Parasitol Res (1987) 73:466–471

Parasitology Research
Founded as Zeitschrift für Parasitenkunde
© Springer-Verlag 1987

Important parasites of dover sole (*Solea solea* L.) kept under mariculture conditions

P. Kirmse *
Unit of Aquatic Pathobiology, Department of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland

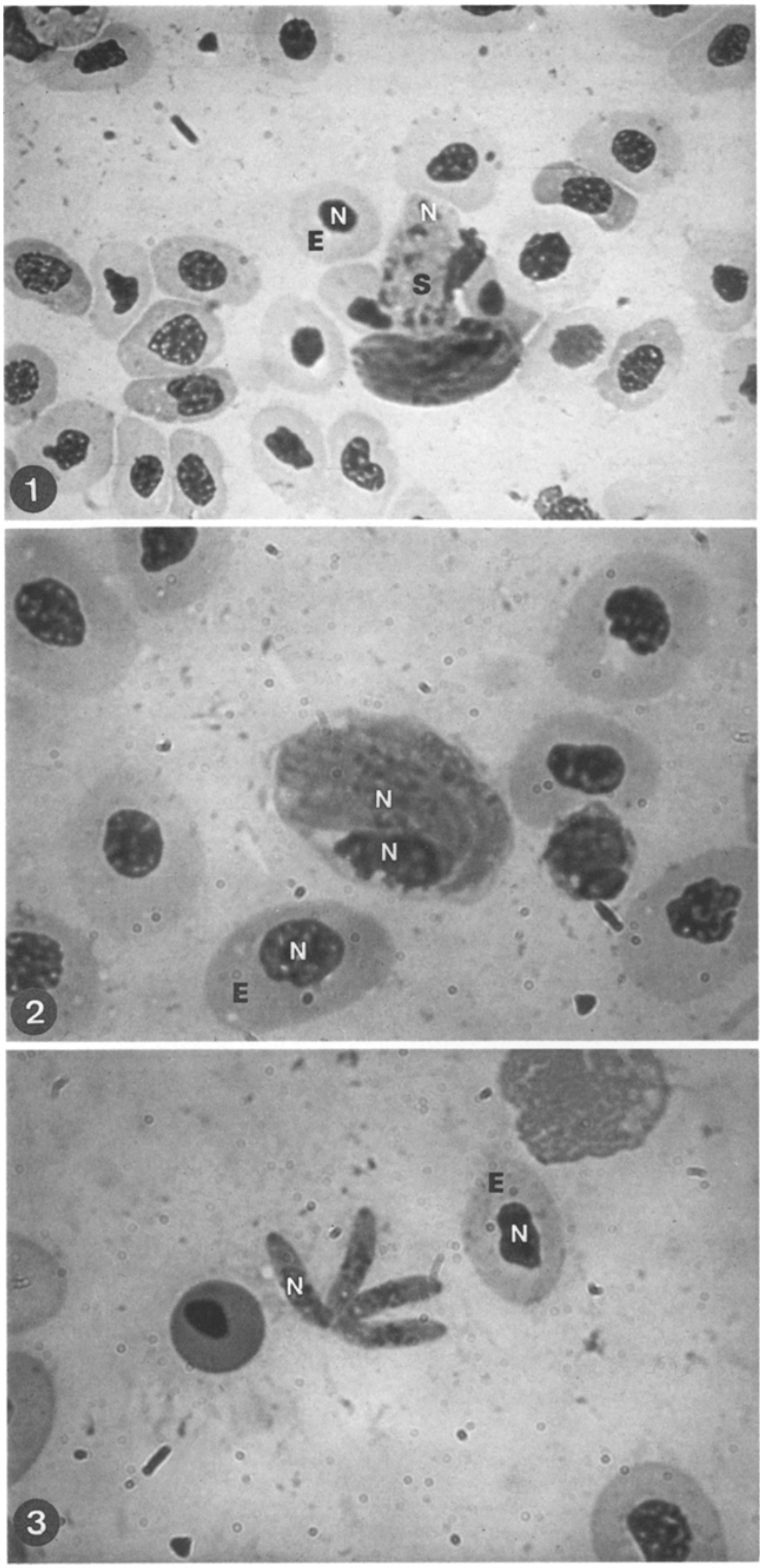
Abstract. Dover sole (*Solea solea* L.) were examined for parasites at the White Fish Authority (WFA) fish farm at Hunterston, Ayrshire, Scotland. The most important parasites appeared to be the two blood-feeding ectoparasites *Lernaeocera* sp., a copepod, and *Hemibdella soleae*, an ichthyobdellid leech, and the haematoprotozoan *Haemogregarina simondi*. The anaemia and general malaise of the sole investigated was attributed partly to general stress and the debilitating effects of transfer from the wild into an aquaculture system and partly to the impact of the parasites observed.

Adult Dover sole (*Solea solea* L.) from the English Channel were introduced as brood stock to the White Fish Authority (WFA) fish farm at Hunterston, Ayrshire, Scotland. All of them had mature gonads and were captured at the spawning grounds at Selsey about 10 miles from Portsmouth, at a depth of 4 fathoms and 1/2 to 5 miles offshore. Only 10 out of 200 fish survived the transport to Scotland. On arrival the sole were separated into two groups of 34 and 36 and accomodated in two square 11 m^3 tanks in a wooden shed. The stocking density was 1.3 kg/m^3 or 15 kg per tank and the flow rate of the seawater was 33 m^3/per day/per tank. The fish were fed mussels (*Mytilus* sp.) and lugworms (*Arenicola* sp.) 3 to 5 times per week and the tanks were cleaned after each feed to get rid of excessive waste. Warm effluent cooling water from the adjacent atomic power station at Hunterston was used during most of the year raising the normal temperature of seawater by 8° C.

An investigation of these fish was undertaken because their general health seemed to be impaired and they were feeding poorly.

The first sign of a general malaise was noticed by the WFA fish farmers at the end of May, one month after the fish had been introduced into the aquaculture system. Almost complete anorexia and cachexia were followed by limited mortality in June, with two fish found suddenly dead after ceasing to feed 2 weeks earlier. After close inspection by the WFA both fish were found to be heavily infested with 20 and 26 copepods respectively, on the gills. These ectoparasites were identified as the blood-feeding adult female stage of the anchorworm (*Lernaeocera* sp.). The fish were all treated at the end of the spawning period (June/July) by immersing them individually in tap water for 20–30 min. This apparently killed the other ectoparasites (leeches and flukes) but did not have any effect on the *Lernaeocera* sp. An additional formalin treatment was also tried without success. No attempt was made to remove the *Lernaeocera* sp. mechanically by crushing them with a pair of forceps as suggested by Slinn (1967). Brood stock caught earlier in the year (February instead of April) and stock from earlier years had never been observed to be infested with *Lernaeocera* sp. although this had been reported elsewhere (Slinn 1967). An examination of the two tanks was conducted for the first time in July when blood samples of infested fish and ectoparasites were collected and together with another moribund fish were taken to the laboratory at Stirling University. Later, through summer and autumn the number of copepods infesting the soles decreased and no egg deposits or juvenile stages were seen in the tanks. However, in late autumn many soles still appeared to be infested with *Hemibdella soleae* and those examined for haemogregarines were still harbouring *Haemogregarina simondi* in their blood. During October, freshly captured adult *Solea solea* from the North Sea near Lowestoft were examined

* *Present address:* P. Kirmse, Ministry of Agriculture and Water, P.O. Box 322, AL-Jouf-Sakaka, Saudi Arabia



Figs. 1–3. *Haemogregarina simondi* in Giemsa stained blood smear. *E*, erythrocyte; *N*, nucleus

Fig. 1. Schizonts with nuclei (*S*). × 850

Fig. 2. Mature schizont with long merozoites within a host cell. × 1360

Fig. 3. Mature schizont in remnants of a host cell. × 1360

at the WFA fish farm and these were equally infested with leeches and haemogregarines but not with the copepods.

Materials and methods

A general examination of all fish in the tanks was done by observing their behaviour in the water and later by handling them individually. Ectoparasites were collected from the upper and lower surfaces of the live fish. Blood was drawn without anaesthesia from either the dorsal or the ventral portal vein. Blood was also taken from the ectoparasites and from control sole from the hatchery at the same location and from adult sole collected later in the year. Wet blood smears were examined using Lugol's or Methylen green as intra vitam stains. Thin blood films were fixed in methanol and stained with Giemsa at pH 6.8. The erythrocytes and leucocytes and the differential blood cell count were counted using conventional methods. In addition, one moribund fish was subjected to a post mortem examination, including histopathology.

Results

General conditions

The sole in both tanks showed an increased ventilation rate easily visible in the water. At close examination, a distinct pallor of the gills suggested an anaemic condition. The fish appeared rather weak and sluggish in their movements and reluctant to swim. They were easily captured with a hand net.

Ectoparasites

All fish were infested with the adult female stages of the marine copepod *Lernaeocera* sp. and from 1 to 22 specimens were detected on individual fish. There were also 1–45 specimens of the ichthyobdellid leech *Hemibdella soleae* infesting individual soles. In addition, the marine trematode *Entobdella soleae* was found in small numbers on these fish.

Haematoprotozoan parasites

Examination of wet and stained blood films revealed a moderate infestation with *Haemogregarina simondi* and parasitaemias ranging from 0.7% to 2.4% of total of blood cells examined. These parasites were found in large lymphocytes, neutrophils, monocytes and mature erythrocytes. From 1 to 8 parasites in various stages of development were counted in individual cells (Figs. 1–3). Both infested leucocytes and erythrocytes, were greatly hypertrophied compared to uninfested blood cells (Table 1). Individual cells were often ruptured, appearently as a consequence to repeated schizogonies, and free parasites were therefore frequently encountered. In addition, wet impressions and stained smears of *Lernaeocera* sp. and *Hemibdella*

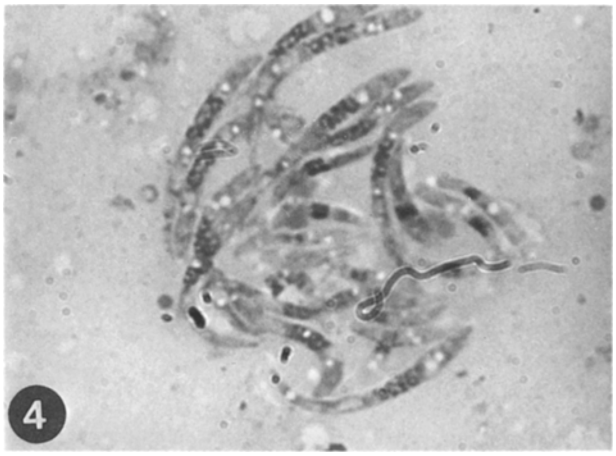


Fig. 4. *H. simondi.* Motile stages in the gut of the vector leech *Hemibdella soleae.* ×960

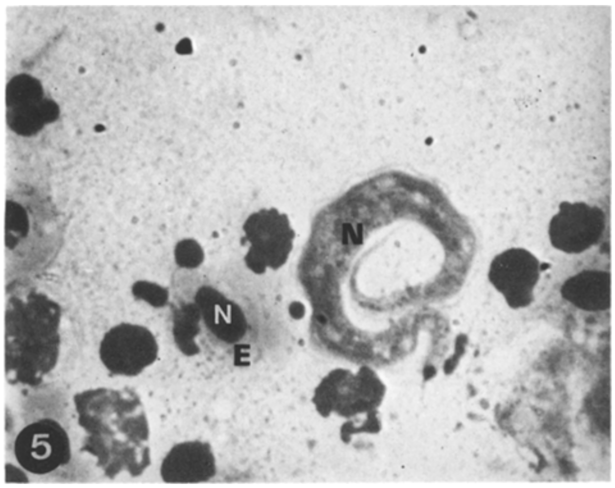


Fig. 5. *Trypanosoma soleae.* Blood smear stages from sole. *E* erythrocyte; *N*, nucleus. ×840

soleae (Fig. 4) collected from infested sole also revealed numerous merozoites and other developing stages of *Haemogregarina simondi.* The haemoflagellate parasite *Trypanosoma soleae* was only detected once in a blood smear (Fig. 5).

Postmortem examination

Parasitology. Seven *Hemibdella soleae* were removed from the upper surface of the fish and two *Entobdella soleae* from the lower surface close to the head. Although this sole had been dipped in Methylen blue to get rid of the copepods there were still five adult female *Lernaeocera* sp. anchored deeply in the gills. Their colour was brown to dark red and they had ripe egg sacks. The copepods induced considerable amounts of haemorrhage when mechanically removed. Ingested blood

Table 1. Size differences between normal and haemogregarine infested blood cells of Dover sole (*Solea solea* L.). Sizes are means in μm of 25 cells from each of 25 fish *L*, mean length (range); *W*, mean width (range)

Normal cell size	Infested cell size
Small lymphocytes	
L 4.1 (3.9–4.5)	Not infested
L 3.9 (3.9–4.1)	
Large lymphocytes	
L 7.8 (6.5–9.5)	L 12.3 (11.8 –12.5)
W 7.3 (6.5–8.5)	W 9.4 (8.75–10.0)
Neutrophils	
L 10.4 (9.1–11.7)	L 15.9 (13.0 –19.5)
W 9.9 (7.8–11.7)	W 7.5 (5.2 –10.4)
Monocytes	
L 14.9 (13.0–19.5)	L 16.5 (14.9 –19.8)
W 11.1 (9.1–14.3)	W 9.1 (6.5 –9.8)
Erythroblasts	
L 12.8 (12.5–13.5)	Not infested
W 12.8 (12.0–13.3)	
Erythrocytes	
L 12.7 (11.7–13.0)	L 19.1 (16.9 –22.1)
W 6.9 (6.5–7.8)	W 9.3 (5.8 –11.7)

Table 2. Differential analysis of blood from haemogregarine infested Dover sole (*Solea solea* L.) and controls, expressed as a percentage of every 100 cells counted

Cell types	Infested fish (25)	Healthy fish (10)
Erythrocytes	76.6	94.6
Erythroblasts	1.8	0.5
Thrombocytes	1.4	3.8
Small lymphocytes	6.0	0.7
Large lymphocytes	3.2	0.1
Neutrophils	3.4	0.1
Monocytes	7.6	0.2

from the fish host was seen pulsating through the parasite when viewed under the dissecting microscope. Scrapings of fins and gills did not reveal any other parasites.

Pathology. The spleen was enlarged and dark brown. The liver was pale yellow, friable and fatty. The posterior kidney was hypertrophied and dark brown in colour. The digestive tract was flaccid and the stomach and intestine were empty and devoid of gross lesions or endoparasites. No specific changes were obvious in any of the other organs examined. However, impressions of spleen, kidney, heart, brain and liver showed numerous schizonts of *Haemogregarina simondi* in capillary spaces and resembled those in the circulating blood.

Histopathology. Examination of the internal organs revealed an excessive haemopoiesis in the kidney and large fibrinous deposits the macrophage centres of the spleen and to a lesser extent the liver. Schizonts of *Haemogregarina simondi* were detected mainly in the lumen of small vessels of the posterior kidney.

Haematology

There was a decrease in erythrocytes and a pronounced leucocytosis with a general increase in lymphocytes, neutrophils and monocytes compared to apparently unaffected sole (Table 2). In addition, the marked increase in progranulocytes and erythroblasts indicated an active haemopoiesis to compensate for the apparently anaemic condition.

Discussion

Lernaeocera sp. In the wild, the Dover sole (*Solea solea* L.) is the intermediate host for the juvenile larval stages of *Lernaeocera* sp. and only gadoid fish are the final hosts for the blood-feeding adult copepods. Possibly this copepod was present in its larval stage on the soles when they were introduced into the WFA fish farm in the spring. A similar aberrant development from the larval to the adult stage on the same host may have taken place here, as described earlier by Slinn (1967), due to the absence of the final host in this mariculture system. Some *Lernaeocera* sp. contained free merozoites and other developing stages of *Haemogregarina simondi*. However, adult female *Lernaeocera* sp. cannot be vectors for this haemogregarine since they are unable to leave their fish host. High infestation with these copepods can, however, cause considerable damage to the fish because of the large amount of blood they extract. Immersing the infested sole in tap water or formalin did not have any effect. However, a trial in August where five infested fish were treated with Dipterex 80 (trichlorphon) at a concentration of 8 ppm apparently cured this. An obvious decline of this ectoparasite was observed in autumn; the fish fed normally again and no further casualities were recorded.

Hemibdella soleae. This leech feeds on blood and seems to be highly specific for sole due to its morphological characteristics. A peripheral modification of the posterior sucker enables it to adhere to the ctenoid scale of *Solea solea* but not to the spines of other *Soleidae* (Llewellyn 1965). *Hemib-*

della soleae is possibly the main vector for *Haemogregarina simondi*, as various developing stages of the parasite are found in its gut and it is host specific. Although its blood intake is considerably less than that of *Lernaeocera* sp. because it is so small, a heavy infestation with *Hemibdella soleae* may debilitate the host.

Entobdella soleae. This monogenean trematode feeds exclusively on the epidermis of its teleost host and on mucus and mucus cells but not on blood (Kearn 1963).

Haemogregarina simondi. This parasite and other haemogregarines appear to be common haematoprotozoan parasites of marine fishes, especially marine flatfish (Kirmse 1978). Their importance as pathogens in fish raised under mariculture conditions has been pointed out recently by observations on the pathogenicity of *Haemogregarina sachai* in cultured turbot (*Scophthalmus maximus*) from several fish farms in Western Scotland (Kirmse 1980). An erythrocytopenia and leucocytosis was also observed earlier in soles infested with *Haemogregarina simondi* (Neumann 1909). A similar decrease in circulating erythrocytes was also observed in other diseases affecting the reticuloendothelial system such as lymphoma in *Esox lucius* (Mulcahy 1975) and vibriosis in *Gadus morhua* and various pleuronectids (Anderson and Conroy 1970). In the present study a considerable deformation of blood cells and their nuclei, possibly as a result of a mechanical action of the haemogregarine, was observed and this had been noted already by Lebailly (1906) in *Solea solea* together with the destruction of a large portion of erythrocytes. Similar changes in blood cell morphology were seen by Henry (1913) in the infestation of marine fish with *Haemogregarina simondi, H. bigemina, H. clavata* and *H. quadrigemina* and were more recently reported by Kirmse (1980) in *Scophthalmus maximus* infested with *H. sachai*. Deformed red cells have been observed also in *Oncorhynchus kisutch* fed a folic acid deficient diet (Smith 1968). B vitamins are apparently essential for parasite multiplication and thus an excessive proliferation of the parasite in the host's system may induce rapid depletion of vitamins which would otherwise be available for the host. In the present study numerous schizonts of *Haemogregarina simondi* were detected in the circulating blood and in most organs, both by wet impressions and histopathology. These results compare favourably with a disease condition in farmed *Scophthalmus maximus*, except that a proliferation of the lymphoid tissue as seen in *H. sachai* infestation (Kirmse 1980) was not observed.

Trypanosoma soleae. Nothing is known about the pathogenicity of this and other haemoflagellates in marine fish but high infestations in *Cyprinus carpio* and other freshwater fish can cause wasting and heavy losses.

The most interesting feature in the histopathology of the sole was the excessive haemopoiesis in the kidney. However, no auxiliary haemopoietic tissue was detected in the heart and liver, as is normally the case in teleosts with longstanding anaemia.

All Dover sole in the two tanks at the WFA fishfarm were infested with *Haemogregarina simondi*. The infection rate in wild sole examined in France varied from 50% in summer to 28% in winter (Kirmse 1978). Noble (1957) found a 24% infection of *Callionymus lyra* with *H. quadrigemina* in summer and only a light infestation of 7% in winter. Laird and Morgan (1973) observed an infestation rate of 23% with *H. platessae* in *Trinectes maculatus* in summer but no infestation in winter. Warmer water temperatures probably encourage the multiplication of the parasites. The low overall infestation rate of 1%–6% in *Scophthalmus maximus* with *H. sachai* (Kirmse 1980) may be explained by the absence of the vector in the mariculture system, whereas a frequent reinfestation of sole with *H. simondi* was assured by the presence of the intermediate host *Hemibdella soleae* in great numbers at the WFA fish farm.

There may be several reasons for the "general weakness" of the adult sole under investigation at the WFA fish farm and their anaemic condition. The resistance of fish newly introduced from the wild into an aquaculture system using warm effluent cooling waters from a power station is certainly lowered by the stress of transportation and by having to adapt to the new environment. Transportation stress is well documented for domestic animals, especially cattle, and is evident in the present case by the 28.6% losses in sole. Under even less favourable conditions of husbandry and management (inadequate handling, reduced flowrate, high stocking density, higher susceptibility because of spawning stress etc.), the percentage of diseased and unmarketable fish may rise to levels of quite important economic consequence (one brood stock of Dover sole costs £ 30–35 Sterling or more). Crowding and high water temperatures in particular in a marine fish monoculture system can facilitate reproduction and transmission rate of parasites (McVicar and MacKenzie 1977). This will increase even further when the intermediate parasitic host and vector can obtain optimal living and reproduction conditions in an aquaculture system used by both the fish and definite host. It is unlikely that the anaemia observed in the soles at the WFA fish farm was due to the haemogregarine infestation alone, since the parasitaemias were much lower than those recorded by Kirmse (1980) of up to 36% from *Scophthalmus maximus* infested with *H. sachai*. The massive infestation with adult blood-feeding stages of *Lernaeocera* sp. and, to a lesser degree, the action of the piscicolid leeches probably contributed a great deal to the general malaise. Debilitating effects from a massive parasite infestation may also result in poor growth rates and higher susceptibility to infections with other agents such as bacteria, viruses and fungi. It may also have an impact on spawning of brood stock, resulting in poor quality eggs and offspring.

The parasites alone cannot be held responsible for the anaemic condition and general weakness observed in the sole under investigation. The various stress factors pointed out (transportation, spawning, raised water temperatures, parasites attacking an aberrant host) during adaptation to the new environment may have debilitated the fish in the first place, so that parasitic infestation and all its consequences were greatly facilitated.

Acknowledgements. I am grateful for the cooperation received from the staff of the WFA fish farm at Hunterston. I further thank Mr. I.H. MacRae and Mr. W. Dunn for assistance rendered with some of the fieldwork.

References

Anderson JIW, Conroy DA (1970) Vibrio disease in marine fishes. In: S.F. Snieszko (ed) Symposium on Diseases of Fishes and Shellfishes American Fishery Society Special Publication No. 5, Washington DC, pp 266–272

Henry H (1913) A summary of the blood parasites of British sea-fish. J Pathol Bacteriol Cambridge 18:218–219

Kearn GC (1963) Feeding in some monogenean skin parasites. J Mar Biol Assoc UK 43:749–766

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

Kirmse P (1980) Observations on the pathogenicity of *Haemogregarina sachai* Kirmse, 1978 in farmed turbot *Scophthalmus maximus* (L.). J Fish Dis 3:101–114

Laird M, Morgan RP (1973) *Haemogregarina platessae* Lebailly (= *H. achiri* Saunders) from the hogchoker in Maryland. J Parasitol 59:736–738

Lebailly C (1960) Recherches sur les hématozoaires parasites de teleostéens marins. Arch Parasitol 10:348–404

Llewellyn LC (1965) Some aspects of the biology of the marine leech *Hemibdella soleae*. Proc Zool Soc London 145:509–528

McVicar AH, MacKenzie K (1977) Effects of different systems of monoculture on marine fish parasites. In: Cherret JM, Sagar GR (eds) Origins of Pest, Parasite, Disease and Weed Problems. Blackwell Sci Publ, pp 163–182

Mulcahy MF (1975) Fish blood changes associated with disease: a haematological study of pike lymphoma and salmon ulcerative dermal necrosis. In: Ribelin WE, Migaki C (eds) The Pathology of Fishes, University of Wisconsin Press

Neumann RO (1909) Studien über protozoische Blutparasiten im Blut von Meeresfischen. Z Hyg Infektionskr 64:1–113

Noble ER (1957) Seasonal variations in hostparasite relations between fish and their protozoa. J Mar Biol Assoc UK 36:143–155

Slinn DJ (1967) An infestation of adult *Lernaeocera* (Copepoda) on wild sole (*Solea solea*) kept under hatchery conditions. J Mar Biol Assoc UK 50:787–800

Smith CE (1968) Haematological changes in Coho Salmon fed a folic acid deficient diet. J Fish Res Board Can 25:151–156

Accepted March 25, 1987