The Biology of Gyrodactylid Monogeneans: The "Russian-Doll Killers"

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ABSTRACT

This article reviews the history of gyrodactylid research focussing on the unique anatomy, behaviour, ecology and evolution of the viviparous forms while identifying gaps in our knowledge and directions for future research. We provide the first summary of research on the oviparous

gyrodactylids from South American catfish, and highlight the plesiomorphic characters shared by gyrodactylids and other primitive monogeneans. Of these, the most important are the crawling, unciliated larva and the spike sensilla of the cephalic lobes. These characters allow gyrodactylids to transfer between hosts at any stage of the life cycle, without a specific transmission stage. We emphasise the importance of progenesis in shaping the evolution of the viviparous genera and discuss the relative extent of progenesis in the different genera. The validity of the familial classification is discussed and we conclude that the most significant division within the family is between the oviparous and the viviparous genera. The older divisions into Isancistrinae and Polyclithrinae should be allowed to lapse. We discuss approaches to the taxonomy of gyrodactylids, and we emphasise the importance of adequate morphological and molecular data in new descriptions. Host specificity patterns in gyrodactylids are discussed extensively and we note the importance of host shifts, revealed by molecular data, in the evolution of gyrodactylids. To date, the most closely related gyrodactylids have not been found on closely related hosts, demonstrating the importance of host shifts in their evolution. The most closely related species pair is that of *G. salaris* and *G. thymalli*, and we provide an account of the patterns of evolution taking place in different mitochondrial clades of this species complex. The host specificity of these clades is reviewed, demonstrating that, although each clade has its preferred host, there is a range of specificity to different salmonids, providing opportunities for complex patterns of survival and interbreeding in Scandinavia. At the same time, we identify trends in systematics and phylogeny relevant to the *G. salaris* epidemics on Atlantic salmon in Norway, which can be applied more generally to parasite epidemiology and evolution. Although much of gyrodactylid research in the last 30 years has been directed towards salmonid parasites, there is great potential in using other experimental systems, such as the gyrodactylids of poeciliids and sticklebacks. We also highlight the role of glacial lakes and modified river systems during the ice ages in gyrodactylid speciation, and suggest that salmon infecting clades of *G. salaris* first arose from *G. thymalli* in such lakes, but failed to spread fully across Scandinavia before further dispersal was ended by rising sea levels. This dispersal has been continued by human activity, leading to the appearance of *G. salaris* as a pathogen in Norway. We

review the history and current status of the epidemic, and current strategies for elimination of the parasite from Norway. Finally, we consider opportunities for further spread of the parasite within and beyond Europe.

1. INTRODUCTION

Monogeneans of the genus *Gyrodactylus* have been known for almost 180 years for their retention of fully grown daughters *in utero* until they themselves contain developing embryos (Figure 1). This ''Russian-doll'' reproduction, dubbed hyperviviparity by Cohen (1977), is extremely rare in the Animal Kingdom and was the focus for intensive study by late 19th century microscopists. We recently reviewed gyrodactylid reproduction (Cable and Harris, 2002) and specificity (Bakke *et al*., 2002) but there are now compelling reasons to re-evaluate their ecology. In the first place, although the viviparous gyrodactylids have attracted most attention, we are becoming increasingly aware of the diversity of oviparous gyrodactylids from South American catfish (Kritsky *et al*., 2007) and a review of their relationship to the viviparous genera is timely. Furthermore, the viviparous gyrodactylids are increasingly reported as pathogens of

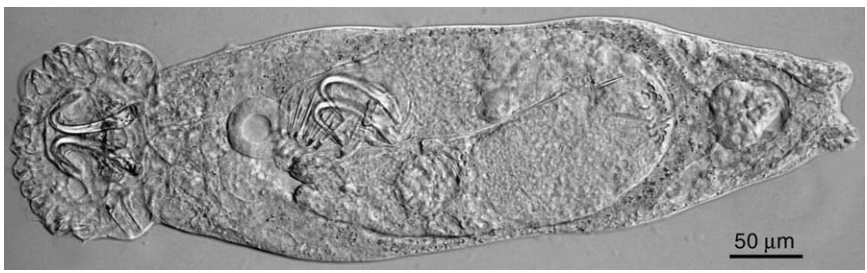


Figure 1 Light micrograph (interference contrast) of a living gravid *Gyrodactylus salaris* with two daughters *in utero* like ''a Russian-doll''. The lack of penis and spermatozoa in the seminal receptacle together with the near term F1 embryo *in utero* indicates that the parasite has not yet given birth for the first time. (G. Robertsen, unpublished.) See coloured version on the front cover.

farmed fish (Lile *et al.*, 1993; Woo, 1995; Mo and Lile, 1998; Jalali *et al.*, 2005; You *et al.*, 2006) and the notoriety of the *Gyrodactylus salaris* epidemic has stimulated research to such an extent that gyrodactylids are now the best studied of all monogeneans. Much research in Europe has focussed on three economically relevant species: *G. salaris* on Atlantic salmon (*Salmo salar*) and its non-pathogenic sibling *G. thymalli* on grayling (*Thymallus thymallus*), and *G. derjavini* on brown trout (*Salmo trutta*). Recent studies suggest that in the former case we are observing the evolution of a fish pathogen complex in real time. However, several other gyrodactylids have also been studied extensively, including those infecting guppies, gobies and sticklebacks, and represent ideal model systems for studying evolutionary and ecological processes in this group, and in parasitic organisms in general.

1.1. Early History

Gyrodactylus was first described from bream (*Abramis brama*) by von Nordmann (1832). Although he observed the embryo *in utero*, he failed to appreciate its significance, and the prominent embryonic hamuli (Figure 1) were interpreted as ''stomach hooks''. Viviparity was first recognised by von Sieboldt (1849) and the value of gyrodactylids for studies on reproduction was established. Gyrodactylids were particularly useful to early microscopists as flatworms without an impervious egg shell. Flatworms attracted much attention at the end of the 19th century because of their apparently basal position close to Haeckel's (1874) hypothetical gastraea ancestor of the Metazoa and their potential for regeneration. The enclosure of several embryos inside each other also represented an attractive model for the study of germ cell lineages, a paradigm which was just becoming established at this time. *Gyrodactylus* was therefore a popular choice for early studies on chromosome and embryonic cell behaviour (e.g. Wagener, 1860; Kathariner, 1893, 1899, 1904; Gille, 1914), remaining important until shortly before the First World War.

1.2. Taxonomic and Faunistic Research

Gyrodactylids soon became well known as pathogens in fish farms (e.g. Atkins, 1901; Embody, 1924; Guberlet *et al*., 1927) and wild fish populations (e.g. Williams, 1964). Fish health textbooks describe ''fluke'' infections, often without differentiating the distinct epidemiology of viviparous gyrodactylids from that of oviparous dactylogyrids and ancyrocephalids. The gyrodactylids were known to be species-rich, and by 1970 some 200 species had been described, mostly from North America and Eurasia (Malmberg, 1970). There was considerable scepticism over the validity of many taxa, and gyrodactylids were largely ignored by evolutionary ecologists because of their complex taxonomy. They were perceived as difficult to work with, which undoubtedly has held back research into their ecology, and by the 1980s only a handful of researchers worldwide retained an interest in the group.

1.3. The *Gyrodactylus salaris* Epidemic

During the mid-1970s, Norwegian researchers began to report a highly pathogenic epidemic disease amongst both farmed and wild salmon populations, which they ascribed to *Gyrodactylus salaris* (see Tanum, 1983). The first international report of the epidemic (Johnsen, 1978) followed internal reports in Norway (Bergsjö and Vassvik, 1977). Research was at first slow, but over the next 20 years the disease was observed in many rivers within Norway (Heggberget and Johnsen, 1982; Johnsen and Jensen, 1986, 1991; Mo, 1994). The generally accepted conclusion is that the parasite was introduced from the Baltic region into East Atlantic stocks of salmon which lacked endogenous resistance to the parasite (but see Halvorsen and Hartvigsen, 1989; and Section 10 below). Subsequently, *G. salaris* has spread to cause epidemic disease in the Russian River Keret (Kola Peninsula) and high infection levels are also recorded in the River Högvadsån on the south west Coast of Sweden. Much effort is devoted to prevent the pathogen spreading to Scotland, Ireland and

other areas with large natural populations of Atlantic salmon. *G. salaris* now represents the most significant threat to the continued existence of large wild populations of East Atlantic stocks of Atlantic salmon, and endangers the reintroduction of salmon into rivers of the North Sea basin. It represents a major drain on resources for the EU and the potential for translocation with salmonid stocks to other parts of the world cannot be ignored. This pathogen has been a research driver which has led to huge improvements in our understanding of gyrodactylid biology. In addition, due to their species richness, ubiquity, economic importance in aquaculture and potential conservation threat, they are the most intensively studied group of monogeneans. From a research backwater, gyrodactylid research is now a growth area within parasitology and wildlife disease ecology.

1.4. Genus, Species Flock or Host Races?

One of the most interesting questions regarding *Gyrodactylus* today concerns the species concept; what is the relationship between the operational taxonomic units (OTU's) that we currently regard as valid species? *Gyrodactylus* is hugely species-rich but lacks obvious morphological diversity. Over 400 species have been described (Harris *et al.*, 2004) but from only ~200 predominantly teleost hosts (Bakke *et al.*, 2002). Extrapolation to the ~24 000 teleost species would suggest around 20 000 gyrodactylid species. The existence of such a megadiverse genus has wider significance. There is no evidence that the rate of discovery of new gyrodactylids is slowing for any reason other than lack of research, and where close attention has been paid to specific host groups, species descriptions have multiplied (on poeciliid fishes, see Harris and Cable, 2000; Cable *et al.*, 2005; on gobiids see Longshaw *et al.*, 2003; Huyse and Malmberg, 2004; Huyse *et al.*, 2004a). The rate of discovery of new gyrodactylid taxa is set to risen again, as the availability of molecular markers reveals previously undetected species and strains (Lautraite *et al.*, 1999; Ziętara and Lumme, 2002; Hansen *et al.*, 2003).

1.5. *Gyrodactylus*: The Drosophilids of the Parasitic World

The discovery of *G. salaris* as a devastating pathogen marked a turning point in gyrodactylid research and by stimulating interest in molecular markers, has reinvigorated gyrodactylid taxonomy and evolutionary biology. Molecular probes (Cunningham *et al.*, 1995a, b; Cunningham, 1997) were initially developed to provide objective and reliable means for identification of *Gyrodactylus* parasitising salmonid fish by non-specialists, and from this the modern field has developed. One of the most exciting observations has been the ability to record evolution in action by an actively adapting species, *G. salaris.* The boundaries between *G. salaris* on Atlantic salmon and on Arctic charr (Olstad *et al.*, 2005, 2007; Robertsen *et al.*, 2007a), and *G. thymalli* on grayling (Hansen *et al.*, 2003; Meinilä *et al.*, 2004) are not distinct, suggesting that we are watching the evolution of a gyrodactylid which has switched hosts and is developing new patterns of host specificity. This has stimulated interest in other gyrodactylids, for example those infecting guppies (Cable and van Oosterhout, 2007; King and Cable, 2007; van Oosterhout *et al.*, 2003, in press a, b) and gobies (Huyse and Volckaert, 2002; Huyse *et al.*, 2003, 2004a, b), and has shown that the patterns of evolution of these organisms are subtle and informative about general evolutionary processes in parasites. It may not be too fanciful to consider the genus *Gyrodactylus* the *Drosophila* of the parasite world. Our strains of *G. turnbulli* have been maintained experimentally for more than 10 years, and *G. salaris* for more than 5 years in the laboratory. With their short generation times and simple culture requirements, the entire trajectory of infection of these ectoparasites can be monitored in real time on a single host. However, the most important resource for evolutionary biologists is the huge diversity of gyrodactylids on bony fishes, representing a wide range of evolutionary interactions. The temporary evasion of host responses by host transfer may increase the abilities of *Gyrodactylus* species to colonise new host species and may partially explain the high rate of diversification demonstrated by viviparous gyrodactylids (Boeger *et al.*, 2005). Amongst the 400 recorded species, there are examples where speciation may have occurred in the last few thousand years, through to species which are

several millions of years old. By reviewing gyrodactylid biology here, we aim to increase the profile of these parasites not just among helminthologists, but also for those with a wider interest in evolution.

Part 1. Gyrodactylid autecology

2. MORPHOLOGY

Gyrodactylids are amongst the smallest monogeneans (Figure 2) and some, such as *Isancistrum* (see Llewellyn, 1984), are similar in size to oncomiracidium larvae (~200 μm). The fusiform body has a posterior opisthaptor armed with marginal hooks, hamuli and bars (see Section 2.3). Anteriorly, two conspicuous cephalic processes bearing

Figure 2 Scanning electron micrograph of a heavy *Gyrodactylus salaris* infection on the skin of an Atlantic salmon parr (K. Kvalsvik, unpublished).

adhesive glands and spike sensilla are involved in attachment but are also important in the sensory biology of gyrodactylids (see Bakke *et al.*, 2004a). The transparent body is dominated by the F1 generation embryo curled within the uterus (Figures 1 and 3); in gravid worms, all other body organs are compressed by the sheer volume of the developing embryonic mass.

The internal anatomy of gyrodactylids has been extensively misinterpreted because they lack many structures present in oviparous monogeneans. Egg-laying monogeneans produce large tanned egg capsules (Smyth, 1954) which hatch to a (normally) swimming oncomiracidium. This larva has external, ciliated locomotory cells and usually four eye spots, and on hatching swims to a new host, where it sheds ciliated cells and settles to a sedentary existence, usually spending its entire life on the same host. The female reproductive system of these oviparous monogeneans is separated into a germarium, producing oöcytes and vitellaria, producing vitelline cells which contribute to the egg shell and provide nourishment for the developing embryo. These cells, with sperms, mix within the oötype to form a mature egg, which is then extruded and laid. Several reviews have been dedicated to different aspects of the biology of oviparous monogeneans, but gyrodactylids have barely any characteristics in common with these organisms, and their structure was consequently poorly understood for many years. Harris (1983) was the first to recognise egg laying in *Ooegyrodactylus farlowellae*, which allowed the identification of derived structures from plesiomorphic characters in gyrodactylids.

2.1. The Importance of Progenesis and Viviparity

The term progenesis is often confused with neoteny. Neoteny is the retention of larval characters beyond the normal developmental stage to allow continued exploitation of a particular habitat. Duration of the life cycle is not shortened (for further clarification, see Gould, 1977). Progenesis, on the other hand, is the acceleration of the life cycle to allow an organism to reproduce as a juvenile or larva. The early-maturing precocious larvae of *Polystoma* spp. are excellent examples of

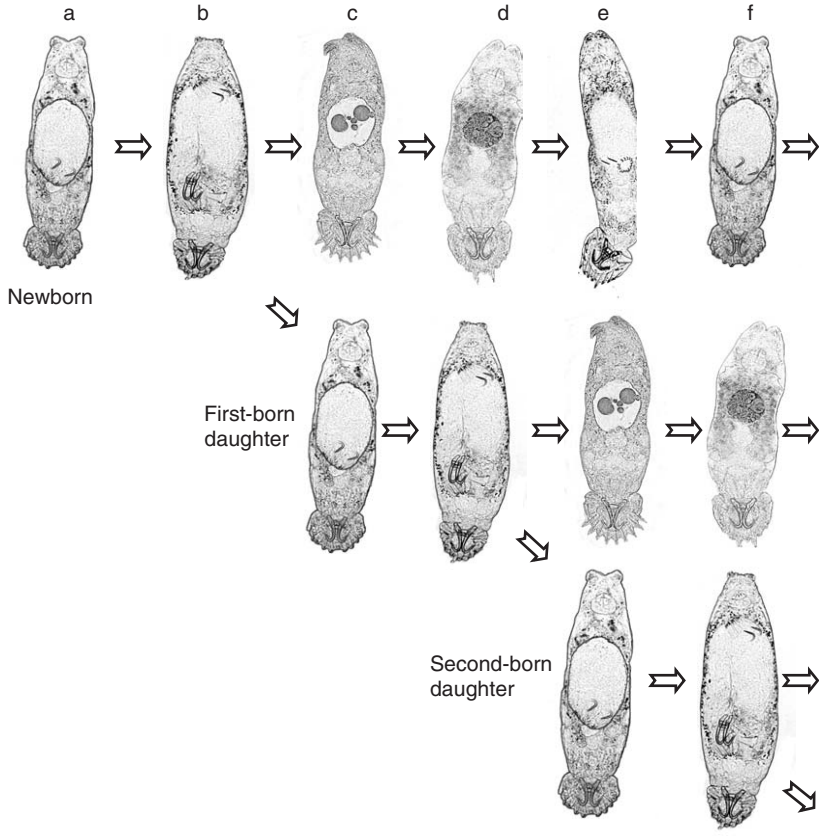


Figure 3 Light micrographs of *Gyrodactylus salaris* showing embryonic development during the life cycle. (a) Following birth (newborn, first born and second born daughters as shown), the hamuli of the next generation are visible towards the posterior axis of the F1 embryo close the Egg Cell Forming Region (ECFR) of the parent. (b) The hamuli of the F2 embryo lie at the anterior pole of the parental uterus, whereas the sclerites of the F1 embryo, at the posterior pole, are fully developed in near term adults. (c) Following birth, the contracted uterus contains two dividing cells which have originated from a single cell that has entered the uterus from the ECFR. (d) F1 embryo is a mass of ~50 cells that fills the uterus. No evidence of embryonic sclerite development. (e) A ring of primordial marginal hooks and rod-like hamuli primordial within the F1 embryo. (f) The marginal hooks have separated so a ring structure is no longer visible and curvative of the hamuli apparent in F1 haptor. The F1 embryo in this post-first birth worm is similar to that of a newborn parasite.

progenesis (although confusingly referred to as neotenics), as they reproduce when only weeks old, whereas "normal adults" (otherwise known as slow-growing worms) reproduce after 3 years. The retention of larval eye spots by the adult *Pseudodiplorchis* (see Cable and Tinsley, 1991) may be a neotenic adaptation allowing a light response when the toad hosts emerge from hibernatory burrows. Paedogenesis is as a general term used to describe reproduction in larvae, but often life-cycle duration is overlooked, so examples (such as salamanders) that have a life cycle of normal length or extended, should actually be considered neotenic. The viviparous gyrodactylids are highly progenetic (Harris, 1983) and the mature *Gyrodactylus* closely resembles larval and young (male) *Ooegyrodactylus*. The first birth of *Gyrodactylus* occurs after days (e.g. Jansen and Bakke, 1991), whereas in *O. farlowellae* there is a generation time of weeks (Harris, 1983). This abbreviation of the life cycle in viviparous gyrodactylids can be attributed to progenesis.

Viviparous gyrodactylids are amongst the most successful progenetic groups. Progenesis is also thought to have been important in the evolution of other minor phyla including rotifers, gnathostomulids and kinorynchs, groups which contain at most hundreds to thousands of species. If we are correct in estimating ~20 000 *Gyrodactylus* species (see above), the gyrodactylids would be one or two orders of magnitude more species-rich than these other groups.

Cable and Harris (2002) described the effects of progenesis on the female reproductive system. In other monogeneans, including the egg-laying gyrodactylids, this system has extensive vitellaria with a complex oötype and glandular apparatus. In the viviparous gyrodactylids however, the female system never fully develops and remains structurally simple, characterised by syncytial structures. Viviparous gyrodactylids are protogynous hermaphrodites, the female system maturing before the male, whereas all other monogeneans, including the oviparous gyrodactylids, are protandrous. This is further evidence of precocious maturation of the simplified female system, which becomes active before the male system has followed its normal trajectory to maturation. However, substantial differences exist in the extent of progenesis in different gyrodactylids. *Macrogyrodactylus* appears least progenetic, undergoing considerable post-embryonic

differentiation, particularly in the putative glandular tissue/subtegumental cells of the posterior body (see Cable *et al*., 1996, 1997, 1998; El-Naggar and Cable, in press). By contrast, *Isancistrum* may represent extreme progenetic abbreviation of the life cycle; the haptor lacks hamuli and bars, and other organ systems appear simplified.

Coincident with progenesis in other organisms, such as bdelloid rotifers, is eutely (a constant number of nuclei, usually established in the juvenile stage), and this is also seen in gyrodactylids. Gyrodactylids such as *G. gasterostei,* contain ~1000 cells, with all non-reproductive cell division being completed before birth. The only major changes which occur post-birth are within the male reproductive system. This may not be the case for all genera, especially for the very large and relatively long-lived *Macrogyrodactylus*. The exact point at which eutely is established must represent the switch from development of the parent to development of the next-generation embryo, and is therefore an important determinant of reproductive rate.

As in other progenetic, eutelic groups, gyrodactylid body size and cell number are insufficient to support the development of extensive internal organ systems. Functions such as internal transport and homeostasis are carried out by syncytial layers; many of the internal boundaries in gyrodactylids are syncytial, allowing transport of material within, but not necessarily across, layers. Syncytia characterise this group and are found throughout the body including the external tegument, the absorptive layer of the intestine, the lining of the Egg Cell Forming Region (ECFR; see Jones *et al*., 1997, 1998; Cable *et al*., 1996, 1997, 1998), and the uterus.

Viviparity is the second major reproductive adaptation of gyrodactylids. Uniquely they give birth to fully grown young which already contain developing embryos *in utero* (Figures 1 and 3). Embryos may originate in one of two ways. The second-born and subsequent daughters develop from mature oöcytes in the same manner as in any other metazoan (although oöcytes may be fertilised by sperm or develop apomictically). However, the first-born daughter always develops asexually in the centre of its parental embryo as a group of cells that become differentiated from their parent. It is impossible therefore to track the first-born daughter back to a single cell. In this respect, viviparity in gyrodactylids, at least the development of the

first-born daughter is another expression of the large potential for asexual reproduction in platyhelminths. A plausible hypothesis for the origin of viviparity is that an oviparous gyrodactylid acquired an adaptation whereby an embryo *in ovo* developed daughter embryos asexually, to be born almost directly after the larva had hatched. Such an adaptation would be favoured because of the reduction in generation time achieved, and eventually loss of egg shell would lead to the assumption of an entirely viviparous life cycle. All other aspects of morphology must be interpreted in the context of the extreme progenesis and viviparity shown by gyrodactylids.

2.2. Tegument

The characteristic syncytial tegument of monogeneans has been described for *Gyrodactylus gasterostei* (see Lyons, 1970; Cable *et al*., 1996), *Gyrodactylus eucaliae* (see Kritsky, 1971; Kritsky and Kruidenier, 1976) and for *G. turnbulli* and *G. bullatarudis* (see Cable *et al*., 1996). Embryonic gyrodactylids have a nucleated epidermis, which in other monogeneans is replaced by an outer anucleated cytoplasmic layer connected to subtegumental cell bodies that usually lie beneath muscle blocks. Surprisingly, no intact cytoplasmic connections linking the outer tegument of mature gyrodactylids to the putative subtegument have been described and there is considerable confusion as to the identity of the subtegumental cells themselves. However, there are a series of lateral cells, which are assumed to be subtegumental cells as they contain secretory vesicles similar to those in the surface layer of the adult tegument (e.g. El-Naggar and Cable, in press). In *G. eucaliae*, a single type of subtegumental cell appears to produce two distinct tegumental vesicles (Kritsky and Kruidenier, 1976) whereas in *Macrogyrodactylus clarii* three different secretory vesicles are each manufactured in a distinct cell type (El-Naggar and Cable, in press). In both gyrodactylids, these putative subtegument cells are restricted to particular regions of the body. The extent of subtegumental variation in other gyrodactylids is unknown, but the basic structure of the tegument appears similar to that of other monogeneans. The outer surface is surrounded by an outer plasma membrane amplified with numerous microvilli, between which

often lies a prominent carbohydrate-rich glycocalyx. The outer cytoplasmic layer is usually devoid of cell organelles, as nuclei, mitochondria, Golgi bodies, and endoplasmic reticulum tends to be restricted to the subtegumental cells. It rests on a basal membrane overlying a basal lamina complex and muscle layers; in gravid worms this tissue is often greatly compressed against the uterus (Cable *et al*., 1996). The contents of tegumental vesicles are secreted on to the surface of *Gyrodactylus* spp. (Cable *et al*., 1996; Bakke *et al*., 2006) and there is presumably quite a high turnover of vesicles as indicated by the manufacture of large numbers of vesicles in the putative subtegumental cells. Even the surface layer of near term F1 embryos contains numerous tegumental vesicles, but their secretion has only been observed after birth.

2.3. Attachment and Musculature

Gyrodactylids primarily attach using the opisthaptor armed with 16 peripheral articulated marginal hooks and usually with a single pair of ventrally orientated hamuli, linked by separate dorsal and ventral bars. As in the acanthocotylids, *Enoplocotyle*, the anoplodiscids, bothitremids and tetraonchoidids, the marginal hooks have articulated blades (sickles), which can move relative to their shafts. The marginal hooks are arranged around the periphery of the haptor within finger-like tegumental papillae (Figure 4). The marginal hooks are capable of considerable mobility, moving freely within each tegumental papilla, in addition to the shafts being capable of extension in and out of the haptor. The papillae have an extensive musculature, allowing each individual marginal hook to work independently of its neighbours, but the roof of the haptor is also well provided with radial fibres which bind the hooks together and allow them to act as a unit. Fibres attaching to the marginal hooks may be strengthened and therefore more visible than normal musculature. The "sickle filament loop" (Figure 5) of Malmberg (1970) is an open-ended loop which fits over the marginal sickle blade and transmits force from effector muscles located deep within the papilla (Shinn *et al*., 1993), while "shank ligaments" connect the marginal hooks into the deeper haptor. The marginal hooks represent the major attachment organs of

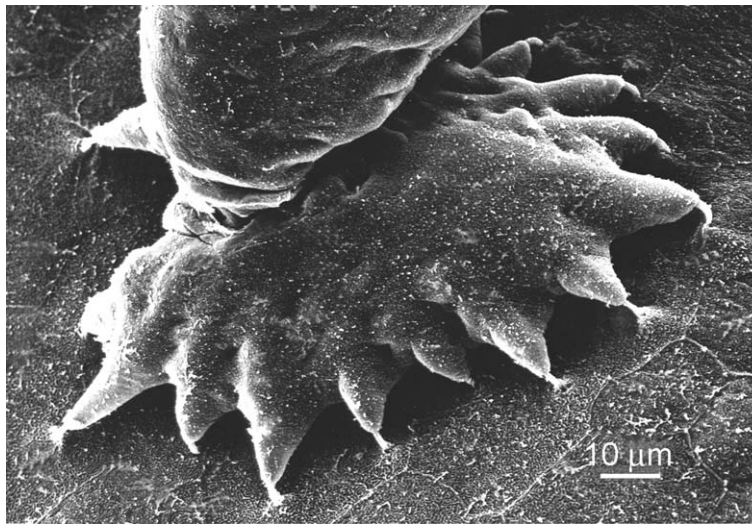


Figure 4 Scanning electron microscopy of the opisthaptor of *Gyrodactylus salaris* attached to the skin of an Atlantic salmon.

gyrodactylids and, with the hamuli and ventral bar, represent the main taxonomic structures. Shinn *et al.* (2003) showed that their gaffing action, taken in concert, firmly attach the gyrodactylid to the fish surface. They are also essential in other behaviours, most notably helping to attach to smooth surfaces, such as glass (personal observation) and the surface water film (Cable *et al.*, 2002a).

The single pair of hamuli lies ventral to the marginal hooks. In skin parasites, the hamuli are fish-hook shaped, connected by the dorsal bar which is fused at its ends to processes on the surface of the hamuli and a ventral bar loosely attached to the hamuli (Figure 6). The anterior tips of the hamulus roots are bound to large fibrous ligaments running anteriorly and eventually join the body wall musculature. Some 30–50 µm anterior of the hamulus roots these ligaments pass through a fibrous yoke which ensures that the force exerted by the body wall musculature acts at an angle to pull the hamulus roots forward and into the mid-line. The dorsal bar antagonises these forces and maintains the spacing between the hamulus shafts (Figure 7). When the body wall musculature relaxes, the hamuli rotate slightly (Shinn *et al.*, 2003) and move relative to the ventral bar. This

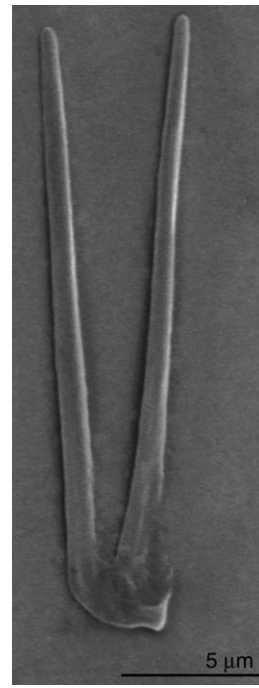


Figure 5 Scanning electron micrograph of the sickle filament loop of the marginal hook of *Gyrodactylus salaris* from Atlantic salmon, often mentioned in species descriptions. In our opinion, this structure, often detached entirely from the sickle during digestion, is of little value in *Gyrodactylus* taxonomy.

sequence of movements forces the hamulus points against the fish skin, exerting pressure to lift the roof of the haptor, while tensioning the marginal hooks. The ventral bar (Figure 8) acts as a pressure pad preventing slippage of the anterior part of the haptor and the hamuli (Shinn *et al*., 2003). In skin parasitic gyrodactylids (on which Shinn *et al*., 2003 based this model of attachment), the hamulus points normally do not extend from the tegument (Wagener, 1860; Cone and Odense, 1984) or gaff the fish skin (Figure 7). Instead, pressure is applied by the whole length of the point, forced down onto the fish skin but not penetrating it. This is therefore similar to the mechanism of attachment of skin-parasitic capsalid monogeneans (e.g. Kearn, 1971) in which damage to the host epithelium is minimised and attachment is achieved while the musculature is relaxed.

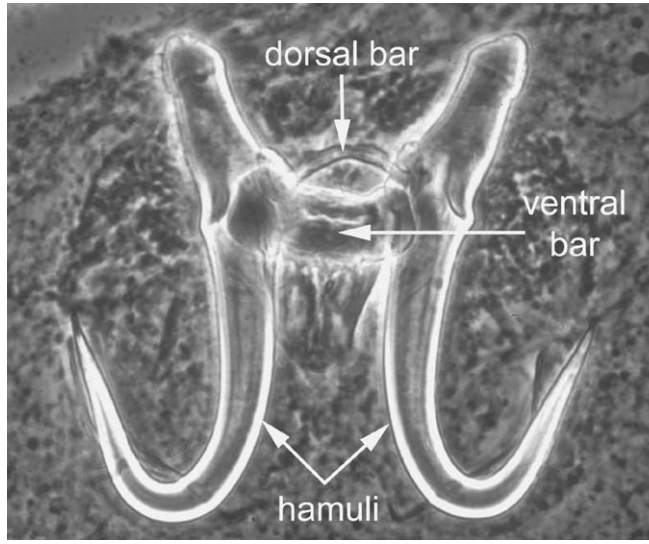


Figure 6 Light micrograph of flat mount preparation of the hamuli and bars of *Gyrodactylus salaris* (Lierelva strain) from Atlantic salmon as seen from the ventral side.

The apparent uniformity of gyrodactylid attachment hides a huge diversity of detail. In gill-parasites, with their characteristic strongly curved hamulus shafts, the hamuli often penetrate the gill tissue to some depth. *Ooegyrodactylus farlowellae*, despite appearing similar to other skin parasitic gyrodactylids, also gaffs skin tissue deeply, and marginal hooks are relatively unimportant in attachment (Harris, 1983). This may be because the large body size requires more robust attachment than the smaller *Gyrodactylus* species or it may be an adaptation to the bony external surface of loricarid catfish. More subtle diversity of attachment also exists. Harris and Cable (2000) noted that *G. poeciliae*, with slender marginal hook sickle points less than 1 μm in length, all fell from the host following fixation, whereas *G. milleri*, with more robust marginal hook sickles, remained attached to the same poeciliid host. Cone and Odense (1984) found that *G. avaloniae*, *G. adspersi*, *G. bullatarudis* (possibly *G. turnbulli*, see Harris, 1986) and *Gyrodactylus* sp. from goldfish (close to *G. gurleyi* according to Cone and Wiles, 1984) all had a superficial attachment mechanism in which the marginal hooks hardly penetrated the

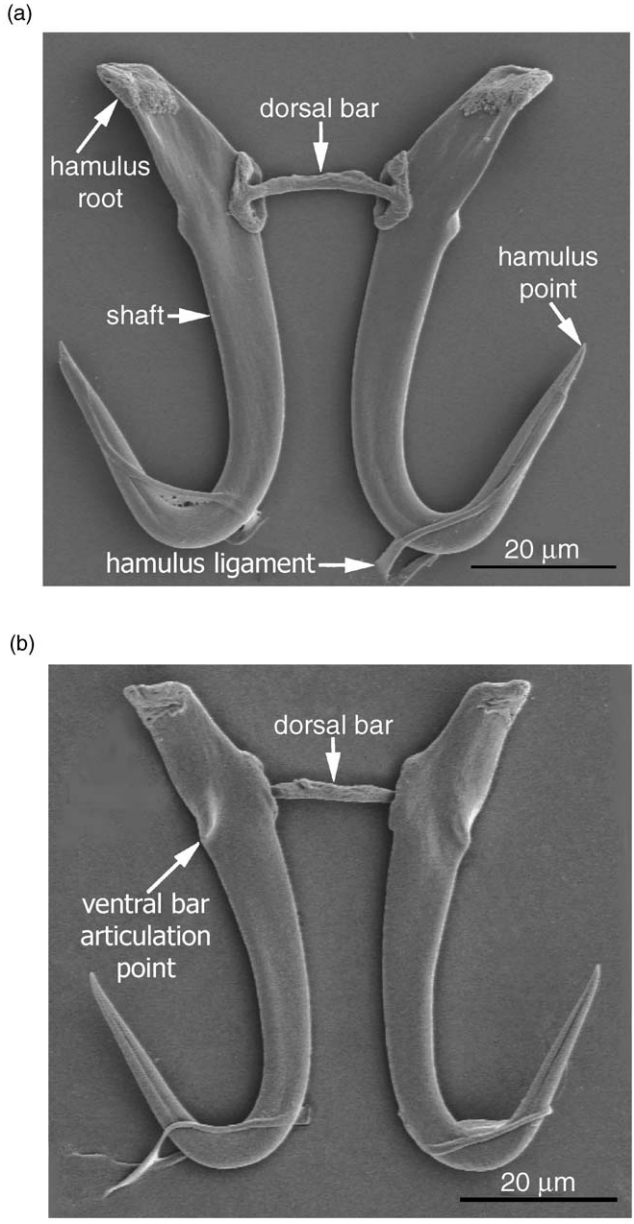


Figure 7 Scanning electron micrographs of the dorsal side (a, Lake Bullaren strain from rainbow trout) and ventral side (b, River Rauma strain from salmon) of the hamuli (anchors) and dorsal bar of *Gyrodactylus salaris*.

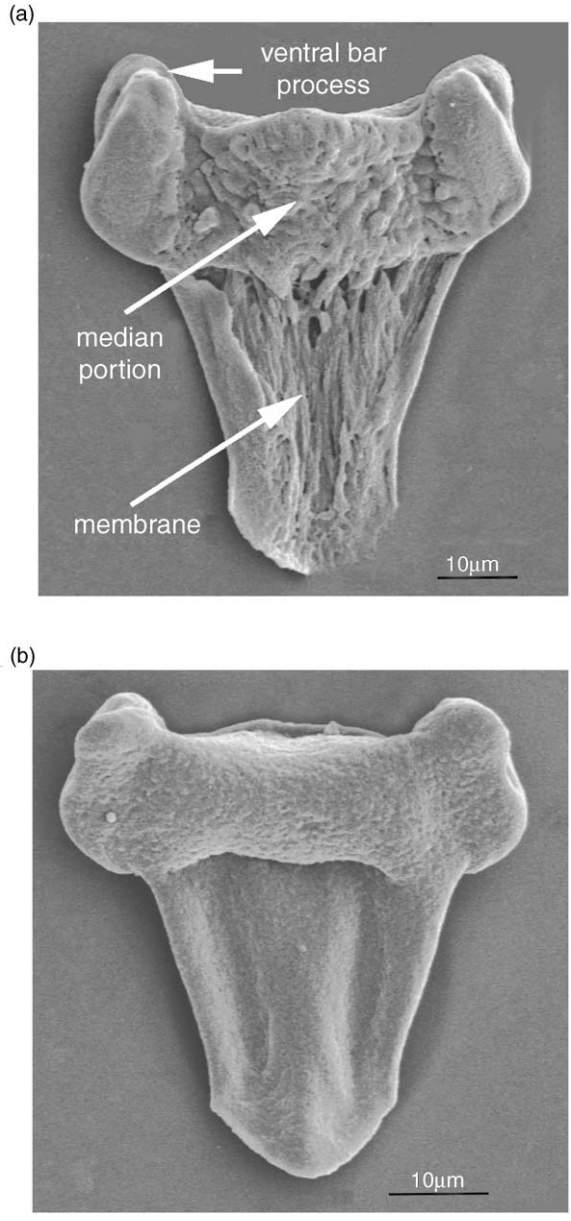


Figure 8 Scanning electron micrographs of the dorsal (a) and ventral (b) sides of the ventral bar of *Gyrodactylus salaris* (River Rauma strain) from Atlantic salmon.

epithelium. In contrast, *G. salmonis* penetrated host cells deeply (Cone and Cusack, 1988). In *G. salaris*, the sickle points are approximately 6 μm in length and too short to penetrate the epidermis into the blood supplied dermis (Figures 9 and 10; see Sterud *et al.*, 1998).

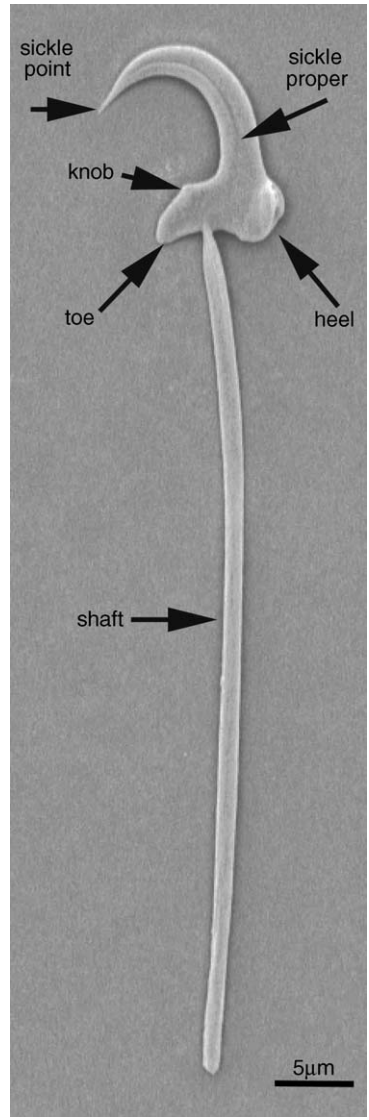


Figure 9 Scanning electron micrograph of a marginal hook of *Gyrodactylus salaris* (River Rauma strain) from Atlantic salmon.

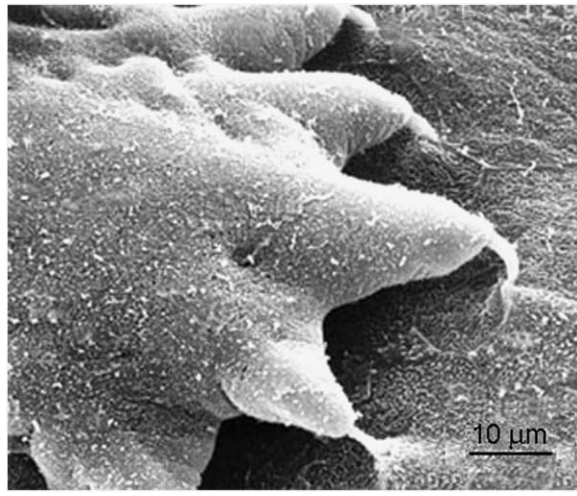


Figure 10 Scanning electron micrograph of the marginal hooks of *Gyrodactylus salaris* penetrating the skin of an Atlantic salmon. The sickle points are ~6 µm in length and too short to penetrate the epidermis into the blood supplied dermis.

Overall, *Gyrodactylus* species seem to have been selected to minimise damage to the host epithelium, trending towards superficial attachment via the marginal hooks and the hamuli used as a pressure pad.

The structure and organisation of the haptor has been the main character used in describing new genera, and different genera therefore have different attachment strategies. The simplest modification is the complete loss of the hamuli and bars. This appears to have occurred independently at least twice; in *Anacanthocotyle* parasitising characins in Central America (Kritsky and Fritts, 1970) and in *Isancistrum* infecting squids in the North Atlantic (de Beauchamp, 1912; Llewellyn, 1984). In these genera attachment relies on the marginal hooks only, in *Isancistrum* possibly because of the very small body size (100–300 µm length; Llewellyn, 1984) or the fragile thin epidermis of the host squid (Polglase *et al.*, 1983). Loss of hamuli and bars may also be due to progenesis, which reduces developmental time available for hamulus development and migration. Progenesis may also explain the peduncular location of hamuli in *Acanthoplacatus* (see Ernst *et al.*, 2001a) which do not migrate into the haptor as the parasite matures. It is difficult to see how the hamuli could

function in their peduncular position and they appear rudimentary in the illustrations of Ernst (1999).

The larger-bodied gyrodactylids such as *Macrogyrodactylus*, *Swingleus*, *Polyclithrum* and *Mormyrogyrodactylus* (Luus-Powell *et al.*, 2003) all use shallow suctorial attachment. The marginal hooks are separated into anterior and posterior groups (recognising the stresses placed on the haptor when the host fish moves), and the haptor may be strengthened with accessory plates or bars. In each of these genera, the haptor has evolved independently for suctorial attachment suggesting strong common selective forces on gyrodactylid evolution. The functional parallels with the capsalid attachment mechanism (Kearn, 1971) are striking. In *Fundulotrema*, *Swingleus* and *Mormyrogyrodactylus*, an accessory peduncular plate is present to allow the anterior of the haptor to be pressed against the host (Cone and Odense, 1988; Billeter *et al.*, 2000; Luus-Powell *et al.*, 2003). Suctorial attachment has also evolved in *Gyrdicotylus gallieni,* from the mouths of clawed toads (*Xenopus*). The entire haptoral rim has become modified into a sucker, partially separated into two units by the hamuli (Harris and Tinsley, 1987). The membraneous edge of the haptor extends beyond the marginal hooks and lacks the papillae characteristic of the genus *Gyrodactylus*, instead being modified into a marginal valve (Harris and Tinsley, 1987). Attachment is to the highly contractile oral epithelium and the suckers have converged upon those of polystomatid monogeneans, which attach to the extensible epithelium of the amphibian urinary bladder.

The attachment of several gyrodactylid genera has not been fully analysed. The haptors of *Gyrodactyloides* and *Laminiscus* were originally thought to be identical, but are clearly not, even though both are adapted for gill attachment. *Laminiscus* is most similar to *Archigyrodactylus* (Mizelle and Kritsky, 1967) and has an additional plate within the haptor, the function of which remains unknown. It is unfortunate that we understand so little about the diversity of gyrodactylid attachment, as the minutiae of hamulus and bar structure still form the basis for classification of the group. There are many descriptions of haptoral structures which we do not understand functionally; *G. anudarinae*, *G. tibetanus* and *G. luckyi* all have a ventral bar membrane (an essential component of the pressure pad)

modified into two filaments; *G. aksuensis* has hamuli barely larger than the marginal hooks; and many species have hamulus roots folded inwards to the mid-line of the haptor. This has evolved independently at least twice (once in loach gyrodactylids such as *G. pavlovskyi*, *G. jiroveci* and *G. incognitus* and the second time in *G. pleuronecti*, *G. flesi* etc. infecting flatfishes), suggesting that it confers specific advantage to skin parasites.

Hamuli and bars of individual species are invariant. In *G. gasterostei,* the variance of hamular dimensions in natural populations was no greater than that within inbred laboratory lines, with variance being only ~5% of the mean (Harris, 1998). This work, and our unpublished observations on the guppy parasites *G. turnbulli* and *G. bullatarudis*, implies that the hamuli and bars are tightly controlled genetically, although their size may vary according to environmental influences. The most important environmental factor affecting hamulus and marginal hook size is temperature, an increase in which results in smaller hamuli and marginal hooks (Kulemina, 1977; Mo, 1991a, b, c, 1993; Dávidová *et al*., 2005; see also Section 9.1). This is general in all species in which it has been studied. Why then is gyrodactylid morphology so constant within, but consistently different between, species? The evidence, particularly from the *G. salaris* species complex is that morphology can change following host switching and isolation, as populations of *G. salaris* from salmon (and rainbow trout) are morphologically different to specimens from grayling (Lindenstrøm *et al*., 2003a; Shinn *et al*., 2004; Olstad *et al.*, unpublished), despite their suggested recent origin by host switching (see Meinilä *et al*., 2004). These anecdotal observations suggest that the rate of morphological evolution in gyrodactylids changes when a host shift occurs, in addition to being influenced by environmental factors.

The body wall musculature in *G. eucaliae* is typical of other invertebrates with one to three discontinuous layers (Kritsky, 1971); an outer circular, an intermediate longitudinal and an inner diagonal layer. The outer two layers were present throughout the body whereas the inner was usually restricted to the cephalic and peduncular regions of the parasite. Two types of myofilaments with an electron-dense sarcoplasm were non-striated and orientated longitudinally in the fibres (Kritsky, 1971). More recently, El-Naggar *et al.* (2004a) confirmed

the triad of muscle layers and using confocal scanning microscopy were able to show the intricate lattice-like arrangement of muscle fibres throughout the body and haptor of *Macrogyrodactylus clarii*.

2.4. Digestive System

All gyrodactylids are epidermal browsers that occasionally will take dermal cells as well. Goblet mucous cells may be digested with epithelial cells, and as a thin layer of mucus covers the epidermis (Whitear, 1970), their diet is usually cited as epidermal cells and mucus. Gyrodactylids with black guts have invariably been grazing on melanocytes (Cable *et al.*, 1997, 2002b), rather than on blood.

The ultrastructure of the bilobed, blind ended gut of gyrodactylids is well documented, especially the characteristic syncytial gastrodermis (Kritsky *et al.*, 1994; Cable *et al.*, 1996, 1997, 1998; El-Said Arafa, 1998; Olstad *et al.*, 2006). This pattern is common throughout the group except in *Isancistrum*, in which the two caeca fuse behind the testis forming a ring. The gastrodermis lies on a thin basal lamina complex, often close to the surface of the parental uterus enclosing the embryo. Nuclei are sparse, and often associated with mitochondria and Golgi bodies, while much of the cytoplasm is packed with a range of different digestive vesicles (Kritsky *et al.*, 1994; Cable *et al.*, 1999). Microvilli project into the gut lumen or are compressed together in newborn worms or specimens which have recently evacuated their gut contents.

The pharynx, lying just above the union of the two gut caeca, is less well described. It consists of a ring of eight large cells, constricted by muscle blocks (Figure 11). Malmberg (1970) made the distinction between species with long pharyngeal processes (e.g. *G. salaris*; Figure 12) and those with short processes. However, the processes extend anteriorly from the pharyngeal cells, their difference in length being one of degree. The pharynx is protruded through a bell-like valve onto the host epithelium (Figure 12) during feeding, and the processes placed against the host epithelium. This can be seen more easily in species with long processes, but the feeding mechanism appears identical irrespective of process length.

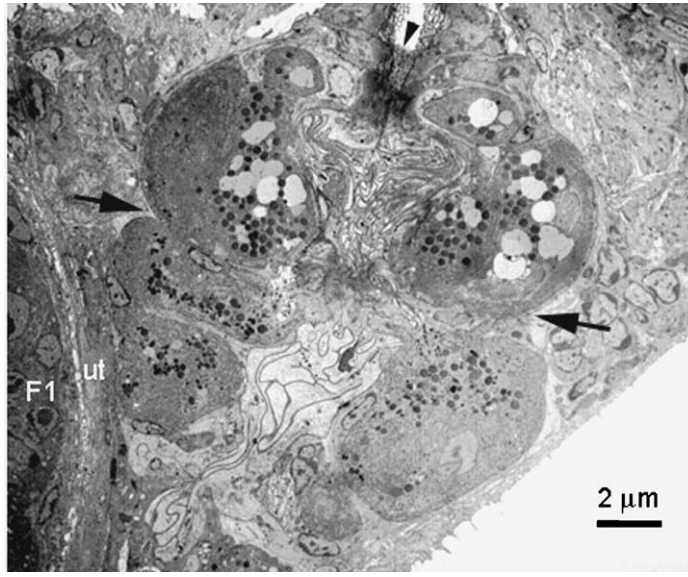


Figure 11 Transmission electron micrograph of the mouth (arrow head) and pharynx of *Gyrodactylus gasterostei*. The pharyngeal bulb constricted by muscle blocks (arrows) lies in close proximity to the parental uterus (ut) and F1 embryo. [Reproduced from Cable *et al*. (2002b) with permission of Cambridge University Press.]

The pharyngeal cells are packed with secretory granules, and, by analogy with *Entobdella soleae* (see Kearn, 1971) and other skin-feeding monogeneans (Halton, 1997), we assume that digestive enzymes are released directly onto the skin of the fish within the chamber sealed off by the pharyngeal valve. This begins digestion, and partly digested epithelial cells are drawn up into the gut for further intra-cellular digestion by the gastrodermis. Proteolytic activity has never been demonstrated in the pharyngeal cells of gyrodactylids, but using SEM potential feeding pits are detectable in the host epidermis (Figure 13). These circular lesions appear to heal rapidly by epidermal growth and regeneration, and probably leave the dermis intact (see Figure 13; Kearn, 1963). Within the intestine, digestion proceeds rapidly. The actively phagocytic intestinal syncytium takes up fragments of host cells. Using previously starved parasites (the only feasible experimental scenario), feeding recommenced within 1 min of starved *G. gasterostei* being returned to a host

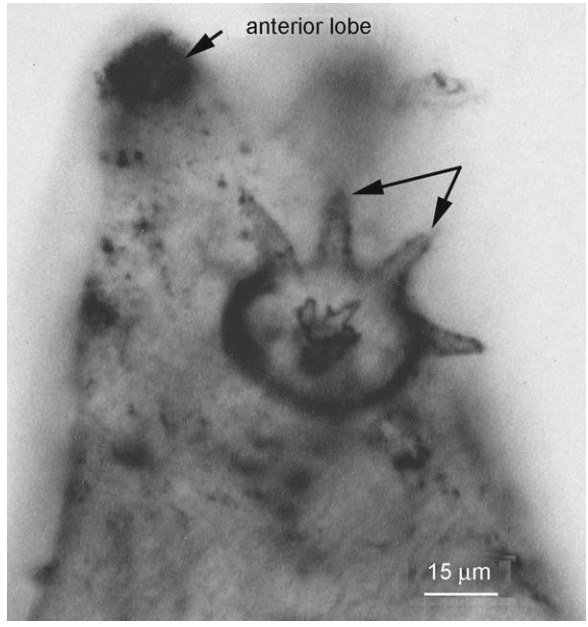


Figure 12 Light micrograph of the extended pharynx with the eight pharyngeal lobes spread out in a *Gyrodactylus salaris* specimen. (K.B. Nilsen, unpublished.)

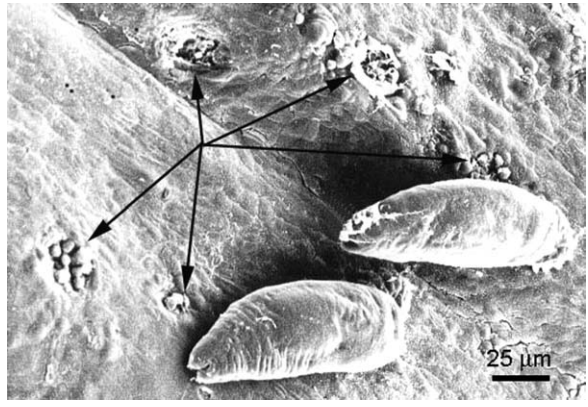


Figure 13 Scanning electron micrograph demonstrating the wounds (see arrows) on the epidermis of an Atlantic salmon parr caused by the feeding activity of *Gyrodactylus salaris*.

(Cable *et al.*, 2002b). The ingested material was highly particulate suggesting that the initial homogenisation of contents during the extra-corporeal phase of digestion is effective at breaking up host cells, although occasionally, almost intact host cells and host melanin are detected in the gut lumen (Figure 14). Within 5 min of first feeding, material was being actively phagocytosed into the intestinal syncytium and a digestive cycle similar to that observed in *Calicotyle kroyeri* (see Halton and Stranock, 1976; Kritsky *et al.*, 1994) was then followed (Cable *et al.*, 2002b). In *G. gasterostei*, feeding occurs once every 15–30 min, a patch of epidermis containing ~30 cells being stripped away on each occasion (Harris, 1982).

Direct physical damage to the host caused by feeding has been most comprehensively documented in *G. salaris* (see Figure 13). Not all ingested material is either absorbed or directly excreted. Observations on the fate of melanin granules in *Macrogyrodactylus polypteri* (see Cable *et al.*, 1997), where these are a common dietary component, and

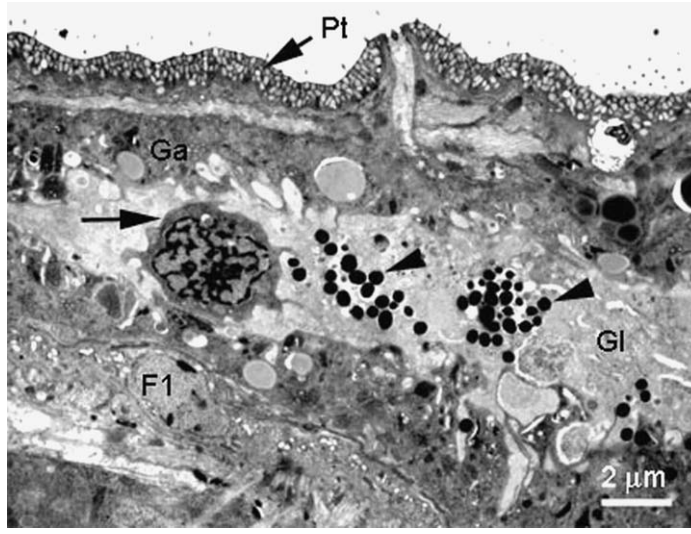


Figure 14 Transmission electron micrograph of the gastrodermis of *Gyrodactylus gasterostei*. The gut lumen (Gl) contains intact host cells (arrow) and melanosomes (arrow heads). The syncytial gastrodermis (Ga) is compressed between the tegumental surface layer of the parent (Pt) and the F1 embryo (F1). [Reproduced from Cable *et al.* (2002b) with permission of Cambridge University Press.]

in *G. gasterostei* (see Cable *et al*., 2002b) where they are not, indicate that the granules become phagocytosed by the gastrodermal syncytium and then remain *in situ* for long periods. The accumulation of host melanosomes in four distinct transverse bands in the intestine of *M. polypteri* is particularly intriguing (Malmberg, 1957; Khalil, 1970; Cable *et al*., 1997). Granules are also phagocytosed elsewhere in the gut, giving old animals an orange colouration between the black bands. Defecation has been observed in *M. polypteri* because melanin granules colours the expelled material.

2.5. Glandular System

Generally, monopisthocotyleans possess groups of bilaterally paired unicellular glands in the cephalic and trunk regions. In gyrodactylids, Wagener (1860) was the first to describe these glands and their distribution. Kathariner (1893) then observed ducts leading to the cephalic lobes and described these as cephalic glands. The cephalic glands release their contents individually onto the surface of the cephalic lobes. The first detailed account of these glands in any monogenean is that of Kritsky (1978, Figures 1–6), who described them in *G. eucaliae*. In this species, they consist of three distinct morphological types: three dorsal bilaterally paired groups producing elongated acidophilic secretions; one paired antero-ventral group producing basophilic secretions; and a group of postero-ventral glands immediately behind the pharynx secreting granular acidophilic secretions. All three glands are merocrine (the cell survives the cycle of secretory activity). The release of the contents of each granule is by union of its limiting membrane with the tegumental unit membrane (Kritsky, 1978). The presence of several types of cephalic glands indicates a multi-purpose role in *G. eucaliae*, but their primary function is considered to be adhesion (Kritsky, 1978; see Section 5.3).

Other components of the glandular system are described in Section 2.2 (tegumental secretions), Section 2.4 (secretions of the pharyngeal glands and the gastrodermis) and Section 2.8.2 (female reproductive system), but there are also unpublished thesis accounts of glands located throughout the body and associated with the haptor

(Kritsky, 1971; El-Said Arafa, 1998). Nothing is known of the chemistry of secretions from tegument and glands in gyrodactylids or in monogeneans in general (Whittington *et al*., 2000a; Whittington and Cribb, 2001).

2.6. Excretory System

The excretory system consists of two longitudinal looped canals draining the two halves of the body and receiving smaller ducts from individual flame cells. The structure of individual flame cells and ducts is identical with that of all other platyhelminths in which they have been studied (Kritsky, 1971; Rohde, 1989; Cable and Harris, 2002) but overall the system is much simpler than in larger flatworms, presumably because of progenesis. Malmberg (1957, 1970) described the excretory system in considerable detail, and used it to subdivide the genus *Gyrodactylus* and establish the phylogeny of the group. The validity of Malmberg's (1970) classification based on excretory systems is discussed in Sections 4.1 and 5.2. A drawback to the use of the excretory system in taxonomy is that it can only be studied in living material, and apart from *Gyrodactylus*, only *Ooegyrodactylus* (see Harris, 1983), *Macrogyrodactylus* (see Malmberg, 1957), *Gyrdicotylus* (Vercammen Grandjean, 1960; Harris and Tinsley, 1987), *Swingleus*, *Polyclithrum* and *Isancistrum* (Malmberg, 1998) have been fully described.

2.7. Nervous System

Despite the wealth of descriptions of platyhelminth nervous systems visualised by immunofluorescence (e.g. Halton and Maule, 2004, and references therein), until recently the only accounts of the gyrodactylid nervous system were an early description of an unidentified species from the three-spined stickleback visualised using the thiocholine method (Lyons, 1969) and immunocytochemical demonstration of two neuroactive substances in *G. salaris* (see Reuter, 1987) revealing the now classical orthogonal pattern of nerve cords (Reuter *et al*., 1998). However, confocal scanning laser microscopy has revealed with superb clarity the spatial arrangement of muscle and associated

cholinergic, peptidergic and aminergic innervations in *Macrogyrodactylus clarii* (see El-Naggar *et al*., 2004a).

Three types of putative sense organ have been described in gyrodactylids. Firstly, there are uniciliate tegumental receptors, consisting of a nerve bulb connected to the tegument by septate desmosomes and a terminal, free 9 + 2 axoneme with no basal body (Lyons, 1969), which in other invertebrates are thought to be mechanoreceptors (Lyons, 1973). In *G. salaris*, three distinct types of uniciliate sensilla occur in the head region of *G. salaris*: a tapering form, a cylindrical form and a form with a terminal bulb (see Bakke *et al*., 2004a). The length of sensilla axonemes is also highly variable reflecting growth or functional differences, and some may be retractable (Bakke *et al*., 2004a). Second, there are compound uniciliate tegumental receptors clustered to form the prominent pair of ''spike sensilla'' on the cephalic lobes of *Gyrodactylus* spp. (Lyons, 1969, 1973; Figures 15 and 16). The phylogenetic distribution of these receptors is unclear, but they are absent from most monogenean groups. Kearn (1993) recorded similar structures from an analogous position in *Enoplocotyle kidakoi* and Lyons (1969) found them in the same position in the oncomiracidium of *Entobdella soleae*, and from the pharynx of adult *Acanthocotyle lobianchi* (see Lyons, 1969). The phylogenetic distribution of these receptors warrants further examination. These

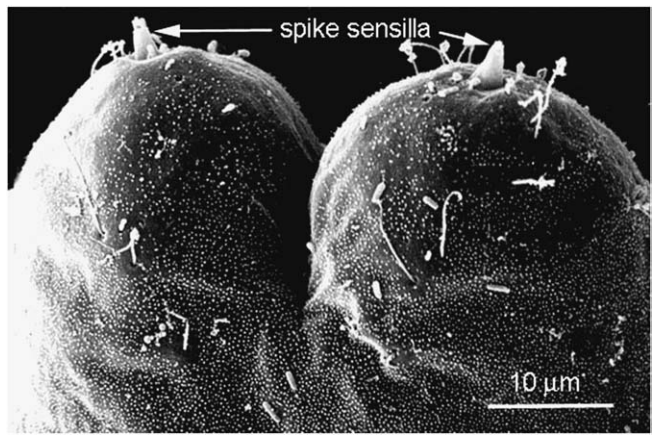


Figure 15 Scanning electron micrograph of the sensory apparatus on the anterior lobes of *Gyrodactylus salaris*, dorsal view.

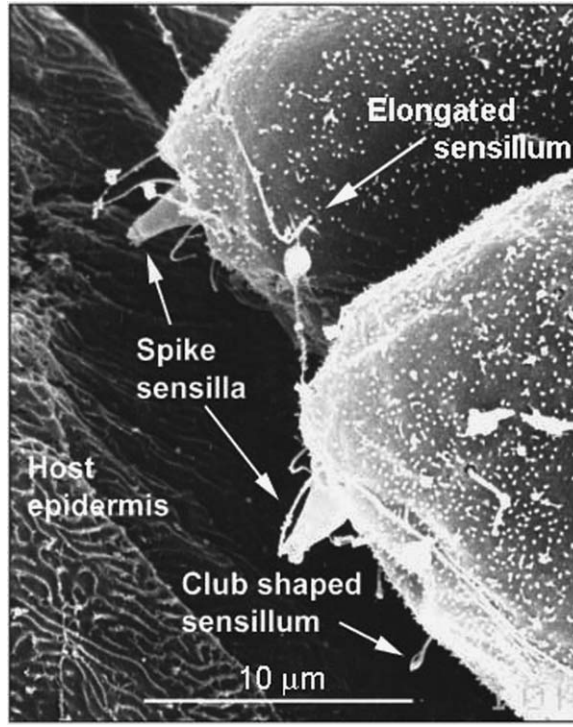


Figure 16 Scanning electron micrograph of the sensory apparatus at the anterior lobes of *Gyrodactylus salaris* actively searching the skin surface of an Atlantic salmon parr, anterior lateral view. (Adapted from Bakke *et al.*, 2004a with permission from *Folia Parasitologica*.)

receptors are thought to be chemosensory and are used extensively when gyrodactylids probe their substrate or surrounding environment prior to movement, feeding or interaction with another gyrodactylid (Figure 16). Third, there are sub-surface ciliary receptors, possibly photoreceptors, described by Watson and Rohde (1994) using TEM from just below the spike sensilla of an unidentified *Gyrodactylus* sp. from swordtails. Each receptor consisted of a single dendrite ending in an extra-cellular cavity containing a small number of modified cilia. Unlike most monogeneans, no pigmented eye spots have been described from *Gyrodactylus* species. In living *Ooegyrodactylus farlowellae*, a pale green, lenticular structure lies in the midline anterior to the pharynx, which persists throughout life although

it is most obvious in larvae (Harris, 1983). In fixed, stained specimens, this structure lies in the same position as the cerebral organ of *Macrogyrodactylus polypteri* (see Malmberg, 1957). Harris (1983) suggested that this may be a photoreceptor, but this has also never been investigated further.

Surface sensilla (uniciliate and compound uniciliate) are symmetrically distributed around the median longitudinal axis and can be mapped using silver nitrate that stains cell junctions. Shinn *et al.* (1997, 1998a, b) and El-Naggar *et al.* (2001) used this method to distinguish *Gyrodactylus* and *Macrogyrodactylus* species, but single specimens cannot be identified reliably. Staining is particularly unreliable in the haptoral region due to the presence of other argentophilic structures and host mucus (Shinn *et al.*, 1997; Bakke *et al.*, 2004a), and the sensilla pattern on the body can sometimes be obscured by a large *in utero* embryo (Shinn *et al.*, 1997). Bakke *et al.* (2004a) questioned the temporal stability of the sensilla pattern at specific locations, and the pattern may change with age. Bakke *et al.* (2004a, b) concluded that mapping sensilla was useful in investigating variation in gyrodactylids, but was not diagnostic. Chaetotaxy may prove to be a reliable indicator of higher taxon relationships (Shinn *et al.*, 1998a; El-Naggar *et al.*, 2001) based on the branching structure of the nerve systems in relation to sensilla pattern, but perhaps more interesting would be to assess whether there is any relationship between the abundance and distribution of sensilla and gyrodactylid life history strategies, such as mode of transmission and behaviour on the host.

2.8. Reproductive System

The reproductive system of viviparous gyrodactylids is highly simplified because of progenesis (see Kritsky, 1971 for ultrastructural details; and Section 2.1). Reproductive structures and modes were reviewed by Kearn (1996) and more recently by Cable and Harris (2002), but here we briefly consider the differences in reproductive morphology between genera, focussing on the viviparous forms unless otherwise specified.

2.8.1. Male Reproductive System

The gyrodactylid male reproductive system consists of a single sac-like post-ovarian testis with flat epithelial cells resting on a thin basal lamina enclosing a central mass of loosely packed germinal cells, from which spermatozoa pass forward to a penis, localised just behind the pharynx. In viviparous genera, the male system only becomes functional after the parasite has given birth once. In *G. gasterostei*, sperms begin to mature just after the first birth and then accumulate in the anterior seminal vesicle, located just behind the penis (Harris, 1985; Cable *et al.*, 2001) (Figure 17, which is plate 3.17 in the separate Colour Plate Section). Malmberg (1970) and Kritsky (1971) depicted a *vas deferens* connecting the testis to the penis (following either the left or right gut caeca, respectively) but we have never identified this in whole mount preparations or sections, although the accumulation of sperms in the seminal vesicle is very clear. We suspect that in some species sperms migrate anteriorly via tissue layers (Harris *et al.*, 1997; Cable *et al.*, 1998, 2001). The structure of the male reproductive system varies in different species and appears more complex in *Macrogyrodactylus polypteri* (see Malmberg, 1957). This genus is least affected by progenesis and therefore additional development of the male system may take place.

Considerable diversity exists in the structure of the male intromittent organ, which forms an important character for generic diagnosis. Previous nomenclature, including Kritsky (1971), refers to the intromittent organ as a cirrus, a structure that is inverted when not in use. Strictly speaking a penis is not inverted when stored, and may be protruded or extended during copulation. The confusion arises from the fact that the spherical intromittent organ of *Gyrodactylus* is similar in structure to that of rhabdocoels (Baer and Joyeux, 1961), which was known to be everted during use and is therefore a cirrus. Observations on copulation in *Gyrodactylus* show that the structure does not evert (Braun, 1966; Cable and Harris, unpublished); it is thrust out of the body and used the hook into the tegument of the partner. The structure is therefore technically a penis. Amongst the egg-laying gyrodactylids, *Ooegyrodactylus* also has a penis, in this case tubular, and Harris (1983) captured images of this structure

extended. We have therefore previously favoured use of the term "penis" for the intromittent organ of gyrodactylids and have considered that an evolutionary trend from tubular to spherical has taken place. The ball-like penis of *Gyrodactylus* comprises a muscular bulb enclosing both the prostatic reservoir and a short ejaculatory duct (Kritsky, 1971), and is armed with a large hook and small spines. This situation has recently become much more complex. Amongst the viviparous genera, published descriptions suggest that some African *Gyrodactylus* species have tubular penes (Paperna, 1979) and we have seen similar structures in undescribed species of *Gyrdicotylus* (Harris, unpublished). *Mormyrogyrodactylus* has a tubular structure characterised as a cirrus (Luus-Powell *et al*., 2003), but this organ may be extended from the body, functioning partly as a penis. The recent description of several new egg-laying gyrodactylids by Kritsky *et al*. (2007) has also demonstrated the diversity of the male intromittent organ. Although *Ooegyrodactylus farlowellae* appears to have a conventional penis (Harris, 1983), the intromittent organ of *Phanerothecium caballeroi* is coiled and covered entirely in a layer of hardened tissue, making it difficult to imagine how it could function as either an eversible cirrus or an extensible penis. In some genera, such as *Hyperopletes*, the intromittent organ is lined with fine spines, suggesting that it everts as a cirrus, much like *Mormyrogyrodactylus* (Luus-Powell *et al*., 2003). Amongst the other genera of egg-laying gyrodactylids, diversity of the intromittent organ approaches the bizarre. *Nothogyrodactylus*, *Aglaiogyrodactylus* and *Onychogyrodactylus* all have a system of accessory sclerites around the penis, the function of which is entirely unknown. Oviparous genera may also have two seminal vesicles of various shapes (e.g. Kritsky *et al*., 2007), in contrast to the single seminal vesicle of viviparous forms. In *Onychogyrodactylus sudis*, copulation was observed (Kritsky *et al*., 2007) similar to that of *O. farlowellae* (see Harris, 1983). The accessory sclerites did not appear to be used for hypodermic impregnation in this case, or to be inserted into the partner's female tract.

The spermatozoa of monopisthocotyleans show considerable morphological variation (Justine *et al*., 1985; Justine, 2001). There is a relatively large database on monogenean spermiogenesis and sperm

ultrastructure covering more than 60 species; however, few attempts have been made to study gyrodactylid spermatozoa (Justine, 1993). The mature 30 μm-long filiform spermatozoa of *G. eucaliae* conform to the typical flatworm structure, being spindle-shaped, of a nearly uniform diameter (0.7 μm) except at its gradually tapering ends (Kritsky, 1971; see also Malmberg and Lilliemarck, 1993). The nucleus extends from within its lobe towards the centre of the spermatozoa, the two axonemes are bilateral on either side of the nucleus and the mitochondrial lobe opposes that of the nucleus (Kritsky, 1971). The sperms are differentiated from those of other flatworms by the lack of free flagellae and/or peripheral or marginal microtubules beneath the plasmalemma (Figure 17); they most closely resemble those of turbellarians and digeneans. More recently, Schmahl and Elwasila (1992) described spermatogenesis of *Macrogyrodactylus polypteri* as similar to that of other monopisthocotyleans, but clearly different from that of polyopisthocotyleans.

2.8.2. Female Reproductive System

The female reproductive system is also apparently simple, belying complexity at a subcellular level. The egg-laying gyrodactylids have a female system similar to that of other oviparous monogeneans, although they possess characters linking them with *Enoplocotyle* and the acanthocotylids: the ovary is in the mid-body and develops after the testis. The testis of *Ooegyrodactylus* becomes less apparent and recrudesces when the female system matures (Harris, 1983; Kritsky and Boeger, 1991). In young *O. farlowellae*, with a mature male system, the female system consists of a small germarium, immediately posterior to a large seminal receptacle. This opens to the exterior via the common female duct, which functions as a vagina in young worms. The male and female systems of *O. farlowellae* open side by side in the mid-region of the body, and insemination occurs when the penis of one individual is inserted into the common female duct of a partner (Harris, 1983). As the male system starts to recrudesce, the female germarium grows substantially. Four rows of post-germarial vitelline follicles appear, and the seminal receptacle and posterior part of the common female duct begins to function as an oötype. The

oötype region is surrounded by large, diffuse glandular tissue, a synapomorphy with *Enoplocotyle* (see Boeger *et al*., 1994). Eggs develop one at the time and are retained in the common female duct before being laid, when they are glued to the substrate via the adhesive droplet (Harris, 1983; Kritsky *et al*., 2007).

In the viviparous forms, the female system is modified, but the basic pattern of oviparous forms can still be discerned. As a result of progenesis, the full developmental expression of the female system is never attained; the ''vitellaria'' never fully developed and never produce egg-shell precursor proteins. The vitelline cells appear reduced to patches of glandular syncytia within the posterior part of the body (Cable *et al*., 1996), which have no connection with the other parts of the female system, but our understanding of the function and physiology of these tissues is rudimentary, and to complicate matters further, some of these ''vitelline'' cells (at least in *Macrogyrodactylus*) may actually be subtegumental cells (El-Naggar and Cable, in press). The most distinctive feature of the female system of the viviparous genera is the uterus, which develops as an expansion of the common female duct, anterior to the large seminal receptacle. The germarium is reduced to a patch of tissue on the posterior wall of the seminal receptacle. The main function of the germarium/seminal receptacle appears to be egg cell maturation, and for this reason it is termed the Egg Cell Forming Region (ECFR) (see Jones *et al*., 1994, 1997).

In *Macrogyrodactylus*, there is much more complexity to the posterior female system, much of which develops after the parasite has given birth for the first time. Patches of tissue in longitudinal rows have the same configuration as the vitellaria of *Ooegyrodactylus*. However, different cell groups have a different microscopic appearance (Malmberg, 1957) suggesting a different function. A further complication in *Macrogyrodactylus* is the appearance of a putative secondary seminal receptacle that lies to one side of the primary receptacle/ECFR. This structure was noted by Malmberg (1957) and has been confirmed by further light microscope studies on *M. clarii* by El-Abbassay (2001). This structure also appears to be present in the other large-bodied genera *Polyclithrum* (Ernst *et al*., 2000, 2001a) and *Swingleus* (Billeter *et al*., 2000).

The diffuse glandular tissue which characterises the oötype region of the egg laying gyrodactylids and *Enoplocotyle* is not seen in viviparous forms. However, there are groups of cells at the junction of the ECFR and the uterus, which may be homologous with this glandular tissue. These cells are not well developed in *Gyrodactylus*, but are noticeable in, for example, *Gyrdicotylus* (see Harris and Tinsley, 1987).

A consequence of progenesis, and a remarkable feature of the gyrodactylid reproductive system, is a general lack of ducts. There appear to be no permanent ducts connecting the separate parts of the female or male reproductive systems. As noted above, at least in some species, self-sperm seem to migrate to the seminal vesicle through body tissues (Cable *et al.*, 1998). Presumably, all sperm acquired from a partner during hypodermal impregnation that are not injected directly into the ECFR, must migrate through tissues. Similarly, different parts of the female system are not directly connected. When a daughter is born, it appears to break through the body wall (Jones *et al.*, 1998; see Figures 3C and 19A in Cable *et al.*, 1996 and Cable and Harris, 2002, respectively, for images of birth pore/plug), which then heals over, leaving no permanent female opening. Similarly, there is no permanent connection between the ECFR and the uterus. After a daughter is born, the next oöcyte enters the uterus via the so-called cap cells at the junction of uterus and ECFR (Cable and Harris, 2002). As noted above, there are also no ducts linking the structures identified as vitelline cell homologues. Any secretory product from these cells must disperse in some other way (Cable *et al.*, 1996).

2.8.3. Embryology

The early research on gyrodactylid germ cell lineages is remarkably accurate. Using mid-19th century light microscopy, Wagener (1860) in particular achieved results which were not surpassed until the advent of electron microscopy some 100 years later. The high quality of early osmium-fixed sectioned material prepared by Kathariner (1893, 1904) and Gille (1914) was demonstrated when Cable and Harris (2002) published comparable images prepared using Feulgen-stained chromosome spreads. The improved technology does not give

substantially better insight into gyrodactylid reproduction, and Gille (1914) was able to show both mitotic and meiotic chromosomes within the gyrodactylid oöcyte.

Kathariner (1904) undertook the work which led to *Gyrodactylus* being considered a classic example of polyembryony. The myth of *Gyrodactylus* development grew from this observation: following cleavage of the zygote, one of the cleavage products became quiescent, while the other forming the F1 generation grew around it. Eventually, the quiescent cell became activated to form the F2, when one cell produced by the first division of the F2 became quiescent, eventually re-activating to form the F3 (see Cable and Harris, 2002). *Gyrodactylus* thus appears a neat example of segregation of the germ cell lineage, which is then transferred intact to the next generation. Braun (1966) revisited this system and more or less confirmed Kathariner's (1904) results (although he claimed to observe meiosis within the quiescent cell) and demonstrated for the first time that reproduction (at least of the first-born daughter) can continue for up to 30 generations without the need for a sexual partner. This has subsequently been repeated with *G. gasterostei* (see Harris, 1998). Our own observations, however, suggest a re-interpretation of Kathariner (1904) and Braun (1966). In particular, we note the difficulty of tracking the F2 generation embryo back to a single cell, and note that the large, pale staining cells of the embryo (which Kathariner and Braun interpreted as quiescent) actually contain up to 4*n* copies of DNA, suggesting they are about to divide (Cable and Harris, 2002). It appears that the F1 generation embryo within the embryo cluster develops when the uterine wall of its parent appears, separating off cells at the centre of the embryo to become the next generation.

This mechanism remains poorly understood, partly because of the small size of gyrodactylids, the fact that two different developmental routes exist within the life cycle (the first daughter develops at the centre of an embryonic mass, whereas all subsequent daughters develop from an oöcyte), and because it is unique with no parallels in the Animal Kingdom. However, several interesting features have emerged over the past 15 years (reviewed in Cable and Harris, 2002). Perhaps, the most interesting concerns the role of "blastomere anarchy" in gyrodactylid development. In most invertebrates,

cleavage is a precise process resulting in an embryo capable of developing into an adult organism. This precision is usually based upon the intra-cellular architecture of the oöcyte, with its specific localised mRNAs allowing structural differentiation. However, in neoophoran platyhelminths (in which yolk and oöcyte functions reside in separate cells), the pattern of cleavage is a property of the precise embryonic architecture and vitelline cells provide "scaffolding" within which the blastomeres can differentiate accurately. Bresslau (1909), coined the term "blastomeren anarchie" to describe this apparent totipotency of the blastomeres in rhabdocoels – only the position of blastomeres, determined by the scaffolding vitelline cells, could limit their developmental pathway. In gyrodactylids, this same phenomenon seems to apply, but vitelline cells are not available to form scaffolding. Instead, the early embryo can be highly plastic, with blastomeres separating and rejoining in different configurations. Only after the embryo has achieved a certain complexity, does re-organisation and re-shaping cease, and development proceed along a fixed pathway. These cellular re-organisations have not been observed during development of the first-born offspring, which occurs within a cell mass where cell movements are restricted. One consequence of blastomere anarchy is that it has proved impossible to identify a particular blastomere as the progenitor of the first-born daughter.

In the absence of scaffolding vitelline cells, the function of organising the embryo appears to have been taken over by the syncytial uterine lining. Cable *et al.* (1996) describe this layer in detail, finding it to be metabolically very active, with a great deal of protein secretion, and, judged by fluorescence microscopy, abundant RNA transcription. At the anterior and posterior poles of the uterus are cell bodies, termed cap cells by Cable *et al.* (1996). The role of these cell bodies and the associated syncytium is unclear, but is almost certainly related to nutrition of the embryo and to co-ordination of its early development (Cable and Harris, 2002).

There are surprisingly few studies on gyrodactylid internal structure from across the spectrum of species, and most studies have concentrated on easily obtainable forms from guppies, goldfish, salmonids and sticklebacks. The diversity of gyrodactylid internal structure has been underestimated, although several studies suggest that it might be

considerable. In particular, the glandular tissue posterior to the ECFR and the extent of the fully formed testis, seem variable (Ernst *et al.*, 2001a, b) as does the construction of the pharynx and penis spines. This diversity has never been fully investigated or placed within a phylogenetic context. There is also diversity in reproductive strategy, an extreme example being *G. gemini*, in which a pair of daughters develops *in utero* (Ferraz *et al.*, 1994). No details are available as to the sequence of development in *G. gemini,* and it is not known whether these offspring are twins (i.e. derived from a single oöcyte) or sisters (originating from separate oöcytes). The intra-uterine generation (i.e. the first born daughters) presumably develop asexually as in conventional *Gyrodactylus* species (see Cable and Harris, 2002), but again whether this is synchronous or asynchronous development is unknown (Ferraz *et al.*, 1994). We have been unable as yet to obtain new material of this parasite, but the factors controlling embryogenesis would be particularly interesting to examine. The original description of *G. trairae*, another South American species, also shows subtle differences in the orientation of the embryo cluster (Boeger and Popazoglo, 1996), which suggest a difference in embryology.

3. ETHOLOGY

3.1. General Behaviour

Almost all behavioural observations have been made on viviparous gyrodactylids. Harris (1983) maintained *Ooegyrodactylus farlowellae* in culture for a short period, and additional behavioural observations have been provided by Kritsky *et al.* (2007). The viviparous gyrodactylid monogeneans exhibit a limited behavioural repertoire and with the morphological uniformity of *Gyrodactylus* spp., researchers might be excused the belief that all species are very similar. In fact, the limited range of behaviours is combined in ways that adapt gyrodactylids to the wide range of hosts and habitats exploited. As the most diverse vertebrate group, fishes may be shoaling or territorial, lotic or lentic, pelagic or benthic, marine, brackish or freshwater (Bakke *et al.*, 1992a). Some hosts are semelparous (e.g.

Oncorhynchus or squid), others are anadromous (e.g. Atlantic salmon) or catadromous (e.g. European eel), while many acquire resistance, forcing gyrodactylids to transfer to other hosts (see Sections 8.1 and 8.2). Wherever investigated, there is growing evidence that the different behaviours are combined into repertoires that maximise transmission even where host ecology is extreme. The range of basic behaviours exhibited by gyrodactylids includes:

3.1.1. Locomotion

When moving on the host, the anterior glands cement the head temporarily to the substrate while the opisthaptor is released and drawn up to the head. The head is then released. This may be repeated for sustained periods, or just one or two steps may be taken before settling down to a single position again.

3.1.2. Swimming

Recently, El-Naggar *et al.* (2004b) reported swimming in *G. rysavyi*. This skin and gill parasite exhibits coordinated, unidirectional wriggling movements when detached from its host, the Nile catfish (*Clarias gariepinus*). This questions the doctrine that gyrodactylids have no specific transmission stage, but so far this behaviour has not been observed in other gyrodactylids, most of which sink in an outstretched position if released in the water column. Specimens of *G. turnbulli* and *G. salaris* if forcibly detached may thrash back and forth until reaching a solid substrate, but such behaviour is not unidirectional. The specific transmission behaviour of *G. turnbulli* (in which detached parasites migrate into the water film, see Section 3.3; Cable *et al.*, 2002a) is not a "swimming behaviour" (cf. Huyse *et al.*, 2003).

3.1.3. Questing

All gyrodactylids spend much of their time questing, in which the body is extended away from the substrate, with the cephalic lobes frequently stretching and spreading out. At intervals, the parasite bends to allow

the cephalic lobes to touch the surrounding substrate. Parasites may attach to another substrate or host, or may interact directly with other parasites leading sometimes to copulation. When questing is undertaken immediately before feeding, brushing of the host's epidermis with the cephalic lobes may involve mechanical or chemical assessment of the host skin. In all cases, questing behaviour seems to allow the spike sensilla and uniciliate receptors of the cephalic lobes to sample the environment, host or another parasite (see Figure 16).

Questing may be spontaneous, but it also increases substantially in response to external stimuli, such as touching the body with a fibre. Questing behaviour has been quantified in *Gyrdicotylus gallieni* (see Harris and Tinsley, 1987), which responds to vibration with increased activity and to water currents with violent thrashing movements. The response to vibration has also been noted anecdotally in other species. Responses to light or shadowing have not been tested. Generally, gyrodactylids alternate between phases of movement with stationary periods and periods of questing.

3.1.4. Feeding

Our understanding of the mechanics of feeding is discussed in Section 2.4. In *G. gasterostei*, *G. turnbulli*, *G. salaris* and *M. polypteri*, individual parasites lie in a characteristic pose with the anterior portion of the body extended and flattened against the host epidermis while firmly attached by the opisthaptor, often with the anterior lobes raised (Harris, 1982; Cable *et al.*, 2002b, unpublished). The pharynx is protruded and brought into close contact with the host's epithelium. Pumping of the pharynx can be observed, with slight waves of contraction passing along the body. Feeding lasts a few minutes, or even seconds, after which the worm straightens up but is contracted and less active for a few minutes.

3.1.5. Copulation

This has been described in detail for viviparous genera (Malmberg, 1957; Braun, 1966) and regularly observed during routine experimental

infections (Harris, 1989; Cable *et al.*, unpublished). Parasites quest with conspecifics until they impale their penis into the chosen partner. Once copulation is initiated, the partner may react by grasping the initiator with its own penis and mutual insemination may follow (Harris, 1989). Normally, viviparous gyrodactylids copulate with their bodies aligned or entwined for a few seconds or minutes, until insemination into the seminal receptacle, or other region of the body, has occurred via hypodermic impregnation. The parasites then part. Unilateral copulations, in which one partner does not reciprocally inseminate, are also common, at least in *G. turnbulli* (Cable *et al.*, unpublished). In oviparous gyrodactylids, the copulatory organ of one copulant is inserted directly into the uterine pore of its partner (Harris, 1983; Kritsky *et al.*, 2007). Considering the diversity of male copulatory organs (Kritsky *et al.*, 2007), it is possible that egg laying gyrodactylids also display a range of associated behaviours.

3.1.6. Birth and Oviposition

Only the viviparous gyrodactylids give birth to fully grown young. The best description of birth in *Gyrodactylus* is Braun (1966). It was also described in *Macrogyrodactylus polypteri* (see Khalil, 1970), but in this case the worms were in suboptimal conditions and the births may have been pathological. In healthy worms attached to a fish, birth occurs rapidly. The daughter breaks out through a ventral birth pore close to the pharynx. A parasite about to give birth can be identified by its gravid appearance and the slow waves of muscular contraction passing along the parent's body. The first part of the daughter to emerge, as a bleb of tissue, is the central region of the folded body (see Figures 1 and 3), which is quickly followed by the anterior portion of the worm (see Figure 18). The head of the daughter attaches using sticky secretions from the cephalic glands and pulls the rest of the body out of the mother onto the skin of the host, where the haptor attaches. The mother then shrinks down and is quiescent for a period before activity is resumed, when she normally moves away from the immediate vicinity of the daughter, often towards the anterior of the host (Cable *et al.*, 2000). If for some

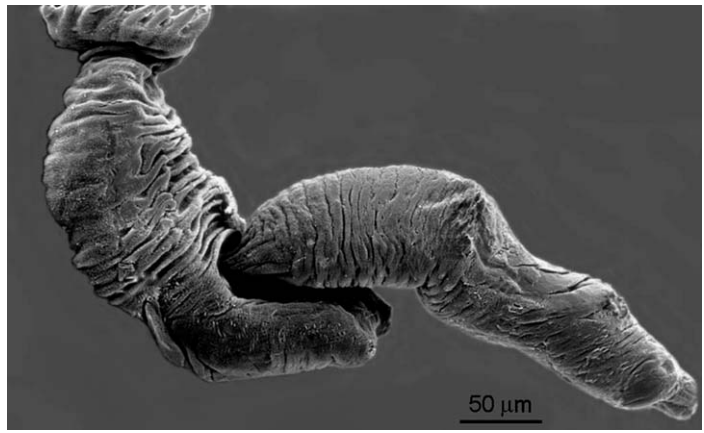


Figure 18 *Gyrodactylus salaris* individual giving birth to a pregnant daughter as large as itself. Such a daughter potentially could give birth itself 1–2 days later depending on temperature. (T.A. Mo, unpublished.)

reason, the mother becomes detached during the early stages of birth and the daughter has no host substrate onto which it can attach its anterior glands, both worms usually die as the daughter is unable to escape from its mother's uterus. If, in these instances, the daughter is manually pulled free using watchmaker's forceps and a fine pin, the mother can survive. Thus, muscular activities of both parent and offspring are essential for successful birth. If parasites are detached for prolonged periods, abortion of young embryos is common (Cable *et al*., 2002b).

Oviparous gyrodactylids lay eggs within the environment, where they adhere by the sticky droplet either to the surrounding substrate (*Ooegyrodactylus farlowellae*; see Harris, 1983) or to the surface of the fish itself (Kritsky *et al*., 2007). Several egg-laying forms retain eggs *in utero* (Kritsky *et al*., 2007) in a manner reminiscent of *Acanthocotyle greeni* (see Llewellyn, 1984).

3.1.7. Transmission

Little is known about transmission of oviparous gyrodactylids. All stages of the life cycle can transmit (at least in *Ooegyrodactylus*; see Harris, 1983), and eggs are placed on (Kritsky *et al*., 2007) or near

(Harris, 1983) the sedentary host, leading to the build up of infrapopulations. The relative importance of individuals of different ages in transmission is unknown although Harris (1983) found both very young larvae and adults able to move between hosts. In viviparous gyrodactylids, the egg stage is entirely absent, and development is curtailed by progenesis at the "young male" stage of the egg-laying gyrodactylids. Part of the selective pressure for progenesis in the viviparous genera may therefore be to maintain maximum transmission potential in the adult parasite. Continuous transmission and the infection of new hosts throughout the life cycle enhances colonisation of new host resources (Boeger *et al.*, 2005) and favours host shifts. Viviparous gyrodactylids are renowned for transferring quickly from host to host, and some can move between fish during the most fleeting of contacts (Bychowsky, 1961). However, if given the opportunity to transfer under optimal conditions, many make no attempt to leave their current host, probably as most parasites only transfer when necessary (see Section 3.2). As noted by Scott (1985a), transmission is risky and results in high mortality. However, transmission may also influence population performance by reducing the death rate caused by host responses (Boeger *et al.*, 2005). Not surprisingly, gyrodactylids show considerable diversity of strategies when detached from the host and we are becoming increasing aware of a range of different transmission strategies related to host ecology.

Following prolonged detachment or host death, individuals of *G. salaris* may hold their extended body (two or three times its length) at right angles to the substrate gently swaying back and forth. They rarely probe the surrounding substrate but can transfer immediately if a suitable host comes into contact. In *G. salaris* attached to dead hosts, two distinctive modes are recognised: (i) stationary transmission mode (STM), in which parasites are motionless with their slightly extended bodies held at right angles to the host tissue; and (ii) search mode (SM), in which the parasites' body is highly extended (at least 4 × its normal length) and makes constant circular movements and contractions around the motionless haptor (Olstad *et al.*, 2006). Thus, individuals of *G. salaris* in the laboratory remain on a dead host (Olstad *et al.*, 2006), possibly similar to *G. pungitii* (see Malmberg, 1970) but in marked contrast to *G. rarus* and

G. cryptarum (see Malmberg, 1970), *G. gasterostei* (see Harris, 1982) or *G. turnbulli* (see Cable *et al*., 2002a), which all leave the host shortly after its death. *G. gasterostei* and *G. pungitii*, infecting the three- (*Gasterosteus aculeatus*) and nine-spined sticklebacks (*Pungitus pungitus*), respectively, thus employ quite different transmission strategies although their hosts occupy the same macro- and microhabitats (e.g. Copp and Kovac, 2003). Future studies should examine the behaviour of these parasites on sympatric hosts and under the same macroenvironmental conditions.

3.2. Site Specificity and Migrations on the Host

Some gyrodactylids show pronounced site specificity, but this is highly variable between species. Most infect the skin and fins, but some also occur on the gills (*G. masu*, see Ogawa, 1986; *G. arcuatus*, see Harris, 1993). Amongst gill specialists, there may be further subdivision. Some (e.g. *G. aeglefini* and *G. unipons*) infect only the gill filaments, others may occur on gill filaments and on gill arches and in the pharynx (*G. branchicus*), some utilise the pharynx but also occur on filaments and arches (*G. cernuae* and *G. perlucidus*), while *G. cryptarum* inhabits the preopercular sensory canals of the head (Malmberg, 1970). *Isancistrum subulata* occurs mainly on the tentacles and suckers of the squid, *Alloteuthis subulata,* and rarely enters the mantle cavity, in contrast to *I. loliginis* which infects the gills (see Llewellyn, 1984). Such segregation of different species on the same host is common and may occur even within skin parasites. The two common gyrodactylids of guppies (*Poecilia reticulata*) display marked site preferences, with *G. bullatarudis* occurring rostrally, especially on the head and mouth at low densities (Harris and Lyles, 1992), while *G. turnbulli* occupies caudal regions (Harris, 1988). In mixed infections on salmonids, *G. colemanensis* almost exclusively attaches to the margin of fins and *G. salmonis* the head, body and the broad surfaces of the fins (Cone and Cusack, 1988).

Initial colonisation appears random, followed by migration to a specific site. In *G. colemanensis* on rainbow trout fry, parasites attach anywhere on the body surface but then relocate posteriorly, with

most migrating to the caudal fin followed by the pectoral and pelvic fins. The length of the fin margin and fin activity appeared to influence this distribution (Cone and Cusack, 1989). Under experimental conditions, *G. turnbulli* most commonly infects guppies via fins (Harris, 1988), but individual parasites then migrate to the peduncle and caudal fin where dense aggregated populations develop. After a few further days a return migration to the fins occurs, possibly to facilitate transmission (Harris, 1988). At low intensities, the dorsal fin of salmon parr was the principal site for attachment for *G. salaris* (see Jensen and Johnsen, 1992; Koski and Malmberg, 1995), followed by pectoral and anal fins. This pattern is also seen in the field. *G. salaris* on salmon parr from Batnfjordselva (Mo, 1992) occurred mainly on the fins (34.4% on the dorsal, 27% on the pectorals and 24.7% on the other fins). The remainder occurred on the body (7.8%), head (3.5%) and gills (2.6%). The parasite population only increased in size significantly on the caudal fin and body, suggesting these are the most suitable areas for parasite feeding. In a later study of Batnfjordselva, Appleby and Mo (1997) found 15% of *G. salaris* on the gills, predominantly on the filaments. In some years, up to 40% of the parasites were found on the gills (up to 300 per fish). In a few fish with small infections, all parasites were found on the gills. The proportion infecting the gills appeared higher in summer than winter and was also greater on older (2+) fish (Appleby and Mo, 1997). The reason for this difference between these two investigations in the same river system is not explained, even though both the intensity and age of infection were similar (see Harris, 1988, 1993). However, pragmatically, these data illustrate the importance of examining the gills for *G. salaris,* which has generally been assumed to be a skin parasite when monitoring epidemics in Norway (Appleby and Mo, 1997) and elsewhere in the EU.

Parasite distribution may also vary with infection intensity. At intensities of less than 100 parasites, more *G. salaris* were located on the dorsal fin, followed by the pectoral and anal fins, but at intensities of greater than 100 parasites more *G. salaris* were located on the caudal fin, and when exceeding 1000 parasites the body of the fish is also heavily infected (Jensen and Johnsen, 1992). Mo (1992, 1997) speculated that the preferred dorsal, pectoral and pelvic fins may be

related to transmission, which is primarily via the substrate by detached parasites while the fish rest, and not via host-to-host contact (Bakke *et al*., 1991a, b; Jensen and Johnsen, 1992). The preference for the dorsal fin might be due to physical contacts between fish as they nip each other while defending the territory against intruders (Mo, 1992). Appleby (1996a) noted increased abundance of *G. callariatis* on the skin in very heavy infections, which he interpreted as a migration of parasites out of the gill chamber for transmission. Conversely, in small infections, most worms were found on fins, suggesting they had recently arrived on the cod.

Individual gyrodactylids tend to move short distances on the host to feed (see Section 3.1), but at high parasite densities, intraspecific competition and resource depletion may induce a switch in site preference on a particular host. *Isancistrum subulatae,* which occurs on the arms and tentacles and suckers of the squid *Alloteuthis subulata*, is found on the surface of the head and the eyes only in heavy infections (>1000), and only in extremely high parasite loads does it enter the mantle cavity (Llewellyn, 1984). Crowding of growing infrapopulations of *G. salaris* on salmon may also result in dispersal of the parasite over wider areas of the host's skin and fins (Appleby and Mo, 1997).

Site specificity may be influenced directly or indirectly by external factors. Salinity influences site selection by *G. callariatis.* In salt water it parasitises the host's body, whereas in brackish water it is found mainly on the gill arches (Malmberg, 1970; Appleby, 1996a). This is also observed in *G. arcuatus*, although in this case, interaction with other gyrodactylid species may be as important as the direct impact of salinity. This species can infect gill arches, filaments, pharynx or skin and fins. In UK freshwater, it is normally restricted to the gills, with *G. gasterostei* present on the skin surface. In UK marine environments, *G. arcuatus* is often found on the skin surface, with *G. branchicus* in the gills (Harris, 1993). An increase in the proportion of *Gyrodactylus* individuals on the gills of cyprinids (goldfish and golden shiners) with increasing temperatures has also been observed (Anthony, 1969; Kirby, 1981). However, in these studies, individuals were not accurately identified, and the most likely explanation of this observation is that different species were present on the gills and skin that responded differently to temperature.

Morphological and physiological host parameters may also influence site specificity. Buchmann and Uldal (1997) infected four different salmonids (rainbow trout, brown trout, and Baltic and Atlantic strains of salmon) and found differences in site selection on each. Similarly, the site preference of the Danish Gx morph of *G. salaris* differed between rainbow trout and salmon (Lindenstrøm *et al.*, 2003a). Microhabitat preferences of *G. salaris* in relation to susceptibility status of different salmonid strains has also been tested, with the highest proportion of parasites found on the fins and head of all fish species and strains (Heinecke, 2005). However, over time they found an increasing percentage of parasites on the caudal fin of susceptible East Atlantic salmon strains and a converse tendency of decreasing proportion on the tail of the responding Swedish Lule salmon. The factors triggering migration of gyrodactylids are unknown but are probably related to resource availability, infrapopulation density, parasite age, reproductive status, avoidance of the host's immune response, and finally inbreeding or hybridisation avoidance.

The age of individual gyrodactylids may be important in migrations. Young *G. gasterostei* placed on the head or pectoral fins of three-spined sticklebacks almost inevitably migrate towards the caudal fin (Harris, unpublished). Activity is closely linked to reproductive status and increases in post-first and post-second birth mothers that contain small embryos. This was observed both from living attached worms (Harris, 1982) and deduced from the age structure of detached parasites in sediment (Harris, 1993). Older *G. gasterostei* (post-second birth) develop a swollen, empty uterus which is filled with a clear fluid (Harris, 1985, 1997; Cable and Harris, 2002), and wander extensively upon the host, being found at widely different points on different days. It is not clear whether this is a change in behaviour as parasites become more active, or whether these older individuals readily become dislodged (see Lester, 1972) but in confined experimental situations can reattach again to different parts of the host. In *G. salaris,* most newborn parasites move only short distances but those which do migrate (defined as movement from one region to another) most commonly re-locate immediately after each birth. The majority migrated anteriorly when placed on the caudal fin

and did not return to posterior sites; the migration and distance from the original site increased with parasite age (Cable *et al*., 2000). Worms migrating from the tail were normally found on the peduncle (42%) or the anal or adipose fin. Only post-second birth or older worms migrated as far as the pectoral or more rarely the pelvic or dorsal fins. In *G. salaris*, the level of activity of individual worms after birth of the first daughter varies according to the particular stock of salmon that is infected (Cable *et al*., 2000), illustrating the complex interplay between factors influencing migration on the host. Movement of gill parasites is particularly interesting as it must be undertaken for transmission to occur. In UK lowland populations of *G. arcuatus*, where the parasite is normally found in the stickleback gill chamber, migration of older specimens onto the skin was noted during epidemic population growth (Harris, 1993). This suggests a movement of parasites from the gill chamber to achieve transmission. For obligate gill parasites (e.g. *G. rarus*), such migrations are a prerequisite for transmission and must take place at some point in the life cycle. *Isancistrum loliginis* from the squid *A. subulata* (see Llewellyn, 1984) must leave the gills to achieve transmission, but has only rarely been found among *I. subulatae* on the arms and tentacles. If transfer occurs during squid copulation both species may transfer to the arms but later migrate to their respective site-specific sites. The only gyrodactylids in which migrations have been directly observed are *Gyrdicotylus* spp., from the African clawed toad *Xenopus* spp. Entry to the mouth is normally via the nostril, and parasites experimentally placed on the skin orientate towards and enter the nostril, emerging into the mouth shortly after (Harris and Tinsley, 1987). The reverse migration, out of the mouth cavity, has not been observed directly, although specimens of *Gyrdicotylus* do appear in the water in old infections, suggesting migration away from the toad (Harris and Tinsley, 1987; Jackson and Tinsley, 1994).

Much migration behaviour may relate to immunological changes in the host epidermis. *G. derjavini* on rainbow trout normally prefers the caudal, pectoral, pelvic and anal fins, but as infections progress the proportion of worms on the pectoral fins declines (Lindenstrøm and Buchmann, 1998). In corticosteroid-treated immunosuppressed fish, this trend is reversed (Lindenstrøm and Buchmann, 1998).

Olafsdóttir *et al.* (2003) noted aggregation of *G. derjavini* on the cornea (said to be an immunologically privileged site), with up to 30% localising on this relatively small area. This aggregation is reduced if hosts are immunosuppressed with dexamethasone (Olafsdóttir *et al.*, 2003). Interestingly, Cable (unpublished) have noted similar behaviour by *G. bullatarudis*, but not by *G. turnbulli* which occurs on the same poeciliid host. Similarly, Pie *et al.* (2006) have suggested that migrations by *G. anisopharynx* on the surface of *Corydoras* sp. are not adaptive in terms of transmission, but may be concerned with evading either the immune response or intra-specific competition. It has been suggested that mucous cell densities, which differ over the fish surface (Pickering, 1974), may influence behaviour and survival of gyrodactylids on rainbow trout, as they may select mucus cell-rich areas during initial colonisation but escape these areas during the host response phase (Buchmann and Bresciani, 1998). These results strongly suggest that mucous cells play a decisive role in gyrodactylid site selection. On abnormal hosts, distribution may change. Mo (1997) found *G. salaris* mainly on the gills and mouth of the strongly resistant brown trout. However, mucous cell density decreases when exponentially growing *G. salaris* populations are present, suggesting that more complex factors may influence site selection (Sterud *et al.*, 1998). Matejusová *et al.* (2006) support this, suggesting very local expression of particular genes in relation to damage by parasites. *G. vimbi* on roach aggregates around the anus, but this species apparently migrates to the gills during the host response, returning to the skin when the acquired immune response has declined (unpublished). In *Macrogyrodactylus polypteri*, parasites may aggregate around inflamed areas of epidermis (Khalil, 1970), while surrounding skin is unexploited. These aggregations move from day to day and periodically disperse, but always appear to be associated with inflamed host tissue (Harris, unpublished). Appleby and Mo (1997) explained the frequent occurrence and relative high intensity of *G. salaris* on salmon gills as a possible evasion of the immune response. Generally, changes in the gyrodactylid site selection have been interpreted as being an escape from localised immune reactions (Richards and Chubb, 1996; Buchmann and Bresciani, 1997; Buchmann and Uldal, 1997; Buchmann, 1998a, b).

There is a temptation to regard site selection by gyrodactylids as niche restriction or specialisation to reduce competition with other species. Site selection can also enhance reproductive isolation, by ensuring that conspecifics find, and mate with, only their peers. This was originally suggested to account for the restriction of egg-laying polyopisthocotylean monogeneans to particular gill arches (Llewellyn, 1956; Rohde, 1979). There is little consensus about site selection and restriction by parasites (e.g. Friggens and Brown, 2005), and it is worth remembering that we know very little of the factors controlling gyrodactylid distributions. There is little consistent evidence that gyrodactylids can exhaust nutritional resources, as, for example, salmon can tolerate infections of several thousand *G. salaris* without immediate death (Mo, 1992), while guppies may support less than 20 *G. turnbulli* before dying (Madhavi and Anderson, 1985; Cable *et al.*, unpublished). Different gyrodactylids all exploit the same carbon source when infecting a fish; over-exploitation leading to host death by one species will kill individuals of another gyrodactylid species on the same host, even if in a different microhabitat. It could be argued that microhabitat segregation allows one species to avoid a host response induced by another gyrodactylid. However, Richards and Chubb (1996) found that the host response affected both *G. turnbulli* and *G. bullatarudis* in similar ways, despite their differences in microhabitat. There is also no evidence that gyrodactylids of minnows, for example, which support more than 15 gyrodactylid species show any greater niche specialisation than do those of fishes, such as tench, which support only two gyrodactylid species. Arguments from reproductive biology are also not compelling; in general, gyrodactylids do not show the extent of microhabitat specialisation of dactylogyrids or ancyrocephalids, and they can also reproduce asexually (Section 2.8). Aggregation for the purposes of sexual reproduction is therefore unlikely.

Adaptive variation of the attachment organs is clearly important in the evolution of site specificity, as skin parasites generally have different haptoral morphology to gill parasites, and host shifts occur more readily than site shifts. Nevertheless, there are members of skin-parasitic groups which have become specialist gill parasites (e.g. *G. tincae*), although in this case there is no pressure to avoid competition with other gyrodactylids. The shift in the distribution of *G. arcuatus* relative to other

species present may on the other hand be due to competitive interactions. However, there are many more distributions which cannot be explained in this way and there is much to learn about gyrodactylid site specificity before we can speculate on issues of niche restriction.

3.3. Migration Between Hosts: Lack of a Dispersive Phase

In most parasites, specific transmission stages or larval adaptations for dispersal have evolved. In contrast, gyrodactylids have no specific transmission stage as the newborn individual attaches itself to the same host as its mother and host transfer can occur at any life stage. Transmission must be linked either to the development of a hostile microenvironment due to host immunity or death, to occasional chance contacts with other hosts but related to the daily and seasonal host behaviour and ecology, to eventual density-dependent mechanisms within parasite infrapopulations themselves, or to accidental dislodgement.

Gyrodactylids may survive varying periods away from the host. Old accounts broadly refer to survival of 48 h (Guberlet *et al.*, 1927; Bychowsky, 1961; Tripathi, 1957), but this depends on temperature, species and physiological condition. At 15°C, Lester and Adams (1974a) observed a mean life expectancy of 1.8 days in *G. alexanderi* detached from *Gasterosteus aculeatus*. In *Gyrodactylus gasterostei* at 10°C, mortality was constant for the first 60 h of detachment, after which it increased substantially until the end of life, suggesting worms could survive until the exhaustion of energy reserves (Cable *et al.*, 2002b). However, embryos of parasites long detached from a host tend to be aborted or show developmental abnormalities, suggesting that energy reserves are withdrawn from the embryo to sustain the parent (Cable *et al.*, 2002b). In *G. salaris*, survival is improved by remaining attached to a dead host. At 18°C, maximum survival of worms attached to glass was only 27 h. On dead salmon, however, worms survived much longer, up to 72 h (Olstad *et al.*, 2006). Parasites were particularly active on dead hosts, moving short distances on the fins during the 24 h after host death. A high proportion of *G. salaris*

remain with their dead hosts, even after decay had begun (cf. *G. turnbulli*; Cable *et al*., 2002a). After 72 h, they could re-infect naïve hosts much more successfully than worms attached to glass. Clearly, *G. salaris* is specialised to remain with the host after death, achieving survival advantage from doing so (Olstad *et al*., 2006), although it can also drift in the water column, attaching to salmon both high in the water column or close to the bottom substrate (e.g. in rivers with water flow 0.25 m/s; Bakke *et al*., 1992a; Soleng *et al*., 1999a).

Detached *Gyrdicotylus gallieni, Gyrodactylus turnbulli* and *G. bullatarudis* survive for similar periods (Harris and Tinsley, 1987; Cable, unpublished) at tropical temperatures (25°C), and *Macrogyrodactylus polypteri* can survive for up to 5 days away from the host (Khalil, 1970; Harris, unpublished) at 25–30°C. Under these circumstances, energy conservation is paramount, and although the swimming behaviour of *G. rysavyi* (see El-Naggar *et al*., 2004b) may be a high-cost transmission strategy, most gyrodactylids adopt a "sit and wait" strategy, with limited movement until a host approaches. A substantial proportion of the total *M. polypteri* population can be detached at any one time (Harris, unpublished), and movement between the fish and the substrate appears a normal part of this host–parasite interaction. This is also true of *G. salaris,* which may survive for prolonged periods off the hosts, for example, attached to the walls (plastic) of fish tanks or net (metal) of grid boxes, and later infect newly introduced fishes (Bakke *et al*., 1991a, 2002, unpublished; Olstad *et al*., 2006). A high proportion of the *G. salaris* suprapopulation may, at any moment, be off the salmon due to transmission hazards in lotic habitats and the death of heavily infected hosts. Normally, however, the proportion of the population detached in other *Gyrodactylus* species is probably small. Harris (1982, 1988) used dishes on the tank floor to quantify detachment in *G. gasterostei* from sticklebacks and *G. turnbulli* from guppies. In both cases, although substantial numbers of parasites were recovered, they still represented a relatively small proportion compared to the number still attached to the hosts. Specific behaviours facilitate transmission in *G. turnbulli* (see Cable *et al*., 2002a). This species crawls into the meniscus of the water column to hang from the surface film, increasing opportunities for transmission when the surface-feeding host guppy skims the surface film for floating food items.

Transmission from living hosts is parasite age and stage dependent. In both *G. turnbulli* on guppies (Harris, 1989) and *G. salaris* on salmon (Harris *et al.*, 1994), the detached parasite population recovered from the water column contains an excess of older (post-first) birth worms. In the case of *G. salaris* (Harris *et al.*, 1994), this could be refined to show excess detachment of individuals 1–2 days after they had given birth for the first, second or third time. This coincides with an increase in activity seen in individuals of this age in *G. gasterostei* (see Harris, 1980, 1982) and presumably results in increased transmission of these age/stage classes. This phenomenon has also been deduced from observations of transmission of *G. sphinx* on the Black Sea Blenny (*Aidablennius sphynx*) where transmission is linked to development of the male reproductive system (Dmitrieva, 2003).

Vertical transfer of gyrodactylids may also occur. *G. salaris* cannot feed on salmon eggs, but they can attach and survive for up to 2 days (Mo, 1987; Bakke *et al.*, 1998) making eggs potentially important in transnational dispersal by Man. However, *G. salaris* display a strong preference for newborn alevins as oppose to salmon eggs (Mo, 1987). Transmission may take place during adult–fry interactions, either during birth (*G. turnbulli* on guppies; Cable, unpublished) or brooding (*G. gasterostei* on three-spined sticklebacks; Cable, unpublished). Gyrodactylids are particularly common on fish with highly developed brood care, and the possibility of transfer from parent to offspring at birth (poeciliids) or within the nest (sticklebacks, gobies, blennies) is likely to be increased compared to fishes that show little parental investment or live in age-structured shoals. However, the introduction and spread of *G. salaris* and *G. vimbi* within the new salmon and roach generation, respectively, in spring occurs early and rapidly (Jansen and Bakke, 1993a; Appleby and Mo, 1997; unpublished). Harris (1982) demonstrated rapid acquisition of *G. gasterostei* and other ectoparasites by young stickleback fry, which presumably took place from male fish to fry in the nest. Transmission from these individuals to other stickleback fry then took place rapidly in the large shoals of young fish in river margins.

Transmission strategies must also take into account the population structure of the host population. This is very clear in *G. salaris*, a

parasite of wild salmon parr (Johnsen, 1978; Johnsen and Jensen, 1988, 1992, 1997, 2003; Jansen and Bakke, 1993a; Appleby and Mo, 1997). The length of the freshwater parr phase varies significantly, from ~9 months in southern Britain and Spain to 5 years in Northern Norway (Nicieza *et al.*, 1994; Bakke and Harris, 1998). Although parasite dispersal to new salmon populations probably occurs via adult ascending and spawning salmon and parr (Soleng *et al.*, 1998), or between precocious male parr, transmission between parr generations is also important in sustaining a *G. salaris* infection in a river. Clearly, there is greater potential for intra-parr transfer in the North of Norway than there is in southern Europe. The pathogenic potential of *G. salaris* is bound to be related to salmon population structure, and the parasite is unlikely to be highly pathogenic in rivers at the southern limit of the range. Here, there is a distinct break between parr generations, salmon are entirely absent from the river for a short period (Bakke and Harris, 1998) and *G. salaris* cannot survive in salt water (see Section 9.2).

These specific transmission behaviours are clearly an important part of gyrodactylid evolutionary biology and worthy of further investigation, especially given the ecological diversity of gyrodactylid hosts. Insufficient parasite species have been examined, but trends towards particular transmission strategies may occur, for example, in species infecting solitary compared to shoaling fishes, lotic vs. lentic hosts, or pelagic vs. benthic hosts. Finally, understanding the processes that facilitate transmission may be important in determining speciation mechanisms, as they are important in host switching.

Part 2. Gyrodactylid systematics, phylogeny and evolution

4. PHYLOGENY: THE FAMILY ALBUM

4.1. Taxonomic History

Attempts to subdivide the viviparous gyrodactylids (see below), have until recently been based entirely on morphology, primarily that of the attachment hooks and bars. This has run parallel with discussions

over the species concept in *Gyrodactylus*. Although most authors tend to split species, a few have taken a wider view. Wagener (1860), for example, grouped all similar gyrodactylids from cyprinids into a single species, which was never formally described. Sproston (1946) similarly recognised only "*G. elegans*" or "*G. medius*", discriminating on hamulus characters now known to be a fixation artefact. Although this approach has long been discredited, it explains many spurious records [e.g. *G. elegans indicus* (see Tripathi, 1957) and *G. medius* from *Ciliata mustela* (see Srivastrava and James, 1967)].

Malmberg (1970) was the first to systematise relationships within *Gyrodactylus* and the Gyrodactylidae based on morphology. His scheme was based on the excretory system, supplemented with observations on marginal hook type, and assumed that the excretory system had evolved from greater to lesser complexity by the loss of flame cells and secondary canals (Malmberg, 1957, 1964, 1969, 1970). Six subgenera (*Gyrodactylus*, *Mesonephrotus*, *Paranephrotus*, *Metanephrotus*, *Neonephrotus* and *Limnonephrotus*) were formally described (Malmberg, 1970) using these characters and host group/habitat. Malmberg (1970, 1993) also established species groups within each subgenus based on marginal hook morphology. The gyrodactylid subgenera were linked to the larger host phylogenetic categories within Greenwood *et al.*'s (1966) teleost classification. For example, species of the subgenus *Gyrodactylus* (according to Malmberg, the most primitive subgenus), infect ostariophysans, placed by Greenwood *et al.* (1966) at the base of the teleost stem. Within this subgenus, two species groups, the *G. elegans* and *G. phoxini* groups were recognised. The *G. elegans* group are gill parasites with a thin, spine-like ventral bar membrane, while *G. phoxini*-type species are skin parasites with a broad, spoon-like ventral bar membrane.

Malmberg's (1970) contribution in establishing the foundation for subsequent phylogenetic and taxonomic studies of this diverse group cannot be over emphasised. However, his scheme suffers from an assumption of host–parasite co-evolution. Originally based on a now outdated fish phylogeny (Greenwood *et al.*, 1966), Malmberg (1998) updated his work using Nelson's (1994) fish classification, but problems remain. The most significant weaknesses are the lack of an independent character system to establish the direction of excretory

system evolution, and the assumption that *Macrogyrodactylus polypteri* has co-evolved with the chondrostean *Polypterus* and therefore the evolution of viviparous gyrodactylids predated the appearance of the teleosts. In fact, most *Macrogyrodactylus* species infect catfishes (Prudhoe, 1957; Paperna, 1979; El-Naggar and Serag, 1987), suggesting the association with chondrosteans is due to a host switch. Bakke *et al.* (2002) could find little convincing evidence for any co-evolutionary relationships between gyrodactylids and their fish hosts, and certainly not before the evolution of the Ostariophysi or Clupeomorphs. Molecular work has shown the importance of host switching which has occurred even between families (Zięta*ra et al.*, 2002) as well as orders (Huyse *et al.*, 2003) of fishes in gyrodactylid evolution (see Section 6.1) and at the species level attempts to identify co-evolutionary trends have failed. Nevertheless, there is some support for groupings based on morphological, ecological and biogeographical trends, and although Malmberg's (1970) species groups have no formal taxonomic status, they have been important for handling the complexity of this genus.

Malmberg's subgeneric analysis was restricted to Scandinavian species and has never been fully adopted by other researchers, probably because the excretory system can only be studied in living worms. Only Gläser (1974) extended the excretory system analysis to describe new species groups within *Gyrodactylus*, again, for North European species and only from gobies. A great number of North American and Eastern Eurasian species may belong to existing species groups, but without redescription or molecular analysis their relationships cannot be confirmed. The danger of basing species groups on morphology and host group only is shown clearly by the *G. salaris* group, created by Malmberg (1993) to accommodate *G. wageneri*-like forms from salmonids. This notion of a separate radiation on salmonids is the simplest co-evolutionary hypothesis and accounts for those few *G. wageneri*-like forms that are found on salmonids in North America (Cone *et al.*, 1983). However, molecular phylogenies fail to support a separate *G. salaris* group (Cable *et al.*, 1999) and infer strongly that salmonids have instead been infected on several occasions by *G. wageneri*-like gyrodactylids (Matejusová *et al.*, 2003; see Section 5.3.2).

4.2. The Family Gyrodactylidae

The Gyrodactylidae (Beneden and Hesse, 1864), as originally constituted included only the viviparous genus *Gyrodactylus*. As explained by Bychowsky (1961), Beneden and Hesse (1864) referred to the ''Gyrodactylides'' rather than the Gyrodactylidae, and it was left to later authors (principally Cobbold, 1864) to implement the formal family name. This explains the confusion in the literature over the authority for the family ''Gyrodactylidae''. We agree with Bychowsky (1961) that the origin of the grouping in the modern sense lies with Beneden and Hesse (1864) and these authors should therefore be credited with establishing the family. A series of subfamilies have been erected piecemeal to accommodate genera based on haptor morphology. The Isancistrinae was erected by Furhmann (1928) for *Isancistrum*, lacking all bars and hamuli. Rogers (1967) erected the Polyclithrinae, for *Polyclithrum*, with numerous supporting bars in the haptor. All other genera have been assigned to the catch-all subfamily Gyrodactylinae. We deprecate the use of these subfamilies, which are based on morphology only and may reflect convergence rather than true relationship. They have received little support in either molecular (e.g. Matejusová *et al*., 2003) or morphological (e.g. Malmberg, 1998) phylogenies. However, we do recognise the division between the viviparous genera and the oviparous forms, which are so profoundly different biologically, and agree with Boeger *et al*. (1994) that viviparity evolved once within one group of the Gyrodactylidae. The family therefore includes both egg-layers and live-bearers, and the family Ooegyrodactylidae of Harris (1983) should be allowed to lapse.

4.3. The Genera

Based on morphological criteria, 30 genera have now been described (7 oviparous and 23 viviparous; see examples of genera in Figures 19 and 20). Several viviparous genera are however invalid. *Paragyrodactylus* Szidat is invalid as the name was preoccupied by *Paragyrodactylus* Gvosdev and Martechov. The genus was renamed as

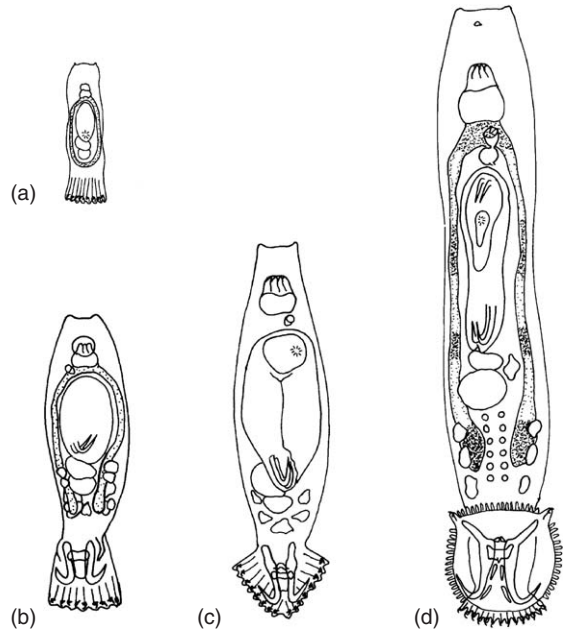


Figure 19 Relative body size and morphological complexity amongst the viviparous gyrodactylids. (A) *Isancistrum*, from squid (body length ~0.1 mm). Redrawn from Llewellyn (1984) and original observations; (B) *Acanthoplacatus*, from tropical marine fish (body length ~0.3 mm). Redrawn from Ernst *et al.* (2001a); (C) *Gyrodactylus* (body length ~0.6 mm, although much smaller and larger species are also known in this genus, 0.1–1 mm). Original; (D) *Macrogyrodactylus* from African fish (body length 1.0–1.5 mm). Redrawn from Malmberg (1956) and El-Naggar and Serag (1987). All drawn to scale.

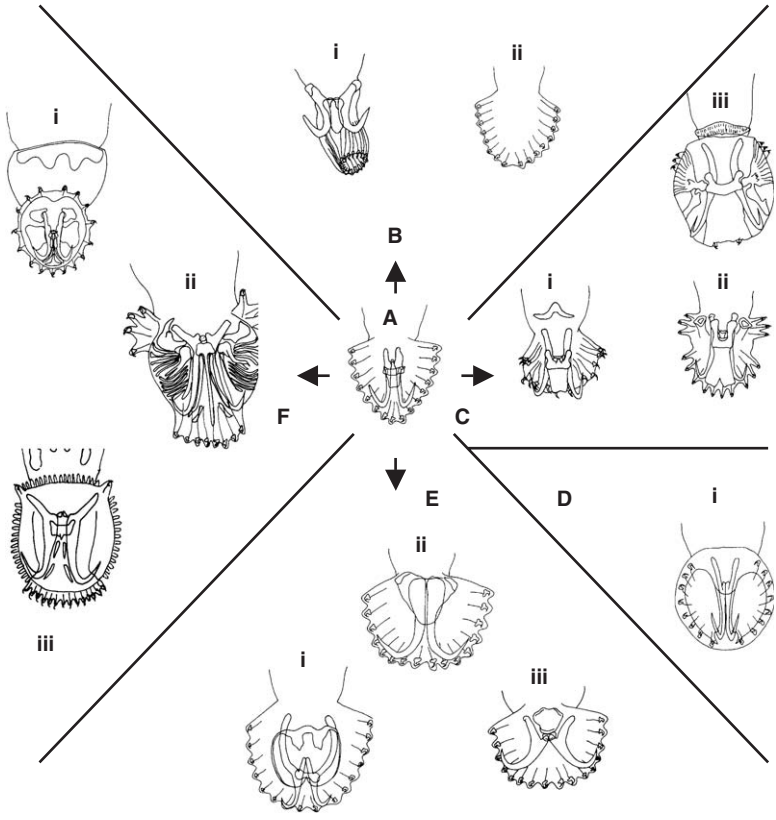
Paragyrodactyloides (Szidat), but the species concerned is now regarded as a *Gyrodactylus* (Popazoglo and Boeger, 2000), making the generic name a junior synonym. *Neogyrodactylus* Prudhoe is similarly a junior synonym of *Macrogyrodactylus. Neogyrodactylus* Baugh, 1957 was pre-occupied and therefore should not be used. Yamaguti introduced *Metagyrodactylus* (Baugh) as a replacement. *Micropolyclithrum* is considered a junior synonym of *Polyclithrum* (Ernst *et al.*, 2000). We also recommend that *Fundulotrema* Kritsky and Thatcher and *Metagyrodactylus* (Baugh) Yamaguti be considered as junior synonyms of *Gyrodactylus* (see below). *Afrogyrodactylus*

was synonymised with *Gyrodactylus* by Paperna (1979). The taxonomic status of *Swingleus* Rogers remains uncertain.

The following genera make up the family Gyrodactylidae Beneden and Hesse, 1864:

4.3.1. Oviparous Genera

The oviparous *Phanerothecium* was originally described as viviparous (Kritsky and Thatcher, 1977), but its true significance was recognised by Harris (1983), who erected the Ooegyrodactylidae to accommodate this genus and *Ooegyrodactylus*. The Ooegyrodactylidae is paraphyletic (Boeger *et al.*, 1994) and so the oviparous forms are considered here as part of the Gyrodactylidae. The egg-laying genera



possess a unique combination of traits (16 articulated marginal hooks, single pair of hamuli with connective dorsal bar, ''spike'' sensilla) characteristic of the gyrodactylids. They are all protandrous and lay eggs with a sticky droplet that hatch to release unciliated crawling larvae with prominent spike sensilla. Seven genera have been described, including three new genera proposed by Kritsky *et al.* (2007), based on differences in the structure of the male reproductive system and the vitelline system [although Kritsky *et al.* (2007) comment that the taxonomic significance of the vitelline follicles and ducts should be re-evaluated].

These parasites infect South American catfish, predominantly loricariids. Some infect pimellodellids and Kritsky *et al.* (2007) also recorded undescribed forms from trichomycterid catfish. These often live within the gill chamber of loricariids and the parasites may be

Figure 20 Haptor diversity in the viviparous gyrodactylids. (A) Basic haptoral type with two hamuli, linking dorsal bar, ventral bar and 16 marginal hooks. Exemplified by *Gyrodactylus*. (B) Successive diminution of the haptor, in (i) *Acanthoplacatus* (hamuli and bars fail to migrate) and (ii) *Isancistrum* and *Anacanthocotyle* (attach to fish and cephalopod skin), hamuli and bars lost. (C) The ''*Swingleus*'' group (attach to fish skin). Hamular and ventral bar morphology suggests these genera are related. (i) *Fundulotrema*, peduncular bar and grouping of marginal hooks (three pairs anteriorly). This genus differs from species of *Gyrodactylus* infecting *Fundulus* only in the peduncular bar (ii). *Swingleus*, as *Fundulotrema*, but grouping of marginal hooks is more prominent, accessory plates present. (iii) *Accessorius*, as *Swingleus* but peduncular bar absent, accessory plates absent and two sucker-like sclerotised structures present. (D) Modification of haptor into discrete suckers in genera that attach to amphibian buccal epithelium. (i) *Gyrdicotylus*. (E) Marine forms (attach to fish gills), hamuli with two roots, accessory plate present in haptor, function unknown. (i) *Gyrodactyloides*; (ii) *Archigyrodactylus*; (iii) *Lamniscus*. (F) Large parasites (attach to fish skin), entire haptor modified into flattened sucker. (i) *Mormyrogyrodactylus*. Peduncle with peduncular bar present and functioning in attachment; (ii) *Polyclithrum*. Marginal hooks grouped anteriorly and posteriorly, accessory bars present; (iii) *Macrogyrodactylus*. Marginal hooks grouped anteriorly and posteriorly, different arrangement of accessory bars present. Note that these groupings are not exhaustive (*Swingleus* could equally be placed within group F), and that phylogenetic relationship is not implied by similarity of haptoral mechanism.

utilising the trichomycterid as a phoretic host. The loricarid catfish were probably one of the most recent groups of bony fishes to differentiate, making them unlikely primary hosts for such a large and diverse group as the gyrodactylids. Boeger *et al.* (2003) use the occurrence of egg-laying genera on loricariids to suggest that gyrodactylids diversified from a South American origin some 60 million years ago. We prefer to suggest that the presence of oviparous gyrodactylids on catfish is the result of an ancient host switch.

(1) *Aglaiogyrodactylus* Kritsky, Vianna and Boeger, 2006: Species of this genus, recovered from loricarid (*Kronichthys*) catfish in Brazil, possess a complex accessory piece and a male copulatory organ enclosed within the copulatory sac.

(2) *Hyperopletes* Boeger, Kritsky and Belmont-Jégu, 1994: Parasites of loricariid catfishes in Brazil, *Hyperopletes* is distinguished from the other egg-laying gyrodactylids by a copulatory organ armed with fine spines and by the arrangement of seminal vesicles; otherwise, it is very similar to the other oviparous forms.

(3) *Ooegyrodactylus* Harris, 1983: Collected from the loricariid catfish (*Farlowella*) from Brazil/Peru, this genus can be distinguished by an entirely muscular penis with small basal bulb. The growth from small crawling larva through male phase to the large egg-laying females was described by Harris (1983) and it is the only oviparous genus so far to have been maintained in laboratory culture. Kritsky *et al.* (2007) consider this genus most similar to *Phanerothecium*, separated by characters including short egg-filament, separate seminal receptacle, absence of pregerminal vitelline follicles and inverted U-shaped vitelline ducts. The original name (*Oögyrodactylus*) was modified to *Ooegyrodactylus* in accordance with the International Code on Zoological Nomenclature.

(4) *Phanerothecium* Kritsky and Thatcher, 1977: *Phanerothecium* differs from *Ooegyrodactylus* in having the intromittent organ supported by a thin external layer of hardened protein. Some species of *Phanerothecium* retain egg clusters *in utero*, have a long egg filament, the seminal receptacle is absent or intra-germarial and pre-germarial vitelline follicles are

present. Species have been described from the loricariid catfish *Plecostomus* (see Kritsky and Boeger, 1991) and *Hypostomus* (see Boeger *et al.*, 1994), suggesting that this genus also primarily infects loricariid catfish, although the type species was described from the pimellodellid *Zungaro zungaro*.

(5) *Phanerothecioides* Kritsky, Vianna and Boeger, 2006: Species from this genus infect *Hypostomus* catfish in Brazil. The parasites lack haptoral bars, there are no pre-germinal vitelline follicles and the vitelline ducts take the form of an inverted U-shape. Species have a conspicuous testis, a syncytial prostatic gland and a reduced copulatory sac.

(6) *Nothogyrodactylus* Kritsky and Boeger, 1991: Described from the loricarid catfish *Ancistrus* from Brazil, this genus was originally distinguished by the presence of accessory sclerites on the penis; these sclerites have subsequently also been found in *Onychogyrodactylus* and *Aglaiogyrodactylus*.

(7) *Onychogyrodactylus* Kritsky, Vianna and Boeger, 2006: This genus is similar to *Nothogyrodactylus* with species of both infecting *Ancistrus* spp. Species are characterised by the spine-like accessory sclerites lying within a separate pouch to the copulatory sac.

4.3.2. Viviparous Genera

The viviparous genera are remarkably uniform, with little variation in internal anatomy. Differences tend to be either in the presence of accessory sclerites on the haptor (*Polyclithrum*, *Swingleus*, *Fundulotrema* and *Macrogyrodactylus*) or loss of hamuli and bars (*Anonchohaptor* and *Isancistrum*), or in penis structure (*Gyrdicotylus*, *Mormyrogyrodactylus* and *Scleroductus*). Molecular evidence (Cable *et al.*, 1999; Ziętara *et al.*, 2002) indicates fundamental differences even within the genus *Gyrodactylus*, which are not reflected in morphology.

(8) *Afrogyrodactylus* Paperna, 1968: Erected for *A. characinis* from *Alestes*, this genus has a tubular, spinous penis and hamuli

with a developed dorsal root. Synonymised with *Gyrodactylus* in Paperna (1979), this species would repay further examination as the penis structure suggests that it may represent a different genus, more akin, for example, to *Mormyrogyrodactylus* described by Luus-Powell *et al.* (2003).

(9) *Accessorius* Jara, An and Cone, 1991: This viviparous taxon from the South American characin, *Lebiasina maculata*, has two tubular sucker-like structures within the haptor. These appear to be reinforced, rather than simply muscular, and their function remains unclear. Jara *et al.* (1991) grouped *Accessorius* with *Polyclithrum* and *Swingleus* in the Polyclithrinae, but there is probably not a close relationship between the latter genera and there is certainly no reason to link *Accessorius* to them. The ventral bar morphology resembles that of *Gyrodactylus costaricensis* and *G. poeciliae*, but the detailed relationships of *Accessorius* remain unclear.

(10) *Acanthoplacatus* Ernst, Jones and Whittington, 2001: Recorded from the skin of *Siganus* spp. from tropical reefs, this genus is characterised by hamuli that remain embryonic and fail to migrate back down the peduncle into the haptor. Surprisingly, this genus is recorded as lacking spike sensilla (Ernst *et al.*, 2001a).

(11) *Anacanthocotyle* Kritsky and Fritts, 1977: A genus which lacks hamuli and bars: the haptor is armed only with 16 marginal hooks. Erected for *Anonchohaptor anonchohaptor* from a South American fish, this genus is poorly characterised and there seems little justification for it. Apart from host group, this genus cannot be distinguished from *Isancistrum*, and it appears to be a *Gyrodactylus*-type in which the hamuli and bars have been secondarily lost.

(12) *Archigyrodactylus* Mizelle and Kritsky, 1967: Recorded from the Pacific tomcod *Microgadus*, this genus has an elaborated ventral bar membrane, with wings spreading back around the haptor, probably to reinforce the suctorial disc. Insufficient is known about this genus to comment on its validity; however, the resemblance with *Laminiscus*, from gadids in the Atlantic, is striking.

(13) *Fundulotrema* Kritsky and Thatcher, 1977: This genus was erected by Kritsky and Thatcher (1977) to accommodate five *Gyrodactylus* species infecting *Fundulus* and related fishes in North America: *G. foxi* Rogers, 1973, *G. megacanthus* Wellborn and Rogers, 1967, *G. stableri* Hathaway and Herlevich, 1973, *G. trematoclithrus* Rogers, 1967 and *G. prolongis* Hargis, 1955. All possess a characteristic peduncular bar, which is thought to act as a pressure plate during attachment (Kritsky and Thatcher, 1977; Cone and Odense, 1988). There is little doubt that *Fundulotrema* is derived from *Gyrodactylus* species; indeed there is a close relationship between the hamulus and bar structure of *Fundulotrema* and that of *G. funduli* or *G. stegurus*, which also infect *Fundulus* but lack the peduncular bar. To this extent *Fundulotrema* cannot be regarded as valid, but should rather be seen (with the species lacking peduncular bars) as part of a species group of *Gyrodactylus* in the sense of Malmberg (1970).

(14) *Gyrdicotylus* Vercammen Grandjean, 1960: First recorded from the stomach of *Xenopus* toads in Kivu by Vercammen Grandjean (1960), this genus was redescribed by Harris and Tinsley (1987). These parasites infect the mouth and pharynx of *Xenopus* and the related pipid toads, *Silurana* and *Hymenochirus* (see Tinsley, 1996), and have a suctorial attachment mechanism in which the ventral and dorsal bars are absent, the hamuli have two roots which form the dorsal wall of the suctoral haptor, and the marginal hooks pin down a marginal valve around the edge of the haptor. The penis has a single complete row of large spines. *Gyrdicotylus* species have now been recovered from throughout sub-Saharan Africa (Harris and Tinsley, 1987; Tinsley 1996; J.A. Jackson, personal communication).

(15) *Gyrodactyloides* Bychowsky, 1947: Bychowsky established this genus for species parasitic from marine teleosts (gadids, osmerids and marine phase salmonids). The ventral bar lacks a membrane, the hamuli have two roots and the dorsal bar is absent. Instead, a thin, membraneous structure extends around from the anterior of the haptor to just posterior to the ventral bar.

(16) *Gyrodactylus* von Nordmann, 1832: The original genus described by von Nordmann (1832). Von Nordmann grouped both *Gyrodactylus* and what we now know as *Tetraonchus* and *Dactylogyrus,* within the same genus. It was left to Wagener (1860) to reformulate the generic description to include only viviparous forms. *Gyrodactylus* now contains over 480 species descriptions, ~400 of which are valid (Harris *et al*., 2004).

(17) *Isancistrum* de Beauchamp, 1912: *Isancistrum loliginis* held the distinction of being the only monogenean from a cephalopod mollusc when described from the squid *Loligo* at Roscoff by de Beauchamp (1912). It was also characterised by the absence of hamuli and bars, the haptor being armed only with 16 marginal hooks. Subsequent workers were unable to rediscover this parasite until Llewellyn (1984) redescribed it from *Alloteuthis subulata*. Llewellyn also described *I. subulatae*, distinguished from the original species on the basis of site of infection. *Isancistrum* is probably derived from *Gyrodactylus*, although it shows a number of intriguing features. It is the smallest known gyrodactylid, with a body size of only 0.1–0.2 mm. Unlike *Anonchohaptor*, *Isancistrum* possesses a ring intestine. The extent to which *Isancistrum* has radiated onto cephalopods is not clear and molecular data are urgently needed for this genus.

(18) *Macrogyrodactylus* Malmberg, 1956: This genus includes the largest known gyrodactylids, attaining a maximum length of ~1.5–2 mm and clearly visible to the naked eye, infecting the skin of African freshwater fishes. Coincident with Malmberg's description, Prudhoe (1957) described a catfish-infecting form as *Neogyrodactylus*, which is therefore a junior synonym. The haptor is armed with two hamuli, ventral and dorsal bars and 16 marginal hooks. However, the anterior marginal hooks are reflected forwards, while the other seven pairs are arranged in a row along the posterior margin of the haptor. Additional hardened struts within the haptor reinforce the suctorial disc, which is also characterised by small, possibly sensory, peg-like tegumental extensions.

The penis is armed with flat plates (one larger than the others), rather than the hooked spines of other gyrodactylids.

There is also a secondary seminal receptacle behind the ECFR (Malmberg, 1957; El-Naggar and Serag, 1987; El-Abbassay, 2001) and numerous unidentified, lateral cells in the body, some of which have been identified as putative subtegumental cells (El-Naggar and Cable, in press), although the function of others remains unknown. In *M. polypteri*, the gut is banded (Malmberg, 1957), due to accumulation of melanin granules from the host *Polypterus* (see Cable *et al*., 1997), but other species of *Macrogyrodactylus* have a transparent intestine. Having described *M. polypteri* from the primitive fish *Polypterus*, Malmberg (1957) considered this genus to be primitive. Most other species, including the form described by Prudhoe (1957), have, however been described from catfish, and it seems most likely that *Polypterus* is infected as the result of a host-switch. At a molecular level, *Macrogyrodactylus* appears to group with the African *Gyrdicotylus* from amphibians (see Matejusová *et al*., 2003), but many more African species need to be included in the analysis to draw conclusions about the primitiveness and relationships of these genera.

(19) *Mormyrogyrodactylus* Luus-Powell, Mashego and Khalil, 2003: Described from the primitive African fish *Marcusenius macrolepidotus* in South Africa, this genus may be close to the forms described as "*Gyreteroncus*" by Euzet and Birgi (1988) in a conference abstract, but never properly described. Unfortunately, the internal structure of this genus was not fully described, and the homology of organ systems to those of other gyrodactylids is unclear. The significance of this genus is that it has many characters which appear primitive and it may be basal to many other genera. The haptor is reinforced to function as a shallow sucker, but anteriorly there is a broad peduncle, including a peduncular bar, which is also involved in attachment. Additional work on this genus is awaited with interest.

(20) *Neogyrodactylus* Prudhoe, 1957: A junior synonym of *Macrogyrodactylus* Malmberg, 1957.

(21) *Neogyrodactylus* Baugh, 1957: This genus was erected for a gyrodactylid from the parasitic crustacean *Argulus* in India.

According to the description, the ventral bar is absent. Current knowledge would suggest that this represents a *Gyrodactylus* species using *Argulus* as a phoretic host. However, the description is poor and does not correspond with any known description of a fish-infecting gyrodactylid. The name is preoccupied by Prudhoe (1957) and was therefore replaced by *Metagyrodactylus* Yamaguti 1963.

(22) *Metagyrodactylus* (Baugh) Yamaguti, 1963: Yamaguti (1963) used this name to replace the preoccupied *Neogyrodactylus* Baugh, 1957. However, there is little to separate this genus from *Gyrodactylus*, and we suggest that this genus be considered a junior synonym of the latter.

(23) *Polyclithrum* Rogers, 1967: Another genus with accessory supporting struts on the haptor and an asymmetrical distribution of marginal hooks (like *Macrogyrodactylus* and *Swingleus*). *Polyclithrum* has been recorded extensively from mullets (Mugilidae). As in *Macrogyrodactylus* (see above), there is a distinct seminal receptacle, lying to one side of the ECFR (Ernst *et al.*, 2000), which was not identified by Rogers (1967). This sets *Polyclithrum* apart from the *Gyrodactylus* species infecting mullets, and links it with *Macrogyrodactylus* and *Swingleus*. This may indicate relationship between these genera, or may simply be because they are the largest and least progenetic of the viviparous genera. Molecular data are needed to answer this question.

(24) *Micropolyclithrum* Skinner, 1975: Described to accommodate *Polyclithrum*-like forms from the mullet *Mugil cephalus* from Florida: considered a synonym of *Polyclithrum* by Ernst *et al.* (2000).

(25) *Scleroductus* Jara and Cone, 1989: A South American form from pimellodellid and auchenipterid catfish, *Scleroductus*, is characterised by two hardened ribs along the ejaculatory duct within the bulbous penis (Jara and Cone, 1989). Although resembling *Gyrodactylus*, Kritsky *et al.* (1995) agreed that the structure of the copulatory organ was sufficiently different to justify the separate genus. The intromittent organ has well-developed prostatic reservoirs and a seminal vesicle, and

appears intermediate between the tubular primitive penes (in *Mormyrogyrodactylus* and the oviparous genera) and the globular copulatory organ of *Gyrodactylus*. Further study of this genus could help resolve important aspects of the evolution of primitive gyrodactylids.

(26) *Swingleus* Rogers, 1969: *Swingleus* was erected for a gyrodactylid from *Fundulus* in which the haptor has one pair of marginal hooks shifted anteriorly while the remainder are grouped posteriorly. The haptor bears support struts and superficially resembles *Macrogyrodactylus* or *Polyclithrum*. However, *Swingleus* also possesses a peduncular bar, linking it with both *Fundulotrema* and those *Gyrodactylu*s species (*G. funduli* and *G. stegurus*), with similar hamulus morphology that infect *Fundulus*. However, *Swingleus* also has an accessory seminal receptacle (Billeter *et al*., 2000), which may link it with *Macrogyrodactylus* and *Polyclithrum. Swingleus* potentially sheds light on the plasticity of the gyrodactylid haptoral apparatus; this radiation onto fundulids has resulted in considerable variation in haptoral armature.

(27) *Paragyrodactylus* Gvosdev and Martechov, 1953: A parasite from loaches from the central Asian Ili River basin, separated from *Gyrodactylus* because of the greater complexity of the attachment apparatus. Further study needed to confirm whether these differences are fundamental, or whether this represents a local diversification within *Gyrodactylus*.

(28) *Paragyrodactylus* Szidat, 1973: Erected for *Paragyrodactylus superbus*, a parasite of *Corydoras* from southern South America, the genus name was preoccupied by *Paragyrodactylus* Gvosdev and Martechov, 1953; the genus was therefore renamed *Paragyrodactyloides* by Nunez (1975).

(29) *Paragyrodactyloides* Nunez, 1975: Renamed because of preoccupation of *Paragyrodactylus*, *Paragyrodactyloides* was synonymised with *Gyrodactylus* by Popazoglo and Boeger (2000).

(30) *Laminiscus* Palsson and Beverly Burton, 1983: The haptor of *Gyrodactyloides gussevi*, from the capelin *Mallotus villosus*, differs significantly from that of other *Gyrodactyloides* species, having a rounded shield-like plate between the hamulus roots.

Palsson and Beverly Burton (1983) erected *Laminiscus* for this species and others (e.g. *G. strelkowi*) with a rounded plate between the hamulus roots. *Laminiscus* closely resembles *Archigyrodactylus* from gadids.

4.4. Evolutionary Affinities of the Gyrodactylids

Gyrodactylids are very different from other monogeneans, lacking structures and organ systems that may help determine their affinities. It is important to distinguish: (i) plesiomorphic characters which differ because ancestral oviparous gyrodactylids were different to other monogeneans, and (ii) apomorphic characters that have arisen due largely to progenesis and viviparity. Monogeneans typically have the highly modified, ciliated, swimming oncomiracidium larva, and Llewellyn (1981) discussed viviparity in *Gyrodactylus* from the viewpoint that this larva had been secondarily lost. Absence of a swimming larva was seen as an uninformative apomorphy resulting from the viviparous lifestyle. However, it then became apparent that gyrodactylids primitively lack a ciliated oncomiracidium (Harris, 1983), and that, like the terrestrial lice, all stages in the life cycle could transfer between hosts. The larva of *Ooegyrodactylus* is therefore plesiomorphic with the primitive unciliated crawling larva of the acanthocotylids, of *Enoplocotyle* and of *Udonella*. Plesiomorphic characters of gyrodactylids include: (i) a pair of multiciliate ''spike'' sensilla, one on each cephalic lobe (Lyons, 1969; see Figures 15 and 16), present throughout life; (ii) male and female reproductive systems with separate openings, but no secondary vaginae. The primitive intromittent organ is tubular, reinforced with rings, accessory sclerites or spines; (iii) an unciliated crawling juvenile, and (iv) pigmented eye spots absent throughout the life cycle. A combination of these plesiomorphic characters is shared with other primitive monogeneans, including the Acanthocotylids, *Enoplocotyle* and *Udonella*, although only *Enoplocotyle* shares all of these characters. Other characters, including the hinged marginal hooks (Boeger and Kritsky, 1997) also link the gyrodactylids with the acanthocotylids and *Enoplocotyle*, but these may be shared, derived characters.

The most striking resemblance is between the egg-laying gyrodactylids and *Enoplocotyle*, a small monogenean from the skin of the moray eel. In this genus, the haptor is armed with 16 small articulated marginal hooks, identical to *Gyrodactylus*. The larva is a primary unciliated ''crawlaway'', lacking eye spots and armed only with marginal hooks. The three species of *Enoplocotyle* are normally linked with the acanthocotylids, which also, have 16 marginal hooks, but develop a large pseudohaptor immediately in front of the haptor proper, armed with rows of plates which, in *Acanthocotyle*, engage with the dermal denticles of the host (Malmberg and Fernholm, 1991). In the related *Myxinocotyle*, the pseudohaptor is suctorial, allowing attachment to the smooth-skinned hagfish (Malmberg and Fernholm, 1989). *Enoplocotyle* has been poorly studied since first described by Tagliani (1912), but *E. kidakoi* is highly reminiscent of the ooegyrodactylids (Kearn, 1993), with four longitudinal rows of vitellaria, a post-germarial testis separate male and female systems, and a large fertilisation chamber (cf. the ECFR of gyrodactylids) adjacent to the germarium. Kearn (1993) also notes the presence of compound ciliary sensilla ''at the anterior tip of each cone-shaped head lobe''. No micrographs are available, but the described resemblance to the spike sensilla of gyrodactylids is considerable. Boeger and Kritsky (1997) also link the diffuse glands surrounding the fertilisation chamber of *Enoplocotyle* with those of egg-laying gyrodactylids. We consider it likely that enoplocotylids are probably the closest described monogeneans to the egg laying and viviparous gyrodactylids.

Frustratingly, acanthocotylids and *Enoplocotyle* (and the ooegyrodactylids) have not yet been included in molecular phylogenies. Using 18S rDNA sequence data, Olson and Littlewood (2002) linked the gyrodactylids with *Udonella*, the hyperparasite of caligid copepods which lacks opisthaptoral hooks and has often been treated as convergent with, rather than closely related to, the Monogenea. The morphology of *Udonella* is also very similar to that of *Enoplocotyle* and the oviparous gyrodactylids although the male and female systems share a common opening. Remarkably, despite all the molecular analyses of *Gyrodactylus*, no deposited 28S rDNA sequences were available to allow Olson and Littlewood (2002) to place *Gyrodactylus* more

precisely. Boeger and Kritsky (1997) using morphology, link the gyrodactylids with the acanthocotylids (including *Enoplocotyle*), forming a sister group to the anoplodiscids, another primitive group with hinged marginal hooks. This analysis, however, is based on the premise that *Enoplocotyle* is indeed an acanthocotylid; *Udonella* was not considered. Matejusová *et al.* (2003), using a wider range of gyrodactylid 18S rDNA sequences, also achieved a close sister group relationship (>90% bootstrap support) between the gyrodactylids and *Udonella*, with capsalids again the sister group to the *Gyrodactylus*/*Udonella* clade. These appear to represent the surviving terminal branches of an earlier monogenean radiation, prior to the appearance of the modern dominant groups such as the microcotylids and polyopisthocotyleans. An argument against the basal position of the ooegyrodactylids in gyrodactylid ancestry is the identity of the host group; the loricariid catfish are relatively recently evolved (Boeger *et al.*, 1994, 2003). However, other apparently primitive egg-laying monogenean groups also occur on modern fishes, presumably as a result of host switching. For example, *Udonella* is a hyperparasite of *Caligus* parasitic on a range of teleosts, *Enoplocotyle* is found on *Muraena*, while the acanthocotylids occur on elasmobranchs and agnathans.

The formal taxonomic position of gyrodactylids remains confused, partly because our perception of the position of these groups within the Monogenea is poorly developed. Today, the Monogenea are usually treated as a class of the Platyhelminthes (e.g. Brusca and Brusca, 1990 (see Tree of Life Project); Olson and Littlewood, 2002), distinct from the class Trematoda, which includes the Aspidogastreans and Digeneans. Although Odhner's (1912) classification into Monopisthocotylea (based on the adults fairly simple attachment mechanisms, and which feed on skin and mucus) and Polyopisthocotylea (based on the adults complex attachment mechanisms involving clamps and suckers, and which are generally gill feeders on blood) is well known and intuitively simple. However, the classification of Bychowsky (1961), who divided the Monogenea into the Polyonchoinea (with numerous marginal hooks in larvae, roughly corresponding to Monopisthocotylea) and the Oligonchoinea (reduced marginal hooks in larvae, roughly corresponding to the Polyopisthocotylea), is also widely used. The

difficulties with all classifications of the Monogenea is that they fail to reflect the fact that the Monopisthocotylea (Polyonchoinea) are a much more diverse group than the Polyopisthocotylea, which most authors accept include only two natural groups, the Oligonchoinea proper and the polystomatids (Polystomatoinea) from amphibians. For an informative and acerbic discussion of these nomenclatural issues, see Olson and Littlewood (2002). The Polyonchoinea include such clearly natural monopisthocotylean groupings as the capsalids and the microcotylids, as well as a wide range of smaller groups, some (*Enoplocotyle, Udonella*) represented by only single taxa. To reflect this, Malmberg (1990) created the subclass Articulonchoinea, to include eight families with articulated marginal hook sickles (the Gyrodactylidae, Enoplocotylidae, Anoplodiscidae, Acanthocotylidae Bothitrematidae, Tetraonchoididae, Ooegyrodactylidae and Sundanonchidae) which he considered primitive. Shinn *et al.* (1998a), using patterns of sensilla distribution, also reflected these ideas, proposing that gyrodactylids were closer to the polyopisthocotylean line of monogenean evolution, and formed a separate subclass between monopisthocotyleans and polyopisthocotyleans. Boeger and Kritsky (1993), in the first cladistic analysis of the Monogenea rejected the Articulonchoinea as polyphyletic, as the Acanthocotylidae (including *Enoplocotyle*) grouped with the Capsalidea Lebedev, 1988. Later, Boeger and Kritsky (2001) demonstrated that the monophyly of the Order Gyrodactylidea Bychowsky, 1937 was supported by six synapomorphies of which three were non-homoplasious: the presence of two seminal vesicles, large vitelline follicles and hinged marginal hooks. Boeger and Kritsky (1993, 1997) additionally suggested that the Gyrodactylidea and the Dactylogyridea were sister groups within the Polyonchoinea. Boeger and Kritsky (2001) suggested an independent origin for the six families of the order Gyrodactylidea which comprises the Gyrodactylidae, Anoplodiscidae, Bothitrematidae, and Tetraonchoididae, with the Udonellidae Taschenberg, 1879 and the Acanthocotylidae. Further molecular and morphological studies on the Gyrodactylidea, including other taxa such as *Enoplocotyle*, and overcoming the prejudice to consider *Udonella* as not a monogenean, are required to resolve these competing hypotheses.

5. SYSTEMATICS: THE BIOLOGICAL BEDROCK

5.1. Morphological Conservatism

Gyrodactylid alpha taxonomy is based on morphology, predominantly upon the morphometrics of the attachment apparatus, but may implicitly rely heavily on the host identity. The hamuli and bars represent a remarkable taxonomic resource. They are composed of keratin-like proteins (Kayton, 1983; Shinn *et al.*, 1995a), secreted with a remarkable degree of precision, and the complexity of their shapes provides much taxonomically useful information. The structures are stable in shape, for the most part fully formed at birth (but see Jackson and Tinsley, 1995), tough and not easily distorted, and resistant to most chemical fixatives. However, as 3D structures they present challenges to capture the subtleties of their shape. They are also composite structures, reflecting differences in the proteins secreted during their synthesis. The hamulus points and marginal hook sickles are particularly resilient, surviving prolonged proteinase digestion. These structures are also rigid, whereas hamulus roots (Figure 7) and the ventral bar membrane and processes (Figure 8) are far more fragile and easily damaged during preparation. These structures are also flexible and may distort badly in formalin or ethanol fixed material, or even in air-dried specimens for SEM, when they may appear hollow and flattened (cf. Veltkamp *et al.*, 1996 for a freeze substitution method which minimises dehydration artefacts). This becomes a particular problem in those species with hamulus roots folded naturally into the midline (e.g. *G. nemacheili* and allies), and hence it is important that observations on preserved specimens are supplemented by observations of living gyrodactylids. Ventral bar shape is particularly crucial in identifying a novel gyrodactylid, although current analyses tend not to capture the complexity of this structure (Figures 8 and 21). Similarly, the shape of the dorsal bar can be diagnostic (Figure 8), but there is little need for measurements. On the other hand, critical examination of marginal hook sickles (Figure 9), and to a lesser extent the hamuli, is necessary to establish the finer points of relationship of a gyrodactylid, especially in such

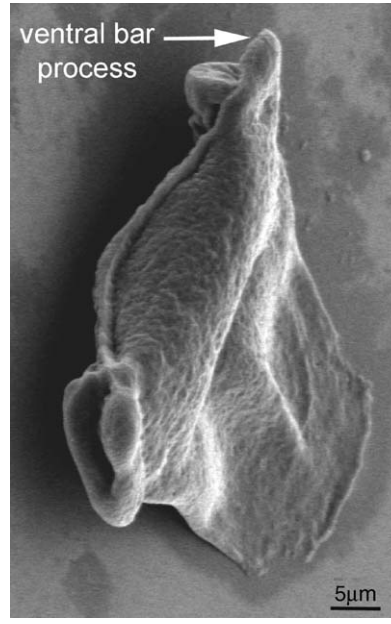


Figure 21 Scanning electron micrograph of a ventral bar (tilted anteriorly) of *Gyrodactylus salaris* (River Rauma strain) from Atlantic salmon.

difficult groups as the *G. wageneri* species complex (Malmberg, 1970), or within variants of *G. salaris* and *G. thymalli.*

Early gyrodactylid taxonomy relied on light microscopy of Canada Balsam mounted specimens. Marginal hooks and hamuli could be resolved with great accuracy in these preparations, and Wagener's (1860) descriptions can be instantly recognised today. However, many other taxonomists lacked this skill and their descriptions are so incomplete (e.g. Kathariner's 1904 description of *G. gracilis*) that the species cannot be re-identified. The viscosity of Canada Balsam makes it difficult to find properly flattened marginal hooks, while the refractive index is too close to that of the marginal hooks to make fine observation easy. American systematists used formalin and other reagents as a bath treatment to remove parasites from hosts prior to mounting (Parker and Haley, 1960; Putz and Hoffman, 1963). This generated many new species descriptions because of the ease with which fish could be screened (and is still in use e.g. Billeter *et al.*, 2000;

Kritsky *et al.*, 2007). However, formalin fixation may distort the hamulus roots, pulling them into the midline and, making their true shape difficult to discern.

With these difficulties in mind, Malmberg (1957, 1964, 1970) experimented with alternative mountants allowing adequate flattening and an appropriate refractive index. He developed saturated ammonium picrate mixed with glycerin as a combined fixative-mountant allowed to seep under the coverslip to fix a living, flattened gyrodactylid at the point of death. This revolutionised gyrodactylid taxonomy, offering significant improvements in flattening, refractive index and visibility, and combined with phase contrast illumination has become the method of choice for taxonomy of lower monogeneans. Although robust, it is not without disadvantages. Ammonium picrate mounts can dry out, but can subsequently be revived by allowing distilled water to seep under the cover slip. Mounts are fragile and contamination with immersion oil is a real problem, and so ringing with varnish is a necessity. They must also be stored flat. There are other disadvantages. Most notably, dried ammonium picrate is highly explosive, with severe restrictions on its supply and transportation and the viscosity of this medium can still be too great to allow full flattening of small marginal hooks. In addition, the limitations of resolution of the light microscope do impose limits on the use of marginal hooks in gyrodactylid taxonomy. For example, *Gyrodactylus poeciliae* has marginal hook sickles which are so small that their tips cannot be resolved with light microscopy (Harris and Cable, 2000). Suggestions for improvements of Malmberg's (1957) method include Ergens (1969) method for transferring *Gyrodactylus* specimens from ammonium picrate-glycerin into Canada balsam, Kritsky *et al.*'s (1978) method for staining the connecting bars of formalin-fixed *Gyrodactylus* specimens with Gomori's trichrome, and Richards and Chubb's (1995) technique using Mallory stain during the transfer of *Gyrodactylus* specimens from ammonium picrate-glycerin into a permanent mountant. Deposition of ammonium picrate-glycerin specimens is permissible within most major museums, a concession to the value of the technique.

With the increasing use of molecular techniques, the main disadvantage of ammonium picrate-glycerin is its inadequacy for

alcohol-fixed specimens, which often show significantly distorted hamulus roots preventing simultaneous high-resolution morphometry of the same specimens. This is particularly noticeable in the description of *G. teuchis* (Lautraite *et al*., 1999), the morphometrics of which are much less adequately described than the molecular sequence (Cunningham *et al*., 2001). This led to experimentation with various methods for examination of haptors of individuals, the bodies of which had previously been used for molecular analysis, and we highly recommend combined morphological and molecular descriptions of gyrodactylids in all taxonomic work (Harris *et al*., 1999). Scanning electron microscopy (SEM) has been used for monogenean taxonomy since Maillard *et al*. (1982) disrupted soft anatomy with sodium carbonate to free the copulatory organ of *Diplectanum aequans*. Mo and Appleby (1990) and Shinn *et al*. (1993) used proteases (principally trypsin), with ultrasound (sonication) to liberate hooks from gyrodactylids. This gave a poor yield, it was impossible to assign individual hooks to particular specimens, and if the body/uterus was not removed hooks from the parental and progeny generations were mixed. Harris *et al*. (1999) finally resolved these difficulties, developing proteinase K/sodium dodecyl sulphate (SDS) digestion of individual worms for both SEM and molecular analysis. This has been used to describe gyrodactylids from poeciliids (Harris and Cable, 2000; Cable *et al*., 2005), gobies (Longshaw *et al*., 2003), bullheads (Winger *et al*., 2005) and salmonids (Shinn *et al*., 2004; Robertsen *et al*., 2007a) including the description of the rainbow trout-infecting *G. salaris* variant (Lindenstrøm *et al*., 2003a). Another advantage is that, providing the surrounding tissue is suitably digested, application of variable pressure when applying the coverslip does not significantly alter measurements. A major drawback of digestion techniques remains their inability to deal with formalin-fixed material. This would be extremely valuable, as gyrodactylids can frequently be collected from museum specimens of their hosts. Analysis of material collected in this way is probably the only feasible method for collection of gyrodactylids from certain host groups, for example, those from the deep sea, from squids or from African freshwater fishes and amphibians.

A number of authors have looked to other morphological characters that could be used in gyrodactylid alpha taxonomy, most notably the mapping of sensory structures (chaetotaxy) (Shinn *et al.*, 1992, 1997, 1998a, b; Bakke *et al.*, 2004a; see Section 2.7). However, the use of haptoral measurements persists with associated advances in statistical analysis. Multivariate statistical analyses and automated methods applicable to a single hook are greatly improving the objectivity of these methods (Shinn *et al.*, 1996, 2000; Kay *et al.*, 1999; Bakke *et al.*, 2004a, b), although the most fundamental issue is to determine the most appropriate measurements to record.

Most authors have a favoured suite of measurements, which have evolved from the work of Malmberg (1957, 1970) and Ergens (1957) and centre on the hamuli and marginal hooks. Prior to this, the Soviet Russian school used dimensions such as ''hamulus length'', ''marginal hook length'', and so on, but these were poorly defined and remain open to interpretation. Malmberg (1957) was the first to use an explicit measurement system. A similar, but slightly different, system evolved in the United States (Mizelle and Kritsky, 1967) and an unresolved problem is that Nearctic and Palaearctic gyrodactylids have been measured using different systems. Eastern Palearctic species described by Gussev (1953, 1955) follow the Soviet measuring system, while most western Palearctic species follow the systems of Malmberg and Ergens. Harris (1985, 1986) followed Malmberg's system fairly closely, but found some characters, for example, dorsal bar measurements, relatively meaningless because they were susceptible to distortion. Shinn (1994) reviewed the measurement of gyrodactylids critically, and revised Malmberg's (1970) range of measurements to give greater precision and utility. In particular, he argued that measurements such as ''marginal hook sickle distal width'' were uninformative because they could not be measured accurately using the light microscope. Others, such as ''marginal hook sickle filament length'' are too variable. Shinn *et al.* (2004; see their Figures 1–4) developed a standard suite of light microscope measurements for gyrodactylids of salmonids with new measurements on the ventral bar added by Olstad *et al.* (unpublished). Light microscopy measurements should, where possible, be supplemented by SEM observations (e.g. Figures 7–10). The use of measurements between established

landmarks allow rapid semi-automatic identification of gyrodactylids by relatively untrained operators (Kay *et al.*, 1999). This has been developed further to utilise the power of PC neural networks to learn identification of single gyrodactylids (McHugh *et al.*, 2000; Shinn *et al.*, 2000). At the same time, shapes, particularly of the marginal hook sickles, can be adequate to discriminate individuals where linear dimensions cannot. This was noted by Malmberg (1970), but had not been addressed objectively until Shinn *et al.* (1996) began to investigate whether computerised image analysis techniques could discriminate aspects of shape, rather than absolute size. However, it is evident that inaccuracy in the specification of landmark positions for linear measurements in gyrodactylid taxonomy still occurs, and non-comparable dimensions of closely related species are still selected by different taxonomists.

Significant seasonal variations occur in the size of gyrodactylid haptoral hooks and bars, and can be generated experimentally using different temperature regimes (see Mo, 1991a, b, c; Appleby, 1994, 1996a, b). Lower temperatures extend embryogenesis leading to the birth of specimens with larger hooks (Kulemina, 1977; Mo, 1991a, b, c; Dávidová *et al.*, 2005; see Section 9.1). This environmental effect can be so marked as to result in samples from the same population having non-overlapping size ranges at the warmest and coldest periods of the year (Mo, 1991b). Morphological variants are known (Harris, 1998; Geets *et al.*, 1999), and there may be significant differences between individual parasites, with quite distinct individuals occasionally present within the same population and molecular clade (Winger *et al.*, 2005; Robertsen *et al.*, 2007a; Olstad *et al.*, unpublished). Breeding of these individuals may lead to a reversion to type morphology (Harris, 1998). Host identity and site of attachment may also influence haptoral morphology (Huyse and Volckaert, 2002; Robertsen *et al.*, 2007a) in genetically characterised taxa. Dmitrieva and Dimitrov (2002) also showed an effect of salinity on hamulus and marginal hook size, but in this case, parasites were not characterised genetically and may have represented different genotypes.

Species descriptions in gyrodactylids present a number of problems which can, in extreme cases, make re-identification almost impossible. This is particularly so for older species descriptions, but can also

apply to recent descriptions which otherwise conform to ICZN protocols. We suggest that all new species descriptions of gyrodactylids include morphometric analysis based on proteolytic enzyme digested specimens using LM, preferably supplemented with SEM, and where possible rDNA sequence data spanning ITS1, 5.8S and ITS2 (Harris *et al.*, 1999; Bakke *et al.*, 2005). To some extent this suggestion has been followed, and recent descriptions such as those of *G. neili* (Le Blanc *et al.*, 2006) and *G. thlapi* (Christison *et al.*, 2005) do show much higher standards of description than many previous accounts.

5.2. Molecular Genetics

Molecular approaches to parasite taxonomy and systematics began in the early 1990s (Wilson, 1991) and with the threat posed by further range extension of *G. salaris*, it became apparent that conventional morphological methodologies were insufficient for identification of this pathogen. Cunningham and co-workers therefore developed molecular probes to differentiate *G. salaris* from other, non-pathogenic species infecting salmonids (Cunningham *et al.*, 1995a, b, c, 2000, 2003; Cunningham, 1997, 2002; Cunningham and Mo, 1997; Cunningham and Johnston, 1998; Collins and Cunningham, 2000; Collins *et al.*, 2000; Matejusová *et al.*, 2001; Sterud *et al.*, 2002; Matejusová and Cunningham, 2004). The first marker developed was the V4 region of the 28S large ribosomal subunit (LSU), chosen because of the availability of highly conserved flanking regions that could be used to design universal primers. This locus contained amplifiable restriction fragments that distinguished *G. salaris* from non-pathogenic *G. truttae* and *G. derjavini* (see Cunningham *et al.*, 1995a, b) but not from *G. thymalli* or *G. teuchis* (see Cunningham *et al.*, 2001). This led to the sequencing of the internal transcribed spacers (ITS1 and ITS2) and 5.8S rDNA of the ribosomal gene cassette (Cunningham, 1997). The 5.8S rRNA sequence is highly conserved and may therefore be a good marker of the deeper divisions within the genus (Zięta ra and Lumme, 2002; Huyse *et al.*, 2003). Cable *et al.* (1999) sequenced ITS from non-pathogenic species, allowing the first molecular phylogeny for the genus. Molecular

phylogenies of the genus *Gyrodactylus* inferred from rDNA ITS regions were used to evaluate the validity of subgenera and species groups (Ziętara *et al*., 2002; Matejusová *et al*., 2003), and the subdivision of species of gyrodactylids into groups with long and short ITS1 (Cable *et al*., 1999; Ziętara *et al*., 2002). ITS1 and ITS2 have proved more useful markers of gyrodactylid genomic variation than the V4 region. They are more variable than the structurally conserved LSU, and because of concerted evolution (Elder and Turner, 1995), they homogenise within sexually panmictic populations. ITS markers have proved remarkably robust and 125 species (over one quarter of the described species) have now been sequenced at this locus, principally by Matejusová *et al*. (2000a, 2001, 2003), Ziętara *et al*. (2000, 2002) and Ziętara and Lumme (2002, 2003, 2004). This data set is particularly useful for its emphasis on the freshwater Eurasian species, especially the *G. wageneri* group species.

ITS sequences have proved sensitive markers of the boundaries between different gyrodactylid taxa, with even small sequence differences signalling different species identity. For example, *G. rarus* and *G. branchicus*, which are almost identical by morphometry but infect different hosts and have different ecologies, differ by only 0.9–1.3% along 774 nucleotides of ITS (Ziętara and Lumme, 2003). Within a species, sequences of PCR amplified ITS regions appear invariant; for example, Ziętara *et al*. (2000) examined populations of several species from White Sea and Baltic Sea drainages without finding any differences in ITS. Cable and Harris (unpublished) have found identical ITS in *G. gasterostei* from different UK sites between which direct interbreeding is impossible. This is in marked contrast to other invertebrates where multiple ITS sequences from the same individual, revealed by cloning, render this data unusable for phylogenetic inference (e.g. Harris and Crandall, 2000). ITS sequences have also proved extremely useful for identifying previously unsuspected species or species complexes. *G. teuchis*, a non-pathogenic gyrodactylid of salmonids, was not differentiated until its ITS was sequenced (Lautraite *et al*., 1999; Cunningham *et al*., 2001). Ziętara and Lumme (2003) in particular have used this approach to great effect, identifying *G. alexgusevi* from within specimens previously identified as *G. lotae* (host *Lota lota*) and *G. jussii* from within specimens

previously identified as *G. macronychus* (host *Phoxinus phoxinus*). In both cases, morphological differences were observed a posteriori with the confidence given by recognition of distinct ITS sequences. None of these cases represent incipient speciation as in all three the difference in sequence identity between pair members is considerable. Interestingly, Ziętara and Lumme (2003) also identified a third variant of *G. macronychus*, also with a sequence difference of some 5% from either *G. macronychus* or *G. jussii*, within the work of Matejusová *et al.* (2003).

The large number of ITS sequences available through GenBank is making *Gyrodactylus* a highly attractive option for testing evolutionary hypotheses. One of the first and most robust findings to emerge is that host switching, rather than co-evolution, is the dominant mechanism for gyrodactylid evolution (Cable *et al.*, 1999; Huyse, 2002; Huyse and Volckaert, 2002; Huyse *et al.*, 2003; Matejusová *et al.*, 2003; Ziętara and Lumme, 2003). This is at odds with the view that co-evolution of hosts and gyrodactylids has dominated (Malmberg, 1970, 1998). For example, *G. gasterostei* on the three-spined stickleback (*Gasterosteus aculeatus*) and *Gyrodactylus pungitii* on the nine-spined stickleback (*Pungitius pungitius*) were regarded as sister species by Harris (1985); ITS variation makes it clear however that the sister species to *G. gasterostei* is *G. aphyae*, infecting the minnow *Phoxinus phoxinus* (see Ziętara and Lumme, 2003). Indeed, all cases of close molecular relationship concern parasites from widely differing fish hosts (Table 1), whereas apparently similar species infecting the same host are found to be only distantly related when ITS loci are sequenced (Table 1). These represent the first objective tests of speciation patterns within *Gyrodactylus*, in which molecular evidence is used to corroborate and test hypotheses generated from morphological and host identity evidence.

Perhaps, the most surprising feature of molecular phylogenies based on ITS1, 5.8S and ITS2 rDNA sequences is the apparent antiquity of the genus *Gyrodactylus*, and the extent of their morphological conservation. Ziętara and Lumme (2002) highlighted this, noting that differences between 5.8S rDNA of *Gyrodactylus* species from different subgenera exceeds that seen between different nematode families. Ziętara and Lumme (2002), perhaps wisely, did not speculate on the

Table 1 Pairwise molecular distances of rDNA internal transcribed spacer sequences between closely related *Gyrodactylus* species

Species pair				Pairwise molecular distance (%)	Author
Species 1	Host 1	Species 2	Host 2		
Gyrodactylid species pairs from the same host					
Gyrodactylus teuchis	*Salmonids*	*G. salaries*		5.6	Lautraite *et al.* (1999)
G. alexgussevi	*Lota lota*	*G. lotae*		8.5	Ziętara and Lumme (2003)
G. macronychus	*Phoxinus phoxinus*	*G. jussii*		21.8	Ziętara and Lumme (2003)
G. macronychus	*Phoxinus phoxinus*	*G. sp.*		19.2	Ziętara and Lumme (2003)
G. rugiensis	*Pomatoschistus microps*	*G. micropsi*		16.2	Huyse and Volckaert (2002)
Gyrodactylid species pairs from different hosts					
G. branchicus	*Gasterosteus aculeatus*	*G. rarus*	*Pungitius pungitius*	0.9–1.3	Ziętara *et al.* (2001)
G. salaris	*Salmo salar*	*G. thymalli*	*Thymallus thymallus*	0	Cunningham (1997)
G. gasterostei	*Gasterosteus aculeatus*	*G. aphyae*	*Phoxinus phoxinus*	1.1–1.2	Ziętara and Lumme (2002)
G. rugiensis	*Pomatoschistus microps*	*G. rugiensoides*	*Pomatischistus minutus*	2.5	Huyse and Volckaert (2002)
G. arcuatoides	*Pomatoschistus pictus*	*G. flavescensis*	*Gobiusculus flavescens*	1.6	Huyse *et al.* (2004a)

Those infecting the same host are invariably less closely related than those infecting different hosts, suggesting that they evolved in different refugia. The most closely related forms infect different hosts, indicating the importance of host switching in gyrodactylid evolution.

reasons for the molecular differentiation with morphological conservation, but it is likely that this is an ancient genus, with origins relating to the differentiation of modern fish groups in the Triassic (Nelson, 1994). Morphological conservation is perhaps surprising, but larvae resemble each other more than their respective adults (Gould, 1977), and as a progenetic group close resemblance between species might be expected.

ITS sequences have proved an objective test of Malmberg's (1970) classification of subgenera and species groups based on excretory system and marginal hooks. Some of Malmberg's (1970) subgenera receive broad support (Ziętara *et al.*, 2002), although there are many differences of detail. Thus, Matejusová *et al.* (2003) reject the subgenus *Gyrodactylus* as paraphyletic because *G. markakulensis* forms a sister clade to the remainder of the genus; however, although Malmberg (1970) assigned *G. markakulensis* to the subgenus *Gyrodactylus*, he was clearly uncertain about its status and considered it somewhat different to other members of the group. All other species placed within subgenus *Gyrodactylus* by Malmberg (1970) which have been included in molecular analyses have formed a monophyletic clade (Matejusová *et al.*, 2003). The subgenus *Limnonephrotus* remains recognisable in molecular analyses, although one paranephrotid species group, the *G. rugiensis* group (Gläser, 1974) also falls within this subgenus according to molecular analyses, and the subgenus *Neonephrotus*, represented by *G. anguillae*, is a separate clade within the *G. rugiensis* group which has evolved following a host switch to eels (Huyse *et al.*, 2003; Matejusová *et al.*, 2003). There is no support for the subgenera *Metanephrotus*, *Mesonephrotus*, or *Paranephrotus* excluding the *G. rugiensis group*, which all group together in molecular analyses. However, despite these differences in detail, we argue that Malmberg's (1970) subgenus concept remains useful, although the precise boundaries of the subgenera are bound to change as molecular re-evaluation of the group continues.

Malmberg's (1970) "species groups", are not as well supported, mainly because of host switching. The notion of a "*G. wageneri*" group as distinct from a "*G. salaris*" species group (Malmberg, 1993) gains no support, as closely related species infect sticklebacks, bullheads, salmonids and cyprinids (Cable *et al.*, 1999; Matejusová *et al.*, 2003). The

problem with ITS phylogenies are that too many of the sequenced species (and perhaps up to one third of the described species) are from the *G. wageneri*/*G. salaris* species group, and too few species groups and subgenera, especially from North America and the tropics, have been included. Nevertheless, sufficient consensus from morphology, molecules and biogeography is possible to allow some crude, but robust grouping of *Gyrodactylus* species at the sub-generic level. We would recognise the following entities:

1) A grouping corresponding roughly to the Malmberg (1970) subgenus *Gyrodactylus*. This includes the gill parasites regarded by Malmberg (1970) as belonging to the *G. elegans* species group (curved hamuli, narrow spine-like ventral bar membrane) and the *G. phoxini* species group (straight hamuli, ventral bar membrane spathulate, often a boss on the ventral bar). Most species occur on ostariophysans (cyprinids and cobitids) in Eurasia, but Le Blanc *et al*. (2006) have confirmed Malmberg's (1970) suspicion that the subgenus has radiated widely in North America also, and host shifts onto the Esocidae have occurred. Some species with this morphology which appear to fall within this group (e.g. *G. markakulensis*) are, according to molecular analyses, not closely related. Further work is needed to establish their status.
2) A grouping corresponding to the Malmberg (1970) subgenus *Limnonephrotus*, including additionally the paranephrotid *G. rugiensis* species group and *G. anguillae*. This grouping is predominantly found in Eurasian freshwater, with extension into brackish water. Most species infect ostariophysans (predominantly cyprinids), but there are examples from most freshwater and brackish fish families in the Northern Hemisphere. There are some putative Nearctic species of this group [e.g. the *G. pungitii*-like form recorded by Cone and Wiles (1985) from Canadian sticklebacks], but these are rare and predominantly infect migratory fish such as salmonids or gasterosteids.
3) A third, catch-all group, including tropical and marine representatives of the subgenera *Mesonephrotus* and *Paranephrotus*, and *Metanephrotus*, many of which have a holarctic distribution,

possibly due to distribution with their hosts. This group undoubtedly will be subdivided further as more sequences become available. *Fundulotrema* may originate in this clade, according to sequence data presented by Kritsky and Boeger (2003). Although the overall grouping is holarctic in distribution, some species groups, such as the *G. eucaliae* group, are found predominantly in the Nearctic and Neotropical.

A problem with establishing robust gyrodactylid phylogenies has been the use of markers inappropriate for the purpose. ITS sequences are too variable for genus-wide phylogenies (although useful within species complexes). The 5.8S rDNA is more useful for genus-wide comparison, but is too short. The V4 region of the 18S rDNA (Cunningham *et al.*, 1995a, b, c) contains sufficient variation to be informative, but is also short. Instead, a greater range of 18S and 28S sequences are needed to establish the wider familial phylogeny. This has not stopped several groups from making genus-wide comparisons using sequences which include both ITS1 and ITS2 (e.g. Matejusová *et al.*, 2001; Kritsky and Boeger, 2003). Small differences in alignment necessitated by the difficulties of obtaining global alignments of ITS with limited numbers of indels, can make large differences to the final phylogeny. Even the use of the 5.8S rDNA for genus-wide phylogenies is not without problems. Ziętara *et al.* (2002) identified sequencing errors in a number of sequences within GenBank which they took time to correct; there is no assumption of correctness in many accessed entries, and there is no curatorial control of accurate morphological identification of species prior to molecular analysis. In only a few cases have voucher specimens of taxa used for molecular analysis been deposited in easily accessible and secure collections.

The absence of any nuclear genomic markers at the intra-specific level has bedevilled molecular analysis of gyrodactylids. This was partly addressed by the studies of the Intergenic Spacer (IGS) of the ribosomal gene cassette (Collins and Cunningham, 2000; Collins *et al.*, 2000; Sterud *et al.*, 2002; Cunningham *et al.*, 2003). IGS remains under the constraint of concerted evolution and therefore does not evolve as rapidly as single locus markers. Cunningham and Mo (1997) employed random amplified polymorphism DNA (RAPD)

markers to differentiate *G. salaris* populations; this did show distinct differences between *G. salaris* populations which would not have been expected if the parasite had been introduced to Norway in the mid-1970s. More recently, Collins *et al.* (2004a) sequenced the β-tubulin gene from *G. salaris*, providing the first single copy nuclear marker for the genus. Variation was found, but predominantly appears to be between gene isoforms within a single genome. Other loci have not been characterised, and attempts to isolate microsatellite markers for the group have been painfully slow. As an alternative we have used amplified fragment length polymorphism PCR (AFLP; Vos *et al.*, 1995) to analyse *G. salaris* stocks from rivers in Southern, Central and Western Norway, and compared this to diversity in *G. turnbulli* from guppies (unpublished). This method has proved highly useful in identifying polymorphisms between stocks, but suffers from the drawback at present that a minimum of 200 gyrodactylids is needed for the initial ligation reaction. Nevertheless, the method has great potential for identifying genomic markers, especially if polymorphic bands are subsequently cloned and sequenced (Cable *et al.*, unpublished).

Mitochondrial markers for gyrodactylids lagged until Meinilä *et al.* (2002) published a preliminary sequence for the *G. salaris* mitochondrial cytochrome oxidase 1 (COI) gene, which was used to generate primers for a variety of species (Meinilä *et al.*, 2002; Hansen *et al.*, 2003, in preparation). This locus is proving indispensable in recreating patterns of gyrodactylid speciation (see Section 5.3.2) and has attracted much interest with the advent of DNA barcoding (Hebert *et al.*, 2003), but two cautionary points should be considered. Firstly, Meinilä *et al.* (2004) examined mitochondrial diversity from a population (Lake Kitka) isolated by post-glacial uplift for 8400 years. Using such data, a rate of mitochondrial gene divergence of some 20% per million years was estimated (Meinilä *et al.*, 2004). This may not be unreasonable considering the short generation time of this parasite, but it compares to a normal rate of 1–2% per million years in other invertebrates (Knowlton and Weigt, 1998; Gomez *et al.*, 2000; Nieberding *et al.*, 2005). This high rate of change has significant implications for the functionality of COI, and several haplotypes have been identified in which amino acid sequence was changed. So

little is known of the structure–function relationships of COI (e.g. Meunier, 2001) that it is unwise to expect all evolution at this locus to be selectively neutral, and with such high rates of mutation, one might expect biologically significant mutants to arise relatively frequently. Given the diffuse and often exotic consequences of mitochondriopathies in humans, the only species in which they have been systematically explored, the likelihood of significant effects from mitochondrial mutations in *Gyrodactylus* is high. Secondly, although the COI mtDNA is an invaluable comparative molecular dataset with which to test the validity of the rDNA ITS data, it is unclear to what extent it is influenced by NUMTs (nuclear copies of mtDNA genes or pseudogenes). Potential NUMT COI sequences are deposited in GenBank (Accession numbers AY225307-08), but Meinilä *et al.* (2004) did not mention the presence of these nuclear copies or discuss their significance. Work is currently ongoing to fully sequence and annotate the mitochondrial genomes of various strains of *G. thymalli* and *G. salaris*, respectively. The complete mitochondrial genome of *G. salaris* from Skibotnelva, Norway, will be published soon (Huyse *et al.*, 2007) and the complete mitochondrial genome of *G. thymalli* from Hnilec, Slovakia, shall follow shortly (Plaisance *et al.*, submitted). Once the sequence data are available, regions showing significant sequence variation within and between species can be determined and subsequently be used for strain differentiation and identification.

Almost all current GenBank gyrodactylid species were originally identified using morphology and host specificity, and it is still on this basis that they are deposited in the database. Only with a more comprehensive whole genomic approach to the entire genus will molecular data begin to rival morphological and experimental studies for understanding the gyrodactylid evolution. However, with appropriate markers there is tremendous potential for studying the molecular ecology of well-characterised gyrodactylids, such as *G. turnbulli* and *G. salaris*, which are readily available from natural populations and easy to maintain experimentally. These microparasitic worms circumvent many of the problems highlighted by Criscione *et al.* (2005), such as small infrapopulations and non-availability of adult stages, and offer almost endless opportunities for testing the impact of

different life history traits (such as reproductive modes and pathogenicity) on genetic diversity.

5.3. Host Specificity Among Gyrodactylids

Most monogeneans are restricted to one or a few host species, and more than 70% are considered to infect a single host species (Bychowsky, 1961; Rohde, 1979). The paradigm that gyrodactylids are narrowly host-specific (Malmberg, 1970) often suggested by published host records, was re-examined by Bakke *et al.* (1992a). After eliminating obvious misidentifications and synonymies and restricting analyses to those gyrodactylids which have featured in two or more field studies or have been used experimentally, the proportion occurring on a single host species declined to 30%, suggesting that gyrodactylids are less host-specific than commonly thought, and that narrow specificity is an artefact based on the numerous descriptions of species collected only once. The prominent differences in susceptibility/resistance between stocks of the same host species (Bakke *et al.*, 1990, 1996) and potential parasite strain differences in infectiousness (Lindenstrøm *et al.*, 2003a; Olstad *et al.*, 2005, 2007; Cable and van Oosterhout, unpublished), reinforces the uncertainty in estimating host utilisation of gyrodactylids based on field observations.

The mechanisms that contribute to host specificity in monogeneans operate at several levels: host localisation, recognition and attachment (ecological and behavioural mechanisms); and establishment, growth and reproduction (physiological mechanisms). Gyrodactylids live within a complex ecosystem on the surface of an aquatic animal. The epidermis of the host, and its products, may attract or be inhospitable to a gyrodactylid or may be refractory due to acquired immunity. Often overlooked, feeding and digestion probably play key roles in determining host specificity in gyrodactylids. These parasites appear to possess acute powers of host discrimination and preferentially infect particular hosts (e.g. Buchmann and Uldal, 1997; Buchmann *et al.*, 2003a, 2004). Given their highly developed sensory system, it is not clear whether they fail to feed on unsuitable hosts or feed but cannot assimilate host molecules. Specific inhibitory

molecules may be present that prevent non-adapted gyrodactylids from utilising a host; but equally it may also be that the parasites are behaviourally specialised not to attempt feeding on sub-optimal hosts.

The cephalic glands have a primary adhesive function (see Section 2.5) and may influence host specificity (Whittington *et al*., 2000a, b; Whittington and Cribb, 2001). Whittington and Cribb (2001) proposes an instant reaction between the adhesive secretions of monogeneans and host mucus to allow firm but temporary attachment during transmission and locomotion on the epidermis (Whittington *et al*., 2000a). During birth, a newborn viviparous gyrodactylid attaches directly to the fish epidermis, initially with its anterior adhesive organs. Whittington *et al*. (2000a) hypothesised that initial contact allowed: (i) mechano- and chemosensory receptors of the parasite to interact with the host's surface, and (ii) a chemical reaction between the host's mucus and the adhesives secreted by the parasite, representing a potential recognition mechanism. As the anterior cephalic lobes of gyrodactylids make initial contact with the host, it is not surprising that Buchmann (1998a, c) found this region immunologically active, being rich in mannose-rich glycoproteins which stimulate the alternative complement pathway (see Harris *et al*., 1998). Both host epidermis and parasite tegument may therefore contribute to maintenance of the specific parasite–host relationship by chemical interactions between them (Whittington *et al*., 2000a, b). An important point about such "recognition" models of host specificity is that only a small change in chemical signature of either host or parasite could bring about a major shift in host specificity, which could not be predicted a priori. As an example, Leberg and Vrijenhoek (1994) noted that *Gyrodactylus turnbulli* could infect pathogenically a single clade of the gynogenetic clone *Poecilopsis* sp. In this case, a presumed minor genetic alteration in the host allowed an entirely unpredicted gyrodactylid to exploit it (but see King and Cable, 2007).

Despite the relative simplicity of a direct life cycle, research on host identification or recognition by monogeneans has been limited since Kearn's (1967) experiments on *Entobdella soleae* (see reviews by Whittington and Cribb, 2001; Whittington *et al*., 2000a, b). Gyrodactylids are particularly suited for experimental studies on host

identification and host specificity (Bakke *et al.*, 2002), and we may expect a diverse range of cues to be involved in host specificity considering their diversity of hosts. However, although gyrodactylids are frequently referred to as generalists and specialists (e.g. Matejusová *et al.*, 2000b), their host specificity, with a few notable exceptions (see Section 5.3.3), remains a neglected area. Several species have been studied experimentally, but most data remain anecdotal, or, as in *Gyrdicotylus*, is restricted to published summaries of a much larger corpus of unpublished data (see Section 5.3.1.). A classic example concerns the common guppy parasites *G. bullatarudis* and *G. turnbulli*, considered generalist and specialist respectively based on their known host range (Harris *et al.*, 2004). Harris (1986) presented anecdotal evidence that *G. bullatarudis* existed as host-specific strains, but although *G. turnbulli* has been the subject of numerous studies (e.g. Madhavi and Anderson, 1985; Scott, 1985a, b; Harris, 1986; van Oosterhout *et al.*, 2003), its host specificity has only recently been experimentally tested (King and Cable, 2007). Even with limited material, Llewellyn (1984) was able to demonstrate that *Isancistrium subulatae* from *Alloteuthis subulata* could infect *Sepiola atlantic*a. Similarly, the specificity of *G. gasterostei,* regarded as a generalist by Matejusová *et al.* (2000b), has been studied, but most data are anecdotal only or unpublished. Gläser (1974) noted that *G. gasterostei* could incidentally infect cyprinids when shoaling with these fishes during the winter months and Harris (1982) showed that it could infect *Pungitius pungitius* and *Phoxinus phoxinus*, but at significantly reduced rates compared to its preferred host, the three-spined stickleback (*Gasterosteus aculeatus*). These observations all suggest this species is a specialist, which can at best establish transient infections on other hosts. It is therefore all the more surprising this parasite is considered common on the cyprinid *Leuciscus cephalus* in the Czech Republic (see Gussev, 1985; Moravec, 2001). Hoffmann and Putz (1964) also presented anecdotal observations on the specificity of *G. macrochiri*, finding this species relatively restricted to *Lepomis macrochirus*. This again is at odds with observations from the field (summarised in Harris *et al.*, 2004), which suggest that this gyrodactylid can infect a wide range of centrarchids. This highlights a common problem with gyrodactylid specificity studies; anecdotal

observations in the laboratory can have little relevance to the field, while accidental infections in the field inevitably broaden observed host ranges. This is best illustrated by Malmberg (1970), who noted species such as *G. errabundus* infecting almost every host with which it came in contact, and by Dmitrieva and Gerasev (1997) and Dmitrieva and Dimitrov (2002), who recorded *G. alviga* from 15 host species from the Black Sea, but again, the precise status of the parasite on most of these hosts is unknown. The three taxa which have been best studied are *Gyrdicotylus gallieni* from the clawed toad *Xenopus* (see Jackson and Tinsley, 1994; Tinsley, 1996), *Gyrodactylus salaris* (see Bakke, 1991; Bakke *et al*., 2002) and *G. derjavini* (see Buchmann and Uldal, 1997) from salmonids.

5.3.1. Case Study: Gyrdicotylus

The host specificity of *Gyrdicotylus* spp., from the clawed toad *Xenopus* has been studied in some detail, although only summaries have been published (Jackson and Tinsley, 1994; Tinsley, 1996). *Xenopus* contains ~30 species (Tinsley, 1996) and gyrdicotylids also infect the related genera *Hymenochirus* and *Silurana* (see Tinsley, 1996). Records from natural populations of these toads suggest that considerable diversity exists within *Gyrdicotylus*, although only one species, *Gyrdicotylus gallieni* from *Xenopus laevis laevis* (see Harris and Tinsley, 1987) and *X. l. victorianus* (Vercammen Grandjean, 1960) has so far been formally described. *G. gallieni* from *X. l. laevis* also infects *X. l. victorianus* (see Harris, 1982; Jackson and Tinsley, 1994), suggesting a single panmictic species infecting *X. laevis* and its subspecies across Central and Southern Africa. It can also infect the endemic South African *X. gilli*, but not *X. borealis* (see Harris, 1982; Jackson and Tinsley, 1994). Infection trials using allopolyploid hybrid *Xenopus* species, such as *X. wittei* and *X. vestitus* are particularly interesting. These taxa originated as hybrids of ancestors from lineages related to *X. laevis* and *X. fraseri* and contain copies of the genome of each parent. *X. wittei* and *X. vestitus* are octoploid, but other taxa are up to dodecaploid (Tinsley, 1996; Evans *et al*., 2005), derived as hybrids between an octoploid and a tetraploid species. These central

African polyploid taxa bear a rich *Gyrdicotylus* fauna, and different populations of *X. wittei* have at least two species of this parasite. Rwandan *X. wittei* is not susceptible to *G. gallieni* from *X. laevis*, although *X. wittei* shares half its genome with a lineage closely related to *X. laevis*. Similarly, parasites from *X. wittei* were unable to infect either of the *X. laevis* subspecies tested or *X. vestitus*, although the latter also shares half a genome with a lineage closely related to *X. wittei*. Parasites from *X. vestitus* were also unable to infect *X. wittei* (J.A. Jackson, personal communication). These experiments are particularly interesting in the light of *Gyrodactylus salaris* and *G. derjavini* infections of salmon-trout hybrids (Bakke *et al.*, 1999; Section 8.2). *Xenopus laevis* develops prolonged acquired immunity to *Gyrdicotylus* spp. (Jackson and Tinsley, 1994), and this gyrodactylid is relatively insensitive to complement (J.A. Jackson, personal communication). It is possible therefore that immunity to *Gyrdicotylus* is mediated in a different way to that developed by fishes to *Gyrodactylus*. Clearly, this host–parasite interaction presents a fascinating model system with which to dissect the mechanisms for host specificity and resistance to gyrodactylids at a molecular level.

5.3.2. Case Study: The G. salaris Species Complex

Host specificity has been most intensively studied experimentally in *G. salaris* and its close relatives including *G. thymalli* and the Danish rainbow trout variants (Lindenstrøm *et al.*, 2003a; Jørgensen *et al.*, 2006). As a consequence, patterns of specificity within this species complex are fairly well known. Bakke *et al.* (1990) developed a protocol for experimental testing of salmonid susceptibility and resistance demonstrating that *G. salaris* (Lierelva strain) was pathogenic to River Lone (South West Norway) and River Altaelva (North Norway) salmon strains, but that infections of River Neva salmon from the Baltic were limited and eventually eliminated. This protocol has been greatly extended and the specificity of *G. salaris* Lierelva strain has been tested on many salmonids (see Tables 3 and 4 in Bakke *et al.*, 2002; Karlsson *et al.*, 2003; Bakke *et al.*, 2004b). However, it is important to note that under aquarium conditions infection of

atypical hosts may be possible, as experimental modulation of the host response in salmonids using cortisol (mimicking aspects of the stress response) significantly influences both innate and acquired resistance to *G. salaris* (see Harris *et al*., 2000), and detached *G. salaris,* frequently observed in aquaria, are found to be less host selective and may attach to any available fish species (Bakke *et al*., 1991a, 1992a).

A fundamental difference exists between the behaviour of Lierelva strain *G. salaris* on salmonids and that on non-salmonids. On brook lamprey, European eel, flounder, minnow, perch, roach, and three- and nine-spined sticklebacks, no population growth occurs and parasites are rapidly lost. On eels (Bakke *et al*., 1991a), survival is only slightly better than on glass (Olstad *et al*., 2006) and parasites may not feed. Generally, parasites transfer poorly to these non-salmonids, and parasites may possess behavioural and physiological mechanisms to avoid these hosts. On all salmonids tested, however, some parasite growth and reproduction was observed, although the population growth rate varied greatly between host species. On salmonids, *G. salaris* can generally transfer, establish, feed and reproduce, although poorly in some species, for example, on lake trout (*Salvelinus namaycush*) and whitefish (*Coregonus lavaretus*) (Bakke *et al*., 1992b; Soleng and Bakke, 2001a). On Atlantic salmon, the usual host in the wild, and on other susceptible salmonids, there is considerable variation in resistance between species, strains and individuals of the same population, even full-sibs (Bakke *et al*., 1996, 1999; see Sections 8.1 and 8.2).

On brown trout (*Salmo trutta*), after *S. salar* the host most likely to be encountered by *G. salaris* Lierelva strain in the wild, population growth is particularly poor (Jansen and Bakke, 1995; Bakke *et al*., 1999, 2002), similar to population growth on the more distantly related *Salvelinus namaycush* and *Coregonus lavaretus* (see Bakke *et al*., 1992b; Soleng and Bakke, 2001a). The grayling, *Thymallus thymallus*, is also a relatively poor host for *G. salaris* Lierelva strain (Soleng and Bakke, 2001b; Sterud *et al*., 2002) compared to salmon, despite the supposed close relationship between *G. salaris* and *G. thymalli* (see Meinilä *et al*., 2004). Soleng and Bakke (2001b) found that *G. salaris* populations survived for only ~35 days on grayling and that infection rarely exceeded 50 parasites per fish, although 0+ fish were more

likely to be susceptible than older (1+) grayling. On rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*, population growth of *G. salaris* Lierelva strain is effective (Bakke *et al.*, 1991b, 1992c) and, although infections eventually self-limit, *O. mykiss* in particular is capable of sustaining population growth for considerable periods. Given the discussion below (Sections 6.5 and 6.6) concerning the "rainbow trout strain" of *G. salaris* from Lierelva (and Drammenselva/Lærdalselva/Bullaren) (Hansen *et al.*, 2003), these experiments need to be repeated with other Norwegian *G. salaris* strains belonging to the other *G. salaris* clades (see Hansen *et al.*, 2003).

The position of the Arctic charr, *Salvelinus alpinus* as a host for *G. salaris* is particularly interesting. An isolated, land-locked Arctic charr population (Korssjøen, central Norway) proved almost entirely refractory to the Lierelva strain of *G. salaris*, eliminating infection within a few days, whereas a northern anadromous population, sustained parasites for long periods; many months in pooled host populations (Bakke *et al.*, 1996). The role of this host in the natural epidemiology of *G. salaris* therefore remains unclear, although Mo (1988) considered Arctic charr a possible reservoir host when rotenone treatment of the Skibotnelva failed. Relatively high infections of Arctic charr were later observed in this river (Kristoffersen *et al.*, 2005). Knudsen *et al.* (2004) have also recorded exceptionally high *G. salaris* infection on Arctic charr in the nearby Signaldalselva, where salmon are also infected by *G. salaris*. The *G. salaris* in these rivers belong to a different clade than the Lierelva strain (Hansen *et al.*, 2003). Arctic charr are also infected with *G. salaris* in five salmon-free lakes in central south Norway (Robertsen *et al.*, 2006, 2007b). This host seems to be able to support *G. salaris* in species-poor fish communities in the absence of Atlantic salmon or rainbow trout. Recent work by Robertsen *et al.* (2007a, b) has shown that the *G. salaris* strain isolated from charr in the lakes had the same mitochondrial haplotype as rainbow trout parasites isolated from Lake Bullaren, Sweden but was non-virulent to salmon (Olstad *et al.*, 2005). However, the ITS of *G. salaris* from Arctic charr showed a difference of one nucleotide to that previously observed in *G. salaris* populations (Olstad *et al.*, 2007).

The specificity of *G. thymalli* has also been examined, although in less detail. This parasite utilises Atlantic salmon even less effectively (Bakke *et al*., 2002) than *G. salaris* can exploit grayling (Soleng and Bakke, 2001b), but also appears less aggressive on its normal host, with slower population growth on grayling than *G. salaris* Lierelva strain exhibits on susceptible salmon strains (Bakke *et al*., 2002; Sterud *et al*., 2002). However, only the Norwegian River Glomma strain of *G. thymalli* has been used in susceptibility testing. Given the molecular heterogeneity of *G. thymalli* in Scandinavia (Hansen *et al*., 2003, 2007; Meinilä *et al*., 2004), and the occurrence of other grayling clades infected with *G. thymalli* outside Scandinavia (e.g. Denham and Longshaw, 1999; Hansen *et al*., 2007), this work should be repeated with other *G. thymalli* strains and grayling stocks. The final members of the *G. salaris* species complex that have been tested experimentally are the rainbow trout variants isolated by Lindenstrøm *et al.* (2003a) and Jørgensen *et al*. (2006) from Danish rainbow trout. These parasites failed to exploit salmon stocks (Baltic and East Atlantic), but reproduced successfully on rainbow trout. Unfortunately, neither grayling nor brown trout were included in these comparisons.

5.3.3. Case Study: Gyrodactylus derjavini

The most frequently encountered gyrodactylid on salmonids in Scandinavia is *G. derjavini*, infecting both *Salmo trutta* and *Oncorhynchus mykiss*. Infections of rainbow trout can only date back to the introduction of this fish into Europe at the end of the 19th century. The widespread occurrence and epidemiology of this parasite on brown trout in southeastern Norway (Mo, 1997; Bakke *et al.*, unpublished) suggests that the original host in Northern Europe is actually *S. trutta*. The host specificity of *G. derjavini* has been extensively tested by Buchmann's group. Buchmann and Uldal (1997) investigated the susceptibility of four salmonids (rainbow trout, brown trout, a Baltic and an Atlantic strain of salmon) to a Danish isolate of *G. derjavini.* They found initial parasite attachment did not differ between host species but that populations

increased significantly faster and to a higher level on rainbow trout compared with other salmonids. Parasite selection of microhabitat also differed between the host species. Salmon are slightly susceptible to *G. derjavini* Sandvikselva strain but this is also dependent upon the salmon stock tested (Bakke *et al.*, 1999, 2002). In an experimental study where detached parasites were offered a choice between salmon, rainbow trout and carp, Buchmann *et al.* (2004) found preferences in both *G. derjavini* and *G. salaris,* as after 2 days 90% of *G. derjavini* infected the rainbow trout and 60% of *G. salaris* were attached to the salmon.

6. EVOLUTION AND PHYLOGENY OF GYRODACTYLIDS

6.1. Models of Parasite Speciation

Gyrodactylus is one of several hyperdiverse monogenean genera that could provide important insights into parasite speciation processes. Originally, parasite speciation was viewed predominantly from the perspective of co-evolution (or co-speciation; Fahrenholz, 1913), a parasite group evolving in parallel with its host group. This was certainly considered so for monogeneans (Bychowsky, 1961; Llewellyn, 1963) and was extended to gyrodactylids by Malmberg (1970). Ironically, it is now realised that there are very few convincing cases of cospeciation because of the exacting requirements needed for its demonstration (see Page *et al.*, 1996) and the difficulties of interpreting topological incongruence between host and parasite phylogenies (Page and Charleston, 1998). Indeed, if Raup (1994) are correct in estimating extinction rates of 10–15% of vertebrate species per million years, then it is hardly surprising that co-evolution is so difficult to recognize, as the probability that two related hosts could be found containing two related parasites more than a few million years after the initial divergence event is small. Even groups such as the lice, originally held to be strictly co-evolutionary (Rothschild and Clay, 1952), are now seen to be much more complex (e.g. Hafner and Nadler, 1988; Page *et al.*, 1996). A more convincing case can be made for the importance of

host-switching in parasite speciation, a concept long accepted by researchers of plant–insect interactions (e.g. Walsh, 1864) and shown by Brooks and McLennan (1993) to be a dominant force in the speciation of animal parasites.

Host switching is counterintuitive as it appears to be an unjustified example of sympatric speciation. Mayr (1963) argued that speciation could only occur when populations were physically separated by extrinsic barriers (allopatric speciation), either by separation into two parts of a previously contiguous population (vicariant allopatric speciation), or by the peripheral isolation of a small part of the initially contiguous population, within which divergence occurs much more rapidly because of its relatively small size (peripheral isolates allopatric speciation). Sympatric speciation, the evolution of intrinsic reproductive barriers between incipient species where the populations are not physically separated, was originally not held to be possible, and is still considered very rare in vertebrate evolution. Lynch (1989) considered over 70% of vertebrate speciation events took place through vicariant allopatric speciation, with 15% via peripheral isolation. Only 6% of cases could possibly be accounted for by sympatric speciation. Host switching within a single geographical locality can be an apparently sympatric isolating mechanism, if gene flow between parasite populations on different hosts is reduced to the point where reproductive barriers can evolve. It has been suggested many times for plant parasitic insects, most notably and persistently in the apple maggot fly *Rhagoletis* (see Jiggins and Bridle, 2004). Populations of this fly, infecting Hawthorn *Crataegus* spp., started to feed on apples after their introduction to America following European colonisation. This began a process in which the phenology of the apple feeding forms changed and now resultant changes in host identification and detection preferences have been detected (Feder *et al.*, 2003). This situation is entirely analogous to, for example, the evolution of the rainbow trout variant of *Gyrodactylus salaris* (see Sections 6.5 and 6.6), an evolutionary change which could not have taken place until after the introduction of rainbow trout into Europe in the 19th century. In *Rhagoletis*, it is argued that the recentness of the evolution of the apple feeding forms is such that sympatric evolution must

be involved (Jiggins and Bridle, 2004). Feder *et al.* (2003), however, have argued that the apple-feeding race of *Rhagoletis* derived from forms which were already reproductively isolated from other hawthorn-feeding forms by chromosomal inversions. This would push the date for the initial divergence much further back in time, and argues again for an allopatric split with reproductive isolation, which preceded the host switch. As Jiggins and Bridle (2004) point out, this does not minimise the importance of sympatric processes in the evolution of the host shift, but does indicate that allopatric processes are initially necessary to establish genetic differences between strains. The relevance of these speciation models to *Gyrodactylus* remains to be seen, but the analogy between *Rhagoletis* and the situation in *G. salaris*/*G. thymalli* appears strong (see Section 6.6), and Kearn (1996) for example, was convinced of the importance of host shifts as a means of gyrodactylid speciation. The role of chromosome inversions in *Gyrodactylus* is uninvestigated; if they occur, they could, as in *Rhagoletis*, lock large parts of the genome from recombination with other parasite genotypes. There have been no investigations of the existence of chromosome races or polyploids in *Gyrodactylus*, although the recent work of Ziętara *et al.* (2006) suggests the existence of potentially triploid races of *G. salaris*. The significance of this finding remains to be evaluated.

There is some evidence of co-evolution at the deeper levels of gyrodactylid speciation. This has been noted above (Section 6.5); the species of the sub-genus *Gyrodactylus* are mostly found on ostariophysans in Eurasia (not in America, where they have radiated onto other groups of fish, see Le Blanc *et al.*, 2006). This may represent a much older co-speciation event, of which only a few deep branches remain, while the terminal clades all represent fairly recent, host-switch driven speciation events. We now go on to discuss three possible speciation scenarios, of slightly different ages. These are: (i) relatively ancient speciation events on guppy hosts in the Caribbean (freshwater, tropical); (ii) more recent speciation events associated with European gobies (marine, temperate and subtropical); and (iii) the most recent events associated with Eurasian salmonids (cold-temperate, freshwater).

6.2. Evolution of Gyrodactylids on Guppies

Several papers (Harris and Lyles, 1992; Harris and Cable, 2000; Cable *et al.*, 2005) have described the natural gyrodactylid fauna of poeciliid fishes in the Caribbean. Poeciliids are excellent models for micro-evolution with some of the most compelling evidence for natural and sexual selection in the wild being provided by the guppy, *Poecilia reticulata* (e.g. Endler, 1986; Magurran *et al.*, 1992; Magurran, 2005). Guppies occur naturally in most freshwater streams in Trinidad (Houde, 1997), with marked phenotypic and genetic differences between upstream and downstream populations within a single river (Endler, 1980; Reznick and Endler, 1982; Shaw *et al.*, 1994; Magurran *et al.*, 1995). For almost two decades, predation pressure has been identified as the key factor driving guppy evolution (Endler, 1980; Reznick and Endler, 1982; Magurran *et al.*, 1992, 1995; Reznick *et al.*, 1997), although recent studies indicate that density-dependent regulation and resource availability may also have been important (Reznick *et al.*, 2002). Surprisingly, selection pressures imposed by parasites have, until recently, been entirely ignored (Cable and van Oosterhout *et al.*, 2003, unpublished; van Oosterhout *et al.*, in press a, b).

Wild guppies sustain a range of fungal, protozoal, helminth and nematode infections and many fish carry multiple infections. The well-studied laboratory models *Gyrodactylus turnbulli* and G. *bullatarudis* (see Scott, 1982, 1985a, b; Scott and Anderson, 1984; Harris, 1986, 1988) are widespread and abundant parasites in natural populations of these fishes (Harris and Lyles, 1992; Cable and van Oosterhout, unpublished). The pathology of even moderate *Gyrodactylus* spp. infections, and their effects on behaviour, reproduction and survival of guppies, can be severe. For example, feeding response and activity of guppies (which are good indicators of health and alertness; Houde, 1997) are significantly reduced in guppies with only relatively small burdens of *G. turnbulli* (see van Oosterhout *et al.*, 2003a). Heavily infected fish, with clamped fins and erratic swimming behaviour (Cable *et al.*, 2002a), are ostracised by uninfected shoal mates (Cable and Griffiths, unpublished), and females discriminate against males infected with *G. turnbulli* which show reduced colour

pattern and courtship activity (Houde and Torio, 1992; Houde, 1997; López, 1998). Experimental infection with an isogenic culture of *G. turnbulli* furthermore showed that upland and lowland population guppies differed significantly in immunocompetence (van Oosterhout *et al*., 2003a), with upland populations experiencing higher infection rates and mortality than lowland fish (van Oosterhout *et al*., 2003). Furthermore, within guppy populations, the parasite-resistance of individual guppies is highly repeatable across infections (Cable and van Oosterhout, 2007).

We are becoming increasingly aware of the complex gyrodactylid fauna infecting poeciliid fishes and there is great potential for studying the interaction between poeciliid evolution and parasitism. The evolution of the poeciliids is partially known, but full molecular phylogenies are unavailable, and morphological phylogenies are uncertain (Poeser, 2003). Cable *et al*. (2005) described *G. pictae* from *Poecilia picta*, with a 5% difference in ITS sequence when compared with *G. turnbulli*. The respective hosts differ by 15% at the mitochondrial ND2 locus (Breden *et al*., 1999). These differences are considerable, and suggest an ancient division between these two fish species and their parasites, which may relate to sea level changes in the Caribbean several million years BP. This system represents an excellent model which can provide a comparison with the relatively much more recent speciation events we observe in salmonid gyrodactylids.

6.3. Species Flocks on Gobies

A second example of gyrodactylid speciation on an evolving host group is illustrated by the gyrodactylids of gobies in the North East Atlantic, which clearly demonstrate that morphological similarity does not reflect relationship between forms. Sand gobies (*Pomatoschistus*, but also other inshore genera) represent a rapidly evolving species flock, the taxonomy of which is still under review. Gyrodactylids have been reported from two sand goby genera, *Pomatoschistus* and *Gobiusculus* (see Huyse *et al*., 2004a, b). Significant events in the evolution of the hosts have included the Messinian

Salinity Crisis (~6 million years ago), which ended with the reopening of the Straights of Gibraltar and led to the separation of the *Pomatoschistus minutus* and *P. microps* clades, and the more recent Pleistocene glaciations that led to the evolution of the *P. minutus* species group within the Mediterranean. As ice retreated from the Last Glacial Maximum (LGM), ~20 000 years ago, gobies migrated North around the European coastline (Huyse, 2002; Huyse *et al.*, 2004b). Huyse *et al.* (2004b) regard sand gobies as being somewhat older than the most recent glaciation, and Gysels *et al.* (2004) make the assumption that *Pomatoschistus* spp. distributions have moved north and south in response to glaciations, including the LGM. Their molecular evidence suggests an expansion of *P. minutus* in the North Atlantic during the Eemian interglacial, 120 000 years BP (Gysels *et al.*, 2004). Both *P. minutus* and *P. microps*, which are not closely related and have been separated since the reopening of the Straights of Gibraltar, retreated southwards after the Eemian, but may have left refugial populations in the Southern North Sea area (Gysels *et al.*, 2004). This detailed and robust phylogeny of the host group, correlated with known geological events such as the Messinian Salinity Crisis and the LGM, allow testing for concordance in the pattern of evolution of their gyrodactylids. Furthermore, given Meinilä *et al.*'s (2004) estimates for mitochondrial evolution in gyrodactylids, these parasites should provide a useful fine-grained marker for evolutionary processes in gobies.

Pomatoschistus gobies are infected by *G. arcuatus*-like forms, noted but not described by Appleby (1996b). The morphometric divergence between stocks of *G.* cf *arcuatus* from different goby species suggested separate species (Geets *et al.*, 1999), which were described by Huyse *et al.* (2003) as *G. branchialis, G. arcuatoides, G. gondae* and *G. flavescensis*. Surprisingly, they are not closely related to *G. arcuatus* with a difference in ITS sequences of 13% (Huyse *et al.*, 2003). Unfortunately, *G. arcuatus*-like forms on Eurasian freshwater and marine fishes are poorly known, although they are common on gadids (Bychowsky and Polyansky, 1953; Malmberg, 1970), and it is not clear which is the sister group to the forms on gobies. The host preference of the four gyrodactylids varies. *G. gondae* occurs on *P. minutus* and the sibling *P. lozanoi* from the North Sea and from

Atlantic watersheds in Norway; *G. arcuatoides* infects only *P. pictus*; *G. branchialis* occurs on *P. microps* from the southern North Sea and Norway, and is closely related to *G. quadratidigitus* from the western Channel (Longshaw *et al*., 2003), while *G. flavescensis* infects *Gobiusculus flavescens* from Norway. Comparison of host and parasite molecular phylogenies (Huyse *et al*., 2003) could not distinguish between host switching and co-evolution for the *G. arcuatus*-like species infecting *Pomatoschistus minutus*, *P. lozanoi* and *P. norvegicus*, but these authors (Huyse *et al*., 2003) note that the evolution of these gyrodactylids can probably be related to the last ice age (i.e. less than 100 000 years BP). If this timing is correct, then the host switch of *G. ostendicus* to the more distantly related host *P. microps* (see Huyse and Malmberg, 2004) is accompanied by a remarkable change in morphology; this species appears more closely related to *G. harengi* based on hook shape, and only molecular analysis reveals the true affinities of this taxon. Similarly, Huyse *et al*. (2003) closely relate *G. anguillae* from the common eel *Anguilla anguilla* to *Gyrodactylus micropsi* from *Pomatoschistus microps*, a further major change in morphology following what seems to have been a fairly recent host shift. Host switching also appears to have been the main factor promoting speciation in the *G. rugiensis*/*G. micropsi* group from the fins of gobies (Huyse and Volckaert, 2002). More recently, Huyse and Volckaert (2005) have used a range of statistical approaches to test for co-evolution, as opposed to host switching, in this goby–gyrodactylid system. Although some of the methods used did generate evidence of co-speciation of host goby and gyrodactylid, particularly amongst the gill parasites, it is very difficult to distinguish genuine co-speciation from host shifts onto closely related hosts. When Huyse and Volckaert (2005) included some estimate of time from divergence in the analysis, it became apparent that all of the gyrodactylid sister species were probably much younger (related to Pleistocene events) than their sister host species, which had diverged in the Pliocene. This also rules out co-speciation and strongly suggests that infection of one host species predilects gyrodactylids to infect other closely related hosts by host shifting (Huyse and Volckaert, 2005). This is also the pattern that is observed in specificity studies (Section 5.3), that gyrodactylids normally infect a spectrum of more or less closely

related fish species although host shifts to unrelated fish species can also occur.

No experimental studies on the host preferences of these gyrodactylids of gobies have been undertaken, and no mitochondrial sequences are available. With evidence from both of these sources, we would be able to infer the evolutionary history of this group with greater confidence.

6.4. Refugia and Pleistocene Dispersals

The history of gyrodactylid speciation, within Eurasia and probably also North America, is closely tied to post-glacial history and the presence of isolated refugia for freshwater fish during the ice ages. The narrow host range of many gyrodactylids, and the molecular information now available, makes these highly attractive organisms for considering patterns of evolution of freshwater fishes in the post-glacial landscape, and can provide important corroboration of mitochondrial DNA evidence in studying evolution of the fishes themselves.

The precise age of gyrodactylid species is unclear, given the lack of a calibration for molecular divergence in the genus. However, several aspects of gyrodactylid evolutionary biology suggest that a major force responsible for their speciation has been the series of climatic upheavals known collectively as the ice ages. Over the past 800 000 years, Eurasia has alternated between phases of cold, dry climate (ice ages) in which ice sheets pushed south and sea level fell, and phases of warm moist climate (interglacials), in which ice retreated and rising sea levels subdivided the land mass. This pattern has never been precisely repeated, and so the location of ice masses and possible refugia has differed between glacial events. As ice sheets have extended south, flora and fauna are thought to have retreated in front of the ice, and to have survived in scattered refugia. During the LGM, three southern European refugia for terrestrial organisms are normally acknowledged: the Iberian Peninsula, Italy/Sicily, and the Balkans (Hewitt, 1999). The relative importance of each varied depending on taxon, but, based on mtDNA evidence, most terrestrial and some aquatic

organisms recolonised northern Europe from one or more of these three areas (Hewitt, 2004). This pattern of recolonisation is still continuing, as organisms with poor dispersive powers failed to colonise northern Europe before rising sea level cut dispersal routes. This is probably the historical reason for the absence of *G. salaris* from Norway and Britain.

Refugia for aquatic organisms are much more complex. Aquatic organisms can retreat southwards only so far before increasingly steep river gradients make habitat unsuitable. Reduction in sea level during glacial maxima increased the distance between header streams of different watersheds (the header streams of Rhine, Rhone and Danube would have been much further apart at the time of the LGM than today), and fundamentally changed the relationships between major watersheds and river systems (Figure 22). During the LGM, the English Channel River, emptying to the west of the European continental shelf somewhere off Brittany (Lericolais *et al.*, 2003), received water from the Thames, the Seine and the Rhine. Finally, and perhaps most importantly, a series of large impounded glacial lakes existed along the edge of the ice sheet. These included the relatively small Lake Humber/Lake Pickering complex in what is today northern England (Clark *et al.*, 2004), but also probably a moderately large lake in the southern North Sea, and a series of very large ice-dammed lakes along the margin of the ice in northern Russia and Siberia (Mangerud *et al.*, 2001, 2004; Svendsen *et al.*, 2003). These lakes, caused by ice-damming of rivers such as the Ob and Yenissei, were of such a size as to potentially have had impacts on global climate (Krinner *et al.*, 2004; Mangerud *et al.*, 2004). From an evolutionary perspective, these were large enough to sustain large, resident populations of many fish, including the anadromous salmonids, sticklebacks, cottids and perhaps gobies. The precise combination of fish within these lakes, and the consequences of genetic drift, would depend in part on their size, and the effective population size of each species within them. These lakes changed their conformation and connections during the Weichselian, from their inception some 90 000 years BP (Krinner *et al.*, 2004; Mangerud *et al.*, 2004) until the LGM ~20 000 years BP. During different glaciations, different combinations of glacial lakes probably arose (there is little evidence of events

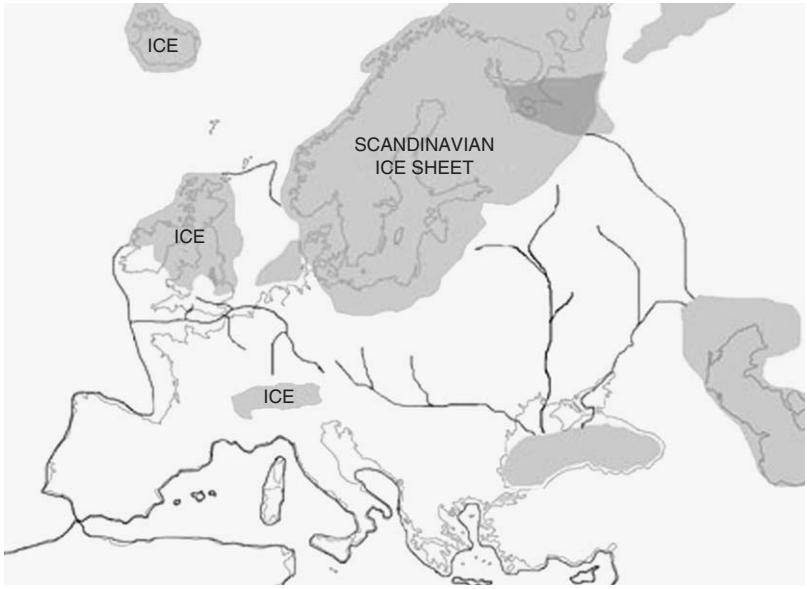


Figure 22 Modern coastline (pale outline), and coastline 20 000 before present (BP) (dark outline) showing significant bodies of freshwater (pale blue). Note that most European watersheds drain into the Black Sea, which at this time was freshwater. The Volga system also connected with the Black Sea and the other European rivers for a short period ~18 000 years BP due to meltwater overflow into the Manych Pass. Consequently, there were opportunities for mixing and exchange of gyrodactylids throughout central and western Europe, with only the Channel River system (Rhine, Thames and northern French rivers) remaining distinct. This should be viewed as a dynamic evolving landscape; prior to this time (60 000 years BP) the rivers of western Siberia (Ob, Yennisei) drained into the Caspian; subsequent to the LGM, the Baltic became a freshwater lake, allowing interchange of fish and gyrodactylids between rivers draining into the Eastern Baltic.

prior to the Weichselian, because later ice sheets have erased traces of previous glacial episodes). During the LGM, for example, drainages of the Danube, Dniepr and Don were all interconnected, draining into the freshwater Black Sea (Mangerud *et al*., 2004), and were linked via the Manych Pass to the Volga drainage into the Caspian Sea. At a previous phase in the last ice age (80 000 years BP), the Siberian rivers Yenissei and Ob were also prevented from draining north, and instead drained south to the Aral, Caspian and Black Seas (Mangerud *et al*., 2004). This mixing of drainage patterns throughout

the last ice age probably accounts for the uniformity and richness of the Central European gyrodactylid fauna, with only the Channel River drainage basin remaining distinct (Figure 22). Ice lakes were freshwater or of very low salinity, and allowed mixing of fish stocks and harsh, possibly immunosuppressive environmental conditions, potentially provided ideal environments for host switching by gyrodactylids, with consequent speciation and evolution. Interestingly, the most closely related parasite species pairs infect different hosts (indicating a host switch, see Table 1), while similar species infecting the same host tend to be more distinct at a molecular level, suggesting evolution in different refugia, possibly during different glaciations (Ziętara and Lumme, 2002). This is best indicated by the gyrodactylids of the minnow, *Phoxinus phoxinus*. This cyprinid has a widespread distribution throughout Eurasia, and may reasonably be expected to have been restricted to several different ice lake refugia. Ziętara and Lumme (2002) noted that host switching triggers adaptation to the new host, and pointed to two phases in the evolution of *G. wageneri*-like species, one phase ~5 million years BP, the other ~1 million years BP. This latter, recent burst of host switching and speciation occurred within the period of glacial and interglacial warmings but we would question the confidence limits for this estimate, suggesting that in some cases, speciation and host switching has occurred as recently as the LGM, perhaps as little as 20–50 000 years BP. This is suggested very strongly by the biogeography of some molecular variants. Ziętara and Lumme (2002, 2003) identified distinctive molecular variants of several species, including *G. aphyae* and *G. macronychus*/*G. jussii* from opposite sides of the Fennoscandian watershed, from Oulu (draining to the Baltic) and from Oulanka (draining to the White Sea). During the Early, Middle and Late Weichselian glaciation (90 000–20 000 years BP), fishes from both the Baltic and White Seas were restricted to distinct ice-dammed lakes (Krinner *et al*., 2004). During the preceding warm phase, the Eemian (~120 000 years BP), the two Seas were linked by a seaway through Lakes Ladoga and Onega, which would have led to the mixing of fish populations and subsequent geographical homogenisation of their gyrodactylids (Funder *et al*., 2002). A slightly older event may have been the evolution of *G. limneus* and *G. phoxini* on minnows in

separate ice-dammed lakes, as these species do occur conspecifically (Malmberg, 1970), suggesting prior mixing during a warm phase. On the other hand, the evolution and shift in host specificity between *G. salaris* and *G. thymalli* appears to have occurred during the LGM and afterwards (see below). Within the large ice lakes (e.g. the Black or Caspian Seas 20 000 years BP and including the eastern Baltic today), conditions favoured the survival of freshwater fish alongside marine species. Within current day Greenland (where conditions probably approximate those within the ice-dammed lakes of the LGM), very low temperatures, and floating wedge-like layers of freshwater on salt at the heads of fjords, allows marine fish such as *Myoxocephalus* to penetrate into almost freshwater. It is under such conditions that the adaptation of marine gyrodactylids to freshwater probably occurred.

Glacial events influencing gyrodactylid speciation were not confined to the Baltic and northern Russia. One of only two gyrodactylids so far considered endemic in the British Isles is *G. rogatensis* from the bullhead *Cottus gobio* (see Harris, 1985). Elsewhere in Europe it is replaced by *G. cotti*, a more widespread species infecting a range of cottids, or by *G. hrabei*, described by Ergens (1957, 1971) as most closely related to marine forms infecting *Myoxocephalus* (e.g. Levinsen, 1881). *G. hrabei*-like forms infect *Cottus poecilopus* across much of its range (Ergens, 1961; Malmberg, 1972; Winger *et al.*, 2005, unpublished). The occurrence of *G. rogatensis* is mirrored by molecular analyses of the host bullheads, which suggest that during the LGM either one group survived within the Channel River system and its tributaries, and has remained distinct within the UK and rivers as far east as the Rhine (Hänfling *et al.*, 2002), or that this clade recolonised from southern Europe but has been subject to serial bottlenecking in the process (Volckaert *et al.*, 2005). *G. rogatensis* appears to have switched to infect this clade within the catchment of the Channel River.

An interesting problem in recent gyrodactylid evolution concerns *G. wageneri*-forms infecting the three-spined stickleback, *Gasterosteus aculeatus*, and small cyprinids. The nine-spined stickleback, *Pungitius pungitius*, is infected by *Gyrodactylus pungitii*, which occurs throughout Scandinavia and is only distantly related to *G. gasterostei*, found on the three-spined stickleback. *G. gasterostei* occurs in northern

Germany (Gläser, 1974), but not in Scandinavia (Malmberg, 1970 failed to find this species), and probably evolved within the Channel River system, spreading eastwards into the Elbe system. The normal host is *Gasterosteus aculeatus*, and in Britain at least, this parasite is specific for this host (Cable *et al*., 2002b; Harris, unpublished). In the Czech Republic, however, *G. gasterostei* is considered a generalist, infecting primarily cyprinids (see Ergens, 1985a, b; Matejusová *et al*., 2001). Furthermore the three-spined stickleback is rare in central Europe (Ahnelt *et al*., 1995), and the molecular evidence of Ziętara and Lumme (2002) indicates that *G. gasterostei* from the stickleback and *G. aphyae* from the minnow are so closely related that the distance between *G. gasterostei* and one *G. aphyae* clade is less than that between the two *G. aphyae* clades. Clearly, *G. gasterostei* and *G. aphyae* are sister species, one the result of a host switch from the other. However, is the Central European *G. gasterostei* of Ergens (1985a, b) simply a misidentification, or is there a more complex situation, with this species infecting a different host in these central European river systems?

6.5. Paraphyly of *Gyrodactylus salaris*

The most complex situation discovered to date is the pathogenic *Gyrodactylus salaris*, and its close relatives, *G. teuchis*, *G. bohemicus* and *G. thymalli*:

(i) *G. teuchis* was described from rainbow trout (the type host) in France (Lautraite *et al*., 1999; Cunningham *et al*., 2001); other hosts include wild Atlantic salmon and brown trout. *G. teuchis* has ITS sequences which are distinct from *G. thymalli* and *G. salaris*, but morphological variation falls within the total range reported for *G. salaris* (see Mo, 1991a, b, c), and few qualitative differences are seen in the hamuli and ventral bar (Cunningham *et al*., 2001). There are small differences in the shape of the marginal hook sickle. In the original description, *G. teuchis* was thought to have evolved in an "Iberian refugium", but this species probably evolved in the Channel River watershed, as it is distributed along the Spanish and French Atlantic seaboards. It has been

reported from farmed rainbow trout in Scotland (Cunningham *et al*., 2001) and from wild brown trout in Denmark (Buchmann *et al*., 2000), but it is difficult to get an idea of the original range of this taxon as it is common on rainbow trout and may have been redistributed with this host. *G. teuchis* is not recorded in Norway and appears to be absent from wild salmon in the UK but this may be because salmon populations in southern rivers are so small, and parr generations do not overlap. Extermination of salmon from rivers around the North Sea in the 19th century also complicates any discussion of recreating the original range of *G. teuchis*.

(ii) *G. bohemicus* from rainbow trout (type host) and brook trout in a trout farm in South Bohemia, Czech Republic, was considered (Ergens, 1992a) most closely related to *G. thymalli* and *G. magnus*. Both hosts were introduced to Europe from America and the parasite has neither been recorded from brown trout in Central Europe nor from any host in North America. The identity of this taxon was a mystery until Lindenstrøm *et al.* (2003a) described the "*G. salaris* variant" (Gx), with slight morphological variation in the marginal hook and a predilection for infecting rainbow trout (Lindenstrøm *et al*., 2000). The ITS rDNA sequence of this rainbow trout variant differed by just three bases from that of *G. salaris sensu stricto,* and one of these bases exhibited intra- and inter-individual polymorphism (Lindenstrøm *et al.*, 2003a, b). Lindenstrøm *et al.* (2003a, b) argued against the rainbow trout variant being *G. bohemicus*; however, perhaps this reverses the burden of proof, and it should be demonstrated that *G. bohemicus* described from a handful of specimens collected during faunistic work, is actually distinct from the Lindenstrøm rainbow trout variant. We propose that, until such evidence is forthcoming, *G. bohemicus* should be regarded as the first record of the Lindenstrøm *et al.* (2003a, b) "Gx" rainbow trout variant. Parsimony would suggest that this is safest course until positive evidence is found to discriminate *G. bohemicus* from the "Gx" variant.

(iii) *G. thymalli,* described from less than a dozen specimens from Slovakian grayling (Žitňan, 1960), was considered to be most

similar to *G. salaris* from salmon. *G. thymalli* can be distinguished from *G. salaris* and *G. teuchis*, being larger than either (Shinn *et al*., 2004), and yet the ribosomal sequences of the V4 and ITS regions of the rRNA gene array are identical to *G. salaris* (see Cunningham, 1997), suggesting that these taxa are more closely related than are any other *Gyrodactylus* species (Cunningham, 2002). The two forms were originally considered to have distinct IGS haplotypes, although they share many of the individual repeat units which make up the haplotypes (Sterud *et al.*, 2002). Recent research, however, shows that this interpretation is not correct (Hansen *et al*., 2005, 2006). The recently sequenced β-tubulin gene also shows differences between them (Collins *et al*., 2004a). These observations, in addition to major differences in pathogenicity and host specificity suggest that these are valid species (Sterud *et al*., 2002). *G. thymalli* from *Thymallus* spp. is widespread in Eurasia, including Kamchatka (Ergens, 1983), Poland (Hansen *et al.*, 2007), Slovakia (Žitňan, 1960; Hansen *et al.*, 2007), Britain (Denham and Longshaw, 1999; Hansen *et al.*, 2007), Norway (Hansen *et al*., 2003, 2006), Finland and the Kola Peninsula (Meinilä *et al*., 2004). The grayling is widespread in NW Europe and was certainly restricted to different ice lake refugia during the LGM (Koskinen *et al*., 2000, 2002; Weiss *et al*., 2002) giving rise to at least four major mtDNA lineages. There is however evidence of extensive mixing in contact zones between drainages (Gum *et al*., 2005) and, as with other salmonids, the distribution has been extensively modified by Man.

Recent work on *G. thymalli* has been based on samples from the Eastern Baltic, particularly Lake Kitka and the River Oulanka system (draining to the White Sea), the River Livojoki (Baltic drainage), and from Pjalma (Lake Onega) in Karelia (see Meinilä *et al*., 2004). Populations from the White Sea drainage had mitochondrial haplotypes belonging to quite different clades to those from the Baltic, again emphasising the importance of different glacial refugia. Meinilä *et al*. (2004) calibrated the divergence between White and Baltic Sea forms to be ~100 000 years, because, as noted above, an earlier

divergence would not have retained geographical separation following the Eemian interglacial. Meinilä *et al.* (2004) also analysed mitochondrial haplotypes collected by Hansen *et al.* (2003) from salmon, grayling and rainbow trout in Norway and Sweden, providing a unique opportunity to examine short-term evolution of *G. salaris* and *G. thymalli* in Scandinavia. These phylogenies (Hansen *et al.*, 2003; Meinilä *et al.*, 2004) may suggest that *G. salaris* on salmon is derived from *G. thymalli*. However, the data clearly show that *G. salaris* was introduced into Norway on several occasions, that it has been dispersed widely by anthropogenic means and that it is polyphyletic. Meinilä *et al.* (2004) consider *G. thymalli* to be a junior synonym of *G. salaris* and that all forms of this taxon from rainbow trout, salmon or grayling (named the *G. salaris* complex) should be referred to as the *G. salaris* cluster or *G. salaris sensu lato*. This mitochondrial evidence is not the full story, and ignores differences in nuclear sequencing data (Sterud *et al.*, 2002; Cunningham *et al.*, 2003; Collins *et al.*, 2004a; Hansen *et al.*, 2006), morphology (McHugh *et al.*, 2000; Shinn *et al.*, 2004) and host preference (Soleng and Bakke, 2001b; Bakke *et al.*, 2002; Sterud *et al.*, 2002) between the forms from grayling and salmon. It also oversimplifies the taxonomic situation. For example, Shulman *et al.* (2005) noted an apparently cold-adapted strain of *G. salaris* from the River Lhizma, in Karelia, while Olstad *et al.* (unpublished) have identified a parasite population from grayling which is morphologically intermediate between *G. salaris* and *G. thymalli.* The former originates from the apparent centre of diversity of *G. salaris* (see Meinilä *et al.*, 2004), but the latter, from Norway, is more problematical. This implies a very rapid host shift and morphological divergence if this clade has indeed originated from the *G. salaris* clades imported into Norway during the 1970s. A final reason for not changing the nomenclatural status quo is that in the case of *G. salaris*/*G. thymalli*, a pure DNA-based taxonomy may result in a species definition which fails to reflect the significant differences in host response and pathology and will scarcely be accepted by the fish management authorities involved in the recognition of such a highly pathogenic notifiable fish disease of European concern (Olson and Tkach, 2005).

There is also great confusion over the ''rainbow trout variant'' of *G. salaris* from Norway described by Mo (1991a) infecting Lierelva and Drammenselva salmon. *G. salaris sensu stricto* (i.e. the salmon infecting forms) is polyphyletic (Meinilä *et al*., 2004), made up of a clade (clade I) which switched to salmon from grayling in North Fennoscandia and a clade (clade II) which switched to salmon and rainbow trout elsewhere, which Meinilä *et al*. (2004) consider genetically relatively invariant. This ignores the fact that clade II contains forms which can be distinguished morphologically (Mo, 1991a); the ''Gx'' form of Lindenstrøm *et al.* (2003a, b) and the variant of Jørgensen *et al*. (2006) are not identical to the ''rainbow trout variant'' of *G. salaris* described by Mo (1991a). More informative evidence about the nature of rainbow trout infecting forms is derived from the IGS data of Sterud *et al*. (2002) and Cunningham *et al.* (2003). This locus, in *G. salaris*, contains two repeat units, containing in turn a series of subrepeats. The pattern of repeats is fairly constrained in *G. salaris* from salmon, as would be expected if, as suggested by Meinilä *et al*. (2004), *G. salaris* is derived from such a restricted origin within clade III of *G. thymalli. G. thymalli* overall showed greater diversity in IGS repeat units than Norwegian *G. salaris* (see Cunningham *et al*., 2003; Hansen *et al*., 2006), but sampling of *G. thymalli* is relatively restricted geographically. Swedish populations of *G. salaris* contain more diversity of IGS haplotypes than Norwegian forms and rival the haplotype diversity seen with limited sampling of *G. thymalli* (see Hansen *et al*., 2006). Parasites from rainbow trout reveal surprising diversity in IGS haplotypes, exceeding that of *G. salaris* from Norwegian salmon populations or *G. thymalli* from grayling. The Sterud *et al*. (2002) study showed that IGS haplotypes from the rainbow trout variant resembles in part repeat variants normally found in salmon-infecting *G. salaris* (repeat region 1), alongside variants normally found in grayling-infecting *G. thymalli* (repeat region 1). Given this diversity, it is impossible to reconcile the rainbow trout variant with the restricted mitochondrial diversity noted by Meinilä *et al*. (2004). The lack of stability in the IGS of the rainbow trout variant is intriguing. Cunningham *et al.* (2003) speculate that the greater diversity of IGS repeats may be due

to selection against many repeat types in either salmon-infecting or grayling-infecting forms. This again seems quite at odds with Meinilä *et al.*'s (2004) hypothesis that the rainbow trout variant is represented by a highly restricted subset of mitochondrial haplotypes, and therefore derived from a very restricted clade of *G. thymalli*. The IGS locus is under the constraint of concerted evolution in a similar way as the other ribosomal gene loci; it might be expected therefore that the variation within the rainbow trout variant will eventually stabilise in a relatively homogenous form. The lack of agreement between mtCOI (Hansen *et al.*, 2003; Meinilä *et al.*, 2004) and IGS phylogenies (Sterud *et al.*, 2002; Cunningham *et al.*, 2003; Hansen *et al.*, 2006) may not be intractable. Mitochondrial haplotypes spread through a population at different rates to nuclear genomic markers because of their maternal mode of transmission. This will be particularly marked in *Gyrodactylus*, with its predominantly parthenogenetic viviparous reproductive strategy. This may go some way to explaining the conflict over the status of the southern Norwegian populations of salmon-infecting *G. salaris*, one of which (River Lierelva) has been the principal strain used in experimental studies of the species (Bakke *et al.*, 2002) and is linked by mtCOI haplotype (Hansen *et al.*, 2003; Meinilä *et al.*, 2004) to the rainbow trout infecting forms. Cunningham *et al.* (2003), on the other hand, using IGS nuclear markers, place the Lierelva strain with the majority of other *G. salaris* strains from salmon. AFLP patterns, also based on nuclear markers (Cable *et al.*, unpublished) link Lierelva strain with the other salmon-infecting forms. There is however a history of introduction of the rainbow trout variant into the area immediately adjacent to the Lierelva/Drammenselva watercourses. The rainbow trout variant (Mo, 1987, 1991a) originally recovered from salmon in both rivers in 1987 might have originated from infected commercial hatcheries in Lake Tyrifjorden which imported salmonids (both rainbow trout and salmon) from Sweden (Mo, 1991a; B.O. Johnsen, personal communication). It has also been identified on escaped rainbow trout in Lake Tyrifjorden, from where it could have spread into both Drammenselva and Lierelva (probably via Holsfjorden; cf. Johnsen *et al.*, 1999). The same parasite was also found in eight rainbow trout hatcheries around Lake Tyrifjorden which may also

represent the origin of the river infections. There has therefore been ample opportunity for hybridisation and introgression of the rainbow trout mitochondrial haplotype into *G. salaris* populations in South East Norway.

Currently, we would have to conclude that the *G. salaris*/*G. thymalli* evolutionary scenario remains highly complex and further research is needed to understand the patchwork of strains of the parasite currently found in Scandinavia. An interesting recent discovery has been of a resident Arctic charr population in lake Pålsbufjorden (Buskerud, County, southern Norway, draining into the commercially important salmon river Numedalslågen), which supports a persistent *G. salaris* infection with a mitochondrial haplotype similar to that of Drammenselva parasites (Robertsen *et al.*, 2006, 2007b). This is the first observation of *G. salaris* maintaining a viable population on charr in the absence of any other susceptible salmonid species. This Arctic charr strain is of limited infectivity to salmon (Olstad *et al.*, 2005, 2007) and may have originated following another host shift from a rainbow trout variant. In Denmark, a low pathogenic strain of *G. salaris* to salmon is reported based on experimental laboratory tests but originally from rainbow trout which experienced high parasite intensities (Jørgensen *et al.*, 2006). All sequenced ITS clones of this Danish strain revealed only one single base substitution when compared to all other known species and strains of *Gyrodactylus* including Gx of Lindenstrøm *et al.* (2003a) and COI data demonstrated it to be closely related to one of the rainbow trout forms in Norway. Mitochondrial COI haplotypes cannot so far be linked with virulence. We must conclude that strains of the parasite were introduced into Norway from elsewhere in Fennoscandia, probably in the 1970s, and distributed widely by Man (Hansen *et al.*, 2003; Robertsen *et al.*, 2006, 2007a). This is clear from the very wide distribution of *G. salaris* clade I haplotypes throughout Norway and in the case of clade II haplotypes throughout Europe (Meinilä *et al.*, 2004; clade III according to Hansen *et al.*, 2003). It is a truism that the global threat to Atlantic salmon posed by strains of salmon-infecting *G. salaris* originating within Europe is not great. Further range extension will probably be by the rainbow trout form, which is not especially pathogenic to rainbow trout, and therefore easily

overlooked, but still pathogenic to salmon. Secondly, we can conclude that the primary evolutionary stimulus for the pathogenic *G. salaris* has been these host switches from grayling to salmon. A very recent origin for these different salmon-infecting clades cannot be ruled out. Numerous salmon, grayling, rainbow trout and charr infecting forms have since arisen, and have been distributed extensively by Man. Introgression of strains have probably been widespread, and the role of the rainbow trout in strain hybridisation and introgression may have been paramount.

6.6. Terminology of the *G. salaris* Species Complex

G. salaris and *G. thymalli* are almost identical at a molecular level and there is no support from mtDNA (CO1) sequences for monophyly of all *G. salaris* and *G. thymalli* haplotypes (Hansen *et al.*, 2003, unpublished data). These taxa therefore probably represent a case of incipient speciation with the sibling taxa representing either a semispecies (one or two polytypic species) or a superspecies (several sibling species; see Mallet, 2001), reproductively isolated by host preference. If we follow Meinilä *et al.* (2004), the relationship between *G. salaris* and *G. thymalli* is so close that they are merely host races of the same species, and both *G. thymalli* Žitňan, 1960 and probably *G. bohemicus* Ergens, 1992 should be treated as junior synonyms. Inclusion of the rainbow trout variant Gx with ITS differing to *G. salaris* at three positions (according to Lindenstrøm *et al.*, 2003a) and two other variants differing in one base substitution (Jørgensen *et al.*, 2006; Robertsen *et al.*, 2006, 2007a) to all other known species and strains of *Gyrodactylus* within *G. salaris* would make the latter polyphyletic as it appears to have evolved independently from different clades within *G. thymalli*. We appreciate that this is a difficult area, and that the names of these currently speciating forms must remain in flux. However, we propose to maintain *G. salaris*, *G. thymalli* and *G. bohemicus* as separate species until further clarification using new molecular markers, knowledge of the impact of different microenvironment factors on *Gyrodactylus* morphology and hybridisation experiments. It is most important that

practice is entirely transparent when depositing specimens or sequences (unfortunately not currently the case) so that future workers can interpret the early 21st century situation in the light of their own theoretical paradigms.

Part 3. Gyrodactylid synecology

7. EPIDEMIOLOGICAL MODELS: BRIDGING THE GAP BETWEEN MICRO- AND MACROPARASITES

The value of gyrodactylids as epidemiological models has been recognised for many years with some of the most elegant studies of host–parasite dynamics being conducted by Scott and co-workers in the 1980s using *G. turnbulli* (at the time called *G. bullatarudis*, see Harris, 1986; Richards and Chubb, 1995; Richards *et al*., 2000) on guppies (Scott, 1982, 1985a, b, 1987; Scott and Anderson, 1984; Scott and Nokes, 1984; Scott and Robinson, 1984; Harris, 1988, 1989; Leberg and Vrijenhoek, 1994; Richards and Chubb, 1996, 1998; Cable *et al*., 2002a). Gyrodactylid reproduction is characterised by a short generation time and completion of the life cycle *in situ* without the need for transmission. The period of infection is also short relative to host life span, due to the acquired host response (Section 8.1). Gyrodactylids bridge the gap between micro- and macroparasites and can potentially model other microparasites of animals and humans. A number of statistical and computer models have also been designed to risk assess the spread of gyrodactylid infections to new rivers (Anonymous, 1996; Paisley *et al*., 1999; Brun and Høgåsen, 2003; Høgåsen and Brun, 2003; Peeler and Thrush, 2004; Peeler *et al*., 2004; Jansen *et al*., 2005, 2007). Host age-structured population models have been used to assess the impact of disease-induced mortality at different stages of the life cycle of salmonids (de Clers, 1993). However, the full potential of gyrodactylid population models has yet to be realised due to the poorly known action of several micro- and macroenvironmental factors, and it remains impossible to predict the likely outcome of gyrodactylid introductions into novel watersheds. In particular, because individual fecundity is so low (normally less than

three daughters per worm), the statistical vagaries of survivorship and mortality make the outcomes of individual infections very difficult to predict. Gyrodactylids can be identified and counted long before their pathological effects are apparent, and so accurate, quantitative data can be collected during the entire infection of a single host (Jansen and Bakke, 1993a, b; van Oosterhout *et al*., 2003). The guppy–gyrodactylid interaction is particularly tractable, and is already a paradigm for studies of host–parasite evolution (López, 1998) because the ecology of both *G. turnbulli* (e.g. Scott and Anderson, 1984; Scott, 1985; Harris, 1988, 1989; Cable *et al*., 2002a) and guppies (Reznick and Endler, 1982; Magurran and Phillip, 2001; van Oosterhout *et al*., 2003) is so well understood. The wealth of information on this system provides an invaluable database with which to test the impact of various host variables on parasite biology (e.g. Cable and van Oosterhout, submitted for publication).

8. HOST IMMUNITY AND PARASITE PATHOGENICITY

8.1. Host Immune Responses

The subject of immunity of fish to monogenean ectoparasites has been reviewed in depth recently (Buchmann, 1999, 2000; Buchmann *et al*., 2001, 2003; Buchmann and Lindenstrøm, 2002) and will only be touched upon here. Understanding immune responses against gyrodactylids was long hampered by the misconceptions that fish and other poikilothermic lower vertebrates possess a primitive and unsophisticated immune system, and that ectoparasites washed by the external milieu would not be significantly affected by blood-borne immune responses. These misconceptions have now been corrected, but there are major differences between the immune response to gyrodactylids and that to other ectoparasitic helminths, indeed that to other ectoparasitic monogeneans. Furthermore, with over 400 *Gyrodactylus* species described, it would be surprising if there were not a range of response mechanisms operating against them.

The first study normally cited as identifying a response against gyrodactylids is that of Lester (1972), who noted shedding of mucoid

material by *Gasterosteus aculeatus* infected with *G. alexanderi*. Parasites became physically entangled within this insoluble slough and became detached from the surface of the fish. They survived dislodgement and were able to reattach to new hosts. Lester and Adams (1974a, b) went on to link this response to the immunological status of the hosts, but this non-specific, physical mechanism of elimination became a paradigm of gyrodactylid immunity for the next 25 years and more subtle involvement of the immune system was not seriously considered until the late 1990s. This was unfortunate because Schechmeister *et al.* (1962) had already shown that irradiated goldfish were far more susceptible to *Gyrodactylus* sp. than un-irradiated controls, suggesting strongly that an intact immune system is needed for a fully functional response against gyrodactylids.

Almost all gyrodactylid–vertebrate interactions which have been examined show evidence of parasite population limitation and decline. Apart from the *G. alexanderi*-stickleback system (Lester and Adams, 1974a), this has also been noted for *G. turnbulli* on guppies (Scott and Robinson, 1984; Madhavi and Anderson, 1985; Harris, 1988; van Oosterhout *et al.*, 2003; Cable and van Oosterhout, 2007), *G. colemanensis* and *G. derjavini* on rainbow trout (Cusack, 1986; Buchmann and Uldal, 1997, respectively), *G. stellatus* on English sole (Kamiso and Olson, 1986), *G. kattharineri* on carp (Gelnar, 1987a, b), *G. gobiensis* on gudgeon (Gelnar, 1987c), and *G. salaris* on rainbow trout, lake trout, brook trout and Arctic charr (Bakke *et al.*, 1991b, 1992b, c, 1996). This phenomenon is also observed in other gyrodactylid genera, including *Gyrdicotylus gallieni* on clawed toads (Harris and Tinsley, 1987; Jackson and Tinsley, 1994). It has not been noted in *Isancistrum subulatae* on the squid *Alloteuthis subulata* (see Llewellyn, 1984). Typically, on an individual fish, gyrodactylid populations initially increase until the rate of population growth slows and declines, parasites are lost and eventually the infection is eliminated. A high proportion of fish may die as a result of infection. The macroenvironment may have a relatively minor effect on this process (but see Section 9) except to modify timing; responses have been noted in marine and freshwater fish, both tropical and cold water. The response can occur at low, potentially immunosuppressive temperatures (Bly and Clem, 1992) and has therefore been regarded as

distinct from a classical immune reaction. However, it shows inter-host heterogeneity (Bakke *et al.*, 2002; van Oosterhout *et al.*, 2003), and memory (Scott and Robinson, 1984; Scott, 1985a, b; Richards and Chubb, 1996; Bakke *et al.*, 2002; Cable and van Oosterhout, 2007), requires an intact immune system (Schechmeister *et al.*, 1962) and can be modulated by immunomodulatory hormones such as cortisol and testosterone (Buchmann, 1997a; Lindenstrøm and Buchmann, 1998; Harris *et al.*, 2000; Nielsen and Buchmann, 2003), all characteristics of an acquired immune response, usually mediated via antibodies. Although antibodies have been detected in natural infections of blood-feeding monogeneans such as *Heterobothrium okamotoi* (see Wang *et al.*, 1997), *Pseudodactylogyrus* (see Mazzanti *et al.*, 1999; Monni and Cognetti-Varriale, 2002) and *Discocotyle sagittata* (see Rubio-Godoy *et al.*, 2004), they have never been demonstrated in gyrodactylid infections. Buchmann (1998a, b, c) failed to find antibodies to *G. derjavini* in rainbow trout and we have similarly failed to find antibodies to *G. salaris* in either mucus or blood of infected salmon using ELISA (unpublished). Negative results are seldom published, but the absence of antibodies in these interactions is of considerable interest.

In the absence of specific antibody responses, inducible non-specific responses may play a part in limiting gyrodactylid population growth. Many species, including *G. derjavini* and *G. salaris*, are extraordinarily sensitive to host complement. Buchmann (1998a) noted binding of complement C3a to *G. derjavini* tegument and Harris *et al.* (1998) showed that living *G. salaris* are killed at physiologically relevant titres as low as 1:200, considerably lower than that needed to kill other parasites (Fishelson, 1989). Complement acts via the alternate (antibody-independent) pathway and pre-incubation in blood from fish exposed to high infections of *G. salaris* (and therefore most likely to possess antibodies against the parasite) failed to enhance killing, further suggesting that antibodies are not involved (Harris *et al.*, 1998). Killing is presumably so efficient because *G. salaris* in a freshwater environment are osmotically stressed when the integrity of the tegument is disrupted by membrane attack complexes (MACs). However, Moore *et al.* (1994) also found a complement-like factor very effective against *G. stellatus* from winter flounder, although in

this case the parasites were maintained in salt water. Buchmann (1998a, b, c) noted the response was not species-specific, an observation confirmed by Harris *et al.* (1998) who noted that salmon complement lysed *G. decorus* from roach, while trout complement lysed *G. salaris*. It would be wrong to suggest that complement is a general mechanism for killing gyrodactylids, particularly as we have noted greater resistance to complement in *G. turnbulli* from guppies (unpublished), and J.A. Jackson (personal communication) has indicated that *Gyrdicotylus* is also relatively resistant. However, we have noted a correlation between complement levels during the course of *G. salaris* infections on salmon and the susceptibility of the strain concerned, with resistant Neva salmon having a C3 complement titre in mucus some ~25% higher than the highly susceptible Alta strain (Bakke *et al.*, 2002). The level of complement, at least in the serum of resistant Neva fish, was also inducible in response to *G. salaris* infection, being some 20% higher 45 days after infection than in naïve fishes (Bakke *et al.*, 2002). These observations do suggest a role for complement in eliminating gyrodactylid infections of salmonids, but clearly there is much further work to be done to confirm this. In particular, salmonids express multiple C3 isoforms and have multiple genes encoding factor B (Sunyer, 2005). To date no functional studies of these different complement isoforms on gyrodactylids have been carried out, although differences in susceptibility to gyrodactylids could be related to complement diversity expressed by particular salmonids (Lindenstrøm, 2005).

As complement is so effective in killing gyrodactylids, a model based on alternate pathway activation can be suggested that does not require additional effectors to explain immunity. Alternative pathway complement activation occurs in response to carbohydrate moieties on the target surface and such molecules (particularly mannose-rich glycoproteins) occur on the gyrodactylid tegument (Buchmann, 2001). Gyrodactylids are always exposed to complement from host mucus and the accumulation of MACs on the tegument must limit their survival. Up to a point, complement-mediated tegument damage can be overcome by repair and this may partially explain the high density of secretory vesicles (Kritsky and Kruidenier, 1976; Bakke *et al.*, 2006) within the tegument. It may also explain the curious

phenomenon that detached gyrodactylids often display an inverted mortality curve, with higher age-specific mortality shortly after detachment. This is very clear in Harris *et al.* (1998; Figure 1), which shows percentage mortality of controls not exposed to complement and of parasites exposed to 1:1000 serum dilution, highest in the first 30 min after beginning the experiment. It is also apparent in *G. gasterostei* (see Cable *et al.*, 2002b), in which mortality increases in the first 10 h after detachment in parasites removed from sticklebacks, but not in the first 10 h of life of parasites born *in vitro*. This suggests mortality due to damage (from MACs or some other mechanism) present when the parasites are first detached from the fish, which is minimised after 10 h, either through denaturation of complement factors or because tegument turnover has removed them from the parasite surface. As an infection builds on a fish, complement titre and hence MAC damage may increase, either killing parasites directly or forcing them to migrate into the environment (Lester and Adams, 1974a, b). As time goes by this effect becomes more marked, until natality is less than mortality and emigration, and the parasite population begins to decline. On unsuitable hosts, the balance between the ability of the parasite to exploit the fish nutritionally and the rate at which complement complexes form is shifted in favour of complement attack, and the parasites fail to thrive.

Although this mechanism can explain observed patterns of gyrodactylid-host population dynamics, it is far from a complete explanation. In the first place, alternate-pathway activated complement is a non-specific, universal response whereas the immune response to gyrodactylids clearly is very specific, but much reduced in some fish strains which nevertheless do possess complement (Bakke *et al.*, 1999). Most importantly, a model based on complement activation fails to explain the evidence for modulation of the response by hormonal status, the evidence for memory, or the results now being obtained using microarray approaches. The response to gyrodactylids can be modulated by the physiological state of the host, particularly by its hormonal status. Testosterone suppresses the response to gyrodactylids (Buchmann, 1997a) and hormonal interventions which mimic stress have the same effect (Lindenstrøm and Buchmann, 1998; Harris *et al.*, 2000). Using hydrocortisone acetate implants,

Harris *et al.* (2000) found that immunosuppression made previously innately resistant brook trout, Arctic charr and brown trout relatively susceptible to *G. salaris*, suggesting that host specificity involves factors which can be modulated in cortisol-treated hosts. However, this treatment did not entirely suppress the response, although it is unlikely that hydrocortisone release from the implants had ceased. There may therefore be a second, late, mechanism, which only becomes effective after several weeks. Natural elevation of cortisol concentrations has been observed in fish infected with *G. derjavini* (see Lindenstrøm and Buchmann, 1998, 2000) and natural stressors have frequently been noted as triggering gyrodactylid population growth. This is perhaps most obvious in *Macrogyrodactylus polypteri* infections on *Polypterus* (see Khalil, 1970; Harris, unpublished observations). If true, then elevation of cortisol by parasite-induced stress could lead to responses against them becoming less efficient, leading to positive feedback and exponential parasite population growth.

A range of other mechanisms may be involved in immune responses to gyrodactylids. The tegumental carbohydrate moieties may be the target of other host defence molecules, particularly lectins (Buchmann, 2001; Jørndrup and Buchmann, 2005). Lysozyme was identified as a potential defence molecule in the early 1980s (Ingram, 1980), but Buchmann and Uldal (1997) were unable to find convincing evidence for its involvement in the response against *G. derjavini*. Similarly, C-reactive protein, pentraxins and low molecular weight anti-microbial peptides have all been suggested as protective against monogeneans including gyrodactylids, but as yet without evidence (Buchmann, 1999, 2000, 2001).

More recent understanding has come from work using microarray technology to dissect the signalling pathways involved in the early immune response to gyrodactylids. Lindenstrøm *et al.* (2004) showed increased transcription of the pro-inflammatory cytokine tumour necrosis factor (TNF)-α1, but not TNF-α2, 8 days post-infection. No increase was noted in secondary infections. Interleukin (IL)-8 transcription did not increase in either primary or secondary infections, but elevation of transforming growth factor (TGF)-β transcripts in secondary infections was dramatic; this transcript was hardly

detectable in uninfected fish or in primary infections, but was substantially elevated by 8 days post infection in secondary infections. The increase appears to have begun as early as 4 days post-secondary infection. Induced nitric oxide synthase (iNOS) was also strongly elevated in primary and secondary infections, while cyclo-oxygenase-2 (COX-2) showed significantly elevated transcription 8 days post-primary infection. In a similar study, Lindenstrøm *et al.* (2003b) demonstrated consistent elevation of two isoforms of IL-1β in primary infections, confirming earlier immunochemical results (Buchmann and Bresciani, 1998, 1999). Lindenstrøm *et al.* (2003b) suggest that IL-1β may activate mucus secretion, as the homologous molecule in mammals is a mucus secretagogue. If this were so, then activation of this cytokine would probably be central to the response. Lindenstrøm *et al.* (2006) have extended this work, suggesting that resistant salmon moderate their mucous cell proliferative response, rendering the microhabitat less suitable for *G. salaris*. Susceptible fish on the other hand are unable to limit their response to pro-inflammatory cytokines such as IL-1β, leading to hypersecretion of mucus and a more suitable host microenvironment. Matejusová *et al.* (2006) also record elevated expression of the myeloid leukaemia differentiation protein (Mcl-1) homologue and the opioid growth factor receptor protein (OGFr) homologue in susceptible salmon; both of which may be linked to epidermal regeneration processes, and in the case of Mcl-1 at least, may be induced by IL-1 produced early in the infection (Matejusová *et al.*, 2006). Later in the infection, a FIP-2-like gene (14 kDa interacting protein) is also induced in susceptible salmon (Collins *et al.*, 2007). The elevation of iNOS and COX-2 is interesting as they represent effector arms of the non-specific immune response, responsible for the release of NO and prostaglandins, respectively. It is difficult to see how such reagents could affect the external surface of a gyrodactylid monogenean, but such metabolites probably have major effects when ingested; Cable *et al.* (2002b) showed the ingestion of intact host cells and organelles, and the action of such metabolites within the gut is likely to be harmful.

Gyrodactylid infections lead to gross changes in epidermal histology which are clearly related to the immune response. Sterud *et al.*

(1998) showed that in *G. salaris* infections, mucous cell density declined in susceptible salmon of the Lierelva stock, but was not affected in resistant brook trout. Similar results were also noted in the case of *G. colemanensis* infections of salmonids (Wells and Cone, 1990). This could relate to differences in the dynamics of epithelial grazing by gyrodactylids, but the evidence above (Lindenstrøm *et al.*, 2003b, 2006; Collins *et al.*, 2007; Matejusová *et al.*, 2006), of specific changes in the expression of genes involved in mucous cell differentiation during infections suggest that this is a specific response. Appleby *et al.* (1997) observed no changes in mucous cell density in *G. salaris*-infected salmon of the relative tolerant Batnfjordelva stock but the epidermis was thicker and contained more cell layers than in uninfected fish. Following this, Buchmann and Uldal (1997) showed that the susceptibility of salmonids to *G. derjavini* was negatively correlated with mucous cell density (the most resistant hosts had the highest mucous cell density) and that parasites aggregated in sites with a poor supply of mucous cells, such as the cornea (Buchmann and Bresciani, 1998). Up to 30% of parasites may aggregate on corneas, which represent less than 1% of the total fish surface (Olafsdóttir *et al.*, 2003), suggesting a significant advantage to such aggregation. Mucous cell density can be manipulated by dexamethasone treatment in a manner correlated with the increased susceptibility of treated fish to *G. derjavini* (see Olafsdóttir and Buchmann, 2004). Heavily infected fish developed a higher density of mucous cells than sham-infected controls, suggesting that, while the parasites induce mucous cell production, dexamethasone inhibits their discharge so that the cells accumulate in the epidermis. In this case, however, it should be borne in mind that the host used, Atlantic salmon, is normally refractive to infection with *G. derjavini* and so this is a somewhat artificial situation. These findings are interesting in that they demonstrate the impact of gyrodactylids on the epithelial architecture of the fish, and that these changes are in some way involved in the host response. The parallel between the changes in epithelial goblet mucous cell density (e.g. Sterud *et al.*, 1998) and processes in the intestine during the mucus-mediated expulsion of gastro-intestinal (GI) nematodes (Ishikawa, 1994; Ishikawa *et al.*, 1995) has been noted previously (e.g. Sterud *et al.*, 1998). However,

similarities with the expulsion of GI nematodes go further. Cliffe *et al.* (2005) showed that changes in intestinal architecture and epithelial turnover have a major role in the elimination of *Trichuris muris*, an effect mediated via immunologically relevant cytokines. If a similar situation can be demonstrated in *Gyrodactylus*, there is potential for linking the "physical" explanations of expulsion (e.g. Lester, 1972) with the evidence for acquired immunity (see above), and the more subtle observations on signalling pathways of Lindenstrøm *et al.* (2004) and Matejusová *et al.* (2006).

8.2. The Heritability of Disease Resistance

Disease resistance in fish, as in most animals (May and Anderson, 1983; Chevassus and Dorson, 1990), is strongly heritable. Pathogens exert selective pressure locally (Lively and Dybdahl, 2000) and parasitism may be an important determinant of local adaptation in host metapopulations. Because disease resistance is physiologically costly, it will be relaxed if a pathogen disappears from a host population, and will not evolve or be sustained in the absence of a pathogen. Significant genetic variation has been found in several immunological parameters in Atlantic salmon (Fevolden *et al.*, 1992; Fjalestad *et al.*, 1996; see Bakke and Harris, 1998) and in overall resistance to a variety of diseases (see Bakke *et al.*, 1999).

Closely related salmonids exhibit remarkable differences in susceptibility to infection with *G. salaris* and *G. derjavini* (see Section 8) ranging from hosts that serve as short-term transport vectors to those that are entirely susceptible to infection without any obvious immune response (Bakke, 1991; Buchmann and Uldal, 1997; Bakke *et al.*, 2002). This range of response can even be demonstrated experimentally between individuals of the same strain (Bakke *et al.*, 1991b, 1996, 1999, 2002; van Oosterhout *et al.*, 2003; Cable and van Oosterhout, 2007). Genetically determined resistance was first demonstrated by Madhavi and Anderson (1985), who selected guppies for resistance to *G. turnbulli* over a 2-year period. By selecting from the most resistant and most susceptible fishes, they were able to breed susceptible and resistant lines and demonstrate heritability of resistance. F1 crosses

between susceptible and resistant fishes had intermediate resistance. Unfortunately, these experiments were not pursued. More recently, the heritability of resistance to *Gyrodactylus* within and between strains of Atlantic salmon subject to different breeding regimes has been re-examined for correlations with genetic markers associated with ectoparasitic resistance (Collins *et al*., 2003, 2004b, 2005). The exact genetic basis of resistance is being investigated using a quantitiative trait loci (QTL) screening approach (Gilbey, 2004; Gilbey *et al*., 2006). In F2 backcrosses of Baltic and Scottish salmon, 10 genomic regions were identified associated with heterogeneity in resistance to *G. salaris*. However, mapping these regions with QTLs could account for only 27% of the total heritability of resistance, suggesting that there are additional, as yet unidentified, regions of the genome that are involved in resistance. Sarginson *et al*. (2004) also reported on the differential expression of immune-related genes in a backcross between susceptible River Conon (Scottish) and resistant River Neva (Russian) salmon. This allowed the identification of 11 immune-related genes with significantly higher expression in susceptible fish.

Infections of hybrids can be a useful way to characterise the nature of genetic resistance. Bakke *et al*. (1999) used salmon X brown trout hybrids to test for susceptibility to two host-specific gyrodactylids, the salmon-specific *G. salaris* and the trout-specific *G. derjavini*. The patterns of susceptibility were complex, including susceptible hosts supporting exponential parasite population growth, responders that showed an increase followed by a decline in infection, and resistant fish on which infections failed to grow, but basically hybrids had susceptibility intermediate to that of their parents. While pure-bred *S. salar* included both highly susceptible and responding individuals which failed to eliminate *G. salaris* infection, pure-bred *S. trutta* were entirely resistant to this species. Pure-bred *S. trutta* ranged in susceptibility to *G. derjavini*; some possessed innate resistance while others were initially susceptible but then mounted a host response, usually eliminating the parasite. Pure-bred *S. salar*, on the other hand, were all susceptible to *G. derjavini*, but population growth rates were reduced and a host response frequently eliminated infections. The abundance of both gyrodactylids was lower on hybrids than on

their respective normal hosts, and a parental sire- and dam-influence on hybrid resistance was observed. When the sire was *S. salar*, the susceptibility of hybrids to *G. salaris* was similar to that of pure *S. trutta*; when the dam was *S. salar* innately resistant, intermediately susceptible and responding individuals were present. In the case of *G. derjavini*, when the sire was *S. trutta*, infections on hybrids were similar to those on pure *S. salar*; when the dam was *S. trutta*, an increased susceptibility was observed. This experiment has provided a fascinating insight into the processes of susceptibility and specificity, and should be followed up in other systems with shorter generation times which are easier to maintain in the laboratory.

8.3. Parasite Pathogenicity

Although gyrodactylids are potentially highly pathogenic (Bauer, 1988; Bakke *et al*., 2002; Jalali *et al*., 2005), little is known about resulting disease in fish populations (Cone and Odense, 1984; van Oosterhout *et al.*, in press b). At the individual level, feeding and attachment wounds destroy the osmotic integrity of the epidermis and encourage potential secondary infections (Snieszko and Bullock, 1968; Cone and Odense, 1984; Bakke *et al.*, 2006; see Figures 10, 13 and 23). *Saprolegnia* infections are occasionally associated with *G. salaris*-induced mortality in salmon parr (unpublished observations). However, Busch *et al*. (2003), who emphasised the pathogenicity of *G. derjavini* on rainbow trout found only weak evidence that virulence is enhanced by concomitant infection with the bacterium *Flavobacterium psychrophilum*. The extent to which different *Gyrodactylus* species are pathogenic is variable. Cone and Odense (1984) who studied the attachment site pathology of five species of *Gyrodactylus*, observed that only *G. salmonis* appeared to cause extensive fin damage due to the activity of the marginal hooks deeply buried into the host epidermis. They observed few bacteria in the wounds.

The impact of *G. salaris* is clearly dependent on host size but larger salmon parr are normally killed when infrapopulations attain thousands (Mo, 1992 observed living fish with up to 12 500 *G. salaris*). Up

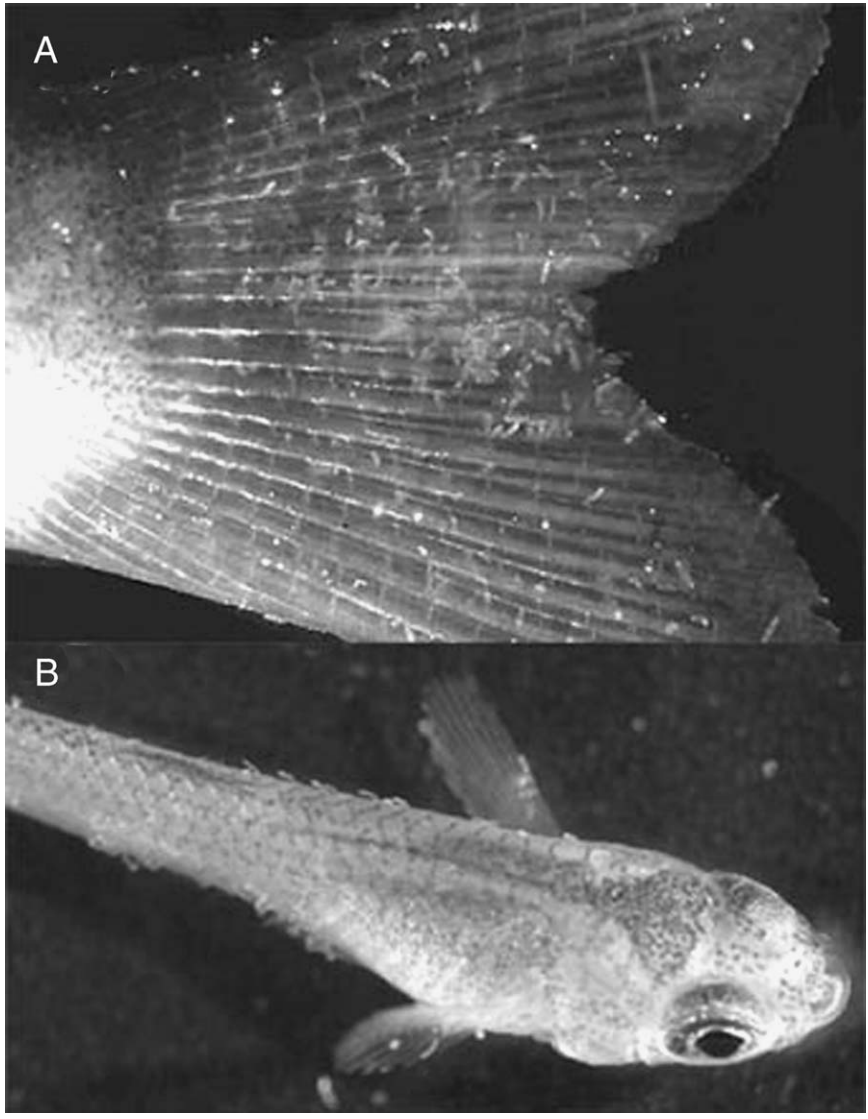


Figure 23 Infected (A) Atlantic salmon tail with *Gyrodactylus salaris* and (B) a guppy with a *G. turnbulli* infection. Small immature fish of both species can harbour several hundred worms.

to ~5000 *G. salaris* per fish did not cause obvious pathology except a thickening of the epidermis in Batnfordselva salmon (Appleby *et al.*, 1997; Bakke, personal observations), suggesting that disease tolerance, in addition to resistance, may occur in this strain. Overall, parasite-induced host death shows huge individual variability, and it is impossible to establish infection thresholds above which death is inevitable. *G. maculosi* may attain population sizes of up to 600 per gill chamber with no evidence of pathology (Cone and Roth, 1993), and *Isancistrum* may number thousands per squid without obvious mortality. By contrast, *G. bullatarudis* or *G. turnbulli* may kill guppies when only tens of parasites are present. Such differences can be apparent even on comparable hosts. *G. colemanensis* on *Salmo gairdneri* caused no pathogenicity, whereas *G. salmonis* on *Salvelinus fontinalis* was highly pathogenic (Cusack and Cone, 1986), possibly because of differences in attachment and feeding strategy (Cone and Odense, 1988; Section 2.3).

Models of gyrodactylid pathogenicity require consideration of parasite population growth rate, as host death becomes almost inevitable when the parasite population grows without check. The virulence of a gyrodactylid species or strain depends in the first place on population growth rate. For example, it is noted above (Section 7) that *G. thymalli* naturally has a lower population growth rate on grayling, than *G. salaris* does on salmon (Bakke *et al.*, 2002; Sterud *et al.*, 2002). Population growth rates for gyrodactylids infecting salmonids decline with age of infection and no correlation between growth rate and the intensity of infection was found (see Figure 9A and B in Bakke *et al.*, 2002). Only hosts supporting the highest initial parasite population growth rates are likely to suffer significant mortality and pathogenicity (e.g. Bakke *et al.*, 2002). There is a negative correlation between time to response in responding host strains and the proportion of innate resistant individuals of salmon (see Figure 13 in Bakke *et al.*, 2002), which also suggests a link between parasite population growth rate and possible pathogenicity. The model of pathogenicity/immunity put forward by Bakke *et al.* (2002; Section 8.1 above) suggested that gyrodactylid infections pose a stress on the host which weakens the immune response. If the response is weakened sufficiently by rapid initial population growth, the host is more

likely to succumb. Growth rate in turn is partly intrinsic to the parasite, but is also dependent on the closeness of the adaptation of the parasite strain to the host and on individual immune status. Local adaptation of parasites and hosts is therefore likely to be important in gyrodactylid infections (Lively and Dybdahl, 2000), tempered by macroenvironmental influences on host immune status.

8.4. Host Specificity and Immunity: Two Sides of the Same Coin?

A paradigm in gyrodactylid research is that host specificity and immunity are closely linked. This has grown from the important studies on salmonid immunity to *G. salaris* and *G. derjavini* and is perhaps inevitable; the simplest and most informative models are unnatural interactions (e.g. *G. salaris* on brook trout, Arctic charr or rainbow trout, *G. derjavini* on rainbow trout) where the boundary between immunity and host specificity becomes blurred. Experiments with immunosuppressants (Buchmann, 1997a, b; Lindenstrøm and Buchmann, 1998; Harris *et al*., 2000), which rendered previously resistant host strains and species susceptible, also strongly link immunity with host specificity. Observations on immunity and specificity using these systems should be interpreted with caution. The natural host of *G. derjavini* is probably the brown trout, *Salmo trutta*, which has seldom been tested except by Bakke *et al*. (1999, 2002). The natural host of *G. salaris* is probably the Baltic race of Atlantic salmon but again only limited studies on this race has been undertaken (Bakke *et al*., 1990, 2002, 2004a, b; Dalgaard *et al*., 2003).

After successful transmission which relies on the parasite and host meeting in space and time, the physiological host specificity depends on the balance between the success of a gyrodactylid in exploiting the particular biochemical composition of the fish epidermis and the success of the fish at eliminating the parasite. Elimination may be via the host response, suggesting that host specificity is related to immunity, but the basic reason for failure is because parasites have been unable to feed properly or breed at a rate outstripping host-mediated loss. Quantifying specificity in gyrodactylids is difficult. Scott and

Anderson (1984) drew attention to the fact that gyrodactylids lie between traditional microparasites, for which a prevalence framework model is normally adequate, and macroparasite models. For macroparasites, which do not reproduce without transmission, specificity can be evaluated as establishment success (no. of adult parasites from a given inoculum) or reproductive output (no. of eggs/parasite). Other measures, such as worm size, or number of uterine eggs, give indirect estimates of host suitability. Gyrodactylid specificity can be estimated as the number of parasites recorded after a certain period of growth from a specific inoculum size, but this omits many subtleties of the infection process. Microparasite reproductive rate is normally estimated as Fisher's R_0, the number of secondary infections arising from a single primary infection (see e.g. Anderson, 1982). This has never been calculated for gyrodactylids, and the impacts of host behaviour or environment on this parameter have never been considered. Instead, gyrodactylid host specificity has usually been considered using the following parameters:

a) The percentage establishment of initial experimental infection;
b) Rate of initial parasite population increase on a host, calculated as:

$$r = \frac{\ln\ N_t - \ln\ N_0}{t}$$

where r is the rate of parasite population growth, N_t the population on day t, N_0 the population size on day 0, and t the time in days between census points (t_0–t_1) (e.g. Jansen and Bakke, 1991; Cable *et al.*, 2000);
c) The number of days before population growth becomes negative. Population counts give the impression of a sudden population crash, usually interpreted as activation of the host reaction (Section 8.1) leading to elimination of the parasites. Recalculation of published data for *G. salaris* indicates that the sudden overturn of the population (the "crash") follows a gradual slowing of population growth rate which begins early in the infection (Bakke *et al.*, 2002). Regression of population growth rate against infection age therefore allows estimation of the time needed for population growth to become negative.

d) The maximum time for which a host remains infected. The number of secondary infections generated is presumably proportional in some way to this period (see Soleng *et al.*, 1999a).
e) The total reproductive output of an infection. Knowing (b), (c) and (d) above, it is possible to calculate the total number of worms produced from a single worm infecting a single fish. Although there have been no attempts to accurately calculate R_0 for gyrodactylid infections on single hosts, it is felt that this is the measure which comes closest to R_0 when estimating the possible contribution of a single parasite infrapopulation to the overall epidemiology of gyrodactylid infections.

9. ENVIRONMENTAL INTERACTIONS

In the microenvironment, host susceptibility and resistance are the main factors which control gyrodactylid occurrence (Bakke *et al.*, 1991a, b), although host species and ecology, including seasonal population dynamics are also important (Bakke *et al.*, 2002; Malmberg and Malmberg, 1993; Soleng *et al.*, 1998, 1999a; Boeger *et al.*, 2005). Experimental studies with regular introduction of naïve hosts into closed, otherwise uniform aquarium systems by Scott and Anderson (1984) and Bakke *et al.* (1991b) have revealed oscillatory parasite population dynamics with cycles of host susceptibility and refractoriness. Macroenvironmental influences may modify these cycles. The most important macroenvironmental factor is probably temperature, followed by water chemistry (e.g. Soleng and Bakke, 1997; Soleng *et al.*, 1999b; Poléo *et al.*, 2004a, b) and water quality generally (see Bauer, 1968). However, biotic macroenvironmental factors, such as micropredation, may also influence gyrodactylid population dynamics.

9.1. Micropredation

Gyrodactylids are large enough to be visible to fish and other visual predators and, like other ectoparasites are vulnerable to micropredation, either by conspecific hosts or other interacting organisms.

This possibility was first suggested by Tyler (1963) who observed that *Gyrodactylus*-infected fish posed for and were cleaned by sticklebacks, and later by Lester and Adams (1974a). The transverse banding of the gut of *Macrogyrodactylus* species has been suggested as an antipredatory device, disrupting their outline for potential visual predators (Khalil, 1970). Harris (1982) found sticklebacks naturally support small numbers of the oligochaete worm *Chaetogaster*, known to feed on digenean cercariae (Khalil, 1961). However, he failed to find gyrodactylid remains within the gut of these worms, which fed instead on diatoms from the fish surface. However, on one occasion, gyrodactylid hooks were found in the faeces of a laboratory-maintained stickleback, suggesting that micropredation can occur. Several workers (Roubal and Quartararo, 1992; Whittington, 1996, 1998; Grutter, 2002; Grutter *et al*., 2002) have drawn attention to the potential importance of cleaner symbionts in removing monogeneans, and have described the cryptic adaptations of monogeneans to avoid this. The susceptibility of gyrodactylids to cleaner symbionts has now been demonstrated specifically between shrimps and fish. In laboratory experiments, the shrimps *Palaemon adspersus* and *P. elegans* removed more than half of a *Gyrodactylus* infrapopulation from plaice within 48 h (Östlund-Nilsson *et al*., 2005).

9.2. Temperature

Gyrodactylids have short life spans, and low fecundity (1–5 offsprings per worm), but still have the highest reproductive rates in the Monogenea (Bychowsky, 1961), approaching those of larger micro-organisms, with doubling times in tropical species such as *G. bullatarudis* or *G. turnbulli* of less than 24 h (Turnbull, 1956; Scott, 1982). Gyrodactylids are sensitive to environmental temperature because growth rates depend not upon fecundity but upon rate of embryo development. Reproductive rate at different temperatures has been examined in *G. gasterostei* (see Harris, 1982) and in *G. salaris* (see Jansen and Bakke, 1991). In the latter, generation time and time between successive births were negatively correlated with temperature; reproductive rate increased with increasing temperature. In *G. turnbulli* (see Scott and Nokes, 1984),

reproductive output also increased with temperature up to 27.5°C. This study is however difficult to compare with Scott (1982), because raw data on the timings of births was not presented, and reproductive rate is compounded of embryo developmental and survivorship. At 30°C, reproductive rate fell, because insufficient worms survived to give birth more than once. Scott and Nokes (1984) note that the relative timing of the births changes with temperature as well, so that the interval between subsequent births is temperature sensitive. Harris (1998) employed degree days to analyse reproduction in *G. gasterostei*, finding that the number of degree days needed for development declined slightly with increasing temperature. This may explain the relationship between the size of the hard parts of gyrodactylids and environmental temperature: as temperature increases the number of degree days needed for full development declines and, therefore, hooks do not attain the size they would reach at lower temperatures.

Environmental temperature also impacts upon gyrodactylid survival, low temperature extending life span both on (e.g. Lester and Adams, 1974a, b; Scott and Nokes, 1984; Jansen and Bakke, 1991; Andersen and Buchmann, 1998) and off the host (Olstad *et al.*, 2006). Cable *et al.* (2002b) showed that survival of detached *G. gasterostei* is temperature dependent with a maximum of 101 h at 4°C but only 67 h at 15°C. *G. salaris* (see Jansen and Bakke, 1991; Olstad *et al.*, 2005, 2006) had a maximum survival of 60 h at 3°C, declining to 27 h at 18°C. Temperature may also influence transmission, which in *G. salaris* on salmon is positively correlated with temperature (range 1.2–12.2°C) during single host-to-host contacts (Soleng *et al.*, 1999a). In *G. salaris*, although survival declined as temperature increased, this was more than compensated by the increase in reproductive rate (Jansen and Bakke, 1991). These changes in reproductive rate and survival have an impact on gyrodactylid population structure, with sub-optimal survival at high temperature characterised by a high proportion of asexual first births in the population. At lower temperatures, when survival is optimal, worms may survive long enough to reproduce sexually. This was noticed by Harris (1993), who showed that the proportion of new-born individuals in *G. gasterostei* populations could transiently rise to more than 60% of the total population, whereas the stable age structure would

predict ~40%. These transient events, leading to clonal reproduction in the parasite population, are probably triggered by short-term changes in weather.

Temperature may also influence the host immune response. Immunity is normally enhanced at high temperatures, as reaction rates of complex proteins such as the complement cascade increase. Gyrodactylid populations may therefore cycle faster on hosts at higher temperatures, and not reach such large sizes. However, it has never been critically established whether the failure of gyrodactylid populations to grow large at high temperatures is due to the host response, or simply to the reduction in survival attendant on high temperature. Gelnar (1991) noted that water temperature markedly affected the growth rate of laboratory populations of *G. gobiensis* on gudgeon. At 12°C, the intensity of *G. gobiensis* gradually increased, reaching a maximum significantly later and at a larger size than at 18°C; populations subsequently declined at both temperatures. A similar phenomenon was noted when temperature was gradually increased or decreased. A field study of *G. rhodei* on bitterling (*Rhodeus sericeus*) demonstrated that both prevalences and intensity of infection increased in autumn and winter months when water temperature decreased (Dávidová *et al.*, 2005). By contrast, *G. katharineri* and *G. rutilensis,* parasitising carp fry and dace respectively, may be more thermophilic, as populations grew faster at higher water temperatures (Gelnar, 1987a, 1990). Anthony (1969) suggested that temperature may have different effects at different sites as skin parasites were more sensitive to temperature changes than gill parasites. However, a more likely explanation is that parasites in different sites were different species (see also Section 3.2).

Seasonal variations in gyrodactylid abundance are frequently attributed to environmental temperature (see review by Chubb, 1977). Such studies have generally formed part of Ph.D. theses and therefore focus on American and European north temperate species, mainly infecting small, experimentally convenient hosts such as sticklebacks. There are no tropical studies, and no studies outside America or northern Eurasia. Apart from the normal concerns over taxonomy, the biggest drawback to such studies is their restriction to a study period of 3 years or less. Gyrodactylid population dynamics are unstable (Scott and Anderson, 1984) and oscillate even when external

conditions are held constant (Scott and Anderson, 1984; Bakke *et al.*, 1991b). Longer studies are therefore needed to gain definitive insights into seasonal processes. There is also, in some studies, a danger that samples are taken too far apart. Because of their high reproductive rate and rapid temperature response, samples taken once per week or once per fortnight are necessary for accurate monitoring, and less intensive sampling reduces confidence in the results. For many north temperate freshwater gyrodactylids, between latitudes 30°N and 70°N, populations remain small through the winter, growing rapidly to form epidemics in spring, before almost disappearing in summer. There may then be a second peak of abundance as water temperature declines in autumn. This is seen in *G. katharineri* on carp in Slovakia (Hanzelová and Zitnan, 1982), for *G. aphyae*, *G. macronychus* and *G. magnificus* infecting minnows in the Kola Peninsula (Shulman, 1977), for *G. arcuatus* on sticklebacks in Poland (Morozinska-Gogol, 2002) and in America for "*G. elegans*" infecting *Notemigonus crysoleucas* in Illinois (Parker, 1965) and for species on *Fundulus* [principally *G.* (*Fundulotrema*) *prolongis*] in Connecticut (Barkman and James, 1979). The same pattern has also been seen in one study from a warmer environment; Rawson and Rogers (1973) noted a spring and autumn peak in abundance for *G. macrochiri* from both *Lepomis macrochirus* and *Micropterus salmioides* in Alabama, where summer water temperature can exceed 30°C. By contrast, Aydogdu (2006), working in Turkey, found *G. carassi* abundance directly correlated with temperature, with no evidence of high temperatures inhibiting parasite population growth. In some cases, for example, *G. stephanus* infecting *Fundulus* within the cold environment of Newfoundland, populations decline throughout the cold winter to a springtime low, and then increase slowly through summer to peak in late summer/early autumn (Dickinson and Threlfall, 1975). This might be an example of low temperature inhibiting gyrodactylid reproduction so much that populations fail to trigger a host immune response before temperatures start to fall again at the end of summer.

An interesting series of seasonal studies relate to the gyrodactylids of intertidal marine fishes. Srivastrava and James (1967) found that "*G. medius*" (not *G. medius* but an undescribed species; see Harris *et al.*, 2004) on rockling (*Onos mustela*) was most abundant in early spring,

declining sharply in July through the autumn period. A negative correlation between the occurrence of *Gyrodactylus pterygialis* and temperature was also observed by Hodneland and Nilsen (1994) in a Norwegian fiord. This decline occurred during the warmest part of the year, again suggesting that this gyrodactylid is either adversely affected directly by summer temperatures, or because the host immune system is more efficient at high temperature (Jansen and Bakke, 1993a). Appleby (1996a), working with gyrodactylids of *Pomatoschistus* in Norway, observed a similar pattern. In a similar study of gyrodactylids of cod, Appleby (1996b) noted some evidence of a bimodal distribution, but it was very clear that gyrodactylid abundance was greatest in the summer period. Similarly, Kamiso and Olson (1986) found that prevalence of *G. stellatus* infecting English sole was unimodal, reaching a maximum in June and declining to a minimum in October.

A final aspect of temperature and seasonality which can affect gyrodactylid epidemiology is the effect on the seasonal biology of the fish. Sticklebacks in southern England, have an annual life cycle, spawning in the summer following hatching. This places a major constraint on gyrodactylid biology, as parasites must transmit between generations during a few weeks in midsummer, giving an apparent midsummer drop in abundance (Chappell, 1969; Harris, 1982). Similarly, salmon in cold oligotrophic streams in North Norway may require 5 years before smoltification, leading to stratification of the parr population in the river and simple transmission between age groups. In southern England and France, on the other hand, smoltification occurs in the summer following hatching. If the river lacks a permanent parr population, it might be assumed that *G. salaris* would be unable to survive permanently. Similarly, in winter at low temperatures salmon parr remain deeper within the interstitial environment at the bottom of rivers, presenting different conditions for the parasite population and offering new opportunities for transmission compared to when these fish are feeding actively in the water column. In these ways, the basic reproductive rate of gyrodactylid infections may be modified in ways which cannot be predicted by a simple understanding of temperature relationships. Complications of this sort influence many of the seasonal studies which have been undertaken, but were not considered or explained by the authors. For example, Srivastrava

and James (1967) found an apparent midsummer decline in the abundance of gyrodactylids on rockling which correlated exactly with the migration of the new cohort of rockling onto the shore. If these fish were uninfected, this would depress gyrodactylid abundance significantly. Similarly, MacKenzie's (1970) study of *G. unicopula* on plaice is sometimes cited as an example of seasonal patterns in gyrodactylid abundance. However, MacKenzie (1970) followed a single cohort of hosts through their first 2 years of life; the steady increase in abundance through the first winter was probably due to epidemic spread of the parasite through a naïve host population, while the contradictory decline in the second winter may have been due to elimination from the now immunocompetent hosts. The most extreme example of the role of host biology perhaps is that of *G. kathariner*i infecting *Varicorhinus steindachneri* in warm springs with a constant temperature (18–20°C). These parasites exhibited a seasonal cycle, despite the constancy of water temperature (Daniyarov, 1975). The confounding effects of temperature and seasonality on host behaviour and ecology may be very important in understanding gyrodactylid seasonal dynamics.

The seasonal dynamics of *G. salaris* on different salmon cohorts has been studied in detail in five Norwegian river systems: the Vefsna (Johnsen and Jensen, 1988) and Lakselva (Johnsen and Jensen, 1992; Johnsen *et al*., 2004) in North Norway, Lierelva in southeastern Norway, both naturally (Jansen and Bakke, 1993a) and in field experiments (Jansen and Bakke, 1993b), and Lærdalselva (Johnsen and Jensen, 1997) and Batnfjordelva in western Norway (Mo, 1992; Appleby and Mo, 1997). In addition, yearly surveillance data are collected (see Johnsen *et al*., 1999). These studies demonstrate a marked seasonality in occurrence, with a spring increase in parasite abundance, peaking in late summer or autumn, followed by a decline throughout winter and early spring when temperatures are close to 0°C. Both geographical and year-to-year differences occur, and the epidemiological mechanisms and external factors controlling the abundance of *G. salaris* remain largely unknown. In Batnfjordselva (Mo, 1992), peak abundance occurred in the late summer (July, August, September) followed by a decline through mid winter and early spring. In one year, there was a small peak in March and April,

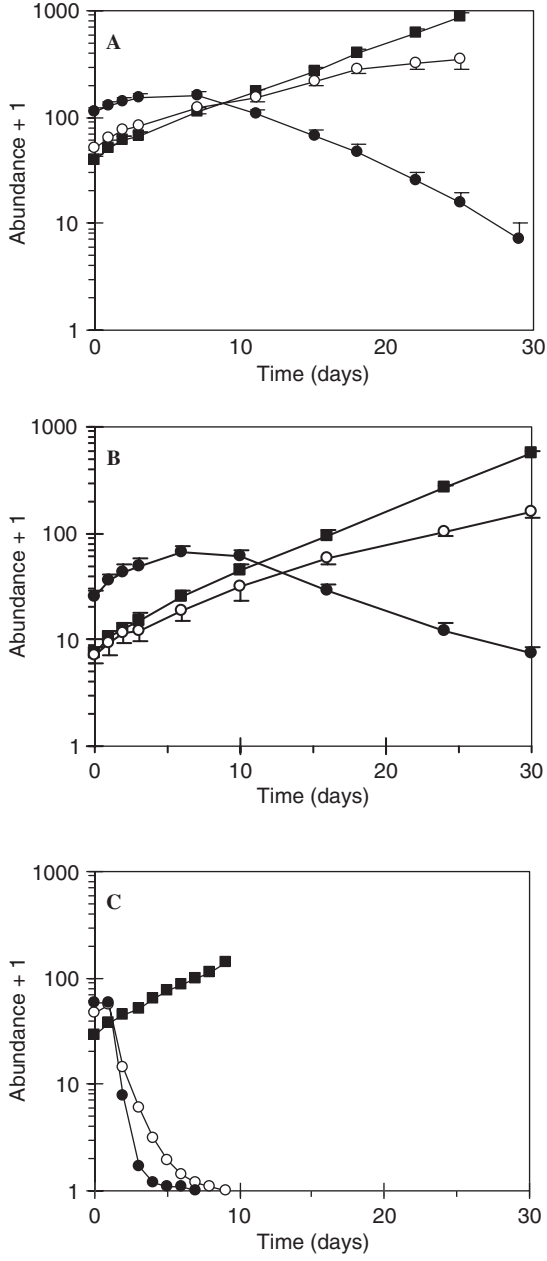
suggesting a bimodal pattern. Mo (1997) saw a similar unimodal pattern in *G. derjavini* on brown trout, peaking in July and August, and declining to a minimum in mid-winter. Transfer of *G. derjavini* onto salmon was most obvious during the midsummer peaks. Jansen and Bakke (1993a, b) on the other hand, working in the slightly warmer southeastern Lierelva river system, saw a pattern closer to the classic bimodal spring and autumn peak. The increase in spring was almost perfectly correlated with water temperature, but the decline in late summer was not related to temperature and may have been due to either immunity or parasite-induced host mortality. *G. salaris* is probably cold-adapted (Malmberg, 1973, 1988), but the discovery of large-scale interspecific genetic variation in this species (Hansen *et al.*, 2003; Meinilä *et al.*, 2004) raises questions about the potential of biological differences between *G. salaris* clades and haplotypes in relation to their temperature optima.

9.3. Salinity and Water Chemistry

Gyrodactylus species occur in freshwater, brackish and marine environments, but relatively few species are euryhaline. *G. arcuatus* is euryhaline (Malmberg, 1970), occurring in natural environments ranging from full seawater down to fairly acid soft water in montane lakes and streams. Molecular evidence suggests that all of these forms of *G. arcuatus* belong to a single panmictic species (Ziętara and Lumme, 2002, 2003, 2004; Cable and Harris, unpublished), and laboratory populations of this parasite derived from freshwater can tolerate seawater with minimal adaptation (Harris, unpublished). Apart from this, only *G. salaris* has been extensively tested for salinity tolerance (Soleng and Bakke, 1997; Soleng *et al.*, 1998). In this species, population growth increased at 5.0‰ salinity at 12°C, but at 7.5‰ salinity the population declined to extinction after 56 days, and at 20‰ salinity, survival was restricted to 12 h. Salinity tolerance was found to be significantly temperature dependent (Soleng *et al.*, 1998). These results suggest that *G. salaris* is a freshwater species, albeit able to survive high salinities for short periods. The salinity tolerance of *G. derjavini* was tested by Buchmann (1997b) who stressed that

G. derjavini seems less euryhaline than *G. salaris*. Parasites in 5‰ NaCl at 11°C survived only 4 days, in contrast to Soleng and Bakke (1997) who found that *G. salaris* survived more than a month (56 days) in 7.5‰ salinity at a comparable temperature. However, as Buchmann (1997b) used dilutions of sodium chloride, rather than of natural seawater, his results require confirmation.

Until recently, other parameters of water chemistry had not been studied. Anecdotally, many freshwater species are difficult to culture in soft water, and Malmberg (1970) alluded to the lack of gyrodactylids in water bodies stained with humic acids. This suggests that gyrodactylid diversity may be reduced in soft water. Following these observations, Soleng *et al.* (1999b) conducted the first experiments testing gyrodactylid survival in soft water and in dilute aluminium solutions (Figure 24). This metal is a frequent contaminant of acidified watercourses in northern Europe as it is leached out of soil and ground rocks. A combination of acidified water and aqueous aluminium (Al) had the most pronounced effects, eliminating *G. salaris* after 4 days at 202 µg Al/l without killing the salmon. The effect was dependent on Al concentration but relatively independent of pH, except at the lowest pH when the effect of Al was enhanced. Acidified water also impaired parasite survival in the absence of metal ions, and at pH 5.0, *G. salaris* was eliminated within 9 days (Figure 24). Similar results have been obtained with Al ions on the survival of *G. derjavini* from trout (Pettersen *et al.*, 2006). These results were confirmed by Poléo *et al.* (2004a) who went on to test the effect of other metal ions on survival. These are rarely found naturally, but are frequently a consequence of human pollution. They may however reach high concentrations in watercourses flowing through metalliferous substrates, such as the serpentine soils of South West England. Poléo *et al.* (2004a) found zinc (Zn) to be effective in controlling *G. salaris* without any apparent influence on salmon. Other heavy metals, including iron (Fe), copper (Cu) and manganese (Mn), had no effect, except at the highest Cu concentrations (Poléo *et al.*, 2004a). The mechanism behind the effect of aluminium on *G. salaris* is unknown, but could be direct (hypoxy-hypothesis) or indirect through the fish skin (repellent hypothesis) on the parasite (Soleng *et al.*, 1997). Gheorghiu *et al.* (2007), studying the effects of waterborne zinc on *G. turnbulli in vitro*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and on isolated guppies, found that concentrations of 120 µg Zn/l are directly toxic to the parasite, and they hypothesised that the host response against *G. turnbulli* may be impaired by high Zn concentrations. The different ecology of the gyrodactylids needs to be considered in evaluating these studies. *G. salaris* infects a host which predominantly inhabits soft-water areas with high water flow, low temperature and minimal human-mediated pollution. *G. turnbulli* is a tropical species inhabiting montane streams, which may also be oligotrophic. The parasite does extend into lowland areas where pollution may be significant (Harris and Lyles, 1992; Cable *et al.*, unpublished). These studies emphasise the importance of a basic knowledge of environmental factors in order to understand population dynamics, range extension and dispersal of gyrodactylids, and have formed the basis of new control strategies. Al is now being used for large-scale control of *G. salaris* in the field (see Section 10.3 below).

9.4. Pollution and Water Quality

Pollutants may directly impact upon the population dynamics, distribution and dispersal of fish ectoparasites (e.g. Møller, 1985, 1987; Khan and Thulin, 1991; Koskivaara, 1992; Poulin, 1992; Overstreet, 1993). Lately, focus is shifting towards the study of chronic exposure to sublethal concentrations not previously considered to have an important effect on fish (Poulin, 1992; Dusek *et al.*, 1998). Because ectoparasites such as gyrodactylids are easily observed, they can be important indicators of host health, reflecting water quality. Monitoring gyrodactylid epidemiology in chronic toxicity tests and in

Figure 24 Course of infection of *Gyrodactylus salaris* on Atlantic salmon (*Salmo salar*) parr ($n = 15$ in each exposure tank at day 0) at 12°C. (A) (■) Untreated control water at pH 6.4; (○) acidified Al-poor water at pH 5.6; (●) acidified Al-enriched water at pH 5.6, 106 µg Al/l. (B) (■) Untreated control water at pH 6.4; (○) acidified Al-poor water at pH 5.2; (●) acidified Al-enriched water at pH 5.2, 93 µg Al/l. (C) (■) Untreated control water at pH 6.4; (○) acidified Al-poor water at pH 5.0; (●) acidified Al-enriched water at pH 5.0, 92 µg Al/l. Bars = standard error of the arithmetic mean. Note $\log_{10}$ scale. [Reproduced from Soleng *et al.* (1999b) with permission of Cambridge University Press.]

polluted areas is an area of potential interest which has so far been largely neglected (see Poulin, 1992a). The impact of environmental factors on gyrodactylid communities has been stressed by Malmberg (1957, 1964, 1970) who indicated that many factors including water chemistry, salinity, and water temperature could influence the occurrence and spread of gyrodactylids. Few studies have considered these interactions, but Koskivaara *et al.* (1991) demonstrated a correlation between gyrodactylid diversity on roach and water quality (see Koskivaara, 1992). The effect of liming strategies to reduce acidification in southern Norway and the West coast of Sweden (Alenäs, 1995), may have inadvertently influence both the potential for gyrodactylid colonisation or level of infection, directly or via interactions with aluminium and pH (Soleng *et al.*, 1999b; Poléo *et al.*, 2004a, b; Soleng *et al.*, 2005).

Part 4. Applied biology of the *Gyrodactylus salaris* epidemic

10. THE *GYRODACTYLUS SALARIS* EPIDEMIC IN NORWAY: IMPLICATIONS FOR OTHER COUNTRIES

10.1. History

The first gyrodactylid described from a Scandinavian salmonid was collected from the Hölle laboratory (now Hölleforsens Laxodling, Indalsälv, Sweden) in 1952, and later described (Malmberg, 1957) as *G. salaris* (syntype later redescribed by Ergens, 1983) (Malmberg *et al.*, 1994). In Norway, *G. salaris* was first observed in 1975, following heavy salmon mortality at the Akvaforsk fish hatchery in Sunndalsøra, Møre and Romsdal County (Bergsjö and Vassvik, 1977; Tanum, 1983; Malmberg, 1989). In this year, the parasite was recorded for the first time from wild salmon parr in the Lakselva and Ranaelva (Nordland County, North-Norway). A significant reduction of salmon parr (but not brown trout) density was noted over the next 3 years in Lakselva (Johnsen, 1978). The epidemic was originally thought to be related to environmental pollution, as agricultural and domestic pollutants entered the river and the river bed was overgrown

with algae (Johnsen, 1978), and it was assumed that *G. salaris* occurred naturally in Norway (Johnsen and Jensen, 2003). However, over the next 20 years evidence accumulated that *G. salaris* had been introduced, probably from Sweden (Heggberget and Johnsen, 1982; Tanum, 1983; Johnsen and Jensen, 1986, 1991; Mo, 1994) and that Norwegian salmon were especially susceptible to this invader.

The discovery of four new infected rivers including some in the mid- and west of Norway (cf. Heggberget and Johnsen, 1982), led to the establishment of a "*Gyrodactylus* committee" to assess the problem, and a surveillance program to track the spread of the parasite. These studies recognised *G. salaris* as a serious threat to Atlantic salmon in Norway, with dramatic declines in salmon parr density in infected rivers, followed after a few years by declines in catches of returning adults by sports fishermen (Johnsen and Jensen, 1991; Mo, 1994; Johnsen *et al*., 1999; Mo *et al*., 2004). The committee concluded in 1982 that *G. salaris* was non-indigenous, probably newly established, spread via hatcheries, restocking and subsequently fish migration in estuaries. Few infected parr survived to the smolt stage, implying that production of adult salmon in infected rivers was strongly threatened. Gyrodactylosis on salmon was declared a notifiable disease (Group B) in Norway in 1983, and recognised a significant fish disease by the Office International des Epizooties (OIE) (Anonymous, 2002a).

Johnsen and Jensen (1986) grouped the 26 rivers then known to be infected into 14 regions. They demonstrated a close correlation between the distribution of *G. salaris* and the stocking of fish from infected hatcheries. Between 1982 and 2003, 23 new river systems were identified as infected, at a rate of 0–3 new rivers per year (Jansen *et al*., 2005). Updated records of the parasite (Autumn 2006) and the actions taken in the rivers infected in Norway are summarised in Figure 25 (which is Plate 3.25 in the separate Colour Plate Section) and Table 2.

Brackish water dispersal has been most important in spreading the parasite (24 river systems), followed by restocking from known infected hatcheries (nine rivers). Dispersal from infected hatcheries into nearby watercourses with escaped salmonids probably accounted for seven river systems, while the origin of infection in four other river

Table 2 *Gyrodactylus salaris* infected rivers in Norway, the years of first observation and the years of extermination of the parasite by use of rotenone or aluminium* and the infection status per August 2006

Infected salmon rivers	Year of 1st observation	Year of chemical treatment	Status
Lakselva	1975	1990	Exterminated 1995
Ranaelva	1975	2003/2004	Uncertain status
Vefsna	1978		Infected
Skibotnelva	1979	1988, 1995	Infected
Røssåga	1980	2003/2004	Uncertain status
Bjerka	1980	2003/2004	Uncertain status
Drevja	1980		Infected
Fusta	1980		Infected
Steinkjervassdraget	1980	1993, 2001/2002, 2005, 2006/2007*	Uncertain status
Figga	1980	1993, 2001/2002, 2005, 2006/2007*	Uncertain status
Batnfjordelva	1980	1994, 2004*	Infected
Driva	1980		Infected
Usma	1980		Infected
Rauma/Istra	1980	1993	Infected
Henselva	1980	1993	Infected
Valldalselva	1980	1990	Exterminated 1994
Beiarelva	1981	1994	Exterminated 2001
Litledalselva	1981		Infected
Tafjordelva	1981	1987	Exterminated 1990
Norddalselva	1981	1990	Exterminated 1994
Eidsdalselva	1981	1990	Exterminated 1994
Vikja	1981	1981/1982	Exterminated 1986
Skorga	1982	1993	Infected
Aureelva	1984	1988	Exterminated 1992
Vikelva	1984	1988	Exterminated 1992
Måna	1985	1993	Exterminated 1989
Korsbrekkelva	1985	1986	Exterminated 1990
Bævra	1986	1989	Exterminated 1994
Drammenselva	1987		Infected
Lierelva	1987		Infected
Vulluelva	1988	1988	Exterminated 1997
Langsteinelva	1988	1988	Exterminated 1997
Sannaelva	1989	2004	Uncertain status
Bardalselva	1989	2004	Uncertain status
Storelva	1989	1991	Exterminated 1994
Innfjordelva	1991	1993	Infected
Hundåla	1992		Infected
Slettenelva	1993	2004	Uncertain status
Leirelva	1996	1996, 2004, 2006	Uncertain status
Lærdalselva	1996	1997, 2005/2006*	Uncertain status
Signaldalselva	2000		Infected
Lundselva	2001	2002, 2005	Uncertain status
Halsanelva	2002	2003	Infected
Hestdalselva	2002	2003	Infected
Sandeelva	2003		Infected
Ranelva	2006	2006	Uncertain status

systems is unknown. One river system was inadvertently infected via a fish transport (Jansen *et al.*, 2005). Norwegian salmon authorities were quick to introduce active measures to combat the epidemic, favouring the ATPase inhibitor, rotenone to kill the fish, and hence the parasite, in infected rivers (Mehli and Dolmen, 1986). Subsequent discovery of the parasite in additional river systems led to an action plan for *G. salaris* eradication (Dolmen and Mehli, 1988), a plan later revised for 1995–1999. In addition, a comprehensive report by the Wild Salmon Committee, established in 1997 (Anonymous, 1999) highlighted reasons for the decline in the Norwegian wild salmon populations and suggested control measures. The Committee's conclusions gained wide political support from The Ministry of the Environment (St.meld. nr. 8, 1999–2000, ''Regjeringens miljøvernpolitikk og rikets miljøtilstand'') which with the Committee plan (Anonymous, 1999) and previous drafts for action plans, formed the basis for the present action plan (Anonymous, 2000). In 2002, following direct commission from the Ministry of Environment, a plan for drastic action was developed by the Directorate for Nature Management (DN) and The Norwegian Animal Health Authority (SDT) which led to the official initiative for rotenone treatment of infected river courses (Anonymous, 2002b). This plan, based on the cost-benefit analysis of Krokan and Mørkved (1994), presented detailed information on disease prevention (e.g. disinfection of equipment, physical barriers for preventing fish migration and rotenone treatment) in each of the eight currently infected regions, with a priority list and a ''cost-use'' analysis. The priorities for action and research/developmental activities were assessed based on the threat of further disease spread within or outside of the infected region, the biological and economical value of the salmon stock in question, the infection status of other salmon rivers in the region, the possibility of eliminating the parasite from all rivers in the region, the local support of stakeholders for treatment, the probability of re-infection and the need for long-lived obstructions within rivers. Because several rivers have been re-infected shortly after treatment (Figure 25; Table 2) the methods for extermination of *G. salaris* have been revised and improved (Haukebø *et al.*, 2000). Risk assessments for inter-river transmission or introduction into Norway (Anonymous, 1996;

Paisley *et al.*, 1999; Brun and Høgåsen, 2003; Høgåsen and Brun, 2003; Jansen *et al.*, 2005) and the North Calotte Region (Brørs, 2002) have also been undertaken. Recently (2001), a Gyromet project was established to develop a methodology using acidified aluminium to exterminate the parasite (see Section 9.3).

In the following account, we assess the current status of *G. salaris* within Europe, and then present an updated description of the distribution of *G. salaris* and the management initiatives to combat the parasite within Norway.

10.2. The Status of *G. salaris* within the EU

G. salaris has been recorded from eight EU member states, although records from wild fish in France and the Iberian Peninsula (Spain, Andorra and Portugal) should be considered doubtful due to confusion with *G. teuchis*. Early German records may also be doubtful. It is worth pointing out that only Norway, Sweden, Iceland, the Faeroes, the UK and Eire contain substantial stocks of salmon within the European Economic area, or contain significant wild populations. France and Spain and the Baltic States contain small, threatened populations of salmon, while plans to return salmon to the Meuse and Rhine would reintroduce the fish to central European states such as Germany, Belgium and Luxembourg. In most European states, the primary host of *G. salaris* is rainbow trout. Translocations of this host, either deliberate or as escapees, present the greatest threat of dissemination of *G. salaris.*

Sweden: Some clades of *G. salaris* from the Baltic-draining Indals älv (Malmberg, 1957) are native to Sweden, where the parasite is generally not a serious pathogen. *G. salaris* became well known in salmon and rainbow trout hatcheries from the mid-1970s onwards in both Baltic and Atlantic watersheds, and could survive for years on rainbow trout (Malmberg and Malmberg, 1987, 1991). No *G. salaris* epidemic is known from the infected Swedish Baltic river systems, Torne älv, Vindelälv and Mörrumsån (Malmberg, 2004). However, 11 rivers of the Swedish West coast, draining into the Kattegat and Skagerak and supporting populations of the East Atlantic salmon

race, are now infected. The parasite was first recorded in the Säveån (a tributary of the Göte älv) in 1989–1991, but it now seems to have disappeared from this river (Malmberg, 2004). Prior to this, there had been no evidence for parasite-induced mortality of salmon parr in Sweden (Malmberg, 1988), but subsequently, high parasite intensities (~1700 per fish) were observed combined with an average decrease in salmon parr density of 90% (Alenäs, 1998; Alenäs *et al*., 1998). On the Swedish West coast, salmon stocks that co-exist with *G. salaris* range from those without obvious fish mortality to those experiencing population decline (Malmberg, 1998). However, in spite of the fact that smolt and precocious males exhibit relatively high infections, especially in Högvadsån (River Âtran system), *G. salaris* is said not to cause problems in Swedish rivers (Malmberg, 2004). Epidemics on the Swedish West coast have not followed the same pattern as that in Norway, and the epidemiology of *G. salaris* in this area would repay closer investigation. The dispersal of *G. salaris* is probably also a result of the spread of infected rainbow trout, as escaped fish from farms in Lake Bullaren on the west coast have been found infected with *G. salaris* (see Malmberg, 2004). The introduction of the parasite to the Swedish West Coast has also proved to be more complicated than originally thought as Hansen *et al*. (2003) demonstrated the presence of different *G. salaris* clades in different rivers. Neither parasite pathogenicity nor host susceptibility can be assumed a priori, but must be experimentally tested.

Finland: *G. salaris* probably has a long history in Finland on salmon, as it shares the infected Torne älv along the border with Sweden and the Russian Neva stock has been heavily used for restocked purposes. After the first record of the parasite in Finland in 1984 (Rintamäki, 1989), the parasite has been found in ~40% of rainbow trout and salmon fish farms (Rimaila-Pärnänen and Wiklund, 1987) in northern Finland, including one farm in brackish water, one rainbow trout hatchery in Lake Inari (River Paatsjoki system) and others within the drainage area of Finnish rivers (Malmberg and Malmberg, 1991; Keränen *et al*., 1992; Aalto and Rahkonen, 1994; Koski and Malmberg, 1995; Koski, 1996; Rintamäki-Kinnunen and Valtonen, 1996). There have been no reports of *G. salaris* epidemics on wild salmon in Finland, which are supposedly resistant to the parasite

(Keränen *et al*., 1992; Aalto and Rahkonen, 1994; Rintamäki-Kinnunen and Valtonen, 1996). However, *G. salaris* can cause serious problems in Finnish fish farms (Malmberg, 2004). *G. salaris* occurs in the Kierettijoki River which flows into the White Sea, and the Rivers Teno and Näätämöjoki, flowing to the Arctic Ocean, are at risk (Aalto and Rahakonen, 1994).

Denmark: Salmonids (salmon, brown trout and rainbow trout) from ponds and fish farms in Denmark, are generally infected with *G. salaris*, *G. derjavini*, *G. teuchis* and *G. truttae* (Malmberg, 1973; Buchmann and Bresciani, 1997; Buchmann *et al*., 2000; Nielsen and Buchmann, 2001; Buchmann, 2005). *G. truttae* [described by Gläser (1974) from *Salmo trutta* (see Ergens, 1992b)] has not been recorded from Sweden, Finland or Norway but occurs frequently south of the Baltic (Poland, Denmark, Germany, Czech Republic, Slovakia and UK). *G. teuchis* also occurs in France and Scotland, and *G. derjavini* is widely distributed in Europe. Only *G. salaris* and *G. derjavini* were found on farmed rainbow trout in Denmark (Buchmann *et al*., 2000). The situation under natural conditions in Denmark is complicated by the presence of *G. teuchis* on wild salmon and rainbow trout escapees, in addition to *G. salaris* variants which infect rainbow trout but exhibit low pathogenicity to salmon (Buchmann *et al*., 2000; Lindenstrøm *et al*., 2003a; Jørgensen *et al*., 2006). No *G. salaris* epidemics on wild salmon have been reported in Denmark (Jørgensen *et al*., 2006), possibly because the rainbow trout variants of *G. salaris* (see Lindenstrøm *et al*., 2003a; Jørgensen *et al*., 2006) do not readily reproduce on salmon, or it may be due to the general scarcity of wild salmon in Danish watersheds.

Italy: The recent molecular confirmation of *G. salaris* on rainbow trout in Northern Italy (H. Hansen and A.P. Shinn, personal communication) indicates that the parasite has probably now spread widely throughout Europe with the rainbow trout trade.

Germany: Lux (1990) recovered *G. salaris* for the first time in Germany and reported infections in 70% of rainbow trout farms (intensity <40 per fish). However, these observations should be considered doubtful pending molecular analysis, which is urgently needed.

UK, Northern Ireland and Eire: *G. salaris* was not observed in the UK by Shinn *et al*. (1995b) despite extensive screening of several

species of salmonid, and it has not been found subsequently as part of routine screening by fish health authorities. Although *G. thymalli* is present in England (Denham and Longshaw, 1989; Hansen *et al.*, 2007), *G. salaris*-like clades appears absent.

France: The first report of *G. salaris* in France was based on heavily infected farmed rainbow trout examined in Oulu, Finland, during an EU Workshop on *G. salaris* (see Johnston *et al.*, 1996). This record was subsequently assumed to be a misidentification due to confusion with *G. teuchis* (Lautraite *et al.*, 1999; Cunningham *et al.*, 2001). During their comprehensive sampling, Lautraite *et al.* (1999) did not find *G. salaris* in Brittany (10 sampling sites from seven main salmon rivers and three restocking farms) or the Western Pyrénées (eight sampling sites from six main salmon rivers and one restocking farm) which are the main French water basins harbouring wild Atlantic salmon populations. Colonisation by *G. salaris* would be expected if the parasite was present in France. *G. derjavini*, in contrast, was frequently recorded on salmon in the area (Lautraite *et al.*, 1999). However, the complete absence of *G. salaris* in rainbow trout hatcheries and farms in France awaits confirmation.

Portugal and Spain: If the records of *G. salaris* in France are incorrect (Lautraite *et al.*, 1999), Johnston *et al.*'s (1996) report of *G. salaris* in Portugal also needs to be confirmed. In Eiras' (1999) studies on rainbow trout and brown trout in several trout farms belonging to different hydrographic basins in Portugal, no *G. salaris* was observed. Johnston *et al.*'s (1996) record may represent *G. teuchis,* but this needs clarification.

The Czech Republic: *G. salaris* is probably absent from the Czech Republic. However, the discovery of *G. bohemicus* by Ergens (1992a) in a rainbow trout and brook trout farm, and the close resemblance of this species to both *G. thymalli* and to the rainbow trout variant of *G. salaris* (see Lindenstrøm *et al.*, 2003a, b) does suggest that further studies to establish absence would be worthwhile.

The status of G. salaris *in areas bordering the EU*: Outside the EU, *G. salaris* is abundant in the Kola Peninsula, Russian Federation, abutting northern Finland. There are however records of *G. salaris* from elsewhere in the Russian Federation, including from Black Sea drainages.

The Kola Peninsula: The first record of *G. salaris* in the River Keret (draining into the White Sea) dates from 1992 when it was probably introduced with stocked fish from the Ladoga-Onega region (Ieshko *et al*., 1995). According to Meinilä *et al*. (2004), infection has been traced to a helicopter-carried canvas bag used to transport salmon parr around Lake Onega and, in the same day, from another hatchery. An epidemic of *G. salaris* has been reported for this river, with a significant reduction in salmon parr density (see Johnsen *et al*., 1999).

Karelia: *G. salaris* has been observed on parr from Lake Ladoga (Ergens, 1983) and in rivers entering Lakes Onega and Ladoga (Ieshko *et al*., 1996). Prevalence and intensity of infection are reported to be low and no epidemics have been observed (Shulman *et al*., 2000, 2005). At least some Karelian populations appear to be cold-adapted (Shulman *et al*., 2005).

Ukraine and Georgia: *G. salaris* was reported on brown trout (*Salmo trutta fario*) in the River Seret, a tributary of the River Dnestr draining into the Black Sea (Malmberg, 1988), and was reported common on rainbow trout in two areas draining to the Black Sea, the River Pliva and the Jezero fish farm (Žitňan and Čankovič, 1970). Although outside the normal geographical range, these records are considered valid.

Bosnia Herzegovina: Žitňan and Čankovič (1970) recorded *G. salaris* from rainbow trout from the Adriatic coast (River Buna and the fish farm Blagaj) of Bosnia Herzegovina. Rainbow trout were widespread and *G. salaris* was common. Although far from the normal range of *G. salaris*, this record is normally considered valid.

10.3. The Current Status of *G. salaris* in Norway

Among the 24 identified *Gyrodactylus* species from freshwater fish in Norway (Sterud, 1999), only *G. salaris* has proved a serious threat to wild salmon (Bakke and Harris, 1998; Mo, 2004). Losses in 1984 were estimated at 520 tonnes (Johnsen and Jensen, 1986), equivalent to 25% of the total salmon catch (Egidius *et al*., 1991). In 14 rivers, the average density of salmon parr and adults was reduced by more than 85% (Johnsen *et al*., 1999; Johnsen and Jensen, 2003). The parasite

has exterminated salmon in six rivers and threatens populations in 34 other rivers. In some infected rivers (Skibotnelva, Røssåga and Vefsna), the relative abundance of salmon/trout hybrids has dramatically increased (by more than 50%), presumably because of the greater resistance of hybrids to *G. salaris* (see Bakke *et al*., 1999; Johnsen *et al*., 2004). Currently, annual loss due to *G. salaris* infections is estimated at 250–500 tonnes of salmon, approximately 15–20% of the natural smolt production (Anonymous, 1999). However, the economic cost of *G. salaris* is twofold: the loss in potential economic output and the direct cost of combating invasions. In Norway, the annual loss in lost fisheries, tourism etc. is calculated to be around USD 34 million per year, with a further expense of ~USD 23 million in surveillance and eradication. Since introduction 30 years ago, the parasite is estimated to have cost a total of USD 450–600 million (Directorate for Natural Resources, May 2002), without including indirect costs due to restrictions on the export of live salmonids within the EU, or the costs of surveillance and control in other countries within (e.g. Scotland) or outside (e.g. the Russian Federation) the EU. Without control measures, *G. salaris* would have reduced the Norwegian salmon fishery by at least 15% (Johnsen *et al*., 1999; Johnsen and Jensen, 2003).

To date, 46 out of 379 salmon rivers and 39 farms (13 coastal salmon hatcheries/farms and 26 rainbow trout hatcheries/farms in Southern Norway) have been infected since in Norway since 1975 (Mo *et al*., 2004; Mo and Nordheim, 2005, unpublished). Eradication using rotenone or acidified aluminium treatment has been attempted in 35 rivers, but of August 2006, 19 remain infected, 12 are under post-treatment surveillance, 15 have been confirmed clear of the parasite and in 8 rivers attempts have proved unsuccessful (Figure 25; Table 2). In 2000, a National surveillance programme was implemented for all uninfected salmon rivers by the Norwegian Animal Health Authority (now part of the Norwegian Food Safety Authority) responsible for sampling salmon in both rivers and fish farms. The spread of *G. salaris* is tracked by routine annual surveillance of 150 salmon rivers and biennial examination of a number of freshwater fish farms. Thirty salmon fingerlings, parr or smolt from each river have been sampled by electro-fishing each year. The fish are

killed, preserved in 96% ethanol and the skin and fins examined by the National Veterinary Institute (NVI, Harstad, North-Norway). This low number of salmon parr examined on a yearly basis makes an immediate record of an infection upon introduction very arbitrary. Additionally, there is no regular or fully satisfactory surveillance control after rotenone treatment and before the 5-year check if the parasite has been exterminated, preventing study of the origin and epidemic spread of *G. salaris* in newly infected rivers. The parasite has reappeared in several rotenone-treated rivers; in some (Skibotnelva, Steinkjervassdraget, Figga, Lundselva, Batnfjordelva, Leirelva and Lærdalselva) more than once. By the mid-1980s, the NVI (OIE reference laboratory for gyrodactylosis) extended surveillance to fish farms, especially rainbow trout farms. In 2004, salmon from 120 rivers (4509 individuals) were screened and *G. salaris* recovered after rotenone treatment in two rivers, the Leirelva in Nordland County (rotenone treated in 1996) and the Halsanelva (rotenone treated in 2003). *G. salaris* was not observed in the 34 fish farms examined in 2004 (Mo and Norheim, 2005). Late autumn 2006, Batnfjordelva as (one of three rivers treated with aluminium) was found reinfected.

Currently infected watercourses and fjords in Norway can be grouped into infection-regions, representing geographic localities where *G. salaris* has been recorded on wild salmon parr, and which are bounded by features limiting natural dispersal by drift or host migration. Originally, 14 infection-regions were identified but only eight (or nine; see Region 8 below) now remain. Official policy (Anonymous, 2002b) is to eradicate the parasite from infected rivers by proceeding from region to region depending on annual funding. Since 1990, *G. salaris* has been introduced to between one and three new regions: Lærdals Region (1996), Halsanelva and Hestdalselva, close to the Vefsna Region and Sandelva in Drammens Region. As of 2006, the eight regions (from north to south) are:

Region 1—Skibotn Region (Troms County): *G. salaris* first observed in 1979 in Skibotnelva. The region consists of two infected river courses, Skibotnvassdraget and Signaldalsvassdraget (and Balsfjordelva which shares an estuary with Signaldalselva), all with natural stocks of salmon and anadromous Arctic charr and trout. Skibotnvassdraget was rotenone-treated in 1988, but re-infected in

1992, re-treated with rotenone in 1995, and re-infected again in 1998. *G. salaris* was recorded for the first time in Signaldalselva in 2000.

Region 2—Rana Region (Nordland County): *G. salaris* was first observed in 1975 in Ranaelva. The region consists of six infected rivers, two large (Ranaelva and Røssåga) and four small, Busteråga (Slettenelva), Bjerka, Sannaelva and Bardalselva. All have natural stocks of salmon and anadromous trout, some also contain Arctic charr. Ranaelva, Røssåga and Bjerka were rotenone treated in 2003 and all rivers in 2004. These rivers will be declared parasite-free if there is no re-occurrence within the next 5 years after treatment.

Region 3—Vefsn Region (Nordland County): *G. salaris* was first observed in 1978 in the large River Vefsnavassdraget. The region also includes seven smaller infected rivers, the Fusta, Drevja, Hundåla, Leirelva, Ranelva, Halsanelva and Hestdalselva. Leirelva was rotenone treated in 1996 and declared parasite-free in 2003, but *G. salaris* re-appeared in 2004. The river was immediately rotenone treated again, and results of the treatment are awaited. However, in 2006 a new river was found infected in this region, Ranelva, close to Leirelva. Accordingly, both rivers were immediately rotenone treated and results of the treatment are awaited. Halsanelva and Hestdalselva were treated in 2003, but the parasite reappeared in Halsanelva in 2004 and in Hestdalselva August 2006. These two rivers may constitute a new infection-region as natural dispersal via the relatively long migration route through saltwater from the Vefsna should be considered unlikely.

Region 4—Beitstad Region (Nord-Trøndelag County): *G. salaris* was first observed in 1980 in the Steinkjervassdraget (Steinkjerelva, Byaelva and Ogna) and Figga. The parasite was first observed in the Lundselva, the third river of this region, in 2001. All river courses have natural stocks of salmon and anadromous trout. Steinkjervassdraget and Figga were rotenone treated in 1993, but the parasite returned in 1997 and the rivers were re-treated in 2001/2002. Re-infection was immediately noted and the rivers re-treated in 2005 to reduce the salmon migration from the rivers. However, despite Steinkjervassdraget being treated twice in spring and autumn 2005, *G. salaris* has again been recovered but upstream from the treated regions (see Hjettnes *et al.*, 2006 for an analysis of the epidemic situation). In August 2006, this river system and some adjacent minor

rivers were treated for the first time with aluminium sulphate based on the previous positive experiences with aluminium against *G. salaris* infections in Batnfjordelva (Region 5) and Lærdalselva (Region 6). This treatment with aluminium will be repeated in 2007. The success of this treatment in Steinkjersvassdraget is still to be seen.

Region 5—Sunndals Region (Møre and Romsdal County): The region consists of four infected river courses, one large (Driva) and three smaller (Usma, Batnfjordselva and Litledalselva), all with natural stocks of salmon and anadromous trout. *G. salaris* was first observed in 1975 in the Driva, Usma and Batnfjordelva. The latter was rotenone treated in 1994, but the parasite re-appeared in 2000. This river was selected for a pilot project for aluminium sulphate treatment (see Section 9.3; Table 2) in 2004. Late autumn 2006 the river was observed reinfected with *G. salaris*.

Region 6—Romsdals Region (Møre and Romsdal County): *G. salaris* was first observed in 1980 in Raumavassdraget and Hensvassdraget. This region consists of four infected river courses, the Raumavassdraget (Rauma and Istra), Innfjordelva, Hensvassdraget (Henselva) and Skorga, all rivers with natural stocks of salmon and anadromous trout. The rivers were rotenone treated in 1993 but the parasite re-occurred in Rauma in 1996, and from there has probably been spread to Innfjordelva (1999), Hensvassdraget (2000) and Skorga (2003).

Region 7—Lærdals Region (Sogn and Fjordane County): *G. salaris* was first observed in 1981 in the Vikja and in Lærdalselva in 1996. Vikja was successfully rotenone treated in 1981/1982. Lærdalsvassdraget was treated in 1997, but the parasite re-occurred in 1999. In 2005, a 24-km infected stretch of the river was treated with aluminium sulphate, a process which was repeated in 2006. Again, confirmation of the success of this trial has still to be announced.

Region 8—Drammens Region (Buskerud County): *G. salaris* was first observed in 1987 in the Drammensvassdraget and Lierelva, which both empty into Drammensfjorden. Both rivers have natural stocks of salmon and anadromous trout. Due to the complexity of this river and fjord system and of the fish fauna, there are no plans for rotenone treatment, but the rivers are restocked annually to maintain the salmon angling industry. In 2003 a new river, Sandeelva (Vesleelva; Vestfold County) was found infected with *G. salaris*, the

only other river with salmon in the area. However, this may represent a new infection-region, but infection from the nearby Drammens Region cannot be excluded.

Predicting host populations at risk of future invasion is problematic. However, three regions are deemed at high risk, as the parasite has previously occurred, or has been found in fish hatcheries but not on wild salmon:

Risk Region 1—Enaresjøen and Pasvikelva (Finland and Troms County, respectively). *G. salaris* was observed in Lake Enare in a rainbow trout hatchery and on escapees in the lake in 1990. The Norwegian Pasvikvassdraget is in the zone at risk;

Risk Region 2—Torneälv and Nord-Troms (Sweden/Finland and Troms County, respectively). Torne älv drains into the Baltic Sea and heavy restocking occurs within the Finnish catchment, where relative high infection levels have been observed on wild salmon parr. The Norwegian salmon rivers Målselva and Reisaelva are particularly at risk;

Risk Region 3—Iddefjorden (Sweden and Østfold County). Enningvassdraget drains several larger lakes in Sweden and Norway, including the Swedish Lake Bullaresjøen where *G. salaris* was observed in a rainbow trout hatchery and on escapees in the lake in 2001. Two Norwegian salmon rivers, Enningdalselva and Tista, are particularly at risk.

10.4. Control Measures

Eradication of a non-indigenous species is sometimes feasible particularly if it is detected early and resources can be applied quickly (Simberloff, 1997; Mack *et al.*, 2000). To eradicate an established invader, such as *G. salaris,* in natural areas is difficult, especially as it can exploit a range of commercially produced fish, and its reproductive rate and dispersal ability are high. Control measures to prevent the further spread vary in their efficacy and are dependent on the continued commitment and diligence of both salmon management authorities and relevant agencies (the fish traders, anglers and general public) to prevent re-introduction (Figure 26). In Norway, the methods considered and employed are designed to eradicate the parasite

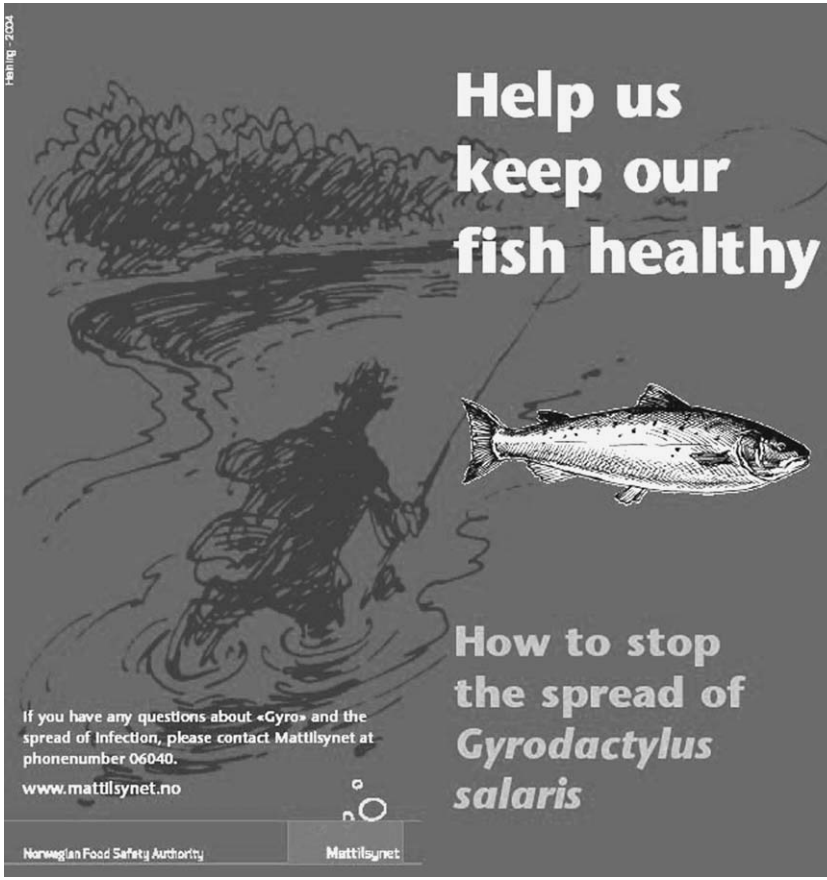

Figure 26 An example of the many information brochures from Norwegian authorities to protect from anthropochore spread of *Gyrodactylus salaris* to uninfected river systems. (Reproduced with permission from Norwegian Food Safety Authority.)

from the respective infection-regions. Hence, the re-occurrence of the parasite may be caused by unsuccessful rotenone treatment, or by new introduction from external regions. The time between eradication and reappearance of the parasite in the largest river in five of the infection-regions has been from 3 to 6 years (Hjeltnes *et al.*, 2006). The available methods for eradication of *G. salaris* include:

Biocides: Rotenone ($C_{23}H_{22}O_6$), an isoflavonoid from the roots of certain leguminous plants. It is insoluble in water and is normally

formulated as a liquid emulsion to allow rapid dispersion and efficacy as a piscicide. However, rotenone is non-specific and apart from being highly toxic to fish it also kills gill-breathing aquatic invertebrates. It has been widely used in Norway to eliminate *G. salaris*. Of the 46 *G. salaris* infected rivers, 28 were treated with rotenone between 1981 and 2003 (Guttvik *et al*., 2004). Different grades of rotenone are used, but CFT-Legumin^{TM}, which contains approximately 5.3% (w/w) of the active substance dissolved in a mixture of diethylene-glycol-monoethyl-ether and 1-methyl-2 pyrrolidone, is recognised as least dangerous for the environment at the concentrations used (25 mg/l) (1.0 ppb) during the first hours, and thereafter at 12.5 mg/l (0.5 ppb). This procedure may be repeated in infected rivers. The limit of rotenone tolerance of salmon parr is 0.2 ppb, the 24 h LC_{50} concentrations for rotenone active substance are between 2 and 10 ppb for salmonids that are among the most susceptible fish species. The lower limit for effective use of rotenone is set at 0.5 ppm (Guttvik *et al*., 2004). It is impossible to ensure 100% success with rotenone treatment (Haukebø *et al*., 2000), and after initial success exterminating the parasite in the River Vikja in 1981/1982, rotenone treatment has frequently failed (e.g. in Skibotnelva, Leirelva, Halsanelva, Steinkjersvassdraget, Figga, Rauma, Innfjordelva, Lærdalselva, Batnfjordelva). The failure is probably due to the complexity of effectively dosing all infected areas within a river system, but recolonisations cannot be excluded. After ~20 years experience, Haukebø *et al*. (2000) reviewed the procedures used and stressed the potential need for more extensive use of river obstructions, updated equipment, high-density rotenone compounds, prolonged periods of application, double treatments, increased focus on the land–water interface and better hydrological expertise as groundwater wells beneath the river beds have been a major problem (Brabrand and Koestler, 1999; Brabrand *et al*., 2005). There is also a concern of escape migration by salmon into groundwater wells or to river mouths or fjord basins, possibly triggered by the presence of rotenone or other substances in the river. After rotenone treatment, the river is artificially and naturally re-stocked with salmon of the local stock. Farms can be easily disinfected using antiparasitic drugs (Santamarina *et al*., 1991; Tojo *et al*., 1992, 1993a, b; Schmahl, 1993), although Norwegian

practice has been to enforce a dry fallow period to be certain of eradication.

Salmon and sea trout quickly recolonise treated rivers provided there is a sea population sufficient for recolonisation (Johnsen *et al.*, 1997; Lund, 1997). Wild salmon stocks preserved in gene banks are used for restocking purposes to supplement the contribution from the marine population and any surviving eggs of both salmon and sea trout buried in the gravel (Hartvigsen, 1997). The first generation salmon fry have a faster growth rate than normal, resulting in earlier smoltification and sea migration (Johnsen *et al.*, 1997). Although the effects on the genetic structure of fish populations are more pronounced in freshwater resident fish species than in anadromous or marine species, there are no empirical data on the genetic impact of rotenone on specific fish populations (Hindar, 1997).

The short-term impacts of rotenone on benthic macroinvertebrates have been studied by Arnekleiv (1997). The impact on estuarine fauna is largely unknown (Wingard and Swanson, 1992), but freshwater invertebrates are severely affected. In the River Steinkjerelva, treatment with rotenone led to an immediate catastrophic loss of invertebrates peaking $^{1}/_{2}$ to 2 h after treatment followed by a decline through the next hours (Arnekleiv, 1997). All common taxa were affected. Amongst the benthic fauna of the upper stream, a reduction of around 90% occurred shortly after rotenone treatment. However, some insect species survived in great numbers and recolonisation by benthic macroinvertebrates occurred rapidly, requiring 6 weeks to restore insect densities to previous levels. One year later, the common species of Ephemeroptera, Plecoptera and Trichoptera had all re-established (Arnekleiv, 1997). However, instability in species composition was observed and probably greatly underestimated due to limited sampling and lack of genetic studies to assess the effects on population structure. Generally, rotenone tolerance and the effects on invertebrates are little known; however, dramatic short turn effects are reported but differing between taxa and life stages (Binns, 1967; Morrison, 1977; Dolmen *et al.*, 1995; Arnekleiv, 1997). Rotenone treatment is reported to have had little long-term impact in Norwegian rivers (Mo, 1994) but criticism of its use is growing because of (i) several unsuccessful attempts to exterminate *G. salaris*; (ii) potential

deleterious founder-effects and genetic drift in restored fish populations; (iii) destruction of a stable host–parasite association based on resistant salmon which could form the basis for breeding natural resistance; (iv) the continuous threat of re-infection (Hessen, 1997); and (v) health concerns for humans. Rotenone formulations are placed in Class II (moderately hazardous) or III (slightly hazardous) of the WHO classification of pesticides. However, rotenone has been suspected of being carcinogenic (Gosalvez, 1983) and involved in the aetiology of human illnesses (see Tables in Ling, 2002), such as Parkinson's disease (Alam and Schmidt, 2002; Sherer *et al*., 2003). Following the EU Biocide Directive (Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998) rotenone must be removed from the market and its use ceased by 1 September 2006. The Norwegian Pollution Control Authority (SFT) in consultation with the Ministry of the Environment has applied to the EU-commission for exceptional permission to continue to use rotenone for *G. salaris* control until 2010. However, attention must focus on other strategies and the Norwegian authorities are seeking to identify alternative methods for *G. salaris* control. In Norway many substances have been assessed (Skjelstad and Simolin, 2001), but only aluminium or zinc show promise. However, most recently, Australian tea tree oil (TTO) has been tested on *Gyrodactylus* infected three-spined sticklebacks (Steverding *et al*., 2005). The results suggest that TTO, in combination with Tween 80 as an emulsifier, might be an effective and potentially environmentally friendly, treatment.

Parasitocides—metal ions: On-going development projects are currently investigating the partial replacement of rotenone by long-term treatment of rivers with aluminium sulphate with simultaneous acidification. The toxicity to *G. salaris* of aluminium and zinc ions in acidified water has been described in Section 9. Following these laboratory experiments (Soleng *et al*., 1999b; Poléo *et al*., 2004a), pilot studies have used aluminium sulphate to control *G. salaris* (see Poléo *et al*., 2004a, b) in Lærdalselva (2002, but full treatment in 2005 and 2006), Batnfjordselva (2003, but full treatment in 2004, see Hytterød *et al*., 2005) and for full treatment of Steinkjersvassdraget in 2006 and 2007. The application of Al is problematic as the concentration has to be balanced exactly to the specific water flow, chemistry and

temperature to kill parasites but not fish. So far, the full-scale treatment of Lærdalselva and Steinkjersvassdraget with Al to eradicate *G. salaris* appears promising but must await appraisal over the next 5–6 years. The macrobenthos community in Lærdalselva and Batnfjordelva was sampled extensively before and after Al treatment, but awaits full analysis.

Barrier methods: Mechanical methods of controlling non-indigenous organisms are sometimes effective. The closure of fish ladders and construction of artificial barriers in infected rivers can prevent spawning salmon re-colonising parts of the river course where the disease is present (Thorstad *et al.*, 2001; Fjeldstad, 2004). Smoltification of salmon parr from above the barrier will then lead, over 2–5 years, to a total disappearance of salmon from the infected part of the river. This approach reduces the area containing infected fish, allowing more restricted and less expensive rotenone treatment. However, when barriers are close to the sea they are likely to be affected by flood, ice and debris, increasing cost and reducing effectiveness.

Selective breeding: Artificial breeding for resistance represents an apparently ideal solution to the problem of *G. salaris* susceptibility, and has been shown to be feasible as resistance has a strong genetic component (e.g. Bakke *et al.*, 1990; Bakke and MacKenzie, 1993; Bakke *et al.*, 1999; Cable *et al.*, 2000; see Section 8.2). However, even once the practicalities are overcome to produce resistant fish for restocking, release of such genetically modified salmon into the environment will be controversial and politically sensitive. Each river system within the range of the Atlantic salmon is populated with a genetically unique fish stock, resulting from repeated bottlenecking and drift caused by the return of only a tiny number of breeding adults relative to the number of eggs laid (Mills, 1989). Conservation of this genetic diversity remains a priority for Norwegian conservation bodies (Anonymous, 1999) and therefore artificial selection for resistance has been ruled out except for complex river systems which are too complicated to be treated chemically (e.g. Drammenselva). Within Drammenselva, artificial mass selection for resistance to *G. salaris* of 50 family groups is in progress with both unexposed parr and the surviving exposed parr being cultured to maturity (Salte and Bentsen, 2004). Progeny from the most resistant fish will then be

tested for resistance and eventually used for restocking the river course. The main arguments against selection for disease resistance are that the heterogeneity of the salmon population will be reduced, escapees will impede the resistance development and artificial selection is time consuming and expensive. Because of the uniqueness of stocks, every infected river system will need its own breeding program. Perhaps most importantly, breeding for resistance will not eradicate the parasite and Norwegian rivers will continue to represent a focus for spread of the parasite. In addition, the evolutionary impact of increased host resistance to a particular parasite species is unknown. However, hopefully, such selective breeding experiments will increase our knowledge of the heterogeneity of Norwegian salmon resistance and of the heritability of resistance to gyrodactylids (Salte and Bentsen, 2004).

Integrated control measures: The governmental strategy in Norway aims for the complete extermination of *G. salaris* from all rivers, but there is still a need for "maintenance control" of the parasite (Schardt, 1997). As the drawbacks of individual control measures, such as rotenone, are realised (Sandodden *et al.*, 2004), integrated control strategies using barriers, aluminium and selective breeding appear increasingly attractive. The treatment of Lærdalselva in 2005 with Al also involved application of rotenone in small quantities where Al treatment was not feasible. The success of this combined treatment will not be fully assessed until 2010. River barriers or closure of salmon ladders are often used as a first step in reducing the area requiring chemical treatment. In Drammenselva, smolt or yearlings of the same stock are restocked in the river mouth and in uninfected tributaries of the river, respectively, to compensate for deaths caused by *G. salaris* and to maintain the number of fish returning to the river for angling. This has been a success in this river (Hansen, 1990, 1991) but poses the danger of reducing the evolutionary development of natural disease resistance.

Biological control: The introduction of natural enemies such as hyperparasitic bacteria or viruses as biological control agents to eliminate *G. salaris* have not been explored, although micro-organisms are frequently observed externally on gyrodactylids (Cone and Odense, 1984; Bakke *et al.*, 2006; Section 7 above).

10.5. Potential for Further Spread of *Gyrodactylus salaris*

Alterations in the distribution of the Earth's biota brought about by human transport and commerce (Mack *et al*., 2000) represent a major threat for the future biological diversity of the biosphere. The potential for further spread of *G. salaris* within Europe, either naturally or by Man, remains significant (Bakke and Harris, 1998). The parasite is not a problem of rainbow trout in aquaculture and it does not cause losses in natural salmon populations across most of its Scandinavian range (see Section 10.2). The perception of *G. salaris* as a pathogen declines further if the suggestion of Meinilä *et al*. (2004) to synonymise salmon- and grayling-infecting clades as *G. salaris* is followed; the parasite then occurs throughout the EU and neighbouring countries but causes problems only in Norway, and perhaps in some infected rivers at the Swedish west coast and the Kola Peninsula. However, experimentally other east Atlantic stocks of salmon, such as those from Scotland and Denmark seem highly susceptible to Norwegian *G. salaris* (see Bakke and MacKenzie, 1993; Dalgaard *et al*., 2003, 2004). Risk analyses show that the potential for spread of *G. salaris* via wild salmon is very sensitive to changes in salinity within estuaries (Paisley *et al*., 1999). However, Peeler and Thrush (2004) and Peeler *et al*. (2004) established that for England and Wales, the greatest risk of introduction was via the movement of rainbow trout. A recent risk analysis of watercourses in which *G. salaris* is already present from the Norwegian Veterinary Institute (Jansen *et al*., 2005) concluded that the major risk factors for the spread of *G. salaris* are in diminishing order: (i) dissemination by migrating fish in brackish water; (ii) spread from infected hatcheries and farms; (iii) restocking with infected fish; (iv) spread by equipment which has been in contact with infected fish; (v) spread by non-predicted human activity; (vi) spread of dislodged parasites with water; and (vii) natural spread by other host species. In this context we would stress the potential of wild Arctic charr to disseminate *G. salaris* amongst natural salmon populations (see Sections 5.3.2 and 6.5). This may also be the case for wild lake trout (*Salvelinus fontinalis*) and rainbow trout which are susceptible to *G. salaris* and

occasionally establish viable populations in Norway. Such populations may represent a reservoir for the spread of *G. salaris* and should be under surveillance (see Mo, 1988; Bakke *et al.*, 1992a, b, c; Hindar *et al.*, 1996; Knudsen *et al.*, 2004; Kristoffersen *et al.*, 2005; Robertsen *et al.*, 2006, 2007b; M. Eken, personal communication).

The trans-national spread of the parasite, particularly to highly sensitive islands as the UK, Ireland, the Faeroes and Iceland, or to the east Atlantic stocks of continental America, is most likely to occur with rainbow trout or other fish species. Recently (1 May 2004), regulations concerning salmon transportation within the EU were relaxed (EC decision 2004/453/EC) such that live salmonids could be moved to *G. salaris*-free areas, as long as the fish originated in coastal sites where the salinity does not drop below 25 parts per thousand. Peeler *et al.* (2006) concluded that this does not directly increase the risk of *G. salaris* introduction into new waterbodies, but that the risk is indirectly increased by the increased volume of salmonid transport. In general, the UK imports only salmonid eggs to ensure the exclusion of salmonid viruses such as Infectious Hepatic Necrosis and Viral Haemorrhagic Septicaemia (Scott, 2004). This should ensure the continued exclusion of *G. salaris* from the British Isles. However, the UK does also import salmonids for processing (Scott, 2004), it is conceivable that *G. salaris* could survive import in this way (see Section 3.3 for an appraisal of survival of *G. salaris* on dead fish) and remain attached to fish packaging materials long enough to gain access to a watershed containing live salmonids. The possibility that the parasite could survive for periods of days attached to eggs (Section 3.3) also raises the possibility of the spread of the disease with egg imports. Rationalisation in the international salmon industry has seen the recent closure of Scottish egg hatcheries with the proposal that eggs will be imported to farms from Scandinavia. Apart from the apparent breach of EU fish hygiene regulations in this proposal, this must represent an unacceptable level of risk for the importation of *G. salaris* into the UK. The illegal import of live fish (principally carp, wels and sturgeon) into the UK is also well known, and is thought to have been a route of origin for Spring Viraemia of Carp (SVC). Most imports do not come from countries where *G. salaris* is likely to be encountered, but clearly a route of entry into

the UK exists which is not easily controllable given existing legislation. The UK is used here to demonstrate that even with a highly developed legislative and monitoring framework to prevent the import of *G. salaris*, biosecurity is by no means assured. In the past 3 years, the import of sleeping disease (SD) syndrome and SVC have both highlighted failures in the established system. Given these failures in a country with a sophisticated and stringent system for assessing fish health, the continued spread of *G. salaris* within continental Europe, where transboundary controls on fish imports could never be as careful, is inevitable. As the parasite spreads within Europe and neighbouring states, the risk of import into the highly sensitive Atlantic Fringe countries increases.

11. CONCLUSIONS AND FUTURE RESEARCH AREAS

We have attempted to provide an overview of the biology of gyrodactylid monogeneans and to highlight the potential importance of this megadiverse group in studies of parasite evolution. The gyrodactylids are best known as viviparous fish parasites which are born already containing a developing daughter; here we highlight that this 19th century view of the group misses much that is biologically interesting and significant about them. In particular, we stress the importance of recognising that viviparity and progenesis are adaptations of only one group of gyrodactylids (albeit the most successful and currently the most species-rich part of the group). Studies on the egg-laying gyrodactylids are in their infancy, but are starting to live up to their early promise. Although Boeger *et al.* (2003) consider the radiation of viviparous gyrodactylids to have occurred recently, we feel that gyrodactylids are a primitive monogenean group, perhaps the remnant of an earlier diversification before the evolution of modern forms such as the capsalids, microbothrids and polyopisthocotyleans. A study of the egg-laying forms is the only way in which potentially plesiomorphic characters, shared with other primitive monogeneans can be identified, because progenesis has modified the viviparous genera so extensively. Further investigations, particularly in the tropics, may also highlight additional variants in gyrodactylid reproductive

biology, behaviour and ecology. We will also point to the general need for more zoogeographic, phylogeographic and population genetic studies which would provide methods to measure for example migration and transmission dynamics, the amount of inbreeding and the effective population size.

The genus *Gyrodactylus* in particular is megadiverse, with over 400 species described out of a potential total of 20 000 species. We have extensively reviewed the taxonomy and sub-generic phylogeny of the genus, and an important question which remains is whether the genus can be subdivided into more manageable natural units. We have identified three such units and have noted that many of the current generic descriptions do not correspond with natural divisions of the viviparous gyrodactylids. The three groups could be elevated to independent generic status. However, there remain many species which lie outside these three groupings and molecular sampling of the genus has been biased towards European species. More effort in sequencing additional gyrodactylids is essential, although such research may no longer appear novel. At present, inclusion of any new molecular sequence can lead to a dramatic re-drawing of phylogenetic relationships although the basic groupings may remain constant. We therefore conclude that it would be premature to fundamentally revise the genus *Gyrodactylus* to take account of molecular evidence, although this may become a feasible project within the next 5 years.

The primary means of evolution in the viviparous gyrodactylids has been host shifts, a phenomenon which has become very clear from molecular phylogenies of *Gyrodactylus*. In Eurasia, and possibly America, speciation and radiation of gyrodactylids has been greatly enhanced by the ice ages, creating and destroying a series of refugia within which host switches could occur. This phenomenon is potentially of particular interest to evolutionary biologists, but an understanding requires much more sophisticated analysis of patterns of host preference and of host specificity. This lack of knowledge is particularly apparent in relation to *G. salaris* in Norway; the precise relationships of the different forms which infect salmonids in Scandinavia, and which are actively evolving via a series of host shifts, remain obscure and elusive. There is a need to be very careful with nomenclature (which can be legally binding) in such a fluid situation,

and we would highlight the potential of this system for evolutionary biologists with an interest in the role of host shifts. The reasons why *G. salaris* is so damaging, when the congener *G. thymalli* and some *G. salaris* strains are not, remain obscure, and much additional research is needed on the role of gyrodactylids as potential biotic invaders. In particular, we need to identify potential future pathogens, particularly of salmonids, to predict their likely impact. This has lent additional urgency by the recent report (You *et al.*, 2006) that *G. brachymystacis* can establish pathogenic infections of rainbow trout in China, with the potential that this may also become a significant pest in aquaculture.

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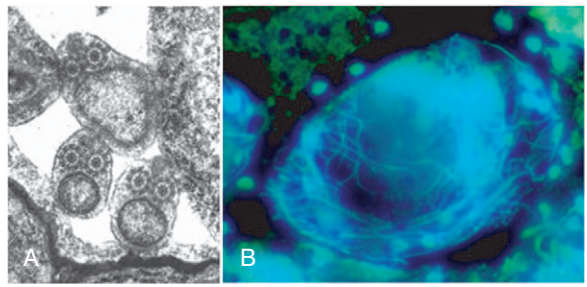

Plate 3.17 (A) Transmission electron micrograph of spermatozoans and of (B) fluorescence microscopy image of inseminated sperm within the ECFR of a bis-Benzimide-stained (see Harris *et al.*, 1997) *Gyrodactylus gasterostei* specimen.

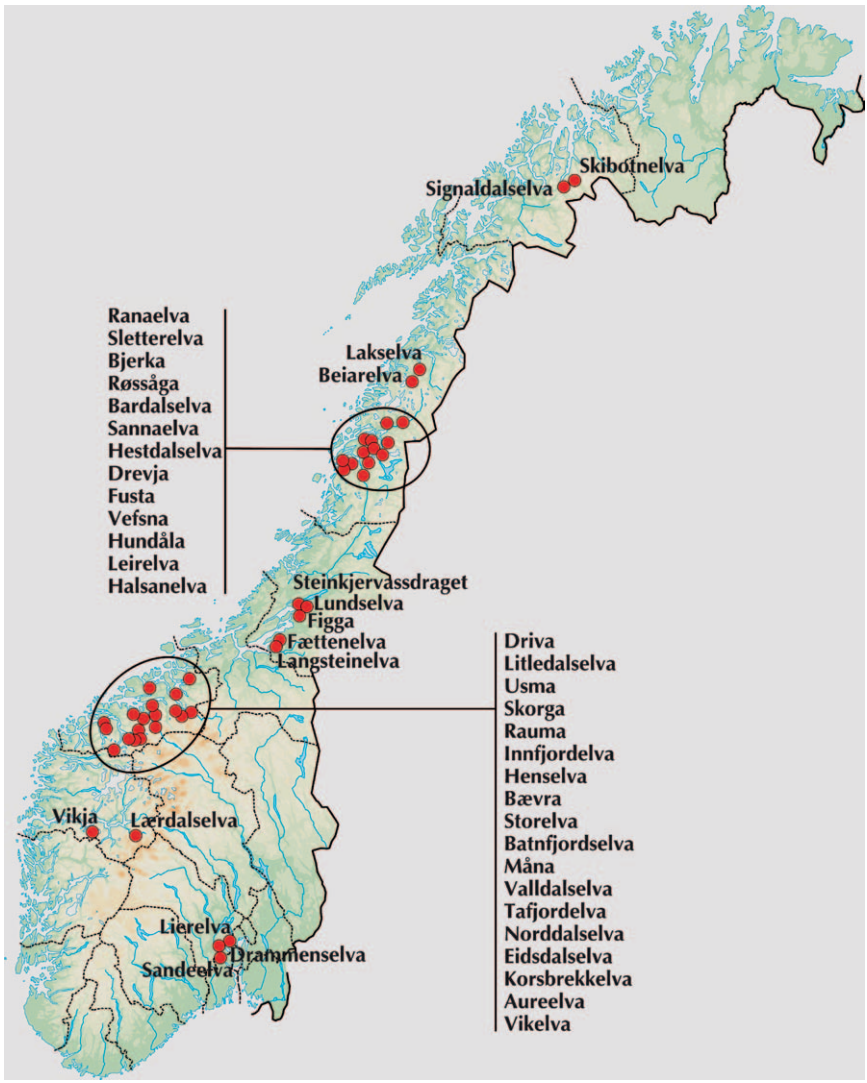

Plate 3.25 The distribution of *Gyrodactylus salaris* on Atlantic salmon in Norwegian river systems per August 2005 since the first observation of the species in mid-1970s. In 2006, one new river is infected, Ranelva, *Region 3—Vefsn Region* (Nordland County); see text. (Reproduced with permission of the Directorate for Natural Resources, Trondheim, Norway.)