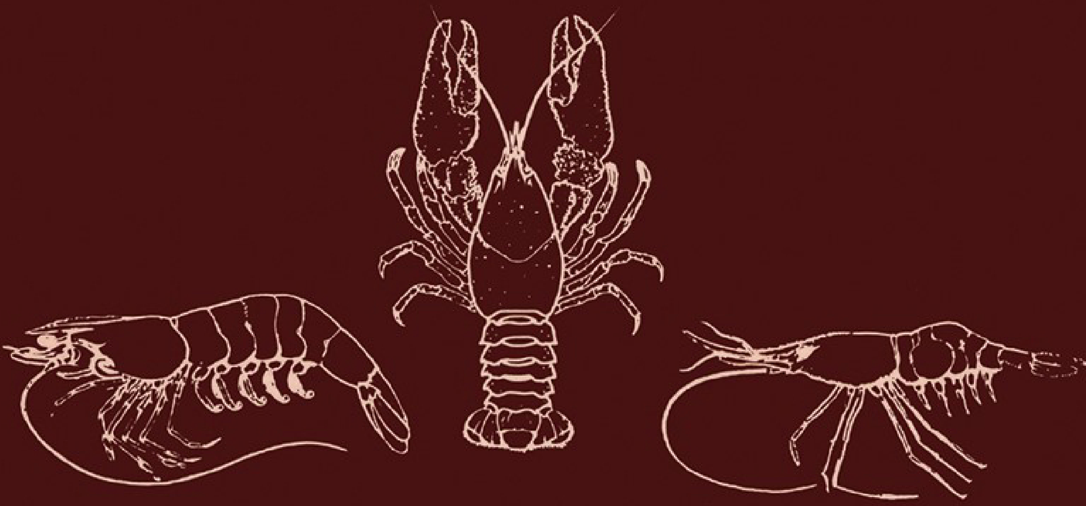

RECENT ADVANCES IN AQUACULTURE

Volume 2

Edited by
James F. Muir & Ronald J. Roberts

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PREFACE

It is a tribute to the vigour of research and development in aquaculture that we are able, in a relatively short time, to provide readers with a second volume in this series, which has such a diversity of high calibre research and developments to report. That the first volume was so well received has been a source of great satisfaction to the editors and supported their conviction as to the need for links to join the research laboratory to the fish farm by making current research available to a wider range of potential users.

In this volume, we have changed the balance somewhat towards more specialised subject areas. We make no apology for this, as the modern aquaculture professional is concerned with a wide range of specialisms within the broader content of management and production, and there is every need to present such work in an acceptable format. Furthermore many of these specialised topics have a significance right across the range of production, are often economically critical, and are therefore of considerable and widespread importance.

The first contribution, by Dr Derek Robertson, manager of the Institute of Aquaculture's Howietoun Fish Farm, covers just such a subject. *Ichthyobodo*, commonly known as *Costia*, can infect a wide range of species in as wide a range of environments, and may cause heavy and sustained losses in affected stocks. An understanding of the biology of this parasite, in relation to aquaculture environments and husbandry practices, surprisingly little studied until recently, is therefore of prime importance, particularly to the burgeoning salmonid culture industry.

The second review, by Laszlo Horváth, of the State Fish Hatchery at Szazhalombatta in Hungary, deals with carp ovogenesis, a particularly important subject for all concerned with hatchery production and developmental characteristics of cultured fish. The target species is one of the most widely cultured in the world. What is particularly valuable is the wealth of scientific knowledge Dr Horváth brings to the practical aspects of hatchery technology.

The physiological changes occurring in the salmonids as they prepare to move onto the different stages of their life cycle, through drastically different aquatic environments, are a source of wonder and a subject of considerable scientific interest, quite apart from being of substantial economic importance to the producer of smolts for farming or 'ranching'. Dr Jeremy Langdon reviews the process of smoltification in the context of current scientific research with particular reference to hormone changes, and relates it to culture conditions.

The grass carp is one of the few species not primarily grown for food supply, its effective handling of larger plant material in overgrown waterways making it pre-eminent as an ideal biological control agent. As such, its use is becoming increasingly widespread. Drs Zonneveld and van Zon relate the principles behind the use of grass carp in this role, and consider the potential and limitations of this extremely useful species, with special reference to their own pioneering work with the species, in Egypt.

The review by Dr Olufemi, of Ibadan, Nigeria, considers both the *Aspergillus* fungi and the role in fish diseases posed by these organisms both in relation to their fatal effects *per se*, and the effects of their poison, aflatoxin, on fish growth and survival. As aquaculture becomes more intensive and producers throughout the world attempt to develop feeds to support higher yields, so the significance of effective food storage, and the quality control, increase, and nowhere more importantly than in Dr Olufemi's native West Africa, where humidity and tropical temperatures combine to make food storage and *Aspergillus* control very difficult.

Dr Simon Davies' review on the role of dietary fibre in fish nutrition concentrates on an often neglected aspect of fish nutrition. Greatest attention in fish nutrition tends to be given to protein, energy and essential element requirements, but as the review shows, the source and quality of fibre can interact very substantially with the other dietary components, and can thus have a significant economic effect on the efficiency of utilisation of the other, highly expensive, components of diets. Fibre also plays an important role in faecal waste output, which in time has a major bearing on the effects of intensive aquaculture on the environment.

The volume is completed by a fascinating account by Dr Sergei Doroshev, of the University of California, Davis, of the biology and culture of the sturgeons. While techniques still have some distance to go to reach levels of production attained in producing other species, culture of several sturgeon species is now a practical reality. This particular review is of additional interest in that it gives access to the relatively little known Russian literature on the subject.

The editors hope that this volume will enjoy the same degree of enthusiastic support as did its predecessor. If it also helps to increase the efficiency and productivity of the world's aquaculture, then it will indeed justify the efforts of all the various contributors.

James F. Muir
Ronald J. Roberts
Stirling

1 A REVIEW OF *ICHTHYOBODO NECATOR* (HENNEGUY, 1883) AN IMPORTANT AND DAMAGING FISH PARASITE

Derek A. Robertson

1. Introduction

Ichthyobodo necator or *Costia necatrix* as it is more commonly known is a very important protozoan parasite of cultured fish throughout the world. Its importance is often underestimated in the scientific literature probably because it is so often missed by scientists and fish farmers alike because of its small size and transparency when observed in the normal light microscope. However, it is probably the major cause of death of cultured salmonid fry in Scottish fish farms; even the viral disease IPN seldom causes such acute mortality as does *Ichthyobodo*. Because of its predilection for first feeding fry salmonid farmers generally assume that an *Ichthyobodo* infection is extant before investigating other possible causes and thus excessive treatment with formalin, often unnecessarily, is the norm in most farms. For such an economically important parasite it is surprising that there has been relatively little research carried out on aspects of its biology. Much of the literature on *Ichthyobodo* was published in the first half of the twentieth century and concentrated on trying to establish the taxonomic status of the parasite. Other reports of *Ichthyobodo* in the literature briefly mention the parasite in species lists for infestations of groups of fish or in parasite lists for specific countries.

2. Taxonomy of *Ichthyobodo necator*

Ichthyobodo necator is a protozoan flagellate of the order kinetoplastida; its currently accepted taxonomic status is shown in Table 1.1. The exact taxonomic status has caused many problems over the last century and was not fully clarified until 1969 by the electron microscopy studies by Joyon and Lom. *Ichthyobodo necator* was first described by Henneguy in 1883 and more fully in 1884 and he named it *Bodo necator*. In 1880 the parasite was relegated to the genus *Costia* by Leclerq who called it *Costia necatrix*, as the genus *Bodo* contained flagellates with only two flagella, whereas Henneguy reported the parasite to have three flagella, two short and one long. Weltner (in Nitsche and Weltner, 1894) described an ectoparasitic flagellate on goldfish, *Carassius auratus* L., and on the basis of four flagella placed it in the Tetramiditae and called it *Tetramitus nitschei*; Moroff in 1904 showed this parasite to be identical to *Costia necatrix*. The name *Costia*, however, was already preoccupied by a genus of *Hymenoptera* created by Kirchner in 1867 and was therefore invalid under the rules of zoological nomenclature. According to Joyon and Lom (1969), Pinto in 1928 proposed replacement of the name *Costia* by the name *Ichthyobodo*. Several other inappropriate generic designations have been used for *Ichthyobodo* but *Costia necatrix* became the accepted synonym and is still used today by most fish farmers and aquarists. *Ichthyobodo necator* is however the correct name according to the strict code of zoological nomenclature.

Recent electron microscope studies by Schubert (1966) and Joyon and Lom (1966, 1969) confirmed that *Ichthyobodo necator* is a kinetoplastid of the family Bodonidae, and not in the Proteromonadidae (Grell, 1956; Reichenow, 1928) or the Tetramitididae (Hall, 1953; Kudo, 1954; Lemmerman, 1914; Minchin, 1922).

However Joyon and Lom (1969) called the parasite *Ichtyobodo necator* and most subsequent authors have dropped the 'h'. Nevertheless as Pinto (1928) had spelt it *Ichthyobodo* and subsequently Grasse in 1952, who

Table 1.1: The Taxonomic Status of *Ichthyobodo necator*

Phylum	:	Protozoa
Class	:	Mastigophora
Order	:	Kinetoplastida
Suborder	:	Bodonina
Family	:	Bodonidae
Genus	:	*Ichthyobodo*
Species	:	*necator*

pointed out the preoccupation of the name *Costia* in his 'Traite de Zoologie', also spelled *Ichthyobodo* with an 'h', this is the correct spelling of this parasite.

Kinetoplastids are well demarcated from the rest of the class Mastigophora by possession of a conspicuous extranuclear deoxyribonucleic acid (DNA) organelle, the kinetoplast. This organelle is still the largest repository of extranuclear genetic material known in any cell (Vickerman, 1976) and consequently has attracted much attention from molecular biologists. The kinetoplast is found within the single mitochondrion usually close to the basal bodies of the flagella.

Bodonine kinetoplastids differ from Trypanosomatina kinetoplastids by possessing two flagella as opposed to the single locomotory flagellum of the latter.

The family Bodonidae are bodonine flagellates in which the recurrent flagellum is free from the body surface, which are phagotrophic and ingest food through a cytostome which opens close to the flagellar pocket.

The number of flagella which *Ichthyobodo* possesses and which caused so many problems for earlier workers has been shown to be two, one long and one short flagellum. The quadriflagellar form of *Ichthyobodo* has been shown to be the predividing form (Joyon and Lom, 1969) and thus the biflagellate normal form establishes *Ichthyobodo* as a true bodonid.

The genus *Ichthyobodo* is unusual in the kinetoplastida in that its members possess several kinetoplasts dispersed throughout the reticular mitochondrion, and not just in the region of the flagellar basal bodies. The only other kinetoplastid reported to have similar multiple kinetoplasts is *Crytobia vaginalis* another bodonine flagellate found in leeches, *Hirudo medicinalis* (Vickerman, 1974).

Only two species of *Ichthyobodo* have been described, *Ichthyobodo necator* and *Ichthyobodo pyriformis*. The latter species was described by Davis in 1943 on rainbow trout, *Salmo gairdneri*, Richardson, and brook trout, *Salvelinus fontinalis* (Mitchill), and by Heckman (1974) on golden trout, *Salmo aquabonita*. This species is supposedly smaller than *Ichthyobodo necator* and pyriform in shape. However, most authors (Becker, 1977; Tavolga and Nigrelli, 1947; Vickerman, 1976) doubt the existence of *Ichthyobodo pyriformis* and consider that this is a small form of *Ichthyobodo necator* as the size range falls within the highly variable size range of *Ichthyobodo necator*. For brevity *Ichthyobodo necator* will be shortened to *Ichthyobodo* from now on.

3. Biology and Life History

3.1 Life Forms

Very little work has been carried out on the life cycle and biology of *Ichthyobodo*, however several forms of the parasite have frequently been described.

Figure 1.1: Interference Phase Contrast Micrograph of Free Swimming Form of *Ichthyobodo*. × 700. f = flagella

The Free Swimming Form (Figures 1.1 and 1.2). This form of the parasite is ovoid or ellipsoid in shape and bears two flagella in most cases but in some cases four. The parasite swims with hesitant spiralling movements by beating the flagella. Most authors think that this is only a transitory stage for swimming from host to host or for moving about on the host.

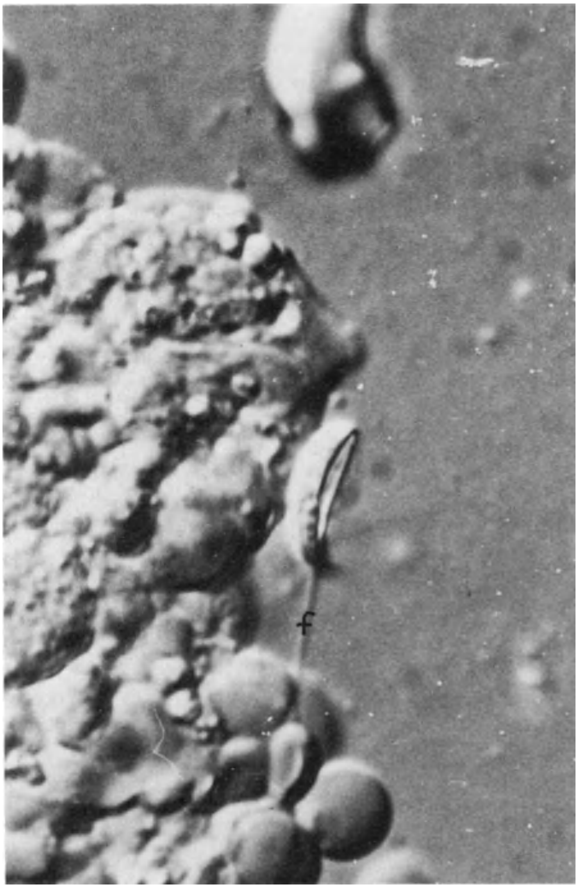

Figure 1.2: Interference Phase Contrast Micrograph of Free Swimming Form of *Ichthyobodo*. Side view. × 700. f = flagella

The Attached Form (Figures 1.3, 1.4, 1.5 and 1.6). The fixed parasitic form is more pyriform in shape and the flagella are less noticeable than in the free living form. The parasite attaches to the epidermal cells of the gills and skin of the host and feeds on the cell contents. The area of skin around the dorsal fin (Tavolga and Nigrelli, 1947) and the tips of the secondary lamellae (Fish, 1940) have been reported to be the most favoured sites of

Figure 1.3: Interference Phase Contrast Micrograph of Attached Form of *Ichthyobodo*. × 700. N = nucleus

attachment. As many as fifteen parasites have been reported attached to a single cell (Fish, 1940); however, one to three are more normal. Becker (1977) has suggested that the parasite can detach or attach to cells readily and that it swims away by twirling the tips of the flagella against the body groove.

Saprophagous Form. A third, saprophagous, form has been suggested by Tavolga and Nigrelli (1947) which they claim feeds on detached and decaying cells and scales on the bottom of the tank or pond in which the host lives. They reported that 10 to 100 such individuals were attached to

Figure 1.4: Scanning Electron Micrograph of *Ichthyobodo* Attached to Epidermal Cells of a Salmonid Host. × 700

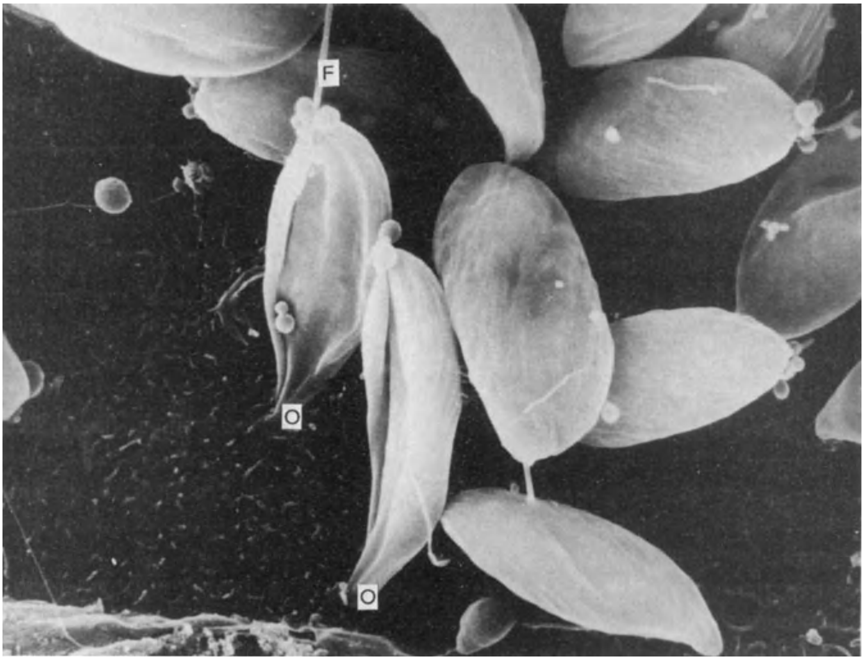

Figure 1.5: Scanning Electron Micrograph of *Ichthyobodo* Showing Ventral View of the Parasite. Note flagella (f) and attachment organelle (O). × 2,625

each scale. No other authors have reported this phenomenon, however, and Bauer (1959) doubted the existence of a saprophagous phase as he thought that the parasites died fairly rapidly after the death of the host.

Cyst Form. The existence of a resistant cyst has been frequently postulated. In 1904 Moroff described a cyst and most authors appear to have repeated his description and drawing without actually describing cysts themselves. Most descriptions of encystment in the Russian literature indicate that the flagella disappear and the parasite rounds up and a cyst wall is produced (Bauer, personal communication). This is similar to the rounding up of the parasite that occurs when the parasite encounters unfavourable conditions, for example in a wet preparation on a microscope slide. However, a cyst wall is not produced in these circumstances; the parasite apparently dies. Neither Benisch (1936) nor Tavolga and Nigrelli (1947) found any evidence for a resistant cyst, although the latter thought

Figure 1.6: Scanning Electron Micrograph of *Ichthyobodo* Showing the Dorsal Surface of the Parasite. Note attachment organelle (O) and epidermal microridges on the surface of the host cell (E); × 10,500

that certain changes in the environment such as temperature, osmotic and light conditions, etc. might bring about encystment. Bauer (1959) quoted Tack (1949) as recording the appearance of *Ichthyobodo* in isolated trout ponds supplied with spring water and in which fish were absent prior to stocking with these trout. However, it is conceivable that *Ichthyobodo* was introduced with the stocked fish as Bauer (1959) and other Russian authors have indicated that *Ichthyobodo* can survive on larvae long before the yolk sac has been absorbed and indeed Hlond (1963) has described the presence of *Ichthyobodo* on eggs of carp. Robertson (unpublished) has observed what appear to be cysts of *Ichthyobodo* (see Figure 1.7) under conditions of rapidly rising temperature and when in the presence of a metabolic

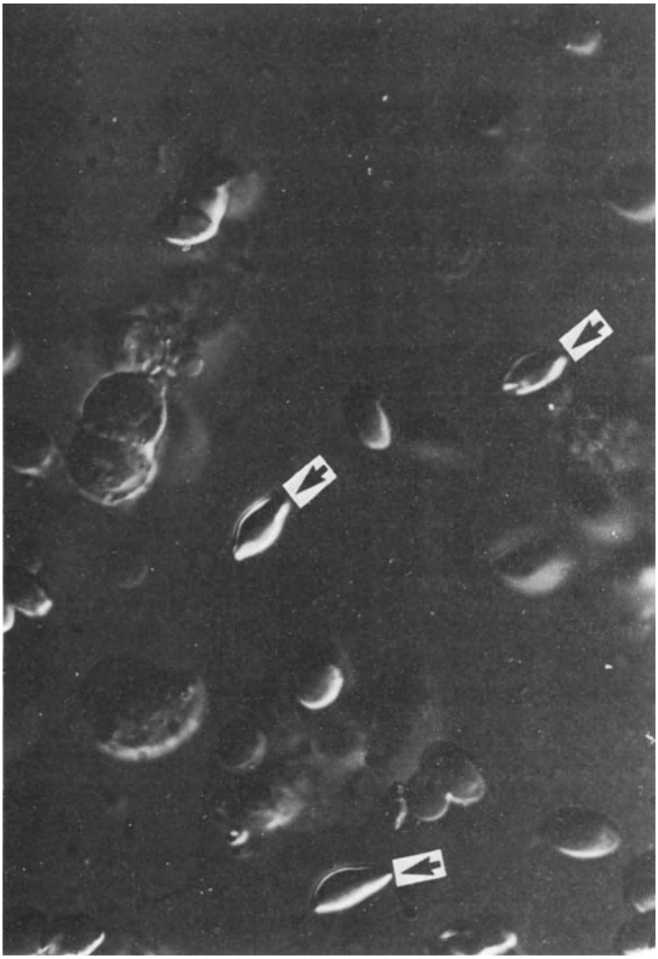

Figure 1.7: Possible Cysts of *Ichthyobodo* x 500

inhibitor (sodium azide), but this has not been confirmed using electron microscopy or other techniques.

Therefore, until the presence of a cyst wall can be convincingly defined, preferably by electron microscopy, the question of whether an *Ichthyobodo* cyst exists or not will remain open. However, the circumstantial evidence existing and the occurrence of cysts in related protozoans such as *Bodo* sp. would indicate that a cyst stage is likely.

3.2 Morphology and Size

Ichthyobodo is a small parasite with a reported size range of 5–18 μm long (mean length of 7.85 μm (Fish, 1940), 9.57 μm (Tavolga and Nigrelli, 1947) and 9.63 μm (Andai, 1933) and 2.5–14.54 μm wide (mean width 4.35 μm (Tavolga and Nigrelli, 1947), 5.12 μm (Fish, 1940), and 7.49 μm (Andai, 1933). The quadriflagellate form is reported to be the largest form with a more uniform size and rounded shape. The mean size for the quadriflagellate form reported by Andai was 14.59 μm long and 14.33 μm wide.

The shape of the parasite varies between the free swimming form and the attached form. The free swimming form is reniform in shape and has a convex dorsal surface and concave ventral surface, whereas the fixed form is more pyriform with a slight twist which gives the parasite a comma-like appearance. Both forms have a prominent groove transversing the posterior two-thirds of the ventral surface, in which lie the flagella. The flagella arise from the kinetoplast at the anterior end of the groove. The biflagellate form has two flagella of unequal length, with the longer being on average 25 μm and the shorter being 20 μm (Andai, 1933). In the quadriflagellate form there are two short and two long flagella, with the short being approximately half the length of the long (12.1 μm versus 24.9 μm).

At the anterior end of the free swimming form of the parasite is a cytostome and this is highly modified in the fixed form to form a flat plate which attaches the parasite to the outer surface of the host cell.

Few structural details can be seen inside the living parasite apart from the contractile vacuole filling and emptying, and a number of dark short bacillary or spherical granules. However, when properly fixed and stained with a suitable stain such as Heidenheim's haematoxylin (Andai, 1933) several internal structures can be seen. The nucleus which measures 1.36 μm – 4.36 μm diameter (Andai, 1933) with a mean of 1.9 μm (Fish, 1940) is found just below the middle of the body, and is composed of a dark spherical block of chromatin surrounded by a clear halo. When stained with Romanowsky's stain a large number of basophilic bodies are seen distributed throughout the cytoplasm. There is also a large basophilic body at the base of the flagellum (Vickerman, 1976). This is the kinetoplast as described in the section on taxonomy. Food vacuoles can also be seen in the cytoplasm.

Studies with the electron microscope by Schubert (1966) and Joyon and