurata* (significant at 95 % confidence level, with *t* values of 5.65 and 7.65 respectively), each form was almost morphologically identical to the parallel form. In the smear the parasites were released from the host cells, but frequently appeared in couples. In addition the smears contained numerous macrophage-like cells, some lymphocytes and red blood cells, none of which contained evidence of intracellular infection.

Discussion

Landau *et al.* (1972) and Landau (1973) describe cyst formation following endodyogeny among haemogregarines of reptiles and mammals in a similar pattern known in *Toxoplasma* (Jacobs, 1967). These cysts within the host cells, hepatocytes or macrophages, usually contain two cystozotes which remain latent and are infective to predators.

Thus among these coccidians there is a fundamental dual cycle, one leading to transmission via invertebrate vector, and another enabling transmission by predation among vertebrate hosts (Landau, 1973, 1973 *a*).

The parasites from *S. aurata* and those found in *Siganus luridus* evidently belong to the genus *Haemogregarina*. In their morphology as well as in their localisation in the host cells and tissues, they closely resemble the intra macrophage stage of *Haemogregarina sachai* described by Kirmse (1978 *a*) in the turbot (*Scophthalmus maximus*). The parasites, found in the turbot and in *S. aurata* (and apparently also in *S. luridus,* although no histological evidence is available) in macrophage-like cells aggregated into encapsulated nodules, are evidently the cystozoite dormant stage of the haemogregarine. Endodyogenous division in the infected cells in *S. aurata* is suggested by our observations on the earliest stages of parasite development in the host cell. Endodyogeny was not reported by Kirmse (1978) from the infected turbot. The intramacrophage parasites in *S. aurata* and in the turbot (as evident from electron microscopic photographs presented by Ferguson and Roberts, 1975) were contained within a common membrane analogous to the « cyst » described in host cells infected with cystozoites of *Toxoplasma* and *Hepatozoon* (Jacobs, 1967 and Landau *et al.,* 1972).

In the infected turbot Ferguson and Roberts (1975) and Kirmse (1978, 1978 *a*) found trophozoites or merozoites in the circulating leucocytes and gametocytes in the red blood cells as well as infection in the tissue macrophages. Kirmse (1978, 1978 *a*) was able to pass infection to and from a leech. In *S. aurata,* infection consisted almost entirely from intramacrophage cystozoites, schizogony was observed rarely and only in tissue macrophages. By analogy to the developmental cycle of *Hepatozoon* (Landau, 1973) it is suggested, however, that this schizogony preceeds the cystozoite formation. In Elat, fish were maintained in a culture system isolated from the sea and therefore from possible leech infestation. *S. aurata* is an aggressive cannibal, thus, infection could presumably be introduced into the system by fingerlings obtained from Bardawil lagoon on the Mediterranean coast of Sinai which perpetuate themselves in the system through the intraspecific predation which occurs among cultured *S. aurata.*

ACKNOWLEDGEMENTS

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References

Ferguson H. W., Roberts R. J. (1975): Myeloid leucosis associated with sporozoan infection in cultured turbot (*Scophthalmus maximus* L.). *J. Comp. Path., 85,* 317-326.

Jacobs L. (1967): Toxoplasma and toxoplasmosis. *Adv. Parasitol.,* 5, 1-45.

Kirmse P. D. (1975): Studies on a proliferative disease of farmed turbot (*Scophthalmus maximus*). M. Sc. thesis, University of Stirling.

Kirmse P. D. (1978): Haematoprotozoan parasites of marine fishes with special reference to mariculture. Ph. D. thesis, University of Stirling.

Kirmse P. D. (1978 *a*): *Haemogregarina sachai* in sp. from cultured turbot, *Scophthalmus maximus* (L.) in Scotland. *J. Fish Diseases, 1,* 337-342.

Kirmse P. D., Ferguson H. W. (1976): Toxoplasma-like organisms as a possible agents of a proliferative condition in farmed turbot (*Scophthalmus maximus*). Wild life Disease. L. A. Page, ed. *Plenun Pub. Co.,* New York, pp. 561-564.

Landau I., Michel J.-C., Chabaud A.-G. (1972): Cycle biologique d'*Hepatozoon domerguei;* discussion sur les caractères fondamentaux d'un cycle de Coccidié. *Z. Parasitenk., 38,* 250-270.

Landau I. (1973): Diversité des mécanismes assurant la pérennité de l'infection chez les sporozoaires coccidiomorphes. *Mem. Mus. nat. d'hist. nat. Nouvelle Sér., Sér. A, Zool.,* 1-62.

Landau I. (1973): A comparison of the life cycle of *Toxoplasma* and *Hepatozoon* with reference to the general phenomenon and the role of the cyst formation in the Coccidia. *Ann. Trop. Med. Parasit., 67,* 403-407.