KATHOLIEKE UNIVERSITEIT LEUVEN
FACULTEIT WETENSCHAPPEN
Departement Biologie
Afdeling Ecologie en Systematiek der Dieren

HOST-PARASITE INTERACTIONS BETWEEN SYMPATRIC *POMATOSCHISTUS* SPECIES (GOBIIDAE, TELEOSTEI) AND THEIR HELMINTH PARASITES: ECOLOGICAL AND PHYLOGENETIC ASPECTS

Aldegonda Geets

Promotor: Prof. Dr. F. Ollevier
Laboratorium voor Ecologie en
Aquacultuur

Proefschrift aangeboden tot
het behalen van de graad van
Doctor in de Wetenschappen

1998

12845

Jan Mees.

KATHOLIEKE UNIVERSITEIT LEUVEN
FACULTEIT WETENSCHAPPEN
Departement Biologie
Afdeling Ecologie en Systematiek der Dieren

HOST-PARASITE INTERACTIONS BETWEEN SYMPATRIC *POMATOSCHISTUS* SPECIES (GOBIIDAE, TELEOSTEI) AND THEIR HELMINTH PARASITES: ECOLOGICAL AND PHYLOGENETIC ASPECTS

Aldegonda Geets

Promotor: Prof. Dr. F. Ollevier
Laboratorium voor Ecologie en
Aquacultuur

Proefschrift aangeboden tot
het behalen van de graad van
Doctor in de Wetenschappen

1998

Ek wou altijd ‘n seeman hê
Sodat hy baie weg is
en dat ek baie kan verlan
en blij wees as hy kom

Marlene Dumas

Haraka haraka
haina baraka

Swahili spreuk

Dit is mijn geheim, het is heel eenvoudig:
alleen met het hart kun je goed zien.
Het wezenlijke is voor de ogen onzichtbaar.

De Kleine Prins

De wetenschap gaat vooruit
in een dubbele beweging
van verzekering en verrassing,
waarvan geen van beiden
ooit het laatste woord heeft.
Ze stelt ons gerust en verontrust ons.
Ze is beurtelings jong en oud,
conformistisch en revolutionair.
Ook wetenschappers zijn avontuurlijke burgers.

Patricia de Martelaere

TABLE OF CONTENTS

Dankwoord, Acknowledgements

Dankwoord, Acknowledgements

Aan het einde van deze lang zeereis, omringd door grondeltjes en hun parasieten, zou ik graag een aantal mensen willen bedanken die me op deze tocht vergezelden.

Vooreerst wil ik Prof. Ollevier bedanken voor de kansen die hij me steeds heeft geboden, zodat ik, ook na enkele omzwervingen, weer in het labo terecht kon en dit onderzoek mocht voltooien. Prof. F. Ollevier, Prof. D. Thys van den Audenaerde, Prof. G. Malmberg, Dr. J. Mees, Prof. L. De Meester, Prof. F. Volckaert were willing to be members of my jury and I thank them for their support and their useful comments on this PhD.

Prof. Malmberg has been a tremendous help during the Gyrodactylus work. His enthousiasm, patience and support have been very valuable and much appreciated. Without 'Malmberg's fixative', I probably would have given up trying to get these tiny worms under the microscope. The hospitality of his family made my stays in Stockholm very enjoyable. Dr. Chris Appleby kindly provided me with Norwegian gyrodactylids, with very useful remarks on the papers and with encouraging e-mails. Prof. Kennedy, Prof. Berland and Prof. Køie inspired me in my parasitological work.

Paul van Damme en Olivier Hamerlynck zijn de verlichtte 'breinen' geweest achter de initiële opzet van dit onderzoek. Zonder hun aanmoediging en enthousiasme zouden mijn eerste stappen in het onderzoek een stuk moeilijker zijn geweest. Dit onderzoek had nooit tot stand kunnen komen zonder de ondersteuning van het CEMO te Yerseke en het labo voor Mariene Biologie van Gent. Aan Prof. Vinckx en haar medewerkers, mijn oprechte dank voor de samenwerking en Prof. Heip van het CEMO voor de logistieke steun. Dankzij het goede zeemanschap van de schipper Wim Röber en de bemanning van de 'Luctor', Co van Sprundel en Piet de Koeyer, hebben we de woeste golven getrotseerd en kilos grondeltjes binnengehaald! De hulp van Hans Francke, Co's eitjes met spek en Piet's interesse voor al wat op de zeebodem leeft maakten de tochten aangenaam. Op Kris Hostens kon ik altijd rekenen, en niet alleen om in weer en wind een zwiepende boomkor binnen te halen. Jan Mees dank ik voor de goeie samenwerking en dit niet alleen op belgische bodem.

De duizenden grondeltjes werden door een schare van (aangeduide) vrijwilligers bijeengeraapt. De zeef van mijn geheugen laat niet toe ze hier allemaal te bedanken, maar eervolle vermelding gaat naar Renato A., Jan M., Ingrid B., Gert B., Isabelle M., Peter P., Luc JdB, Luc G., Bart G. en Angelo V. Luc De Meester, Bart Geets en Angelo Vermeulen hebben hun 'mankracht' ingezet om grondeltjes te proberen slepen langs de belgische stranden, maar die hadden ons van ver zien komen... De weken op de 'Belgica' waren een belevenis dankzij het team van het Rijksstation voor Zeevisserij. Speciale dank aan Kris Cooreman voor de hulp en aan Omer Decock voor het aanvoeren van springlevende grondeltjes van de Oostendse Banken na een paar nachtjes vissen.

De SEM-foto's zouden nooit zo prachtig zijn geweest zonder de professionele aanpak van Julien Cillis van het KBIN. Dr. Boudewijn Goddeeris en Angelo Vermeulen dank ik voor het gebruik van het videoprint systeem, wat het meetwerk aanzienlijk vergemakkelijkte. Karl Cottenie zette me af en toe op het rechte pad als ik weer een of andere statistische hersenkronkel maakte. Giovanna kindly helped me as the 'slide-specialist'. Luc De Meester dank ik voor zijn niet-'versagend' enthousiasme en voor het lezen van de zoveelste versie.

Filip Volckaert en Marek Zietara for the courage to start DNA work on the tiny gyrodactylids, and for the hopeful results which boosted my enthousiasm.

Prof. Ollevier, Prof. Thys van den Audenaerde en Prof. Billen schonken me het vertrouwen om te assisteren in de practica en gunden me de vrijheid om deze taak op mijn manier in te vullen. De job als assistente maakte het doctoreren ook financiëel leefbaar. Prof. Thys van den Audenaerde dank ik voor het delen van zijn Afrika interesse, voor de aanmoediging en de leerrijke gesprekken over biologie en Afrika, de zin en onzin van ontwikkelingssamenwerking en Afrika, kunst en Afrika,...

Veel vrienden en collega's maakten het leven en werken in het labo aangenaam. Ria, de innemende, stond altijd klaar met praktische oplossingen, Gaston bouwde een viersterrenhotel voor grondels en Eddy was een onmisbare hulp en een goeie ploegmaat bij de organisatie van de practica. Maria, Conny, Martine en Stef, bedankt voor de schijnbaar evidente practische hulp. Heidi, bedankt voor de sukuma's, de leute en de Kenya avonturen. Hanne, bedankt voor de Stuc-uurtjes, Olivier voor de fylosofische gesprekken, Erik, Karl, Joost, Geert, Vanessa, Bob, Jimi, Eugène, Giovanna, Christoph, Peter, Els, Tine hebben allen op hun manier een steentje bijgedragen. Bedankt allemaal!

Als de moed me af en toe in de schoenen zonk stonden er steeds goede vrienden klaar. Pol wil ik bedanken voor zijn stroom aan ideeën en zijn pioniersgeest. Luc Janssens de Bisthoven voor zijn steun tijdens mijn studententijd en bij mijn eerste stappen als 'doctorandus- in-spe', voor het samen opgroeien en voor de prachtige tijd in Kenya. Luc Grisez was een 'unicum' op het labo! Luc, bedankt voor je steun over de jaren, de gezellige babbels, de opkikkers, de duikavonturen, de beresnoet en nog veel meer en Ann bedankt je voor enthousiasme en vitaliteit, het werkt aanstekelijk! Krista, bedankt voor je gedeelde 'parasieten-smart' in de beginjaren, je levensvreugde en je vriendschap. Viève voor de fijne jaren vanaf de F. Lintsstraat, voor het 'leven vanuit je buik'en de gastvrijheid van Villa Kakelbont! Ingrid G. beurende me op met telefoontjes en de lange gesprekken. Jan S., bedankt voor de prachtige Mombasa tijd en voor het delen van mijn 'gekke' ideeën. Stein, my viking friend, thanks for being around. Els en Michel dank ik voor hun onvoorwaardelijke steun aan deze Calimero, en Koen en Bie voor de Kenyatta periode en al wat volgde. Ingrid en Suzanne, bedankt voor de ultieme steun en voor de kunstzinnige inspiratie. Angelo, bedankt voor de vele maffe en intense momenten van de voorbije jaren, voor je kritische kijk op de wereld, voor de 'ontsnappingen' als de druk van het doctoreren ons beiden teveel werd en omdat ik een stukje mocht meegaan op je fotografishe avonturen.

De fameuze 'Geets-brothers' stonden altijd paraat. Bart, bedankt voor je "hé zus, hoe is't"-telefoontjes en voor het Mali-avontuur en Lieven voor het ontdekken van de 'vijfhonderd-frank-woorden' in mijn doctoraat en omdat je altijd als 'handy-man' klaar stond als ik weer met een of ander practisch probleem zat.

Ik wil dit doctoraat opdragen aan mijn ouders die me alle kansen boden om me te kunnen ontplooien tot wat ik nu ben. Ze hebben deze tocht van in het prille begin meegemaakt en steunden me niet aflatend in al mijn 'wilde' ideeën. Woorden schieten hier tekort maar dit is voor jullie! Bedankt!

Gonda

Introduction and objectives

The observed parasite fauna of a host species, also called the component community, is the result of the influence of different abiotic and biotic screens or filters on the potential parasite fauna of a certain area (Holmes 1987, 1990). In a marine environment, abiotic factors such as differences in temperature, salinity, inshore-offshore habitats and depth are important in determining which local parasite fauna can be expected. Furthermore, natural disturbances (El Niño, epidemics affecting keystone species,...) and antropogenic effects (e.g. pollution; Khan *et al.*, 1991, Siddall *et al.* 1994, Bagge & Valtonen, 1996) may have an important direct or indirect effect through the host community. The biotic factors influencing the local parasite fauna depend on the composition and functioning of the host community. In an aquatic environment, the host community not only includes fish and other vertebrates, but also invertebrates, which play an important role as intermediate hosts.

The ecology of parasites is unique in the sense that their biotic environment, namely the host, plays a paramount role (Price,1990). For a parasite community, the resource base is the host population, in many cases involving definitive as well as one or more intermediate and paratenic hosts. The characteristics and dynamics of the host population will have a major influence on the parasite community. Host-dependent factors which influence the composition of the component community of parasites are: the host's geographical range, its population density, its mobility, age and life-span, its position in the food web and diet, and its physiology (including immune responses).

Towards the different hosts in an ecosystem, parasites exhibit differences in their ability to form stable relationships. Parasites seem to be restricted to certain hosts, a phenomenon called host specificity. Host specificity largely varies among different parasite groups and species. In assessing host specificity, not only the host range should be taken into account, but also the differences in intensity and prevalence of the infection. Host specificity can have a phylogenetic basis, where only hosts belonging to specific taxa are infected by a specific parasite species, as well as an ecological basis, in which case the parasite will only infect hosts with a certain ecological niche (Rohde, 1982).

In this thesis, the influence of ecological and phylogenetic factors on host-parasite relationships between sympatric *Pomatoschistus* species (Pisces, Gobiidae) and their helminth parasites has been studied.

Gobies of the genus *Pomatoschistus* are very common along the European coasts. In shallow coastal areas they can reach very high densities and are important food items for many piscivorous fish, birds and mammals. Although their place in the food web of estuarine and marine areas has been studied extensively, their role as hosts for parasites has largely been overlooked. Our study focused mainly on the two most common sympatric species of the shallow coastal waters of the Dutch Grevelingen Voordelta Area, *Pomatoschistus minutus* and *P. lozanoi*, as hosts for helminth parasites. Because of their morphological similarity, they were only recently recognised as distinct species. Wallis & Beardmore (1984) showed that *P. minutus* and *P. lozanoi* are phylogenetically very closely related. Most earlier parasitological studies on gobies tend to lump both species together as *P. minutus*, which is unfortunate since they have a distinct ecological niche (Hamerlynck, 1993, Hamerlynck & Cattrijsse, 1994). *P. minutus* is a generalist, feeding largely on benthic and epibenthic food items, while *P. lozanoi* specialised on hyperbenthic and pelagic prey. This difference in food niche is associated with a difference in spatial niche. Furthermore, they have different spawning periods in the Southern Bight of the North Sea, with *P. lozanoi* spawning two months later than *P. minutus*. Both gobies are fast growing and have a short life span of about one year. The ecological differences of *P. minutus* and *P. lozanoi*, combined with the fact that they are sympatric and phylogenetically closely related, make them very interesting study objects from a parasitological point of view.

Our main aim was to determine the impact of ecological and phylogenetic aspects on the composition of the parasite component community of *P. minutus* and *P. lozanoi*. Differences in component community do not only include parasite species richness, but also differences in abundance and prevalence of the different parasite species.

In a first part, we investigate to what extent the phylogenetic similarity of both gobies influenced the presence of similar parasite species. We concentrated the study on monogeneans of the genus *Gyrodactylus*. Monogeneans are known to be very host specific and homoxenous, having only one host in their life cycle. High phylogenetic host specificity and a close relationship between host and parasite promote co-evolution (Connell, 1980). In this part of the study, two other *Pomatoschistus* species were involved: *P. pictus* and *P. microps*. They both occurred in the same sampling area, and were used as reference material. A lot of research in parasitology was triggered by the Fahrenholz's rule: 'parasite phylogeny mirrors host phylogeny' (Eichler, 1948). In our study, the hypothesis was put forward that the closest related gobies (*in casu P. minutus* and *P. lozanoi*) would carry the (phylogenetically) most closely related *Gyrodactylus* species.

The second question we focused on was, to what extent ecological differences between *P. minutus* and *P. lozanoi* influenced their respective component communities. The ecological niche of *P. minutus* and *P. lozanoi* differs with respect to diet, spatial distribution and reproductive period. Each of these aspects should be taken into account when interpreting differences in parasite species richness and parasite load. Since many heteroxenous parasite species (parasites with more than one host in their life cycle) enter the host through the food web, differences in food niche will most probably be reflected in changes of endoparasite fauna. To analyse this issue, we focused on the most abundant endoparasitic helminths occurring in the two goby species studied. Differences in spatial niche (one host being more benthic, the other more pelagic) may also influence the parasite fauna. For example, if the infection strategy of the parasites is based on free-living non-swimming stages (like in the Gyrodactylidae), close contact between the host and the substrate on which the parasites are dispersed, is essential for transmission. In the case of the Gyrodactylidae, we expect a benthic fish to have a higher chance of getting infected than a pelagic one. Finally, the differences in reproductive period between both gobies results in a temporal shift in occurrence of their young and a temporal overlap of adults of *P. lozanoi* with the juveniles of *P. minutus*. This was expected to influence the transmission of parasites and the composition of the parasite community of the hosts. In addition, the ecological niche of the fish hosts is not fixed, but subject to changes in time. These changes are correlated with ageing of the fish and different behaviour patterns during spawning. Therefore, it was a prerequisite to study the seasonal dynamics of the parasite fauna.

References

Bagge, A. M., & Valtonen, E. T. (1996) Experimental study on the influence of paper and pulp mill effluent on the gill parasite communities of roach (*Rutilus rutilus*). *Parasitology*, **112,** 499-508.

Connell, J. H. (1980) Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos*, **35,** 131-138.

Eichler, W. (1948) Some rules in ectoparasitism. *Ann. Mag. Nat. Hist.*, **1,** 588-598.

Hamerlynck, O. (1993) *The ecology of Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) in the shallow coastal waters and estuaries of the Southern Bight of the North Sea*. PhD thesis, University Gent, 187 pp.

Hamerlynck, O., Cattrijsse, A., & Arellano, R. V. (1993) Daily ration of juvenile *Pomatoschistus lozanoi* de Buen (Pisces: Gobiidae). *ICES J. Mar. Sci.*, **50,** 471-480.

Hamerlynck, O., & Cattrijsse, A. (1994) The food of *Pomatoschistus minutus* (Pisces, Gobiidae) in Belgian coastal waters, and a comparison with the food of its potential competitor *P. lozanoi*. *Journal of Fish Biology*, **44,** 753-771.

Holmes, J. C. (1987) The structure of helminth communities. *Intern. J. Parasitol.*, **17,** 203-208.

Holmes, J. C. (1990) Helminth communities in marine fishes. in: G. Esch, A. Bush, & J. Aho *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 5, pp. 101-130.

Khan, R. A., Overstreet, R., & Thulin, J. (1991) Influence of pollution on parasites of aquatic animals. *Ann. Parasitol. Hum. Comp.*, **66 Suppl. I,** 49-51.

Price, P. W. (1990) Host populations as resources defining parasite community organization. in: G. Esch, A. Bush, & J. Aho *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 5, pp. 21-40.

Rohde, K. (1982) *Ecology of Marine Parasites*. St. Lucia, University of Queensland Press, 245 pp.

Siddall, R., Pike, A. W., & McVicar, A. H. (1994) Parasites of flatfish in relation to sewage sludge dumping. *Journal of Fish Biology*, **45,** 193-209.

Wallis, G. P., & Beardmore, J. A. (1984) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces, Gobiidae). *Biological Journal of the Linnean Society*, **22,** 107-123.

Chapter 1

***Pomatoschistus* species: their life history and role as hosts for parasites: a short review of literature.**

Introduction

The family of the Gobiidae belongs to the suborder of the Gobioidea which comprises an extensive group of acanthopterygian teleosts. The Gobiidae, with about 200 genera and an estimated 1800 species, is the largest family of marine fishes (Nelson, 1984, Miller, 1986). At least 52 species of Gobiidae are recorded from the north-eastern Atlantic and Mediterranean coasts (Miller, 1986).

Gobies from European waters are small-sized fish, mostly smaller than 15 cm. They have typical moderately elongated cylindrical body forms and dorsolateral eyes. There are two dorsal fins, one anal and two large pectoral fins and a rounded caudal fin. The pelvic fins are thoracic and fused in a weakly suctorial disk with an anterior transverse membrane (frenum). This allows them to remain stationary even in moderately high currents. They have intermittent swimming behaviour with short darting movements (Miller, 1986).

In marine and brackish waters, gobies are usually very abundant. Except for a few neritic forms, most gobies have a benthic lifestyle. Due to their small size, gobies are efficient predators on small prey items and they have the possibility to exploit a diverse range of spatially restricted habitats (Hoese, 1984). They form the dominant element in the small-sized fish fauna in tropical and subtropical areas, especially on coral reefs and in mangroves (Little *et al.*, 1988, Blaber & Milton, 1990, Winterbottom & Emery, 1986). Among fish families reported from the global mangroves, the Gobiidae enjoy having the maximal number (110) of species (Krisnamurthy *et al.*, 1984). In the Red Sea, 96 goby species were reported, outnumbering other families (Goren & Dor, 1994). Certain goby species have a strong association with specific corals for food, shelter and spawning sites, e.g. *Paragobiodon* *echinocephalus* and the branching coral *Stylophora pistilla* (Kuwamura *et al.*, 1994). Commensalism with alpheid shrimps is often reported (Polulin & Lubock, 1979). Certain species have adapted to extreme environmental conditions such as the Australian desert goby *Chlamydogobius eremius* occurring in artesian springs in arid Central Australia (Miller, 1987). Temperate waters also harbour quite high diversities of gobies. Lough Hyne (Southwest Ireland), for instance, has a species richness of not less than 11 goby species (Costello, 1992, Miller, 1986).

The genus *Pomatoschistus* Gill, 1864

Along the European coasts, the most common and widely distributed species of the Gobiidae belong to the Genus *Pomatoschistus* Gill, 1864. In the Belgian and Dutch shallow coastal waters, six goby species occur, of which only two species do not belong to the genus *Pomatoschistus*. The transparent goby, *Aphia minuta* (Risso, 1810), a pelagic species, is occasionally caught in bottom trawls, but spends most of its time close to the water surface. It becomes sexually mature after 1 year and dies after spawning (Miller, 1986). The black goby, *Gobius niger* Linnaeus, 1758, is the largest species (maximal size 15 cm). It inhabits inshore waters and lagoons and has a life span of at least 4 years (Hamerlynck & Janssens, 1985, Miller, 1986, Doornbos & Twisk, 1987). The four *Pomatoschistus* species are abundant in shallow coastal waters: the sand goby *P. minutus* (Pallas, 1770), his close relative Lozano's goby *P. lozanoi* (de Buen, 1923), the common goby *P. microps* (Krøyer, 1838) and the painted goby *P. pictus* (Malm, 1865). From deeper waters (>18 m) *P. norvegicus* (Collett, 1903) has been reported (Fonds, 1971).

Systematics and identification of *Pomatoschistus* spp.

The small size and morphological similarity between the members of the genus *Pomatoschistus* have generated considerable taxonomic confusion, not only between species within the genus but also with the closely related genus *Knipowitschia*. The arrangement of the head sensory papillae provide important criteria for classification. Miller (1986) describes

11 species of *Pomatoschistus* from the North-eastern Atlantic and the Mediterranean and provides a dichotomous key for determination.

Pomatoschistus minutus (Pallas, 1770)
Sand goby

Pomatoschistus lozanoi (de Buen, 1923)
Lozano's goby

Pomatoschistus microps (Krøyer, 1838)
Common goby

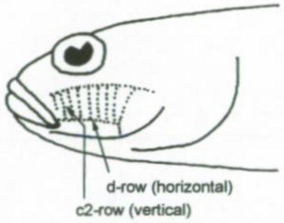
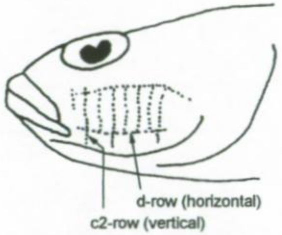
Pomatoschistus pictus (Malm, 1865)
Painted goby

Figure 1.1: The four most abundant *Pomatoschistus* species of the Voordelta Area (after Fonds & Veldhuis, 1973). Gobies were not drawn to scale.

A description of the characteristics used for the determination of the four most abundant *Pomatoschistus* species (Fig. 1.1) of our shallow coastal areas, *P. minutus, P. lozanoi, P. pictus* and *P. microps* are provided by Webb (1980), Edlund *et al.* (1980) and Hamerlynck (1990). A summary of the most important morphological differences between the species is given in Table 1.1.

In a comprehensive study, Webb (1980) solved the taxonomic problems of the members of the *Pomatoschistus minutus* complex: *P. minutus, P. lozanoi* and *P. norvegicus*. Because of its intermediate characteristics, *P. lozanoi* was first seen as a hybrid between *P. minutus* and *P. norvegicus* (Webb & Miller, 1975). The three gobies were finally regarded as separate species on morphological, biochemical, karyological and ecological grounds (Fonds, 1973, Webb, 1980). Under experimental conditions, Fonds (1970, 1971) succeeded in rearing hybrids between *P. minutus* and *P. lozanoi*. Evidence of the existence of naturally occurring hybrids between *P. minutus* and *P. lozanoi* and between *P. lozanoi* and *P. norvegicus* is provided by Webb (1980) and Wallis & Beardmore (1980). In nature, however, these hybrids seem to occur only in very low frequencies (0.3 % - 0.9 %). This suggests that there must be a selection mechanism working against hybridisation. Differences in spawning time, courtship

Table 1.1: Summary of main characteristics for the identification of four *Pomatoschistus* species (adapted from Webb, 1980, Edlund *et al*., 1980, Hamerlynck, 1990).

	P. minutus Sand goby	*P. lozanoi* Lozano's goby	*P. microps* Common goby	*P. pictus* Painted goby
Colour	• grey, reticulation on dorsal side	• pale with small red-brownish spots	• dark with reticulation on dorsal side	• dark with reticulation on dorsal side
Pigmentation	• flanks few vertical double bands in mature males • double spots on lateral line in juveniles • first dorsal fin with dark blue spot in both sexes, spot not reaching the edge of the finmembrane • no dark line (moustache) between eye and mouth in ripe females	• flanks with seven to nine single bands • single spots on the lateral line in juveniles • first dorsal fin with dark blue spot only in spawning males, spot reaching the edge of the finmembrane • dark line between eye and mouth in ripe females (moustache) and dark chin	• dark angle at the basis of the pectoral fins	• dark spot at dorsal base of pectoral fin • two rows of black spots on the first dorsal fin
Attachment of branchiostegal membrane	• attachted only to the anterior half of the isthmus side	• attachted only to the anterior half of the isthmus side	• attachted to the entire isthmus side	• attachted to the entire isthmus side
Pigmentation of spleen	• no pigmentation	• no pigmentation	• spleen with dark chromatophores	• spleen without dark chromatophores
Sensory papillae	• second c-row not below d-row  	• second c-row below d-row  		

behaviour and selection of nesting sites as well as a reduced viability or fertility of the hybrids are possible factors reducing hybridisation on a large scale in nature (Fonds, 1971).

Phylogenetic relationships between some *Pomatoschistus* spp.

The close resemblance of *P. minutus* and *P. lozanoi* as well as their cross-breeding in captivity resulting in viable larvae, indicated they are closely related and probably speciated recently (Fonds, 1973). The high degree of endemism in the Gobiidae of the Ponto-Caspian area indicates that the western Mediterranean may have been an isolated refugium during the Pleistocene and possibly a centre of speciation (Wallis & Beardmore, 1984a, Penzo *et al.,* 1998). Furthermore, Fonds (1973) noted that *P. microps* and *P. pictus*, which are more specialised than *P. lozanoi* and *P. minutus*, may have speciated earlier. Basically, the ideas of Fonds (1973) were confirmed by Wallis & Beardmore (1984a). An allozyme electrophoresis of the genetic variation at 31 loci carried out by these authors, assessed the systematic relationships in nine closely related goby species, including *P. minutus*, *P. lozanoi*, *P. pictus* and *P. microps*. Figures 1.2 and 1.3 summarize their results. Their findings essentially agreed with the relationships established using classical taxonomic approaches. *P. minutus* and *P. lozanoi* showed the highest identity values and *P. norvegicus* is a highly related with both species. In the phenetic dendrogram (Fig. 1.2), *P. minutus* and *P. norvegicus* are more closely related to the other goby species than *P. lozanoi*. *P. pictus* seems to be phylogenetically closer to the members of the *P. minutus* complex than *P. microps*. The formation of the *P. minutus* complex and the speciation of *P. microps* was estimated at 4 to 6 million years B.P., while *P. minutus* and *P. lozanoi* shared a common ancestor as recently as 2.5 million years B.P.

Geographical distribution and microhabitat preference of *Pomatoschistus* spp.

P. minutus occurs in the eastern Atlantic from Tromsø (Norway) to Spain, in the Baltic and in parts of the Mediterranean and the Black Sea (Miller, 1986). The sand goby prefers sandy and muddy sediments with cover (*Zostrea marina*, eelgrass), to depths of about 20 m (Miller, 1986*)*. *P. minutus* is described as an epibenthic species (Wilkins & Myers, 1992).

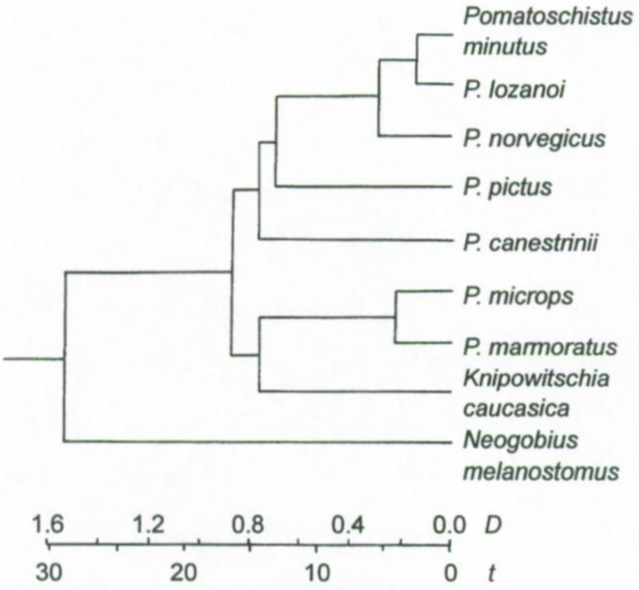


Figure 1.2: Phenetic dendrogram of gobies of the genera *Pomatoschistus, Knipowitschia* and *Neogobius* produced using the UPGMA procedure on Nei's *D* values (genetic distances) adapted from Wallis & Beardmore (1984) (For table of genetic distances see Wallis & Beardmore ,1984). *t* = estimated time of divergence in million years (Vawter *et al.,* 1980).

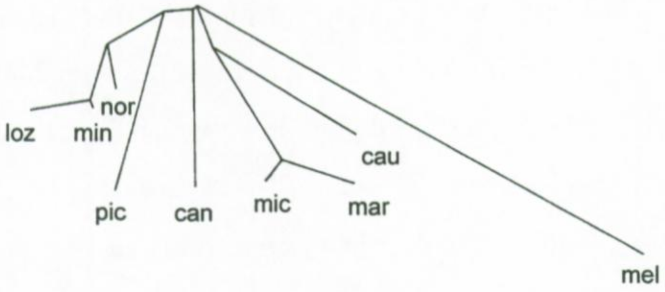


Figure 1.3: Phylogenetic tree constructed using the Fitch-Margoliash procedure of the matrix of Nei's *D* values, from Wallis & Beardmore (1984). (for matrix of *D* values see Wallis & Beardmore, 1984a). Abbreviations: loz: *Pomatoschistus lozanoi*, min: *P. minutus*, nor: *P. norvegicus*, pic: *P. pictus*, can: *P. canestrinii*, mic: *P. microps*, mar: *P. marmoratus*, cau: *Knipowitschia caucasica*, mel: *Neogobius melanostomus*.

P. lozanoi has been reported in the eastern Atlantic from the North Sea (at least 50°30'N) along the East and West coast of Scotland to the west coast of Portugal (40°N) (Miller, 1986, Arruda & Azevedo, 1987, Hamerlynck, 1990). Its distribution is marine inshore and off the mouth of estuaries, this species is rarely found at depths over 35 m (Wallis & Beardmore, 1984b) Although *P. lozanoi* has been regarded as more stenohaline than *P. minutus* (Fonds, 1973), it is common in the estuary of the Zeeschelde where low salinities occur (Maes *et al.*, 1997, Maes *et al.*, 1998). Fonds (1973) suggested that, in comparison with *P. minutus*, *P. lozanoi* is a more neritic species.

P. pictus has been reported in the eastern Atlantic from Tromsø to northern Spain and in the Adriatic Sea and the Sea of Marmara (Koefoed, 1979, Miller, 1986). The painted goby preferably inhabits inshore open habitats with gravel or sandy bottom, up to 55 m depth (Miller, 1986, Costello, 1992). Wilkins & Myers (1992), however, describe the microhabitat of the painted goby as semi-hyperbenthic with a preference of muddy areas with a small amount of hard substrate. In general, this species avoids areas with reduced salinity (Wallis & Beardmore, 1984b).

P. microps is the most euryhaline of the four species described here, and is typically found in estuaries and brackish water. Its distribution includes the Eastern Atlantic from Trondheimsfjord and the Baltic to Southern Portugal and the North-western Mediterranean. The common goby is mostly found in open mud-sand habitats and shallow waters of even less than 1 m depth (Fonds, 1971, Nellbring, 1985, 1986). Interspecific interference competition with other goby species (*P. minutus*, *Gobius niger*) might play a role in the habitat choice of this small goby species (Wiederholm, 1987).

Seasonal distribution and migration patterns of *Pomatoschistus* spp.

The population densities of *P. minutus* and *P. lozanoi* fluctuate seasonally. In the Dutch Wadden Sea, sand gobies have the highest densities in autumn and spring and the lowest densities in winter and early summer (Fonds, 1973). Young pelagic gobies are abundant in the Wadden Sea in summer. After a pelagic phase of approximately 4 to 6 weeks (at a water temperature of about 15°C) they switch to demersal life at a size of about 12 mm. The appearance of juveniles of *P. minutus* and *P. lozanoi* is not simultaneous: *P. minutus* juveniles emerge in June while *P. lozanoi* young are found from September onwards (Fonds, 1973, Arellano, 1995). During winter, the gobies from the Wadden Sea migrate offshore to warmer waters (Fonds, 1973). In the Oslo fjord, *P. minutus* migrates from the littoral in

October - November to the open water, and abundances were very low until April, when they returned to spawn. The new yearclass was recruited in August (Hesthagen, 1975, 1977). Jones & Miller (1966) suggested that the offshore winter migration only takes place in areas where water temperature falls below 5°C. Fonds (1973) reported that sand gobies avoid water temperatures below 2.5 °C. Healey (1971, 1972), studying the population ecology of *P. microps* and *P. minutus* in the Ythan estuary (Scotland), claimed that changes in abundance in winter are not due to the avoidance of low temperature or to breeding behaviour but he did not provide an alternative explanation for the migration.

Important differences in migration patterns exist between populations of gobies in the Mediterranean and the North-Atlantic (Bouchereau *et al.*, 1989, Iglesias, 1981). In the southern populations of gobies, winter migrations are not observed. Populations of gobies, including *P. minutus*, *P. microps* and *P. pictus*, in the Ria de Arosa (South West Spain) are resident and no important seasonal changes in abundance occur (Iglesias, 1981). The sedentary nature of the Mediterranean gobies observed by Iglesias (1981) was not completely true for the *P. minutus* populations in the Gulf of Lyon (Bouchereau *et al.*, 1989). In the Gulf of Lyon, young sand gobies arrived in spring (February - April) and used the lagoon for feeding and growth until the mature gobies migrated towards the sea from November - December onwards, where they spawn and die. The population of sand gobies in the Gulf of Lyon was completely renewed each year. The offshore migration of *P. minutus* in the Gulf of Lyon between December and April was therefore interpreted as a breeding migration.

An interesting observation in the Oslo fjord showed that *P. minutus* does not only avoid extreme low temperatures but also high temperatures. In the Oslo fjord, low tidal ranges and a dry inland climate result in water temperatures of 19 °C up to 5 - 6 m depth in summer. *P. minutus* migrates towards the sea to avoid these warm temperatures (Hesthagen, 1977, 1979). Fonds & Veldhuizen (1973) mentioned that *P. minutus* tolerates less high temperatures than its relative *P. microps*.

In summary, the seasonal distribution of *P. minutus* and *P. microps* shows that these gobies are more sedentary in the southern Atlantic and the Mediterranean than in the northern populations. The seasonal fluctuations in abundance are related to migration and reproduction.

Reproduction of *Pomatoschistus* spp.

In the North-eastern Atlantic, the breeding period of *Pomatoschistus* species falls in spring and early summer. The reproductive biology of gobioid fishes has been reviewed by Miller (1984).

* Reproduction period

P. minutus eggs have been collected in the Dutch Wadden Sea and the Belgian coastal waters from late April to early June and *P. lozanoi* eggs from late May to mid August (Fonds, 1973, Hamerlynck & Cattrijsse, 1994). Both *Pomatoschistus* species mature after their first winter. In the Belgian coastal waters, females of *P. minutus* and *P. lozanoi* have a maximal gonadosomatic index (GSI) in April and May (25 % *for P. minutus* and 20 % for *P. lozanoi*). Although the period of maximal GSI coincided for both goby species, the spawning season is later for *P. lozanoi* than for *P. minutus*. Hamerlynck & Cattrijse (1994) suggested a possible delayed spawning of *P. lozanoi* because of interspecific competition for nest sites. The temporal changes of the GSI over the year were the same in female and male *P. minutus* and *P. lozanoi*. Remarkably, the maximal GSI of *P. lozanoi* males was about half the maximal GSI of *P. minutus* males. Occasionally, sneaker males with aberrantly high testis weights and external pigmentation of females are observed for both *P. minutus* and *P. lozanoi* (Hamerlynck & Cattrijsse, 1994). These sneaker males represent about 2 % of the males in both species. Magnhagen (1992) concluded sneaking to be an opportunistic strategy of small males in *P. microps*. The breeding season of *P. microps* is from May till August on the Swedish west coast (Magnhagen, 1992) and from April to August along the British west coast (Rogers, 1988, 1989). The size of the males depend on the time of hatching in the breeding season. Small male *P. microps* would sneak until they are large enough to defend their own nest. Some *P. microps* acted as both sneakers and nest builders in different environments (Magnhagen, 1992).

Most authors report spawning periods in the North-eastern Atlantic populations of *P. minutus* from April till August (Fonds, 1973, Hesthagen, 1977, Hamerlynck & Cattrijsse, 1994). Temperature and salinity are important factors in the regulation of spawning (Fonds, 1973). As expected, spawning starts earlier in populations from areas with higher water temperature. In the Gulf of Lyon (Mediterranean), the reproduction period starts already in December and runs till April (Bouchereau *et al.*, 1990).

* Reproductive behaviour

Gobies exhibit a typical reproductive behaviour. During the mating season, males develop distinct nuptial colours, while females do not change much in pigmentation. Specific courting behaviour has been described for various *Pomatoschistus* species (Nyman, 1953, Fonds, 1973). Males make nests under shells and stones and defend territories of about 20 - 30 cm around them (Fonds, 1973, Nellbring, 1993). In the North sea, *P. minutus* prefers the shells of *Ostrea edulis, Cyprina islandica* and *Mya arenaria* for nesting, while shells of smaller size of *Mactra corallina, Laevicardium crassum* and *Cardium echinatum* were more often used by *P. lozanoi* (Fonds, 1973). Most nests are found between 10 m and 25 m depth. In the Oslo fjord, where only minor tidal oscillations occur, *P. minutus* builds nests in shallow water of less than 1 m depth (Hesthagen, 1977). Hamerlynck & Cattrijsse (1994) formulated the hypothesis that interspecific competition for nest sites between *P. minutus* and *P. lozanoi* caused delayed spawning of *P. lozanoi*. From field experiments with two other sympatric gobies, *P. minutus* and *P. microps* in the Northern Baltic, Nellbring (1993) noted that interspecific competition for nest sites was minimized, because spawning was separated in time, choice of shell sizes and preferred depth.

Female sand gobies prefer to mate with larger males, especially when the male possesses a high-quality (large) nest (Lindström, 1992a, 1992b). Male sand gobies show a preference for large nest sites (Lindström, 1988). Females will attach the eggs to the ceiling of the nest. One nest may contain batches of different females, and one female may use different nests to deposit her eggs. The males fertilize the eggs and guard them until they hatch. The pelagic young will switch to a demersal life after about 4 to 6 weeks. Parental guarding behaviour of

P. minutus and *P. microps* and its relation to reproductive investment and success has been thoroughly investigated by Magnhagen & Vestergaard (1991) and Lindström & Wennström (1994).

* Fecundity

Female *P. minutus* and *P. lozanoi* can produce several batches of eggs in one breeding season (batch spawners). The fecundity is function of the size of the female (Healey, 1972, Bouchereau *et al.*, 1990). Per batch, 2000 to 4000 eggs are laid. A female can produce 3 to 4 batches in the course of one or two months (Fonds, 1973). In the Mediterranean, however, Bouchereau *et al.* (1990) report for *P. minutus* a maximum of 12 batches in four months, with an estimated annual fecundity of 11976 to 61200 eggs. A female *P. microps*, averaging 35 mm, would produce about 7800 eggs by spawning 6 times over the season (Miller, 1979).

Growth, maximum size and life span of *Pomatoschistus* spp.

In the Belgian and Dutch coastal waters, growth of *P. minutus* and *P. lozanoi* is fastest from July to October and slowest from January to March (Hamerlynck, 1993). *P. minutus* males grow faster than females in the Voordelta Area (SW Netherlands), but this difference in growth rate was not observed in *P. lozanoi* (Arellano, 1995). The sex-related difference in growth rate of *P. minutus* were not confirmed for populations of the Oslo fjord (Hesthagen, 1977).

Theoretical maximum size (standard length) for *P. minutus* in the Voordelta was 79 mm for males and 70 mm for females (Arellano, 1995). *P. lozanoi* males were estimated to reach a theoretical maximal size of 59 mm, while females grow only to 49 mm. Maximal total length of *P. minutus* in the Oslo fjord was 91 mm, which is much larger than reported from other localities of the same latitude (Hesthagen, 1977).

Pomatoschistus species have a short life span, mostly of less than 2 years. A high percentage of the adults die after their first spawning. The theoretical maximal longevity is higher for females of *P. minutus* and *P. lozanoi* (both 650 days) than for males (620 days for *P. minutus* and 600 days for *P. lozanoi*) (Arellano, 1995). Doornbos & Twisk (1987) estimated an annual mortality of 99.9 % for both *P. minutus* and *P. microps* in the saline lake Grevelingen (The Netherlands). *P. microps* reaches a maximal total length of 64 mm. This species also has a short life-span, with few individuals surviving their second winter (Jones & Miller, 1966, Fouda & Miller, 1981). *P. pictus*, with a maximal size to 57 mm, is sexually mature after one year and has a life-span of about two years (Miller, 1986).

Food niche and diet of *Pomatoschistus* spp.

Miller (1979) assumed that the success of the small-sized Mediterranean - Atlantic gobies of the genus *Pomatoschistus* was partly due to their ability to exploit the rich meiobenthos of soft bottoms as a food source. Detailed studies on the diet of gobies revealed that meiofauna prey items are much less important than generally expected (Gee, 1987, Hamerlynck, 1993, Hamerlynck *et al.* , 1993, Hamerlynck & Cattrijsse, 1994). However, in the Baltic, with its marked seasonality, low salinity (approximately 5.7 pro mille) and low species richness, meiofauna is an important food source for gobies (*P. minutus*) less than 40 mm (Aarnio & Bonsdorff, 1993). When Hamerlynck & Vanreusel (1993) observed the selective feeding of *P. minutus* on *Mesacanthion diplechma*, the importance of this meiobenthic nematode in the foodweb of the Oosterschelde was questioned. In spite of its low abundance in the meiofauna of that area, this large nematode was about the only free-living nematode found in the stomach of *P. minutus*. But, its importance as a link to higher trophic levels seemed limited. Food selection of gobies depends on several factors, of which availability of the prey is of major importance. Prey density, mobility and visibility (gobies are visual predators) determine availability (Zander, 1979, Magnhagen & Wiederholm, 1982).

* Diet of *P. minutus* and *P. lozanoi*

The diet composition of *Pomatoschistus* species has been the aim of several studies in different geographical areas. The diet of *P. minutus* was studied by Aarnio & Bonsdorf (1993) in the Northern and the Western Baltic by Zander (1990). Because of their high abundance, *Pomatoschistus* species are often included in studies on feeding relationships of small-sized fish species, as in the Tagus estuary (Portugal) (Costa, 1988), the Baltic fjord Schlei (Germany) (Zander & Westphal, 1992) and the Southern Bothnian Sea (Sweden) (Thorman & Wiederholm, 1983). Fonds (1973) also included a chapter on diet of gobies in the Wadden Sea in his extensive work. He noticed some differences in feeding habit between *P. minutus and P. lozanoi:* the sand goby consumed more benthic prey while Lozano's goby had more mysids on its menu.

In the Belgian coastal waters, the food niche of *P. minutus* and *P. lozanoi* and the possible competition between these two closely related species has been thoroughly investigated by Hamerlynck and his colleagues (Hamerlynck *et. al*., 1985, 1986, 1990, 1993, Hamerlynck, 1993, Hamerlynck & Cattrijse, 1994). *P. minutus* has a diverse diet with predominantly epibenthic and benthic animals, but zooplankton and hyperbenthic prey items also occurred. The composition of the diet changed with the season and with the size of the fish. 0+ juveniles fed mainly on the epibenthic caprellid amphipod *Pariambus typicus* and on the hyperbenthic mysid *Schistomysis spiritus* in July. With increasing size, a more diverse diet with more bivalve siphons, benthic polychaetes and mysids was recorded. In number, the pelagic copepod *Temora longicornis* is also an important food item throughout the year (Hamerlynck & Cattrijsse, 1994). The gradual change in diet from meiobenthos to macrobenthos with an increasing size of the gobies has been reported by Aarnio & Bonsdorff (1993) in the Baltic and for the sand goby and the common goby in the Wadden Sea by del Norte-Campos & Temming (1994).

In the North Sea, differences in food between male and female *P. minutus* are pronounced in April, which is the beginning of the spawning season for this species (Hamerlynck & Cattrijsse, 1994). Males depend mainly on benthic prey and high numbers of *Pomatoschistus* eggs. Females fed mainly on bivalve siphons and *Temora longicornis* (Hamerlynck & Cattrijsse, 1994). Magnhagen (1986) also observed clear differences in diet of male and female common gobies in Gullmar Fjord (Sweden) during their breeding season. After reproduction, the differences in food intake and diet between the sexes disappeared. Since male gobies guard their nests, they will be restricted to take whatever prey item is available in the vicinity of the nest. Females have a higher mobility and hence a higher prey encounter rate in that period, so they can be more selective in the food they take.

P. lozanoi from the Belgian coastal waters has a more specialized diet, feeding mainly on a few pelagic and hyperbenthic species. *Temora longicornis*, *Schistomysis* species and juvenile *Pomatoschistus minutus* were important supplies of energy (Hamerlynck *et al.*, 1990). Unlike *P. minutus*, there was very little tendency towards more benthic feeding at larger size. Lozano's goby shifted to benthic and epibenthic feeding only in August. Magnhagen (1986) and Hamerlynck & Cattrijsse (1994) point out that this shift in food preference was possibly linked to the confinement of gobies to the bottom during the spawning season, but a sudden decrease of nearly all mysid populations (the preferred food of *P. lozanoi*) in early summer in the Voordelta could also play part in *P. lozanoi*'s food shift (Mees *et al.*, 1993). With regard to the food niche, *P. minutus* can be considered to be a generalist, while *P. lozanoi* is a more specialized feeder (Hamerlynck & Cattrijsse, 1994). Concerning their spatial niche, *P. minutus* can be regarded as being predominantly benthic, while *P. lozanoi* will spend more time in the hyperbenthic region to look for prey.

* Competition for food between *Pomatoschistus* spp.

Hamerlynck & Cattrijsse (1994) concluded that their data from the field did not give clear evidence for interference competition between *Pomatoschistus minutus* and *P. lozanoi*. The high local abundances of the shared food resources made competition unlikely. In the Bothnian Sea, no support was found for resource partitioning as a structuring mechanism in a fish assemblage of which *P. minutus* was the most abundant species (Thorman & Wiederholm, 1983).

P. microps and *P. minutus* are sympatric in the shallow waters of the Swedish west coast and were used in several experiments to test which mechanisms permit these two species to coexist (Edlund & Magnhagen, 1981, Magnhagen & Wiederholm, 1982). Habitat, food and time are the most important separating niche dimensions according to Schoener (1974). *P. microps* and *P. minutus* segregated in diet when in a uniform habitat (no shelter provided) (Edlund & Magnhagen, 1981). However, in a more complex environment (open and vegetated habitat provided), the two species retained a high diet overlap but displayed a certain amount of segregation of habitat when together (Magnhagen & Wiederholm, 1982).

* Importance of gobies in the food web

What is the ecological importance of gobies both as predators of meio- and macrobenthos and as prey in the marine and estuarine ecosystems? Zander & Hagemann (1987) evaluated the predation impact of *P. minutus* and *P. pictus* on their prey populations and concluded that these small-sized predators save their prey resources by selective feeding and avoiding the largest, reproductive stages of crustaceans such as *Gammarus*. Also in Gullmar Fjord (Sweden) and in the Ythan estuary (Scotland), sand gobies do not seem capable of regulating prey numbers (Evans, 1983, Jaquet & Raffaelli, 1989). Cage experiments with *P. microps* as a predator in the Oslo fjord yielded the same result (Berge & Hesthagen, 1981). On the contrary, the populations of harpacticoids along the German Baltic coast would be regulated by the grazing of young *P. microps* and *P. minutus* (Schmidt-Moser & Westphal, 1981).

Because of the abundance in food, goby populations in marine and estuarine habitats are in most areas probably more limited by predation than by lack of resources (Evans, 1983). Higher susceptibility to predation is a consequence of the small size of gobies. The danger is of course faced by all young developmental stages of fish, but the small adult size makes gobies vulnerable throughout their entire life. Many small fish species that are abundant in open habitats form essential intermediate components in the foodweb (Miller, 1979). In Lake Grevelingen it was estimated that at least 60 % of the decline in gobiid biomass from late summer to spring would be accounted for by predation of fish and birds, and 17 % of the total goby production would be used by predators (Doornbos & Twisk, 1987). *Pomatoschistus* species are indispensable food items for young gadoids. In a western Norwegian fjord, up to 70 % of the food biomass of *Gadus morhua* (15-25 cm) in summer consists of gobies (Salvanes & Nordeide, 1993). *P. minutus* is the most important fish species in the diet of adult cod in the Western Baltic (Arntz, 1978). In the Voordelta (South-West Netherlands), 14% of the diet of of bib (*Trisopterus luscus*) and 35% of the diet of whiting (*Merlangius merlangus*) consists of *P. minutus* and *P. lozanoi* (Hamerlynck & Hostens, 1993). The lesser weever (*Trachinus vipera*), a demersal predator, feeds mainly on fish (85.6%) and almost

Table 1.2: List of predators of *Pomatoschistus* species.

Predator	Prey	Locality	Author
Fish			
Trisopterus luscus	*P. minutus*	Tagus estuary (Portugal)	Costa, 1988
"	*P. minutus, P. lozanoi*	Voordelta (SW Netherlands)	Hamerlynck and Hostens, 1993
"	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973
Trisopterus minutus	*Pomatoschistus sp.*	Masjfjorden (Western Norway)	Salvanes and Nordeide, 1993
Gadus morhua	*Pomatoschistus sp.*	Masjfjorden (Western Norway)	Salvanes and Nordeide, 1993
"	*P. minutus*	Western Baltic	Arntz, 1978
Merlangius merlangus	*P. minutus, P. lozanoi*	Voordelta (SW Netherlands)	Hamerlynck and Hostens, 1993
Pollachius virens	*Pomatoschistus sp.*	Masjfjorden (Western Norway)	Salvanes and Nordeide, 1993
Pollachius pollachius	*Pomatoschistus sp.*	Masjfjorden (Western Norway)	Salvanes and Nordeide, 1993
Alosa fallax	*Pomatoschistus sp.*	Tagus estuary (Portugal)	Assis *et al.*, 1992
Ciliata mustela	*P. minutus*	Tagus estuary (Portugal)	Costa, 1988
Gobius niger	*P. minutus*	Tagus estuary (Portugal)	Costa, 1988
Trigla lucerna	*P. minutus*	Tagus estuary (Portugal)	Costa, 1988
Trigla sp.	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973
Anguilla anguilla	*P. minutus*	Tagus estuary (Portugal)	Costa, 1988
"	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Pleuronectes platessa	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Plathichtys flesus	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Limanda limanda	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
"	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973
Scophthalmus maximus	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
"	*Pomatoschistus sp.*	Southern North Sea (Netherlands)	Braber and de Groot, 1973
Scophthalmus rhombus	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
"	*Pomatoschistus sp.*	Southern North Sea (Netherlands)	Braber and de Groot, 1973
Scophthalmus sp.	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973
Arnoglossus laterna	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973

Table 1.2 (continued): List of predators of *Pomatoschistus* species.

Predator	Prey	Locality	Author
Fish			
Trachinus draco	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973
Trachinus (Echiichthys) vipera	*Pomatoschistus sp.*	Southern North Sea (Netherlands)	Creutzberg and Witte, 1989
Myoxocephalus scorpius	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
"	*P. minutus, P. lozanoi*	Waddensea, North Sea (Netherlands)	Fonds, 1973
Zoarches viviparus	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Birds			
Phalacrocorax carbo	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Podiceps cristatus	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Mergus serrator	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Numenius arquata	*P. minutus, P. microps*	Lake Grevelingen (Netherlands)	Doornbos and Twisk, 1987
Larus canus	*P. minutus, P. microps*	Baltic Sea (Germany)	Zander and Döring, 1989
Larus argentatus	*P. minutus, P. microps*	Baltic Sea (Germany)	Zander and Döring, 1989
Larus ridibundus	*P. minutus, P. microps*	Baltic Sea (Germany)	Zander *et al.*,1984
Rissa tridactyla	*P. minutus, P. microps*	Baltic Sea (Germany)	Zander and Döring, 1989
Mammals			
Phoca vitulina (harbour seal)	*Pomatoschistus sp.*	Wadden Sea (Germany)	Behrends, 1985

exclusively on *Pomatoschistus* sp. (94% of the fish biomass in the diet) in the southern North Sea. Since gobies do not only utilize benthic but also pelagic prey, they are able to act as suppliers of secondary production from the water column to demersal predators (Creutzberg & Witte, 1989). In Table 1.2 , the extensive (and probably still incomplete) list of predators on gobies mentioned in literature is given, to give a first impression of the important role of gobies as a food resource for higher trophic levels.

Host-parasite relationships in marine fish

Literature on parasites of marine fish is voluminous and diverse. A comprehensive four volume book dealing with all parasite groups infecting marine organisms has been compiled by Kinne (1984). Research topics range from purely taxonomical and descriptive studies to studies on elucidation of life cycles, the effects of parasites on the host both at the individual and population level, the use of parasites as biological tags in zoogeography and phylogeny, their use as bioindicators for pollution, studies on microhabitat use of parasites and the role of competition. Basic concepts on the ecology of the parasite-host relationships and the factors structuring them are discussed by Kennedy (1975) and Rohde (1982). Patterns and processes structuring helminth communities have been the topic of an excellent book edited by Esch *et al.* (1990). Because of the close relationship between fish hosts and parasites, parasites are often studied for their possible use as biological indicators for separating fish populations, or as indicators for migration of their host, the diet of the host or the host's phylogeny. These aspects of the research in fish parasitology have been reviewed by Williams *et al.* (1992). It is striking that the bulk of the literature deals with only a small proportion of marine fish species as hosts for parasites. According to Kinne (1984), our knowledge covers only 2 % of the known fish species. Research on fish parasites was mainly focused on economically important fish species with an emphasize on the northern temperate seas. In addition, despite the numerous studies in different fields of fish parasitology, the knowledge on population dynamics of parasites within their hosts' communities and the processes determining these dynamics is still rudimentary (Holmes, 1990, Kennedy, 1990).

Three major models to explain species richness in a parasite component community have been put forward; these models are not mutually exclusive and are as a matter of fact, to a large extent complementary:

1) The Island Distance hypothesis, which is based on the theory of Island Biogeography (MacArthur & Wilson, 1967 *fide* Esch *et al.*, 1990), states that parasite species richness is related to the difficulty of invation. This hypothesis predicts that isolationist communities will develop when parasite colonization is limited, such as in the case of the depauperate infracommunities (community of parasite species on an individual host) of many fish helminth communities (Kennedy *et al.*, 1986).

2) The 'Co-speciation' Model of Brooks (1980) which predicts that speciation in the host lineage often results in the cospeciation in their parasite lineage.

3) The phenomenon of 'host capture', where a parasite infects a different host by 'accident'. This hypothesis implies that parasites are acquired from ecologically associated hosts and then subsequently become adapted to the new host, perhaps speciating in the process.

Holmes (1987, 1990) suggested that the observed parasite fauna of the individual host (infracommunity) or of a host species (component community) is the result of the action of different abiotic and biotic screens or filters on the potential parasite fauna. Environmental factors, including differences in depth (Køie, 1993), temperature, salinity, inshore-offshore habitats (Shotter, 1973) are important in determining which local parasite fauna can be expected. Furthermore, natural disturbances (El Niño, epidemics affecting keystone species,...) and antropogenic effects (e.g. pollution; Khan *et al.*, 1991, Siddall *et al.* 1994, Bagge & Valtonen, 1996) may have an important direct or indirect effect through the host community. Biotic factors influencing the local parasite fauna depend on the composition and functioning of the host community. In aquatic systems, this community not only include fish and other vertebrate hosts, but also invertebrates which often play an important role as intermediate hosts. For the parasite community, the host is the resource base. For homoxenous parasites, the host population only includes one definitive host. For many parasites, however, one or more intermediate hosts play a role in the completion of their life cycle (heteroxenous parasites). In heteroxenous parasites, the local food webs will determine the possibilities of the parasite to fulfil their life cycles. In addition to the phylogenetic specificity and interactions (e.g. competition) amongst parasites, food habits, behaviour and physiology (including immune response) of the hosts will all have an influence on the observed infra- and component communities (Rohde, 1982, 1984).

The ecology of parasites is unique in the sense that their biotic environment (host) plays a paramount role, more so than in non-parasitic organisms. The host *is* the environment of the parasite, especially for endoparasites (Rohde, 1982). The characteristics and dynamics of the host population will therefore have a major influence on its parasite community. Some of the main characteristics of hosts influencing the parasite community, its variety and intensity of infection, are: (a) the host's geographical range, (b) its population density, ensuring contact between host specimens and determining the potential of horizontal transfer of (ecto-)parasites and the chance of parasites to meet the host, (c) the mobility of the host, including the variety of habitats encountered in its life, (d) the adult size attained (Guégan *et al.* 1992), (e) the life span of the host (a long life span allows accumulation of parasites) and (f) the diet of the host (possibly including intermediate hosts) (Polyanski, 1970, Rohde, 1979). Cod (*Gadus morhua*) can be taken as an example of a marine host which fulfils all these conditions. This results in a parasite fauna which is very rich (Appy & Burt, 1982). In Danish waters, 18 different species of digenean trematodes were recovered from cod (Køie, 1984). Each of the above factors influencing the parasite fauna will be discussed in more detail below, using gobies as an example.

Host specificity and its role in determining parasite communities

The formation of an infracommunity in a certain host not only depends on the chance of the parasite to encounter the host, but also on the suitability of the host for the parasite (Kennedy, 1975). Towards the available hosts in an ecosystem, parasites differ in their ability to form a stable parasite-host relationship. Host specificity, which is the restriction of a parasite species to certain host species, is universal. The degree of host specificity varies largely among different parasite species and groups (Rohde, 1982).

In relation to host specificity, Odening (1976) defines monoxeny when a parasite has only one host species (in a certain stage of its life cycle), oligoxeny when only a few host species are infected and polyxeny when a parasite species can infect many host species. Euzet & Combes (1980) differentiated between oioxenic parasites, with host specificity towards a single host species (overlapping with the term monoxeny of Odening, 1976), stenoxenic parasites, with different but phylogenetically related hosts, and euryxenic parasites which are found in numerous hosts of unrelated taxa.

Parasites infecting only a single host taxon or related taxa are said to exhibit **phylogenetic host specificity** (Rohde, 1979, 1982). Phylogenetic host specificity is more likely in parasite species manifesting (1) low pathogenicity (2) high host specificity maintained through time (3) extensive colonization of a host group and (4) a life cycle showing 'closeness' with the host (Humpery-Smith, 1989).

High phylogenetic host specificity and a close relationship between hosts and parasites (e.g. in homoxenous parasites with one host in the parasite's life cycle, like monogeneans) would promote coevolution (Connell, 1980). Janzen (1980) referred to coevolution in cases where two interactive species show reciprocal adaptive responses. Brooks & McLennan (1991) define coevolution as encompassing both cospeciation (joint speciation of host and parasite) and coadaptation (mutual adaptation of host and parasite to each other). Coevolution may lead to a complete agreement of the phylogenies of the host species and their parasite species. Hence, it would be possible to use parasites for tracing host phylogenies of taxonomically difficult groups (Euzet *et al.*, 1989).

Eichler (1948) formulated three rules which are thought to govern the evolution of parasites in relation to their hosts. (1) Szidat's rule states that primitive hosts are parasitized by primitive parasites and more specialized host by specialized parasites. (2) The divergence rule (sometimes called Eichler's rule) predicts that large host groups have more genera of parasites than small host groups. (3) The Fahrenholz's rule states that 'parasite phylogeny mirrors host phylogeny'. Several studies tried to find out to what extent the latter rule holds for specific host-parasite systems. The digeneans of crocodilians, for instance, clearly coevolved as a faunal unit with their host group (Brooks, 1979). In the *Labeo* (Cyprinidae) - *Dactylogyrus* (Monogenea) system, phylogenetic evolution (Farenholz's rule) explained part of the associations, but nonphylogenetic evolution by colonization and host switching were also displayed (Guégan & Agnèse, 1991)

The major groups of parasites differ clearly in the degree of host specificity. Monogeneans are generally accepted as having the highest degree of host specificity (Rohde, 1982). Therefore, they are often used as biological markers to identify a specific host group or species. Euzet *et al.* (1989) investigated the value of monogeneans of the genus *Protoancylodiscoïdes* as biological indicators for different species of *Chrysichthys*. The *Chrysichtys* species were first separated using allozyme electrophoresis. Each species was found to be characterized by two host-specific monogenean species. Therefore, Euzet *et al.* (1989) concluded that the monogeneans were as good an indicator of the host species as the allozymes. *Dactylogyrus* sp. and *Dogielius* sp. equally were highly host specific for cyprinids of the genus *Labeo* (Guégan *et al.*, 1988, 1989). Rohde (1978) showed marine digeneans to be significantly less host specific than monogeneans. The digeneans' specificity decreased also from the tropics towards the temperate areas. Acanthocephalans, nematodes and cestodes of marine teleosts also appear to be less host specific than Monogenea. In marine fish, host specificity plays a relatively minor role in determining the intestinal helminth communities. The core species (Hanski, 1982) are mostly generalists and the species diversity is not largely influenced by the satellite species (most of them being more host specific; Kennedy, 1990). The infracommunities of gastrointestinal helminths in marine fish are highly variable in number of individuals and species and seem more complex (having more species of a given number of individuals) than communities in freshwater fish (Holmes, 1990).

It should be stressed that even parasites with a wide host range show preferences for certain hosts. These hosts may not be phylogenetically closely related, but often have certain aspects of their ecology in common. In this case, **ecological host specificity** is exhibited (Rohde, 1979, 1982). Ecological host specificity largely depends on the niche of the hosts, with the food niche being a major determinant. Odening (1976) creates a third group of **physiological host specific** parasites, who's hosts are neither phylogenetically nor ecologically related.

Host specificity should not only take the host range (number of host species for a certain parasite) into account, but also the intensity and frequency of infection, since even generalist parasites tend to infect one or a few host species more heavily than others. Rohde (1980) developed host specificity indices including these parameters.

Pomatoschistus species as hosts for parasites: some predictions

Because of the abundance of gobies in the Belgian and Dutch coastal waters and their intermediate position in the coastal food web, being predators of a diverse invertebrate fauna and prey for a number of piscivorous fish and birds, it is likely that they are important as (intermediate, paratenic and definitive) hosts for parasites. From the parasite's point of view, gobies must be ideal hosts in a number of ways:

- *Geographical range*

Gobies of the genus *Pomatoschistus* cover a large geographical range. Mostly, the larger the host's geographical range, the higher the number of parasite species recorded (Price *et al.*, 1988).

- *Host densities*

In many coastal waters, goby populations reach high densities (Fonds, 1973, Arellano, 1995). High host abundance increases the chances of the parasites to meet the host and increase possibilities for horizontal transfer. Furthermore, high host abundance provides the parasites with a large effective host population and a low probability of local extinction rates for the parasite population.

- *Diet of the host*

The diet of the fish is of major importance for the composition of the endoparasite community. Gobies are mainly benthic and use a variety of invertebrates as food. In general, benthic feeders have more parasite species than pelagic or benthopelagic feeders (Holmes, 1990). Differences in food niche between *Pomatoschistus minutus* and *P. lozanoi*, where *P. minutus* is described as a generalist feeding on more benthic prey and *P. lozanoi* as a specialist preferring hyperbenthic invertebrates as food, is expected to lead to differences in parasite communities and parasite abundance, especially for parasites exhibiting ecological host specificity. The extent to which the gobies will concentrate on temporarily or locally dominant food items will certainly play a role in determining the parasite community.

- *Adult size and life-span of the host*

Gobies have a small adult size, high reproductive rate and short life-span. This rapid turnover of individual hosts will provide less time for equilibrium communities to develop, or for individual hosts to become saturated with parasite species.

- *Mobility of the host*

A high mobility of a host seems to lead to a diverse parasite fauna. Migration behaviour of the gobies during winter or during the reproduction period may bring them in contact with parasite species specific for the area to which they migrate. In a study of *Sebastes nebulosus*, which is a territorial, not very vagile fish species of the Pacific, Holmes (1990) suggested that the introduction of the parasites through more vagile hosts is probably more important than through the settling of juveniles or occasionally migrating adults of *Sebastes* itself. The same might be true for *Pomatoschistus* species which are known to be rather sedentary fish.

Most the above issues point to the possibilities of finding differences in the parasite communities among goby species and populations, in relation to the ecological host specificity of the parasites. However, phylogenetic host specificity could also be involved in defining the parasite community. Because their phylogeny is well-studied (Wallis & Beardmore, 1984a), and because of the sympatric occurrence of different species, *Pomatoschistus* species form an interesting host system to check for phylogenetic host specificity and possible coevolution of hosts and parasites. Since *Pomatoschistus minutus* and *P. lozanoi* are very closely related and *P. microps* and *P. pictus* are more distant relatives, predictions can be made on the species composition of their monogenean fauna. If the Farenholz rule holds, than the same phylogenetic relationships between their monogenean parasites should be expected.

Pomatoschistus **spp. in parasitological reseach**

Because of the important role of gobies in the marine and estuarine ecosystem, a lot of attention has been paid to their biology and role in the food web. Since most research on parasites of fish has been focused on commercially important species, however, gobies received little attention in this field.

The first parasitological reports on gobies are mainly descriptive. Markowski (1935) investigated sand gobies (*Pomatoschistus minutus*) from the Polish Baltic Sea for their parasite fauna and described nine parasite species: Cestoda were best represented (4 species), besides Trematoda (2 species), Nematoda (2 species) and Acanthocephala (1 species). The environmental conditions in that part of the Baltic, with its low salinity, explained partly the presence of both marine and freshwater parasites. Koter (1962) expanded the study in the Baltic and included *P. microps* and *Gobius niger* in addition to *P. minutus*. She also detected nine species of parasites, of which three were different from Markowski's study. The work comprised a complete morphological description of the parasites, enlightened the status of the gobies as hosts in the life cycles of the parasites and provided some concise data on infection intensities. In our Belgian coastal waters, one of the first reports on parasites of gobies was from Vaes (1978) describing the infection of *P. microps* with a trematode *Aphalloides coelomica*. This digenean remarkably inhabited the body cavity of its final host. Its eggs could therefore only be released by the death of the host. The author considered this parasite to have a regulatory role on the host population. Concerning ectoparasites, the only description of monogeneans specific for gobies is from Gläser (1974), describing *Gyrodactylus rugiensis* and *G. micropsi* from the sand goby and the common goby.

The first step towards a more ecological approach of the host-parasite relationship in gobies was taken with the work of Mann (1964) focusing on the distribution, intensity of infection and seasonality of the parasitic copepod *Lernaeocera minuta* (currently *L. lusci;* Van Damme & Ollevier, 1995). The influence on the health of the host was loss of weight, lowered fat content and anaemia. *Lernaeocera minuta* has been part of an extensive study on *Lernaeocera* species by Van Damme (1993). A morphometrical study and comparison of host specificity revealed that *L. minuta* from *P. minutus* and *P. lozanoi* was a junior synonym of *L.*

lusci (Van Damme & Ollevier, 1995). During the spawning season, the intensity of infection of *L. lusci* on sand gobies depended on the sex of the host, and this was linked to the differences in behaviour of male and female sand gobies (Van Damme & Ollevier, 1994). Two distinct transmission waves of *L. lusci* on sand gobies were distinguished, one in autumn and one in spring (Van Damme *et al.*, 1997). Petersen (1992) studied the nutritional condition of *P. minutus* in relation to the presence of the copepod *L. minuta* (*L. lusci*) and the nematode *Hysterothylacium* sp. in the German Wadden Sea. A lower condition factor of infected host was noticed, but variability among individuals was high.

In the Elbe estuary, Möller-Buchner (1981) conducted a parasitological survey on *P. microps*. The parasite species diversity was low, with only 4 species detected. Nevertheless, high rates of infection were recorded with about 100 % of the common gobies infected with one or several parasite species. The author stated that this was caused by the specific environmental conditions of eutrophication and low oxygen content in the Elbe estuary, but no proof for this hypothesis was given.

Claridge *et al.* (1985) included a comparison of the infection of *P. minutus* and *P. lozanoi* with the cestode *Ligula intestinalis* in a broad study on the abundance and life history of the gobies from the Severn Estuary. A negative effect of the cestode on the feeding and growth rate in both hosts was noted. *P. lozanoi* was more heavily parasitized than *P. minutus*, but no explanation was provided.

Gobies are important intermediate hosts in the life cycle of an array of parasite species. In solving the life cycles, mainly of digenean trematodes, and in describing the morphology of the different developmental stages of the digenea, the work of Køie has been of paramount importance. Besides other small-sized fish (like the three-spined sticklebacks), she often used the sand goby and the common goby in experimental infections. For example, *P. microps* proved to be a suitable second intermediate host for *Pygidiopsis ardeae*, both in the laboratory and the field (Køie, 1990). *Mesorchis denticulatus* uses *P. microps*, *P. minutus* and *Gobius niger* as second intermediate host (Køie, 1986). *P. minutus, P. pictus* and *Gobiusculus flavescens* were highly susceptible to experimental infections with cercaria of *Stephanostomum caduncum* (Køie, 1978). In the life cycle of the cestode *Bothriocephalus* *gregarius* along the French coast, *P. minutus* and *P. marmoratus* are important paratenic hosts, facilitating the infection to the final host, the turbot, *Psetta maxima* (Robert *et al.*, 1988). In the western Baltic, *P. microps* and (to a lesser extent) *P. minutus* both serve as second intermediate hosts for the trematode *Cryptocotyle concavum,* which forms cysts of metacercaria in the kidney. Infection of the snail *Hydrobia* (first intermediate host) and of the gobies was dependent on the ambient salinity. *P. microps* showed to be the most optimal host because it tolerats very high numbers of the parasite in the kidney, inhabits shallow waters and is small in size and thus easily available for the predating birds which are final hosts (Zander *et al.*, 1984).

The importance of gobies as hosts and transmitters for parasites in marine and estuarine ecosystems have been largely studied in relation to their role in the food web. A first study on the infracommunity of metazoan parasites of *P. minutus* and *P. lozanoi* along the Belgian coast comprised four species (Hamerlynck *et al.*, 1989). *Asymphylodora demeli* (Trematoda), previously reported as adults from *Nereis diversicolor* (Reimer, 1973, Vaes, 1974) and from gobies from the Baltic (Markowski, 1935, Szidat, 1943, Koter, 1962), was present in the intestine. *Lernaeocera minuta* (Copepoda), for which the gobies are final hosts, infected the gills and occasionally the fins. Both gobies are intermediate hosts for *Bothriocephalus scorpii* plerocercoids (Cestoda) and a larval stage of *Hysterothylacium aduncum* (Nematoda). The hypothesis of a link between the food resource partitioning of *P. minutus* and *P. lozanoi* and the differences in infection rate of juvenile *P. minutus* with *A. demeli* was put forward (Hamerlynck *et al.*, 1989).

The important role of gobies as transmitters for different helminth species in the Baltic and a link with the availability of their food items and the seasonal differences in diet of the gobies has been thoroughly investigated by Zander (1988), Zander & Döring (1989) and Zander *et al.* (1993). A large number of factors determined the composition of the infracommunities of the parasites of gobies in the Baltic, of which seasonality of the prey abundance, age and life-span of the gobies and the differences in diet of the fish are of major importance (Zander *et al.*, 1993).

Because of the complexity of the factors determining parasite transfer, Zander *et al.*, (1994) used four helminth species as models to clarify the different mechanisms involved: two cestodes, *Bothriocephalus* sp. and *Schistocephalus* sp., both using planktonic copepods as first hosts and the nematode *Hysterothylacium* sp. and digenean trematode *Podocotyle atomon* having *Gammarus* spp. (Amphipoda) as their first intermediate host. *P. minutus*, *P. pictus* and *Gobiusculus flavescens* were screened as final hosts. The selective feeding on certain size classes of gammarids influenced strongly the parasite transfer. The gobies fed more on smaller size classes which were less infected. For the cestode model, where the gobies had no preference for a specific size class of the intermediate host (copepods), the way of life (planktonic or benthic) of the three gobies and seasonal differences in food intake largely explained the differences in infection rate with the cestodes. *Gobiusculus flavescens* feeding predominantly on planktonic prey (and thus ingesting more copepods) was more infected than *Pomatoschistus minutus* which preferred benthic prey.

References

Aarnio, K., & Bonsdorff, E. (1993) Seasonal variation in abundance and diet of the sand goby *Pomatoschistus minutus* (Pallas) in a northern Baltic archipelago. *Ophelia*, **37,** 1, 19-30.

Appy, R. G. & Burt, M. D. B. (1982) Metazoan parasites of cod, *Gadus morhua* L., in Canadian Atlantic waters. *Can. J. Zool.*, **60**, 1573-1579.

Arellano, R. V. (1995) *Age and growth of two sympatric goby species Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) using daily growth increments in the otoliths*. PhD thesis, University Gent, 178 pp.

Arntz, W. E. (1978) The food of adult cod (*Gadus morhua* L.) in the western Baltic. *Meeresforsch.*, **26,** 60-69.

Arruda, L. M., & Azevedo, J. N. (1987) The occurrence of Lozano's goby *Pomatoschistus lozanoi* (De Buen, 1923) (Pisces, Gobiidae), on the Portuguese coast. *Miscellenia Zoologica*, **11,** 387-389.

Assis, C. A., Almeida, P. R., Moreira, F., Costa, J. L., & Costa, M. J. (1992) Diet of the twaite shad *Alosa fallax* (Lacépède) (Clupeidae) in the river Tagus estuary, Portugal. *Journal of Fish Biology*, **41,** 1049-1050.

Bagge, A. M., & Valtonen, E. T. (1996) Experimental study on the influence of paper and pulp mill effluent on the gill parasite communities of roach (*Rutilus rutilus*). *Parasitology*, **112,** 499-508.

Behrends, G. (1985) Zur Nahrungswahl von Seehunden (*Phoca vitulina* L.) im Wattenmeer Schleswig-Holsteins. *Zeitschrift Für Jagdwissenschaft*, **31,** 3-14.

Berge, J. A., & Hesthagen, I. H. (1981) Effects of epibenthic macropredators on community structure in an eutrophicated shallow water area, with special reference to food consumption by the common goby *Pomatoschistus microps*. *Kieler Meeresforsch.*, **5,** 462-470.

Blaber, S. J. M., & Milton, D. A. (1990) Species composition, community structure and zoogeography of fishes of mangrove estuaries in the Solomon Islands. *Marine Biology*, **105,** 259-267.

Bouchereau, J.-L., Quignard, J.-P., Tomasini, J.-A., Joyeux, J.-C., & Capape, C. (1990) Cycle sexuel, condition, fécondité et ponte de *Pomatoschistus minutus* (Pallas, 1770) (Gobiidae) du Golfe du Lion, France. *Cybium*, **14,** 3, 251-267.

Bouchereau, J. L., Quignard, J. P., Tomasini, J. A., Joyeux, J. C., & Capape, Ch. (1989) La population de *Pomatoschistus minutus* (Pallas, 1770) de la lagune de Mauguio, France. Paramètres démographiques et croissance individuelle. *Cah. Biol. Mar.*, **30,** 487-514.

Braber, L., & de Groot, S. J. (1973) The food of five flatfish species (Pleuronectiformes) in the Southern North Sea. *Netherlands Journal of Sea Research*, **6,** 163-172.

Brooks, D. R. (1979) Testing hypothesis of evolutionary relationships amongst parasites: the digeneans of crocodilians. *Amer. Zool.*, **19,** 1225-1238.

Brooks, D. R. (1980) Allopatric speciation and non-interactive parasite community structure. *Syst. Zool.*, **30,** 192-203.

Brooks, D. R., & McLennan, D. A. (1991) Preamble to cospeciation and coadaptation. in: D. R. Brooks, & D. A. McLennan (eds) *Phylogeny, Ecology, and behavior*. Chicago, The University of Chicago Vol. 6, pp. 189-204.

Claridge, P. N., Hardisty, M. W., Potter, I. C., & Williams, C. V. (1985) Abundance, life history and ligulosis in the gobies (Teleostei) of the inner Severn estuary. *J. Mar. Biol. Ass. U.K.*, **65,** 951-968.

Connell, J. H. (1980) Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos*, **35,** 131-138.

Costa, M. J. (1988) Ecologie alimentaire des poissons de l'estuaire du Tage. *Cybium*, **12,** 4, 301-320.

Costello, M. J. (1992) Abundance and spatial overlap of gobies (Gobiidae) in Lough Hyne, Ireland. *Environmental Biology of Fishes*, **33,** 239-248.

Creutzberg, F., & Witte, J. I. (1989) An attempt to estimate the predatory pressure exerted by the lesser weever, *Trachinus vipera* Cuvier, in the southern North Sea. *J. Fish Biol.*, **34,** 429-449.

del Norte-Campos, A. G. C., & Temming, A. (1994) Daily activity, feeding and rations in gobies and brown shrimp in the northern Wadden Sea. *Marine Ecology Progress Series*, **115,** 41-53.

Doornbos, G., & Twisk, F. (1987) Density, growth and annual food consumption of gobiid fish in the saline Lake Grevelingen, the Netherlands. *Netherlands Journal of Sea Research*, **21,** 1, 45-74.

Edlund, A.-M., & Magnhagen, C. (1981) Food segregation and consumption suppression in two coexisting fishes, *Pomatoschistus minutus* and *P. microps* : an experimental demonstration of competition. *Oikos*, **36,** 23-27.

Edlund, A.-M., Sundmark, G., & Thorman, S. (1980) The identification of *Pomatoschistus pictus*, *P. microps* and *P. minutus* (Gobiidae, Pisces). *Sarsia*, **65,** 239-242.

Eichler, W. (1948) Some rules in ectoparasitism. *Ann. Mag. Nat. Hist.*, **1,** 588-598.

Esch, G. W., Bush, A. O., & Aho, J. M. (1990) *Parasite Communities: Patterns and Processes*. London, Chapman and Hall Ltd, 335 pp.

Esch, G. W., Shostak, A. W., Marcogliese, D. J., & Goater, T. M. (1990) Patterns and processes in helminth parasite communities: an overview. in: G. Esch, A. Bush, & J. Aho (eds) *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 1, pp. 1-19.

Euzet, L., Agnèse, J.-F., & Lambert, A. (1989) Valeur des parasites comme critère d'identification de l'espèce hôte. Démonstration convergente par l'étude parasitologique des monogènes branchiaux et l'analyse génétique des poissons hôtes. *C R Acad. Sci. Paris*, **308,** 3, 385-388.

Euzet, L., & Combes, C. (1980) Les problèmes de l'espèce chez les animaux parasites. in: Sociéte Zoologique de France (ed.) *Les problèmes de l'espèce dans le regne animal.* Paris, Sociéte Zoologique de France Vol. 6, pp. 239-285.

Evans, S. (1983) Production, predation and food niche segregation in a marine shallow soft-bottom community. *Mar. Ecol. Prog. Ser.*, **10,** 147-157.

Fonds, M. (1970) Remarks on the rearing of gobies (*Pomatoschistus minutus* and *P. lozanoi*) for experimental purposes. *Helgoländer Meeresunters.*, **20,** 620-628.

Fonds, M. (1971) The seasonal abundance and vertebral variation of *Pomatoschistus minutus minutus* and *lozanoi* (Gobiidae) in the Dutch Wadden Sea. *Vie et Milieu*, **22,** suppl., 393-408.

Fonds, M. (1973) Sand gobies in the Dutch Wadden Sea (*Pomatoschistus*, Gobiidae, Pisces). *Neth. J. Sea Res.*, **6,** 4, 417-478.

Fonds, M., & Veldhuis, C. (1973) The oxygen consumption of four *Pomatoschistus* species (Pisces, Gobiidae) in relation to water temperature. *Neth. J. of Sea Res.*, **7,** 376-386.

Forsgren, E. (1992) Predation risk affects mate choice in a gobiid fish. *The American Naturalist*, **140,** 6, 1041-1049.

Fouda, M. M., & Miller, P. J. (1981) Age and growth of the common goby, Pomatoschistus microps, on the South coast of England. *Estuarine, Coastal and Shelf Science*, **12,** 121-129.

Gee, J. M. (1987) Impact of epibenthic predation on estuarine intertidal harpacticoid copepod populations. *Marine Biology*, **96,** 497-510.

Gläser, H.-J. (1974) Eine neue Artengruppe des Subgenus *Gyrodactylus* (*Paranephrotus*) (Monogenea, Gyrodactylidae). *Zool. Anz*, **192,** 271-278.

Goren, M., & Dor, M. (1994) An updated checklist of fishes of the Red Sea. CLOFRES II: Introduction. *Israel Academy of Sciences and Humanities, Interuniversity Institute for Marine Sciences, Elat, Israel*, iv-ix.

Guégan, J.-F., & Agnèse, J.-F. (1991) Parasite evolutionary events inferred from host phylogeny: the case of *Labeo* species (Teleostei, Cyprinidae) and their dactylogyrid parasites (Monogenea, Dactylogyridae). *Can. J. Zool.*, **69,** 595-603.

Guégan, J.-F., Lambert, A., & Euzet, L. (1988) Etude des monogènes des Cyprinidae du genre *Labeo* en Afriqua de l'Ouest. I. Genre *Dactylogyrus* Diesing, 1850. *Rev. Hydrobiol. Trop.*, **21,** 2, 135-151.

Guégan, J.-F., Lambert, A., & Euzet, L. (1989) Etude des monogènes des Cyprinidae du genre *Labeo* en Afrique de l'Ouest. II. Genre *Dogielius* Bykhowski, 1936. *Rev. Hydrobiol. Trop.*, **22,** 1, 35-48.

Guégan, J.-F., Lambert, A., Lévêque, C., Combes, C., & Euzet, L. (1992) Can host body size explain the parasite species richness in tropical freshwater fish? *Oecologia*, **90,** 197-204.

Hamerlynck, O. (1990) The identification of *Pomatoschistus minutus* (Pallas) and *Pomatoschistus lozanoi* (de Buen) (Pisces, Gobiidae). *J. Fish Biol.*, **37,** 723-728.

Hamerlynck, O. (1993) *The ecology of Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) in the shallow coastal waters and estuaries of the Southern Bight of the North Sea*. PhD thesis, University Gent, 187 pp.

Hamerlynck, O., & Cattrijsse, A. (1994) The food of *Pomatoschistus minutus* (Pisces, Gobiidae) in Belgian coastal waters, and a comparison with the food of its potential competitor *P. lozanoi*. *Journal of Fish Biology*, **44,** 753-771.

Hamerlynck, O., Cattrijsse, A., & Arellano, R. V. (1993) Daily ration of juvenile *Pomatoschistus lozanoi* de Buen (Pisces: Gobiidae). *ICES J. Mar. Sci.*, **50,** 471-480.

Hamerlynck, O., Geets, A., & Van Damme, P. (1989) The parasites of two sympatric gobies *Pomatoschistus minutus* and *P. lozanoi* in the Belgian coastal waters. in: *Verhandeling van het symposium "Invertebraten van Belgie".* Proceedings of the Invertebrate symposium 25-26 November 1988, Brussels, Koninklijk Belgisch Instituut voor Natuurwetenschappen, Royal Belgian Institute for Natural Sciences, pp. 27-30.

Hamerlynck, O., Heip, C., & Redant, F. (1986) Life history, food consumption and resource partitioning in two sympatric gobies *Pomatoschistus minutus* and *P. lozanoi* in the Belgian coastal waters. *ICES*, **14**.

Hamerlynck, O., & Janssen, C. R. (1985) De grondels (Pisces, Gobiidae) van de Belgische kustwateren en de aangrenzende estuaria. *De Strandvlo*, **5,** 3, 72-78.

Hamerlynck, O., Janssen, C. R., & Heip, C. (1985) A preliminary note on the food of gobies (Pisces, Gobiidae) in the belgian coastal waters. *Proceedings 'Progress in Belgian Oceanographic Research' Brussels*, 314-320.

Hamerlynck, O., van de Vyver, P., & Janssen, C. R. (1990) The trophic position of *Pomatoschistus lozanoi* (Pisces: Gobiidae) in the Southern Bight. in: *Proc. 24th Europ. Mar. Biol. Symp. :Trophic Relationships in the Marine Environment.* Barnes, M. and Gibson, R. N. (eds), Aberdeen University press, pp. 183-190.

Hamerlynck, O., & Vanreusel, A. (1993) *Mesacanthion diplechma* (Nematoda: Thoracostomopsidae), a link to higher trophic levels? *J. Mar. Biol. Ass. U.K.*, **73,** 453-456.

Hanski, I. (1982) Dynamics of regional distribution: the core and satellite species hypothesis. *Oikos*, **38,** 210-221.

Healey, M. C. (1971) The distribution and abundance of sand gobies, *Gobius minutus,* in the Ythan estuary. *J. Zool. Lond.*, **163,** 177-229.

Healey, M. C. (1972) On the population ecology of the common goby in the Ythan estuary. *J. Nat. Hist.*, **6,** 133-145.

Hesthagen, I. H. (1975) Seasonal occurence and length variation in the Sand Goby, *Pomatoschistus minutus* (Pallas), in the shore zone of the inner Oslofjord. *Norw. J. Zool.*, **23,** 235-242.

Hesthagen, I. H. (1977) Migrations, breeding, and growth in *Pomatoschistus minutus* (Pallas) (Pisces, Gobiidae) in Oslofjorden, Norway. *Sarsia*, **63,** 17-26.

Hesthagen, I. H. (1979) Temperature selection and avoidance in the sand goby, *Pomatoschistus minutus* (Pallas), collected at different seasons. *Env. Biol. Fish.*, **4,** 4, 369-377.

Hoese, D. F. (1984) Gobioidei: Relationships. *Ontogeny and Systematics of Fishes, Special Publication*, **1,** 588-591.

Holmes, J. C. (1990) Helminth communities in marine fishes. in: G. Esch, A. Bush, & J. Aho (eds) *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 5, pp. 101-130.

Humphery-Smith, I. (1989) The evolution of phylogenetic specificity among parasitic organisms. *Parasitology Today*, **5,** 12, 385-387.

Iglesias, J. (1981) Spatial and temporal changes in the demersal fish community of the Ria de Arosa (NW Spain). *Marine Biology*, **65,** 199-208.

Janzen, D. H. (1980) What is coevolution? *Evolution*, **34,** 611-612.

Jaquet, N., & Raffaelli, D. (1989) The ecological importance of the sand goby *Pomatoschistus minutus* (Pallas). *J. Exp. Mar. Biol. Ecol.*, **128,** 147-156.

Jones, D., & Miler, P. J. (1966) Seasonal migrations of the Common Goby, *Pomatoschistus microps* (Kroyer), in Morecambe Bay and elsewhere. *Hydrobiologia*, **27,** 515-528.

Kennedy, C. R. (1975) *Ecological Animal Parasitology*. Oxford, Blackwell Scientific Publications, 163 pp.

Kennedy, C. R. (1990) Helminth communities in freshwater fish: structured communities or stochastic assemblages? in: G. Esch, A. Bush, & J. Aho (eds) *Parasite communities: patterns and processes*. London, New York, Chapman and Hall , pp. 131-156.

Kennedy, C. R., Bush, A. O., & Aho, J. M. (1986) Patterns in helminth communities: why are fish and birds different? *Parasitology*, **93,** 205-215.

Khan, R. A., Overstreet, R., & Thulin, J. (1991) Influence of pollution on parasites of aquatic animals. *Ann. Parasitol. Hum. Comp.*, **66 Suppl. I,** 49-51.

Kinne, O. (1984) *Diseases of marine animals: Introduction, Pisces*. Hamburg, Biologische Anstalt Helgoland, 541 pp.

Koefoed, J. H. (1979) Gruntvannskutlingene i Norge. *Fauna*, **32,** 112-120.

Køie, M. (1978) On the morphology and life-history of *Stephanostomum caduncum* (Looss1901) Manter1934 (Trematoda, Acanthocolpidae). *Ophelia*, **17,** 1, 121-133.

Køie, M. (1984) Digenetic trematodes from *Gadus morhua* L. (Osteichthyes, Gadidae) from Danish and adjacent waters with special reference to their life-histories. *Ophelia*, **23,** 2, 195-222.

Køie, M. (1986) The life-history of *Mesorchis denticulatus* (Rudolphi, 1802) Dietz, 1909 (Trematoda, Echinostomatidae). *Z. F. Parasitenkunde*, **72,** 335-343.

Køie, M. (1990) The life cycle of *Pygidiopsis ardeae* Køie, 1990 (Digenea, Heterophyidae). *J. Parasitol.*, **76,** 4, 537-541.

Køie, M. (1993) Nematode parasites in teleosts from 0 m to 1540 m depth off the Faroe Islands (The North Atlantic). *Ophelia*, **38,** 3, 217-243.

Koter, M. (1962) Helminth parasites in *Gobiidae* of the Puck Bay. *Acta Parasitologica Polonica*, **10,** 1-13.

Krishnamurthy, K., Prince Jeyaseelan, M. J., & Sultan Ali, M. A. (1984) The distribution of fishes in the global mangroves. *Current Science*, **53,** September 5, 17, 901-906.

Kuwamura, T., Yogo, Y., & Nakashima, Y. (1994) Population dynamics of *Paragobiodon echinocephalus* and host coral *Stylophora pistilla*. *Marine Ecology Progress Series*, **103,** 17-23.

Lindström, K. (1988) Male-male competition for nest sites in the sand goby, *Pomatoschistus minutus*. *Oikos*, **53,** 67-73.

Lindström, K. (1992) The effect of resource holding potential, nest size and information about resource quality on the outcome of intruder-owner conflicts in the sand goby. *Behavioral Ecology and Sociobiology*, **30,** 53-58.

Lindström, K. (1992) Female spawning patterns and male mating success in the sand goby *Pomatoschistus minutus*. *Marine Biology*, **113,** 475-480.

Lindström, K., & Wennström, C. (1994) Expected future reproductive success and paternal behaviour in the sand goby, *Pomatoschistus minutus* (Pisces, Gobiidae). *Journal of Fish Biology*, **44,** 469-477.

Little, M. C., Reay, P. J., & Grove, S. J. (1988) The fish community of an East African mangrove creek. *J. Fish Biol.*, **32,** 729-747.

Maes, J., Tallieu, A., Van Damme, P. A., & Ollevier, F. (1997) The composition of the fish and crustacean community of the Zeeschelde Estuary (Belgium). *Belgian Journal of Zoology*, **127,** 1, 47-55.

Maes, J., Tallieu, A., Van Damme, P., Cottenie, K., & Ollevier, F. (1998) Seasonal patterns in the fish and crustacean community of a turbid temperate estuary (Zeeschelde Estuary, Belgium). *Estuarine, Coastal and Shelf Science*, **47,** in press.

Magnhagen, C. (1986) Activity differences influencing food selection in the marine fish *Pomatoschistus microps*. *Can. J. Fish. Aquat. Sci.*, **43,** 223-227.

Magnhagen, C. (1992) Alternative reproductive behaviour in the common goby, *Pomatoschistus microps*: an ontogenetic gradient? *Anim. Behav.*, **44,** 182-184.

Magnhagen, C., & Vestergaard, K. (1991) Risk taking in relation to reproductive investments and future reproductive opportunities: field experiments on nest-guarding common gobies, *Pomatoschistus microps*. *Behav Ecol*, **2,** 351-359.

Magnhagen, C., & Wiederholm, A.-M. (1982) Habitat and food preferences of *Pomatoschistus minutus* and *P. microps* (Gobiidae) when alone and together: an experimental study. *Oikos*, **39,** 152-156.

Mann, H. (1964) Vorkommen, Verbreitung und Schadwirkung von *Lernaeocera minuta* T. Scott (*Copepoda parasitica*). *Veröff. Inst. Meeresf. Bremerhafen*, **9,** 79-83.

Markowski, St. (1935) Die parasitischen Würmer von *Gobius minutus* pall. des Polnischen Balticums. *Sciences Naturelles*, **2,** 251-260.

Mees, J., Cattrijsse, A. Hamerlynck, O. (1993) Distribution and abundance of shallow-water hyperbenthic mysids (Crustacea, Mysidacea) and euphausiids (Crustacea, Euphausiacea) in the Voordelta and the Westerschelde, southwest Netherlands. *Cah. Biol. Mar.*, **34**, 165-186.

Miller, P. J. (1979) Adaptiveness and implications of small size in teleosts. *Symp. Zool. Soc. Lond.*, **44,** 263-306.

Miller, P. J. (1984) The tokology of gobioid fishes. in: G. W. Potts, & R. J. Wootton (eds) *Fish reproduction. Strategies and tactics*. London, Academic Press Inc. Vol. 8, pp. 119-153.

Miller, P. J. (1986) Gobiidae. in: P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, & E. Tortonese (eds) *Fishes of the North-eastern Atlantic and the Mediterranean.* UNESCO Vol. III, pp. 1019-1085.

Miller, P. J. (1987) Affinities, origin and adaptive features of the Australian Desert Goby *Chlamydogobius eremius* (Zietz, 1896) (Teleostei: Gobiidae). *Journal of Natural History*, **21,** 687-705.

Möller-Buchner, J. (1981) Untersuchungen zum parasitenbefall von *Pomatoschistus microps* (Gobiidae, Pisces) in der Unterelbe. *Arch. Hydrobiol.*, **61,** 5, 59-83.

Nellbring, S. (1985) Abundance, biomass and seasonal variation of fish on shallow soft bottoms in the Askö area, Northern Baltic proper. *Sarsia*, **70,** 217-225.

Nellbring, S. (1986) A quantitative and experimental study of goby (*Pomatoschistus* spp.) nests on a shallow soft bottom, Northern Baltic proper. *Ophelia*, **25,** 1, 15-23.

Nellbring, S. (1993) Spawning of two *Pomatoschistus* species (Gobiidae) in relation to nest availability and depth: a field experiment. *Netherlands Journal of Sea Research*, **31,** 2, 173-179.

Nelson, J. S. (1984) Family Gobiidae. in: *Fishes of the world.* 3th edition, New York, John Wiley and Sons, Inc., pp. 355-356.

Nyman, K.-J. (1953) Observations on the behaviour of *Gobius microps*. *Acta Soc. Pro Fauna Et Flora Fenn.* , **69,** 5, 1-11.

Odening, K. (1976) Conception and terminology of hosts in parasitology. *Advances in Parasitology*, **14,** 1-93.

Penzo, E., Gandolfi, G., Bargelloni, L. Colombo, L. & Patarnello, T. (1998) Messinian salinity crises and the origin of freshwater lifestyle in western maditerranean gobies. Molecular Biology and Evolution, (in press).

Petersen, F. (1992) Nutritional condition and parasites of the sand goby *Pomatoschistus minutus* (Pallas) from the German Wadden Sea. *Ophelia*, **35,** 3, 197-207.

Polulin, N. V. C., & Lubbock, R. (1979) Five new prawn-associated gobies (Teleostei : Gobiidae) of the genus *Amblyeleotris*. *Bull. Br. Mus. Nat. Hist. (Zool)*, **36,** 4, 239-249.

Polyanski, Yu. I. (1970) Ecology of parasites of marine fishes. in: V. A. Dogiel, G. K. Petrushevski, & Yu. I. Polyanski (eds) *Parasitology of Fishes*. T.H.F. Publications Inc. Translation: Z. Kabata ed., pp. 48-83.

Price, P. W., Westoby, M., & Rice, B. (1988) Parasite-mediated competition: some predictions and tests. *Am. Nat.*, **131,** 544-555.

Reimer, L. W. (1973) Das Auftreten eines Fischtrematoden der Gattung *Asymphylodora* Looss, 1899, bei *Nereis diversicolor* O. F. Müller als Beispiel für einen Alternativzyklus. *Zool. Anz. , Leipzig*, **191,** 3/4, 187-196.

Robert, F., Renaud, F., Mathieu, E., & Gabrion, C. (1988) Importance of the paratenic host in the biology of *Bothriocephalus gregarius* (*Cestoda, Pseudophyllidea*), a parasite of the turbot. *International Journal for Parasitology*, **18,** 5, 611-621.

Rogers, S. I. (1988) The seasonal partitioning of energy in an estuarine fish, the common goby, *Pomatoschistus microps* Krøyer. *J. Fish Biol.*, **33,** suppl.A, 45-50.

Rogers, S. I. (1989) Seasonal variations in fecundity and egg size of the common goby, *Pomatoschistus microps*. *J. Mar. Biol. Ass. U.K.*, **69,** 535-543.

Rohde, K. (1978) Latitudinal differences in host-specificity of marine Monogenea and Digenea. *Marine Biology*, **47,** 125-134.

Rohde, K. (1979) A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. *The American Naturalist*, **114,** 5, 648-671.

Rohde, K. (1980) Host specificity indices of parasites and their application. *Experientia*, **36,** 1370-1371.

Rohde, K. (1982) *Ecology of Marine Parasites*. St. Lucia, University of Queensland Press, 245 pp.

Rohde, K. (1984) Ecology of marine parasites. *Helgoländer Meeresunters.*, **37,** 5-33.

Salvanes, A. G. V., & Nordeide, J. T. (1993) Dominating sublittoral fish species in a west Norwegian fjord and their trophic links to cod (*Gadus morhua* L.). *Sarsia*, **78,** 221-234.

Schmidt-Moser, R., & Westphal, D. (191) Predation of *Pomatoschistus microps* Krøyer and *P. minutus* Pallas (Gobiidae, Pisces) on macro- and meiofauna in the brackish fjord Schlei. *Kieler Meeresforsch.*, **5,** 471-478.

Schoener, T. W. (1974) Resource partitioning in ecological communities. *Science*, **185,** 27-39.

Shotter, R. A. (1973) A comparison of the parasite fauna of young whiting, *Odontogadus merlangus* (L.) (Gadidae) from an inshore and an offshore location off the Isle of Man. *J. Fish Biol.*, **5,** 185-195.

Siddall, R., Pike, A. W., & McVicar, A. H. (1994) Parasites of flatfish in relation to sewage sludge dumping. *Journal of Fish Biology*, **45,** 193-209.

Szidat, L. (1943) Die Fischtrematoden der Gattung *Asymphylodora* Looss 1899 und Verwandte. *Zeitschrift Für Parasitenkunde*, **13,** 25-61.

Thorman, S., & Wiederholm, A.-M. (1983) Seasonal occurrence and food resource use of nearshore fish species in the Bothnian Sea, Sweden. *Mar. Ecol. Prog. Ser.*, **10,** 223-229.

Vaes, F. (1974) A new type of trematode life-cycle: an invertebrate as final host. *Proceedings of the 3th Int. Congress on Parasitology, Munchen*, **1,** B2, 351.

Vaes, M. (1978) Infection of the common goby, *Pomatoschistus microps,* with *Aphalloides coelomicola* (Trematoda Digenea). *Vlaams Dierengeneeskundig Tijdschrift*, **47,** 3, 274-278.

Van Damme, P. (1993) *The suprapopulation dynamics of the marine fish parasites Lernaeocera lusci and L. branchialis (Copepoda, Pennellidae)*. PhD thesis, Katholieke Universiteit Leuven, 254 pp.

Van Damme, P. A., Geets, A., Hamerlynck, O., & Ollevier, F. (1997) The suprapopulation dynamics of *Lernaeocera branchialis* and *L. lusci* in the Oosterschelde: seasonal abundance on three definitive host species. *ICES Journal of Marine Science*, **54,** 24-31.

Van Damme, P. A., & Olleveir, F. (1994) Infection dynamics of *Lernaeocera lusci* on sand goby *Pomatoschistus minutus* in the Oosterschelde (The Netherlands). *Diseases of Aquatic Organisms*, **19,** 83-87.

Van Damme, P. A., & Ollevier, F. (1995) Morphological and morphometric study of crustacean parasites within the genus *Lernaeocera*. *International Journal for Parasitology*, **25,** 12, 1401-1411.

Wallis, G. P., & Beardmore, J. A. (1980) Genetic evidence for naturally occuring fertile hybrids between two goby species, *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). *Marine Ecology Progress Series*, **3,** 309-315.

Wallis, G. P., & Beardmore, J. A. (1984a) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces, Gobiidae). *Biological Journal of the Linnean Society*, **22,** 107-123.

Wallis, G. P., & Beardmore, J. A. (1984b) Genetic variation and environmental heterogeneity in some closely related goby species. *Genetica*, **62,** 223-237.

Webb, C. J. (1980) Systematics of the *Pomatoschistus minutus* complex (Teleostei: Gobiidae). *Philos. Trans. R. Soc. Lond., Ser. B*, **291,** 201-241.

Wiederholm, A.-M. (1987) Habitat selection and interactions between three marine fish species (Gobiidae). *Oikos*, **48,** 28-32.

Wilkins, H. K. A., & Myers, A. A. (1992) Microhabitat utilisation by an assemblage of temperate Gobiidae (Pisces: Teleostei). *Marine Ecology Progress Series*, **90,** 103-112.

Williams, H. H., MacKenzie, K., & McCarthy, A. M. (1992) Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. *Reviews in Fish Biology and Fisheries*, **2,** 144-176.

Winterbottom, R., & Emery, A. R. (1986) Review of the gobiod fishes of the Chanos Archipelago, Central Indian Ocean. *Life Science Contributions, Royal Ontario Museum*, **142,** 1-82.

Zander, C. D. (1979) On the biology and food of small-sized fish from the North and Baltic Sea area. I. Investigations on *Pomatoschistus pictus* (Malm) (Gobiidae) from Helgoland. *Zool. Anz. Jena*, **202,** 5/6, 413-424.

Zander, C. D. (1988) Zur Bedeutung von Kleinfischen in Økosystemen der Ostsee. *Seevögel*, **9,** 4, 51-55.

Zander, C. D. (1990) Prey selection of the shallow water fish *Pomatoschistus minutus* (Gobiidae, Teleostei) in the SW Baltic. *Helgoländer Meeresunters*, **44,** 147-157.

Zander, C. D., & Döring, W. (1989) The role of gobies (Gobiidae, Teleostei) in the food web of shallow habitats of the Baltic Sea. in: *Proceedings of the 21st EMBS.* 14-19 September, 1986, Gdansk, Polish Academy of Sciences - Institute of Oceanology, pp. 499-508.

Zander, C. D., Groenewold, S., & Strohbach, U. (1994) Parasite transfer from crustacean to fish hosts in the Lübeck Bight, SW Baltic Sea. *Helgoländer Meeresunters.*, **48,** 89-105.

Zander, C. D., & Hagemann, T. (1987) Predation impact and ecological efficiency of *Pomatoschistus spp.* (Gobiidae, Pisces) from a clay/sand ecotone of the Western Baltic Sea. *Zool. Anz* , **218,** 33-48.

Zander, C. D., Meyer, W., Antholz, B., Westphal, D., & Kollra, H.-G. (1984) Small-sized euryhaline fish as intermediate hosts of the digenetic trematode *Cryptocotyle concavum. Helgoländer Meeresunters.*, **37,** 433-443.

Zander, C. D., Strohbach, U., & Groenewold, S. (1993) The importance of gobies (Gobiidae, Teleostei) as hosts and transmitters of parasites in the SW Baltic. *Helgoländer Meeresunters.*, **47,** 81-111.

Zander, C. D., & Westphal, D. (1992) Nahrungsbeziehungen von vier euryhalinen Kleinfischarten der Ostseeförde Schlei (Feeding relationships of the Baltic fjord Schlei). *Zool. Jb. Syst.*, **119,** 339-364.

Chapter 2

The population dynamics of *Pomatoschistus* spp. of the Grevelingen Voordelta area as a basis for the investigation of host-parasite relationships.

Abstract

The population dynamics of *Pomatoschistus minutus*, *P. lozanoi*, *P. microps* and *P. pictus*, sympatric goby species from the Grevelingen Voordelta area, are studied. Monthly beam trawl samples were taken from September 1992 to July 1994. Of a total of 28173 gobies, 76.4 % were *P. minutus*, 21.6 % *P. lozanoi*, 1.5 % *P. pictus* and 0.1 % *P. microps*. Densities of *P. minutus* were highest in autumn (657.4 individuals/1000 m² in September 1992) and in late winter (657.4 ind./1000 m² in February 1993). Densities declined from spring to early summer. The seasonal fluctuations in densities of *P. lozanoi* largely followed the same pattern as *P. minutus*, but densities peaked two months later, which is related to their delayed spawning period. Highest densities of *P. lozanoi* were recorded in November 1993 (159.7 ind./1000 m²) and in April 1994 (352.3 ind./1000 m²). Length frequency distributions showed that specimens from three cohorts of *P. minutus* and two of *P. lozanoi* were present in the samples. Most gobies survived only one year. Sex ratios of both goby species indicated that males were less vulnerable for catching than females during the spawning season. Growth curves indicated a fast growth of *P. minutus* from June till October. The sand gobies reached adult size before winter, which was not the case for *P. lozanoi*. Temporal segregation of spawning together with their life span of about one year resulted in the presence of adult *P. lozanoi* together with juveniles of *P. minutus* in July in the Grevelingen Voordelta.

Introduction

Gobies are an important component in the shallow coastal ecosystems of the Mediterranean and Atlantic coasts of Europe (Miller, 1986). In the North Sea, they are the most abundant demersal species (Fonds, 1973, Hamerlynck *et al.* 1990). Although they are not of any direct commercial value, their role in the food web of several economically important fish species should not be underestimated. They are main prey items for young bib (*Trisopterus luscus*), whiting (*Merlangius merlangus*) (Hamerlynck & Hostens, 1993) and cod (*Gadus morhua*) (Arntz, 1978, Salvanes & Nordeide, 1993) besides several other demersal fish (Doornbos & Twisk, 1987, Costa, 1988, Creutzberg & Witte, 1989). Six species occur in the Belgian and Dutch coastal waters: the transparent goby, *Aphia minuta* Risso, 1810, which is a more pelagic species, the black goby, *Gobius niger* Linnaeus, 1758, an inshore species and four *Pomatoschistus* species, *Pomatoschistus minutus* (Pallas, 1770), the sand goby and *P. lozanoi* (de Buen, 1923), Lozano's goby, which are both very closely related, *P. microps* (Krøyer, 1838), the common goby and *P. pictus* (Malm, 1865), the painted goby.

Most studies on population dynamics of gobies in coastal areas and estuaries of the Northern countries are focused on *P. minutus* (Jones & Miller, 1966, Healey, 1971, Fonds, 1973, Hesthagen, 1975, 1977, Claridge *et al.*, 1985) and *P. microps* (Jones & Miller, 1966, Healey, 1972, Fouda & Miller, 1981). Since *P. minutus* and *P. lozanoi* are morphologically very similar and are only recently recognised as distinct species (Fonds, 1973, Webb, 1980), some authors probably lumped both species together in studies on population dynamics and seasonal occurrence (Healey, 1971, Doornbos & Twisk, 1987, Henderson, 1989). Hamerlynck (1993) and Hamerlynck & Catrijsse (1994) pointed to this problem when dealing with the difference in food niche of both species, and possible competition between them. Because of their abundance and their importance in the food web of marine coastal areas, gobies seem to be ideal models to study host-parasite relationships. Since the composition of the parasite component community is largely influenced by the ecology of the host (Rohde, 1984, Holmes, 1990), a sound understanding of the population dynamics of the host is a prerequisite for studying these relationships.

This chapter will focus on the population dynamics of four *Pomatoschistus* species of the Grevelingen Voordelta, with special emphasis on *P. minutus* and *P. lozanoi*. It is not our aim to give a comprehensive analysis of all population dynamical aspects in detail in this chapter, but merely to present the basic data on density fluctuations, length-frequency distribution, sex ratios and growth necessary to explain the choice of the samples analysed during parasitological research and as a background for their interpretation (see chapter 6 and chapter 7). Arellano (1995) conducted an extensive study on the age structure and growth of *P. minutus* and *P. lozanoi* in the Voordelta area on the basis of daily growth increments in the otoliths using subsamples from the same catches. Therefore, for details on age and growth parameters, we refer to his work.

Material and methods

Study area

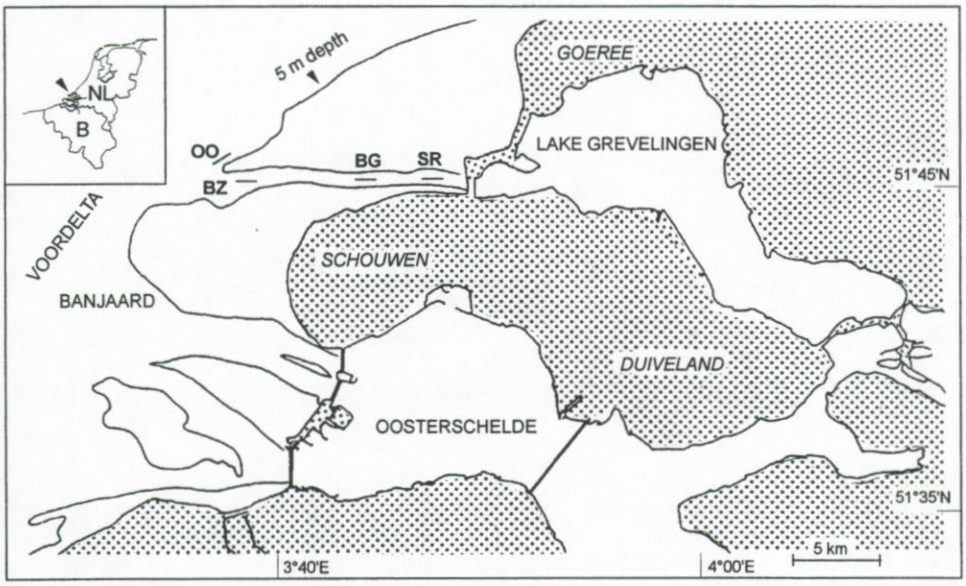


Figure 2.1: Map of the sampling area indicating the four sampling stations in the Grevelingen Voordelta: Schaar van Renesse (SR), Brouwershavense gat (BG), Bollen van het Zand (BZ) and Ooster (OO).

The Voordelta (51°45'N, 3°45'E) is the shallow coastal area between the Belgian-Dutch border in the South and the Hoek van Holland in the North (Fig.2.1). It is formed by the tidal deltas of the (former) estuaries of the Dutch delta. In the Dutch Delta, rich and varied communities exist in the Voordelta (Hamerlynck *et al.*, 1993). Hamerlynck & Mees (1991) studied the temporal and spatial structure in the hyperbenthos community of the shallow coastal Voordelta area. The area could be split up in three geographically defined communities: the ebb-tidal delta of the Grevelingen, the Banjaard area and the ebb-tidal delta of the Oosterschelde. The composition of the biological communities in these three areas differed substantially. The ebb-tidal delta of the Grevelingen had the highest total biomass, the highest density and biomass of macrobenthic larvae, mysids, fish eggs and larvae (Hamerlynck & Mees, 1991). The assemblage of the inshore stations in the ebb tidal delta of the Grevelingen (VIG) had the highest total abundance of epibenthic fauna, including high densities of *P. minutus* and *P. lozanoi* (Hamerlynck *et al.*, 1993). Therefore, four sampling stations (Fig. 2.1) in the ebb-tidal delta of the Grevelingen were selected: Schaar van Renesse (SR) (51°45N, 3°46'E), Brouwershavense Gat (BG) (51°45'N,3°43'E), Bollen van het Zand (BZ) (51°45'N, 3°39'E) en Ooster (OO) (51°45'30"N,3°38'E). The first three stations are situated in the inshore tidal channel of the Grevelingen while the Ooster station is located on the seaward side of the Ooster bank.

Sampling

From September 1992 till July 1994, monthly samples were taken with the research vessel 'Luctor' (34 m, 500 hp) at two depth strata (5 m and 10 m) in the four sampling places. Trawling was carried out during daytime, with a beam trawl of 2.7 m x 0.5 m, equipped with a 6 m long net with a 5 x 5 mm mesh size in the cod-end. One trickler chain and a chain in the groundrope were used. Hauls were 1000 m in length (radar readings from fixed points).

Gobies were collected from all stations. When enough gobies were present (>60) in a trawl, the sample was divided in two subsamples, one for the study of age and growth using otoliths (Arellano, 1995) and another for further parasitological work (cfr. next chapters). In case not enough gobies were caught to split up the sample, an extra trawl for gobies for the parasitological study was carried out at the sampling place with the highest abundance. On board, gobies were first anaesthetised in a saturated solution of Benzocaïne (ethyl amino-benzoaat) in seawater to prevent regurgitation, before preserving them in a 4 % formaldehyde solution.

Identification of the gobies was based on the pattern of head sensory papillae and pigmentation patterns (Edlund *et al.* 1980, Hamerlynck, 1990). Sexing was done on the basis of the difference in morphology of the genital papilla, which is elongated and pointed in the male and blunt in the female (Fonds, 1973). In small specimens (< 25 mm standard length) sexing was not always possible and these animals were classified as 'juveniles'. Standard length to the nearest mm and wet weight to 0.01 g were measured in all individuals. In the results, the term adult length refers to the mean length where the growth curve starts to level off.

At each sampling site, salinity (‰), water temperature (°C), oxygen saturation (%), dissolved oxygen concentration (mg/l), pH, conductivity (µS/cm), Secchi depth (cm) and sampling depth (m) were recorded (Table 2.1). Changes in mean monthly temperature (averaged over all four sampling stations) are shown in Figure 2.2.

Table 2.1: Environmental variables recorded in the Grevelingen Voordelta Area (mean of measurements at four sampling stations.).

Date	Salinity (‰)	Temperature (°C)	Oxygen (%)	Oxygen (mg/l)	pH	Conductivity (µS/cm)	Secchi (cm)
09/09/92	36.1	15.6	93.0	7.5	7.9	54.5	111.2
01/10/92	34.2	16.4	90.5	7.2	7.8	51.9	156.3
30/10/92	34.4	9.8	90.0	8.2	7.7	52.2	88.7
08/12/92	34.2	7.4	89.7	8.6	7.6	51.9	58.7
09/02/93	33.3	6.0	92.4	9.5	7.9	50.7	57.5
11/03/93	33.0	5.5	92.2	9.4	7.8	50.2	126.7
08/04/93	33.6	7.4	98.9	9.6	7.9	51.1	110.0
11/05/93	31.0	12.0	112.8	10.1	7.9	47.5	48.3
08/06/93	32.9	16.8	106.2	8.5	8.2	50.1	175.0
16/07/93	33.5	17.7	92.9	7.2	8.0	50.9	217.5
07/09/93	31.6	16.4	100.9	8.1	7.9	48.3	131.2
11/10/93	33.7	14.1	98.7	8.2	7.7	51.2	270.0
09/11/93	32.5	10.3	89.5	8.2	8.0	49.7	245.0
10/01/94	33.5	5.1	92.7	9.4	7.8	50.9	61.2
02/02/94	32.4	5.8	-	-	7.8	49.6	37.5
01/03/94	33.4	4.4	96.6	10.3	7.7	50.8	105.0
19/04/94	30.7	8.2	97.3	9.4	8.2	47.1	103.7
10/05/94	32.6	12.1	122.4	10.9	8.5	49.6	150.0
14/06/94	30.1	14.5	94.8	8.1	7.9	46.3	225.0

Data analysis

For the present study, the data of the subsamples used for the study of otoliths and the parasitological study were combined. Densities of gobies were expressed as number of gobies per 1000 m² using the following formula:

$$Density = \frac{N * 5 * 1000}{n * 2.7 * 1000}$$

with N = number of gobies, and n = number of trawls. Net efficiency of bottom gear in different studies on small sized fish ranges from 15.7 % to 85 % (Healey, 1971, Fonds, 1973, Kuipers, 1975, Doornbos & Twisk, 1986, Hamerlynck *et al.,* 1986, 1992, 1993). A net efficiency of 20 % was assumed, therefore we multiply the number of gobies caught * 5 (Kuipers, 1975, Hamerlynck *et al*., 1992, 1993). The beam trawl was 2.7 m wide and we trawled 1000 m.

Possible differences in densities of *P. minutus* and *P. lozanoi* throughout the year were tested using Wilcoxon matched pairs test, since the data did not follow the assumptions of parametric tests. Differences in abundance of gobies between the two depth strata (5 m and 10 m depth) have already been reported by Arellano (1995). The present study will analyse the differences in abundance of the gobies between stations, which is complementary to the previously mentioned work. The data of the two depth strata are pooled, unless indicated otherwise. A nonparametric Friedman ANOVA was used to test for overall differences in densities of a given goby species among the four sampling stations. Posthoc pairwise comparisons were calculated using the following inequality:

$$\left|\overline{R}_u - \overline{R}_v\right| \geq z_{\alpha / k(k-1)} \sqrt{\frac{k(k+1)}{6N}}$$

where $\overline{R}_u$ and $\overline{R}_v$ are average ranks within each condition (station), k is the number of conditions and N the number of cases. The value of $z_{\alpha / k(k-1)}$, obtained from tables, is the abscissa value from the unit normal distribution above which lies $\alpha / k(k-1)$ percent of the distribution (Siegel & Castellan, 1988). Thus, if the difference between the average ranks exceeds the corresponding critical value, we may conclude that the samples from the two stations contain significantly different numbers of gobies.

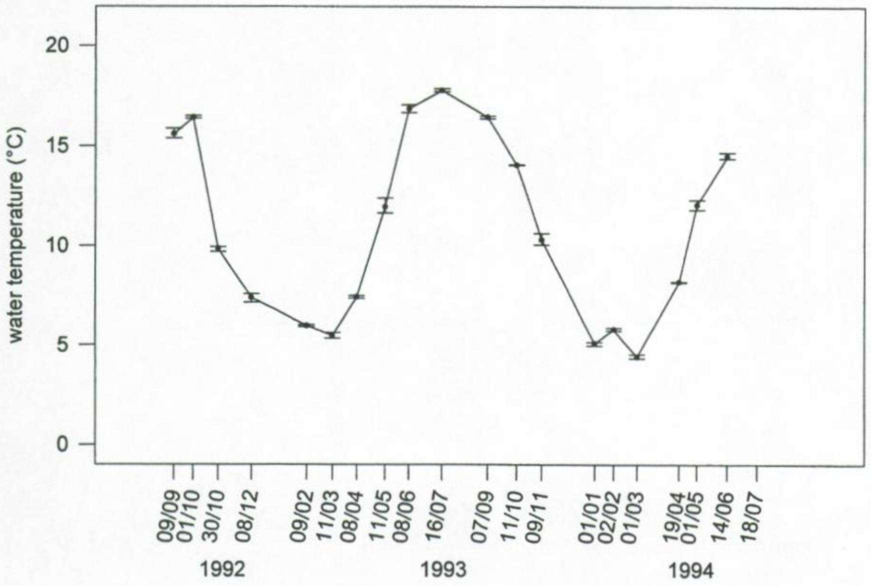


Figure 2.2: Seasonal variation in water temperature in the Grevelingen Voordelta. Mean temperature in four sampling stations (circles) and minimum and maximum are shown.

Results

Densities

Of a total of 28173 *Pomatoschistus* spp. caught in the four sampling stations from September 1992 till July 1994, *P. minutus* was most abundant (76.4 %), *P. lozanoi* was second most important (21.9 %), only 1.5 % were *P. pictus* and 0.1 % *P. microps*. *Aphia minuta* was only caught occasionally. Of 21532 sand gobies, 11257 were males (52.3 %), 10224 females (47.5 %) and 51 could not be sexed (0.24 %). Also for Lozanoi's goby the majority were males (3544 individuals (ind.) or 57.5 %), against 2082 females (38.2 %) and 262 individuals which could not be sexed (4.25 %). In the subsamples of Arellano (1995), *P. pictus* or *P. microps* were not sexed. Sex ratios for these species in our subsamples were 65.2 % females and 34.8 % male *P. pictus* (on 23 ind.) and 26.7 % female and 73.3 % male *P. microps* (on 15 ind.), but the small sample size probably biases these results. Densities calculated for *P. minutus* and *P.*

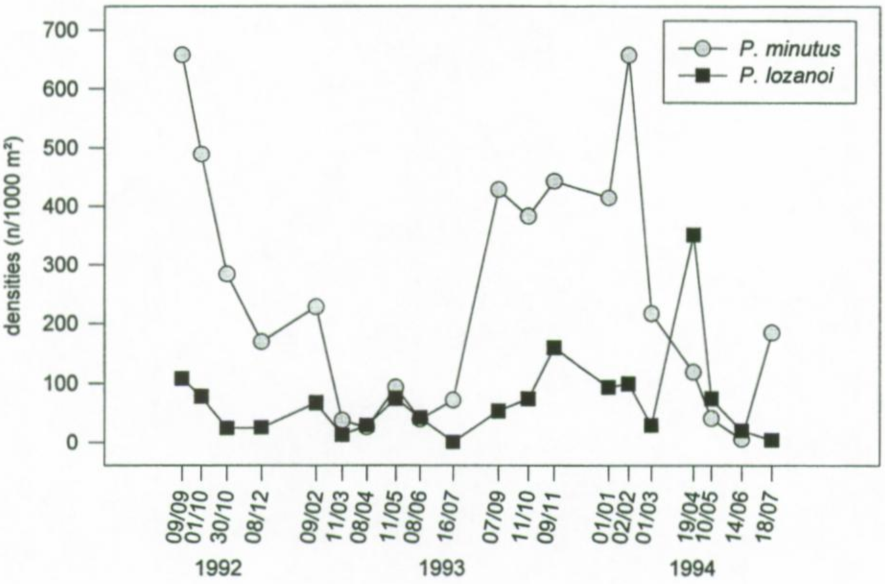


Figure 2.3: Mean densities of *Pomatoschistus minutus* and *P. lozanoi* in the Grevelingen Voordelta.

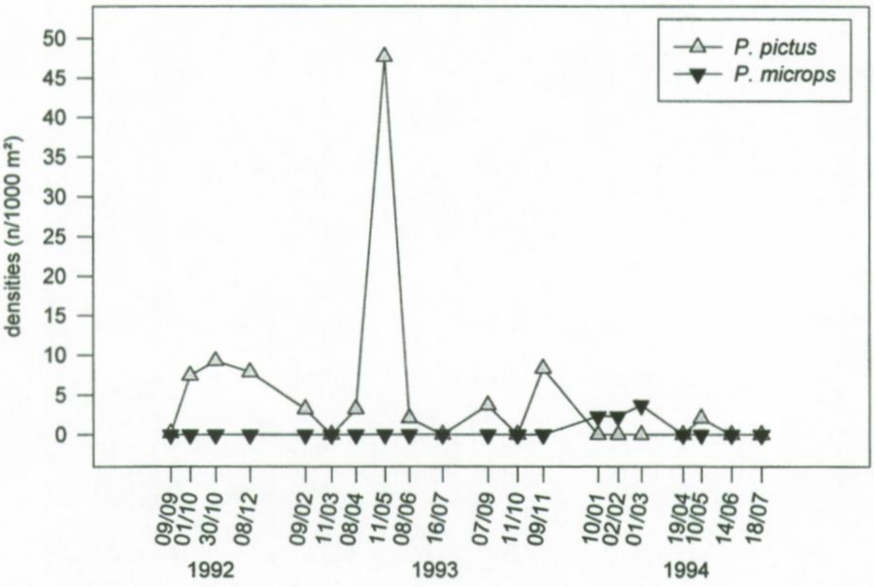


Figure 2.4: Mean densities of *Pomatoschistus pictus* and *P. microps* in the Grevelingen Voordelta.
(Note difference in scale compared to Figure 2.3)

lozanoi (males, females, juveniles and total number of gobies) per sampling station and per month are given in an addendum to this chapter.

Seasonal fluctuations in densities of *Pomatoschistus* species are shown in Figures 2.3 and 2.4. The highest densities of *P. minutus* were recorded in autumn and late winter, with a peak density of 657.4 sand gobies per 1000 m^2 in September 1992 and exactly the same high density in February 1994. Densities declined from spring to early summer. From April to June, densities were low (26.3 ind. per 1000 m^2 in April 1993 and 5.1 in June 1994).

Densities of *P. lozanoi* in the Grevelingen Voordelta were generally lower than those of *P. minutus*. In July 1993 and 1994, very few Lozano's gobies occurred in the catches (0.69 and 4.17 gobies per 1000 m^2 respectively). Densities increased towards autumn (107.8 ind. in September 1992 and 159.7 ind. in November 1993) and decreased slightly in winter time to peak again in May 1993 (74.8 ind.) and in April 1994 (352.3 ind.). The high abundance of Lozano's gobies in April 1994 was mainly due to very high densities caught in the shallow trawls (5 m depth) in Schaar van Renesse (densities of 2281.5 Lozano's gobies per 1000 m^2) and in Brouwershavense gat (1637.1 ind. per 1000 m^2).

P. pictus densities were generally low, reaching a peak of 47.7 painted gobies per 1000 m^2 in May 1993, due to a large catch in Brouwershavense Gat in the 10 m depth stratum (329.6 ind./1000 m^2). The painted goby occurred more often in the deeper strata (0.9 ind./1000 m^2 at 5 m depth, 9.0 ind./1000 m^2 at 10 m depth, all samples from the four stations pooled).

P. microps, a brackish water species, was only caught in January, February and March 1994, in very low numbers (maximum of 18.5/1000 m^2 in BG, 5 m depth).

P. minutus densities were significantly higher than *P. lozanoi* densities in all four sampling stations (Wilcoxon matched pairs test, $p<0.05$). Geographical differences in densities between the four sampling stations largely showed the same trends for *P. minutus* and *P. lozanoi*. Most gobies of both species were caught in Schaar van Renesse (SR), the sampling station which is closest to the Brouwersdam, and in Bollen van het Zand (BZ) (Fig. 2.5). *P. minutus* had significantly higher densities in both stations than in Ooster (OO), the most seaward station (Friedman Anova, $p<0.05$ and multiple comparisons). Brouwershavense Gat (BG) had an intermediate position for *P. minutus*. *P. lozanoi*, although more abundant in SR and BZ, had a more equal distribution between the sampling stations than *P. minutus* and no significant differences were observed among stations (Friedman Anova, $p>0.05$).

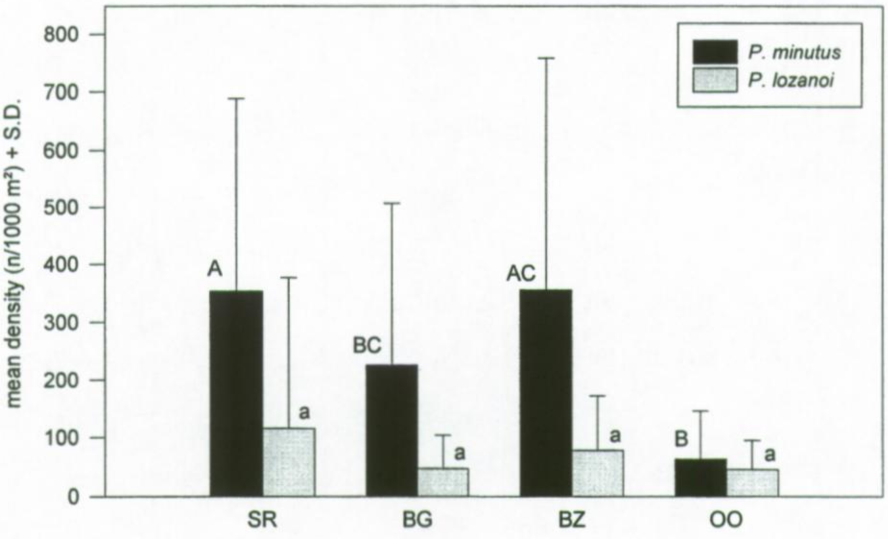


Figure 2.5: Mean densities of *Pomatoschistus minutus* and *P. lozanoi* at four stations in the Grevelingen Voordelta (SR: Schaar van Renesse, BG: Brouwershavense Gat, BZ: Bollen van het Zand, OO: Ooster). Means sharing the same character are not significantly different. Means were calculated from 40 catches per station, except for OO where 39 catches were taken (see addendum of Chapter 2).

Length-frequency distribution and sex ratios

Length-frequency distributions and sex ratios of *P. minutus* and *P. lozanoi,* for all four stations pooled, are shown in Figures 2.6 and 2.7. The length-frequency distributions for male and female showed similar patterns in both goby species. The recruitment of the young gobies and their growth can be clearly followed for both goby species. Both gobies produced only one cohort per year.

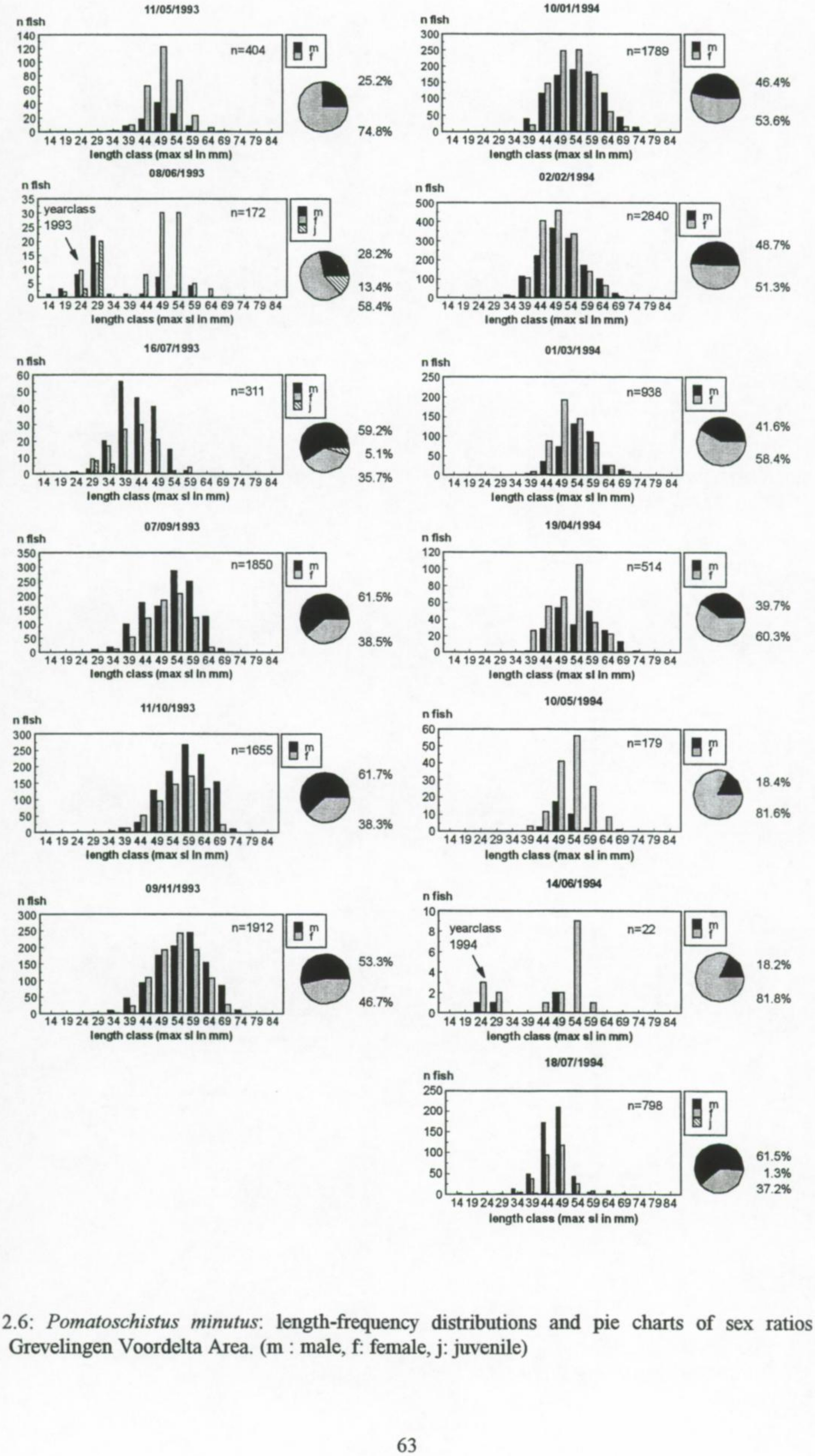


Figure 2.6: *Pomatoschistus minutus*: length-frequency distributions and pie charts of sex ratios in the Grevelingen Voordelta Area. (m : male, f: female, j: juvenile)

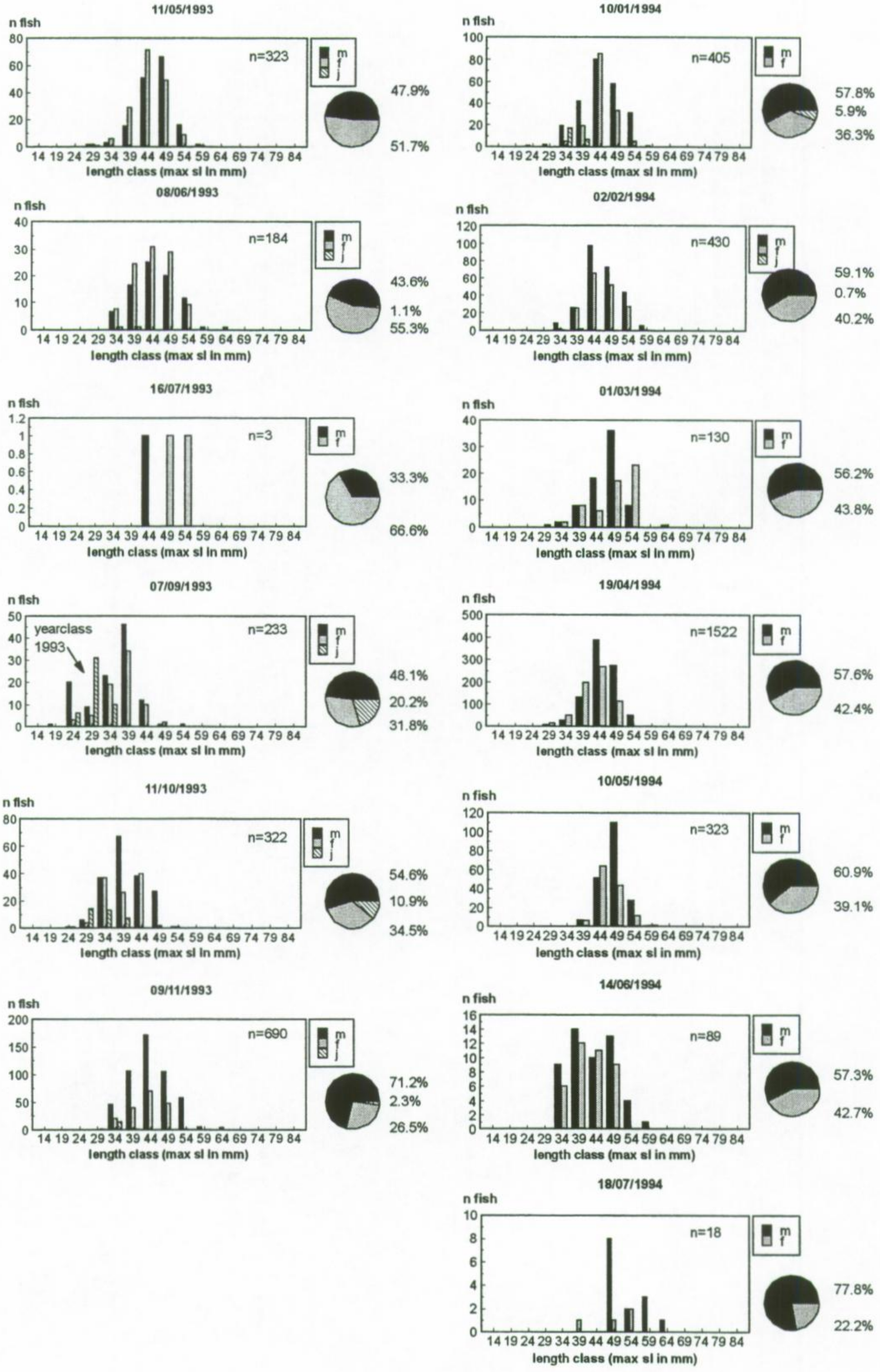


Figure 2.7: *Pomatoschistus lozanoi*: length-frequency distributions and pie charts of sex ratios in the Grevelingen Voordelta area (m: male, f: female, j: juvenile).

Young *P. minutus* were caught in June 1993 and 1994, and can clearly be separated from the co-occuring 'old' cohort (yearclass 1992) of adult gobies. In July, most sand gobies seemed to belong to the new yearclass, judging from the length frequency distribution. The sex ratio during the spawning season (March to June) was largely in favour of female sand gobies (up to 74.8 % in May 1993 and 81.6 % in June 1994), while an excess of male gobies was caught from July to October, changing back to a slight dominance of females in winter.

P. lozanoi young of the 1993 yearclass were first caught in September, two months later than *P. minutus*. No clear overlap of the juveniles with adults of the previous yearclass was observed. Sex ratios were mostly in favour of males, but, during the 1993 spawning season (June 1993), we caught slightly more females than males. This observation was not confirmed in the 1994 spawning season. In July 1993 and 1994, very low numbers of Lozano's gobies were caught, so sex ratios for these months may be biased.

Growth

Growth curves based on mean standard lengths of *P. minutus* and *P. lozanoi* are represented in Figures 2.8 and 2.9. Cohorts indicated in the figures were separated based on the length-frequency distributions (Figs 2.6 and 2.7). For more detailed growth parameters, we refer to Arellano (1995). During the course of the sampling period, sand gobies belonging to three different yearclasses and Lozano's gobies of two cohorts were present. In May and June 1993, the adult *P. minutus* of the 1992 cohort overlapped with the young of 1993, and the same pattern was observed in June 1994. Growth was very fast from June till October. Maximum mean standard length was reached in October (57.8 ± 6.8 mm). Mean standard length declined slightly during winter time.

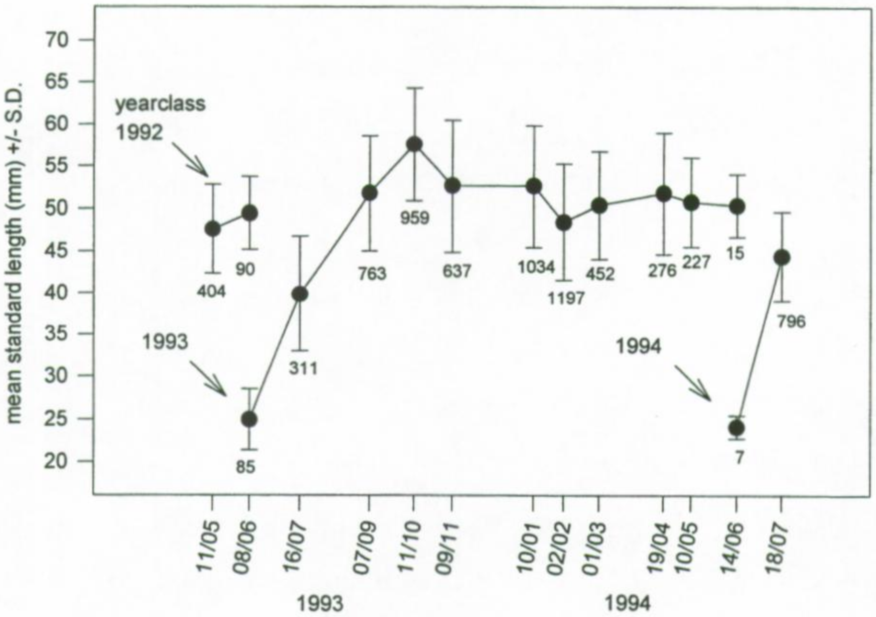


Figure 2.8: Growth curve of *Pomatoschistus minutus* in the Grevelingen Voordelta. Number of fish measured are indicated.

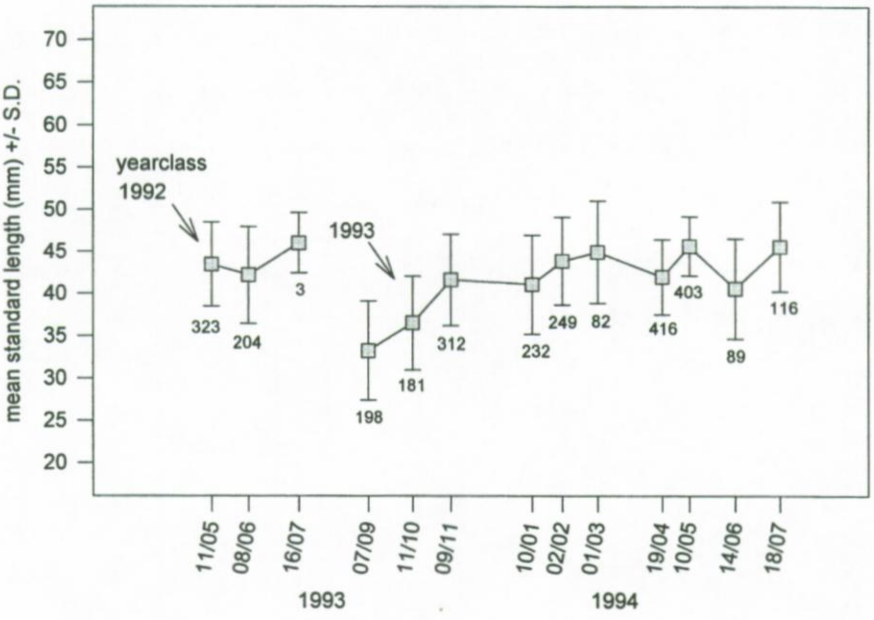


Figure 2.9: Growth curve of *Pomatoschistus lozanoi* in the Grevelingen Voordelta. Number of fish measured are indicated.

P. lozanoi young, which were born two months later than *P. minutus* in the Grevelingen Voordelta, grew fast till November, but most Lozano's gobies did not reach adult length before winter. A growth stop was seen from November to January. Adult standard length was reached from February onwards. Body length was maximal in May 1994 (45.6 ± 3.5 mm) and oscillations in mean standard length were noticed in the subsequent months.

Discussion

Densities

When comparing densities of gobies with literature, it is necessary to take into account the assumed net efficiency used in the different studies for calculating densities. Net efficiencies cited in literature for beam trawls with one trickling chain average around 20 to 30 %. To be able to compare our data with goby densities reported in the Belgian coastal waters (Hamerlynck *et al*., 1986) and in the Oosterschelde and Dutch Delta area (Hamerlynck *et al*., 1992, Hostens & Hamerlynck, 1994) the same estimate of net efficiency as reported by these authors (namely 20 %) was used for all size classes of the fish. However, this assumption has its implications towards the accuracy of the calculated densities. Net efficiency changes with size of the fish (Kuipers, 1975, Doornbos & Twisk, 1986). Larger gobies (> 30 mm) are probably caught in a more efficient way than assumed in this study. Therefore, densities of the larger gobies are probably slightly overestimated. Net efficiency can also vary between catches. When high densities of fish and invertebrates are present, the net will be filled more quickly and net efficiency, also for smaller size classes, will increase. On the other hand, 20 % net efficiency, is probably too high for the juvenile gobies (< 20 mm), which can easily escape through the meshes. Furthermore, Healey (1971) argued that there are two basic ways of gobies to avoid capture by trawls: by swimming out of the path of the net, and by burying themselves in the sand or mud. Since gobies are poor swimmers and the trawling was done at a relatively high speed (4.5 knots relative to the bottom), the first mechanism is unlikely to chance the catchability of the gobies. Healey (1971) mentioned that sand gobies are masters in burying themselves in sand and mud. He observed sand gobies in aquaria literally dive into the bottom when excited. In the present study, males seemed less liable to capture during the spawning season. Male gobies guard the nests during the spawning season and will reside more on the bottom than females in this period. Therefore males might have a better chance to escape the beam trawl by burying themselves. The low densities of juveniles and of males in the spawning season is probably an underestimation.

Catch efficiency can also differ between species. For instance, the difference in feeding behaviour between *P. minutus* and *P. lozanoi* (the first species feeding more on benthic prey and the last species preying more on pelagic organisms and hence spending more time swimming in the water column) may have resulted in a lower catch efficiency of *P. lozanoi*, since the top of the beam trawl reaches only about 50 cm above the bottom.

The dominance of *P. minutus* in the goby community of the Grevelingen Voordelta stations, was also observed in the northern North Sea and the Wadden Sea (Fonds, 1971, 1973). But local factors certainly play a role, since in the southern part of the North Sea towards the Belgian Coast, *P. lozanoi* is more abundant than *P. minutus* (Fonds, 1971, 1973). Of eight species of Gobiidae recorded in the Severn estuary, the most numerous gobies belong to the *P. minutus* complex, with an excess of *P. lozanoi* (80.6 %). Even in sampling stations in the inner Severn estuary, where low salinities (down to 6 ‰) occur, *P. lozanoi* is more abundant than *P. minutus* and *P. microps* (Claridge *et al*., 1985). This observation conflicts with the views of Fonds (1973), who considers *P. lozanoi* to be less adapted to reduced salinities than *P. minutus*. In experiments, where the salinity was gradually lowered starting from 30 ‰, *P. lozanoi* died at a salinity of 5 ‰ at 5 °C and at 3 ‰ at 13 °C, while *P. minutus* died when salinity dropped to 2 ‰ (5 °C), but survived a salinity of 0.9 ‰ at 13 °C. *P. microps* survived 0.9 ‰ at both temperatures (Fonds, 1973). In the goby community of the brackish (4.59 ‰-7.99 ‰) part of Zeeschelde estuary, *P. minutus* has the highest mean densities over a year (74.33 ind./1000 m^3), followed by *P. microps* (46.29 ind./1000 m^3) and *P. lozanoi* (6.20/1000 m^3) (Maes *et al*., 1998).

Geographical differences between the four sampling stations were more pronounced for *P. minutus* than for *P. lozanoi*, but both species had the highest yearly average densities in the three stations closest to the Grevelingen dam and lowest in Ooster, which is the most seaward station. These observations are in accordance with Hamerlynck (1993) who reported a decreasing gradient in macrobenthic, hyperbenthic and epibenthic and fish biomass in autumn (November) from the sheltered lagoonal area close to the Grevelingen dam (Schaar van Renesse) towards the more wave-exposed area (Ooster). The sheltered Grevelingen Voordelta with its low current velocities acts as a sink for various sorts of passively transported material i.e. silt, macrobenthic larvae and fish eggs. The richness and high primary production of the area attracts various species of fish and invertebrates (Hamerlynck & Mees, 1991). Since Ooster is the most exposed of the four sampling stations, lower densities and less diversity of invertebrates could be expected, which is probably reflected in lower average densities of both gobies. However, yearly densities of the mysid *Schistomysis spiritus* were about equally high in all four sampling stations (Mees *et al.*, 1993). Mysidacea are the bulk of the food of *P. lozanoi* (Hamerlynck *et al.*, 1993) while *P. minutus* feeds on a larger variety of prey items (Hamerlynck & Cattrijsse, 1994). This difference in food preference may explain why *P. lozanoi* was more equally distributed over the four sampling stations than *P. minutus*.

The overall yearly average densities of *P. minutus* (248.0 ind./1000 m^2 and 281.4 ind./1000 m^2 for the 1992 and 1993 yearclass respectively) and *P. lozanoi* (57.2 and 96.3 ind./1000 m^2) in the Grevelingen Voordelta are rather high compared to the average yearly densities of 80 *P. minutus* per 1000 m^2 and 40 *P. lozanoi* per 1000 m^2 found in Belgian coastal waters (Westdiep-Trapegeer) in 1984 (Hamerlynck *et al.*, 1986). Besides the geographical differences between communities and the differences in strength of yearclasses, the use of a larger mesh size in the latter study (18 mm stretched), a lower trawling speed (which may lead to a higher escape rate of adult gobies) and an assumed net efficiency of 25 % might have resulted in lower calculated densities. The overall average yearly abundance (from November 1987 to December 1988) in twelve sampling stations of the Voordelta Area was slightly higher than in our samples: 387 ind./1000 m^2 for *P. minutus* and 330 ind./1000 m^2 for *P. lozanoi* (Hostens & Hamerlynck, 1994). Since the same sampling gear and net efficiency were used as in the present study, the differences in densities are probably due to the appearance of stronger cohorts of both species in 1987 and 1988.

For details on the differences in densities of *P. minutus* and *P. lozanoi* between the depth strata, we refer to the study of Arellano (1995). In summary, *P. minutus* showed a significant preference for the deeper strata of the sampling stations, which was mainly explained by the high abundance of males at the 10 m depth stations during the spawning season. *P. lozanoi* had higher total densities in the shallow strata.

P. microps, the common goby, occurred only sporadically in the Grevelingen Voordelta, and only in the winter samples of 1994 (January, February and March). The common goby is known to be an estuarine species, also occurring in shallow coastal waters (0.2-2 m depth) (Fonds, 1971, Nellbring, 1993). This explains its absence in most of our samples. Hostens & Hamerlynck (1994) compared the average yearly abundance of epifaunal species in the Oosterschelde, Westerschelde and two groups of stations in the Voordelta (VD A: which included our sampling stations SR05, SR10, BG10, BZ10, besides other sampling stations, and VD B: including OO05, OO10, BG05 and BZ05 and more offshore stations). In their study, *P. microps* was not observed in the VD A community. The common goby was present in low numbers (0.1 or less per 1000 m²) in the Oosterschelde and the offshore Voordelta stations (VD B) and most abundant (3.0 ind./1000m²) in the Westerschelde estuary (Hostens & Hamerlynck, 1994). We did not catch any *P. microps* in Ooster, which is our most offshore station. In the Severn estuary too, the common goby was not recorded from the most seawards stations (Claridge *et al.*, 1985). Densities in the Severn estuary were highest in wintertime, except for one harsh winter with temperatures of around 4 °C. Jones & Miller (1966) observed a seaward migration of common gobies towards warmer waters when temperatures fell below 5° C in the estuary. In our samples, the lowest temperatures were recorded in January (5.0° C) and March 1994 (4.4° C), which coincided with the presence of *P. microps* in the samples, possibly migrating seawards from the adjacent estuaries.

P. pictus is not abundant along the Dutch and Belgian coast. *P. pictus* was reported from the Dutch Wadden Sea only quite recently (Fonds, 1964). It appears in more open coastal waters, has a depth distribution from the intertidal to 55 m (Collins, 1982, Miller, 1986) and is described as hyperbenthic, with a preference for rocky, stony bottom or gravel and shells (Wilkins & Meyers, 1992). The painted goby generally avoids areas with reduced salinity (Fonds, 1964, 1971, Wallis & Beardmore, 1984, Nijssen & De Groot, 1987). In an extensive survey of the fish species of the brackish part of the Lower Zeeschelde, only one record of *P. pictus* was made (Van Damme *et al.*, 1994). Collins (1982) collected *P. pictus* with a handnet along the Connemara coast to study its biology, but because of the sampling technique, no data on densities were given. Hostens & Hamerlynck (1994) noted an average abundance of one painted goby per 1000 m² in that part of the Voordelta area which includes the Grevelingen Voordelta, and they were even less abundant in the Oosterschelde and the more offshore stations of the Voordelta, and absent in the Westerschelde. The shallow Grevelingen Voordelta with its sandy, muddy bottom probably does not provide a very suitable habitat for *P. pictus*.

Seasonal fluctuations

In Southern Europe, *Pomatoschistus* populations are resident and do not show major density fluctuations throughout the year (Iglesias, 1981, Bouchereau *et al*., 1989). Goby populations in estuaries and coastal zones of Northwest Europe, where winter temperatures fall below 5 °C, show distinct seasonal fluctuations (Doornbos & Twisk, 1987, Aarnio & Bonsdorf, 1993). Seasonal fluctuations in densities of *P. minutus* and *P. lozanoi* in the Grevelingen Voordelta were generally in agreement with the literature on gobiids from the studied area (Fonds, 1973, Hamerlynck *et al*. 1986, Doornbos & Twisk, 1987) and were clearly linked to the reproductive biology and winter migration patterns of the gobies. The temporal variation in densities revealed that 1993 was a better 'goby year' than in 1992, showing a higher recruitment.

The high densities of sand gobies recorded in autumn are a result of the recruitment of the young gobies, which are first spotted in June, and are caught with an increasing net efficiency because of their growth. As explained earlier, densities of young gobies are probably underestimated. Densities lowered in October - November 1992, while they fluctuated around the same level in that period in the 1993 cohort. Overall recruitment was higher in 1993 than in 1992. In both years, an increased density of gobies was observed in February. This increase may be due to return of gobies from deeper waters to spawn in the Grevelingen Voordelta, as well as to the presence of small (last born) sand gobies from the 1993 cohort in the catches. In the growth curve, a decline in standard length is indeed observed in February, which confirms that a group of smaller gobies was caught. In the Wadden Sea and in the Oslo fjord, an increase in densities of gobies was also noted in spring (Fonds, 1973, Hesthagen, 1975, 1977). From March onwards, less sand gobies were caught. This coincided with the start of the reproductive period which lasts until June. During the spawning period, male gobies build nests under shells and stones, which makes them less easy to catch with the beam trawl (Fonds, 1973). Because most gobies have a life span of one year (most adults die after spawning) and juvenile gobies of less than 20 mm are not efficiency caught with the used mess size, it was only from June onwards that densities started to increase again, due to increasing net efficiency for the growing juveniles. The observed length-frequency distributions confirmed the appearance of juvenile gobies in June.

The seasonal fluctuations in densities of Lozano's gobies largely followed the same patterns as those of *P. minutus*, but densities peaked about two months later. This is largely due to their delayed spawning period. In the Wadden Sea and in the Belgian coastal waters, *P. lozanoi* eggs were collected from late May to mid-August, while those of *P. minutus* were observed from late April to early June (Fonds, 1973, Hamerlynck & Cattrijsse, 1994). Hostens *et al.* (1996) reported the occurrence of two peaks of postlarvae of *Pomatoschistus* sp. in the hyperbenthos of the Westerschelde estuary: one peak in May which they allocated to *P. minutus* and one in July, August from *P. lozanoi*. For *P. lozanoi*, a continuous but low recruitment of postlarvae was observed in the Westerschelde (Hostens *et al.*, 1996). This could also be the case in the Grevelingen Voordelta, and might explain the oscillation in the growth curve of this species from March onwards. From September onwards, densities of *P. lozanoi* increased due to growth and hence higher catchability of the juvenile Lozano's gobies. The slight decrease in wintertime would be due to migration towards warmer waters and death of most of the adults of the previous yearclass after spawning. The peak densities in May 1993 and April 1994 are caused by small adult fish, probably returning for spawning. In the Westerschelde estuary, especially in the polyhaline part, two smaller density peaks of *P. lozanoi* were observed each spring (April, May 1988-1992) (Hostens *et al.*, 1996).

Sex ratio

Sex ratios of both *P. minutus* and *P. lozanoi* suggest that males were less likely to be caught than females during the spawning season. This was also observed in other geographical areas and is generally explained by the fact that males hide under shells while guarding the eggs, making them less vulnerable to be caught with a beam trawl. When collecting shells for eggs of gobies, Fonds (1973) reported that a beam trawl was less efficient than a dredge.

Growth

Growth of the gobies is largely dependent on water temperature (Fonds, 1973). *P. minutus* juveniles showed a fast growth from June until October and most of them reached full grown size before winter. Also in more northern areas (Baltic Sea), *P. minutus* reaches adult size before winter (Aarnio & Bonsdorf, 1993) but the average adult size reported by these authors is smaller (35 mm) than in our study (approximately 52 mm standard length). In the Oslo fjord (Norway), *P. minutus* showed an almost identical growth pattern as observed by us, except that juveniles appeared from July, one month later than in our regions, since reproduction at this latitude starts later. The observed decline in mean standard length from October to November 1993 in the Grevelingen Voordelta also occurred in the Oslo fjord gobies. This was probably due to the disappearance of the last few adult gobies of the previous yearclass. Because separation of cohorts was done on sight using the length frequency distributions, possibly some older (from a previous cohort) and thus larger gobies were erroneously included in the 1993 cohort when calculating the mean length for the October sample. Arellano (1995), using the ELEFAN program for cohort separation, estimated that a low number of adults from the 1992 yearclass could still be present at that time. Given that the distinction in yearclasses is important for parasitological research (cfr. infra), gonad development and age (using otoliths) was assessed whenever there was a doubt about the yearclass to which a specimen belonged.

P. lozanoi juveniles did not reach adult size before winter, so they still had to invest some energy into somatic growth in spring, before ripening of the eggs could start. Hamerlynck *et al.* (1986) confirmed this for *P. lozanoi* of the Belgian coastal waters. The decline in mean length in February 1994 for sand gobies and in April 1994 for Lozano's gobies coincided with a marked decrease in density. The same pattern was noted for *P. minutus* in the inner Oslo Fjord, but in May (Hesthagen, 1975). Pethon (1975, *fide* Hesthagen, 1975) explained the same decline in mean length observed for the common goby in the Øra estuary in early summer as a result of immigration by younger, presumably smaller and not fully ripe fish into the estuary, later than the older ones, for breeding. Temporal segregation in spawning together with their one year life span results in the presence of adult *P. lozanoi* together with juveniles of *P. minutus* in July in the Grevelingen Voordelta. Predation by the large *P. lozanoi* on the smaller sand gobies has been observed by Hamerlynck *et al*. (1990) in July.

Aspects of population dynamics of gobies important for parasitological research

The present study on the population dynamics of the goby species of the Grevelingen Voordelta area led to basic information which was taken into account during parasitological research. Firstly, *P. minutus* and *P. lozanoi* are numerically dominating the goby community, and therefore parasitological research was mainly focused on these two species. Overall higher densities of *P. minutus* in Schaar van Renesse and Bollen van het Zand made that gobies from these two sampling stations were preferably used. The observation that overlapping cohorts of gobies occur in summer pointed out the necessity to differentiate between the individuals of the different yearclasses for the interpretation of the parasite loads. Lowering densities of gobies caught during winter time could indicate their migration to deeper waters, where possibly a different parasite community could be present. Temporal differences in spawning period, and thus in appearance of juvenile *P. minutus* and *P. lozanoi*, resulting in an overlap of *P. minutus* juveniles with adult *P. lozanoi* and post-spawning *P. minutus,* might influence horizontal transmission of parasites.

References

Aarnio, K., & Bonsdorff, E. (1993) Seasonal variation in abundance and diet of the sand goby *Pomatoschistus minutus* (Pallas) in a northern Baltic archipelago. *Ophelia*, **37,** 1, 19-30.

Arellano, R. V. (1995) *Age and growth of two sympatric goby species Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) using daily growth increments in the otoliths*. PhD thesis, University Gent, Belgium, 178 pp.

Arntz, W. E. (1978) The food of adult cod (*Gadus morhua* L.) in the western Baltic. *Meeresforschungen*, **26,** 60-69.

Bouchereau, J. L., Quignard, J. P., Tomasini, J. A., Joyeux, J. C., & Capape, Ch. (1989) La population de *Pomatoschistus minutus* (Pallas, 1770) de la lagune de Mauguio, France. Paramètres démographiques et croissance individuelle. *Cah. Biol. Mar.*, **30,** 487-514.

Claridge, P. N., Hardisty, M. W., Potter, I. C., & Williams, C. V. (1985) Abundance, life history and ligulosis in the gobies (Teleostei) of the inner Severn estuary. *J. mar. biol. Ass. U.K.*, **65,** 951-968.

Costa, M. J. (1988) Ecologie alimentaire des poissons de l'estuaire du Tage. *Cybium*, **12,** 4, 301-320.

Collins, S. P. (1982) Littoral and benthic investigations on the west coast of Ireland. XIV: The biology of the painted goby, *Pomatoschistus pictus* (Malm) (Teleostei: Gobiidae), on the Connemara Coast. *Proceedings of the Royal Irish Academy*, section B, **82B**, 21-37

Creutzberg, F., & Witte, J. I. (1989) An attempt to estimate the predatory pressure exerted by the lesser weever, *Trachinus vipera* Cuvier, in the southern North Sea. *J. Fish Biol.*, **34,** 429-449.

Doornbos, G., & Twisk, F. (1987) Density, growth and annual food consumption of gobiid fish in the saline lake Grevelingen, the Netherlands. *Neth. J. Sea Res.*, **21,** 1, 45-74.

Edlund, A.-M., Sundmark, G., & Thorman, S. (1980) The identification of *Pomatoschistus pictus*, *P. microps* and *P. minutus* (Gobiidae, Pisces). *Sarsia*, **65,** 239-242.

Fonds, M. (1964) The occurence of *Gobius pictus* Malm and *Onos septentrionalis* Collett in the Dutch Wadden Sea. *Neth. J. Sea Res.*, **2,** 2, 250-257.

Fonds, M. (1971) The seasonal abundance and vertebral variation of *Pomatoschistus minutus minutus* and *lozanoi* (Gobiidae) in the Dutch Wadden Sea. *Vie et Milieu*, **22,** suppl., 393-408.

Fonds, M. (1973) Sand gobies in the Dutch Wadden Sea (*Pomatoschistus*, Gobiidae, Pisces). *Neth. J. Sea Res.*, **6,** 4, 417-478.

Fouda, M. M., & Miller, P. J. (1981) Age and growth of the common goby, *Pomatoschistus microps*, on the South coast of England. *Estuarine, Coastal and Shelf Science*, **12,** 121-129.

Hamerlynck, O. (1990) The identification of *Pomatoschistus minutus* (Pallas) and *Pomatoschistus lozanoi* (de Buen) (Pisces, Gobiidae). *J. Fish Biol.*, **37,** 723-728.

Hamerlynck, O. (1993) *The ecology of Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) in the shallow coastal waters and estuaries of the Southern Bight of the North Sea*. PhD thesis, University Gent, Belgium, 187 pp.

Hamerlynck, O., Cattrijsse, A., & Arellano, R. V. (1993) Daily ration of juvenile *Pomatoschistus lozanoi* de Buen (Pisces: Gobiidae). *ICES J. Mar. Sci.*, **50,** 471-480.

Hamerlynck, O., & Cattrijsse, A. (1994) The food of *Pomatoschistus minutus* (Pisces, Gobiidae) in Belgian coastal waters, and a comparison with the food of its potential competitor *P. lozanoi*. *J. of Fish Biol.*, **44,** 753-771.

Hamerlynck, O., Heip, C., & Redant, F. (1986) Life history, food consumption and resource partitioning in two sympatric gobies *Pomatoschistus minutus* and *P. lozanoi* in the Belgian coastal waters. *ICES*, **14**

Hamerlynck, O. & Hostens, K. (1993) Growth, production and consumption in 0-group bib *Trisopterus luscus* and whiting *Merlangius merlangus* in a shallow coastal area of the SW Netherlands. *ICES Journal of Marine Science*, **50,** 81-93.

Hamerlynck, O., Hostens, K., Arellano, R. V., Mees, J., & Van Damme, P. A. (1993) The mobile epibenthic fauna of soft bottoms in the Dutch delta (South-West Netherlands): spatial structure. *Netherlands Journal of Aquatic Ecology*, **27,** 2-4, 343-358.

Hamerlynck, O., Hostens, K., Mees, J., Arellano, R. V., Cattrijsse, A., Van De Vyver, P., & Craeymeersch, J. A. (1992) The ebb tidal delta of the Grevelingen: a man-made nursery for flatfish. *Netherlands Journal of Sea Research*, **30,** 191-200.

Hamerlynck, O., & Mees, J. (1991) Temporal and spatial structure in the hyperbenthic community of a shallow coastal area and its relation to environmental variables. *Oceanologica Acta*, **11,** 205-212.

Hamerlynck, O., van de Vyver, P., & Janssen, C. R. (1990) The trophic position of *Pomatoschistus lozanoi* (Pisces: Gobiidae) in the Southern Bight. *Proc. 24th Europ. Mar. Biol. Symp.: Trophic Relationships in the Marine Environment.* (Eds.) Barnes, M. and Gibson, R. N., Aberdeen University Press, 183-190.

Healey, M. C. (1971) The distribution and abundance of sand gobies, *Gobius minutus,* in the Ythan estuary. *J. Zool. Lond.*, **163,** 177-229.

Healey, M. C. (1972) On the population ecology of the common goby in the Ythan estuary. *J. Nat. Hist.*, **6,** 133-145.

Henderson, P. A. (1989) On the structure of the inshore fish community of England and Wales. *J. Mar. Biol. Ass. U.K.*, **69,** 145-163.

Hesthagen, I. H. (1975) Seasonal occurence and length variation in the Sand Goby, *Pomatoschistus minutus* (Pallas), in the shore zone of the inner Oslofjord. *Norw. J. Zool.*, **23,** 235-242.

Hesthagen, I. H. (1977) Migrations, breeding, and growth in *Pomatoschistus minutus* (Pallas) (Pisces, Gobiidae) in Oslofjorden, Norway. *Sarsia*, **63,** 17-26.

Hesthagen, I. H. (1980) Locomotor activity in the painted goby, *Pomatoschistus pictus* (Malm) (Pisces), in relation to light intensity. *Sarsia*, **65,** 13-18.

Holmes, J. C. (1990) Helminth communities in marine fishes. in: G. Esch, A. Bush, & J. Aho *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 5, pp. 101-130.

Hostens, K., Mees, J., Beyst, B., & Cattrijsse, A. (1996) *Het vis- en garnaalbestand in de Westerschelde: soortensamenstelling, ruimtelijke verspreiding en seizoenaliteit (periode 1988-1992).* Report for: Rijkswaterstaat Directie Zeeland Afdeling AXW, Nederland, from: Sektie Mariene Biologie, Instituut voor Dierkunde, Universiteit Gent, Belgium, 106 pp.

Hostens, K., & Hamerlynck, O. (1994) The mobile epifauna of the soft bottoms in the subtidal Oosterschelde estuary: structure, function and impact of the storm-surge barrier. *Hydrobiologia*, **282/283,** 479-496.

Iglesias, J. (1981) Spatial and temporal changes in the demersal fish community of the Ria de Arosa (NW Spain). *Marine Biology*, **65,** 199-208.

Jones, D., & Miller, P. J. (1966) Seasonal migrations of the common goby, *Pomatoschistus microps* (Kroyer), in Morecambe Bay and elsewhere. *Hydrobiologia*, **27,** 515-528.

Kuipers, B. (1975) On the efficiency of a two-metre beam trawl for juvenile plaice (*Pleuronectes platessa*). *Netherlands Journal of Sea Research*, **9,** 1, 69-85.

Maes, J., Tallieu, A., Van Damme, P., Cottenie, K., & Ollevier, F. (1998) Seasonal patterns in the fish and crustacean community of a turbid temperate estuary (Zeeschelde Estuary, Belgium). *Estuarine, Coastal and Shelf Science*, **47,** in press.

Mees, J., Cattrijsse, A. & Hamerlynck, O. (1993) Distribution and abundance of shallow-water hyperbenthic mysids (Crustacea, Mysidacea) and euphausiids (Crustacea, Euphausiacea) in the Voordelta and the Westerschelde, southwest Netherlands. *Cah. Biol. Mar*. **34**, 165-186

Miller, P. J. (1986) Gobiidae. in: P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, & E. Tortonese (Eds) *Fishes of the North-eastern Atlantic and the Mediterranean*. pp. 1019-1085.

Nellbring, S. (1993) Spawning of two *Pomatoschistus* species (Gobiidae) in relation to nest availability and depth: a field experiment. *Netherlands Journal of Sea Research*, **31,** 2, 173-179.

Nijssen, H., & De Groot, S. J. (1987) *De vissen van Nederland*. Utrecht, Koninklijke Nederlandse Natuurhistorische Vereniging, 223 pp.

Pethon, P. (1975) Leirkutlingen i Øra-området. *Fauna, Oslo*, **28**, 23-30.

Rohde, K. (1984) Ecology of marine parasites. *Helgoländer Meeresunters.*, **37,** 5-33.

Salvanes, A. G. V., & Nordeide, J. T. (1993) Dominating sublittoral fish species in a west Norwegian fjord and their trophic links to cod (*Gadus morhua* L.). *Sarsia*, **78,** 221-234.

Siegel, S., & Castellan, N. J. (1988) *Nonparametric Statistics for the Behavioural Sciences* (2nd edition). McGraw-Hill Book Company, 399 pp.

Van Damme, P. A., Hostens, K., & Ollevier, F. (1994) Fish species of the lower Zeeschelde (Belgium): a comparison with historical checklists. *Belg. J. Zool.*, **124,** 2, 93-103.

Wallis, G. P., & Beardmore, J. A. (1984) Genetic variation and environmental heterogeneity in some closely related goby species. *Genetica*, **62,** 223-237.

Webb, C. J. (1980) Systematics of the *Pomatoschistus minutus* complex (Teleostei: Gobiidae). *Philos. Trans. R. Soc. Lond., Ser. B*, **291,** 201-241.

Wilkins, H. K. A., & Myers, A. A. (1992) Microhabitat utilisation by an assemblage of temperate Gobiidae (Pisces: Teleostei). *Marine Ecology Progress Series*, **90,** 103-112.

Addendum to chapter 2

Densities (n / 1000 m²) of *Pomatoschistus minutus* and *P. lozanoi*, per sampling station and sample date.

Pomatoschistus minutus

Male	Station								
date	OO5	OO10	BZ05	BZ10	BG05	BG10	SR05	SR10	mean
09/09/92	166.7	209.3	294.4	1377.8	29.6	237.0	96.3	459.3	358.8
01/10/92	25.9	16.7	29.6	459.3	42.6	718.5	187.0	1081.5	320.1
30/10/92	70.4	64.8	153.7	214.8	5.6	125.9	100.0	370.4	138.2
08/12/92	50.0	27.8	16.7	29.6	70.4	220.4	101.9	87.0	75.5
09/02/93	13.0	11.1	1.9	107.4	33.3	227.8	275.9	229.6	112.5
11/03/93	3.7		1.9	35.2	11.1	13.0	35.2	18.5	16.9
08/04/93	0.0	0.0	0.0	25.9	0.0	9.3	13.0	5.6	6.7
11/05/93	1.9	0.0	0.0	40.7	0.0	124.1	13.0	9.3	23.6
08/06/93	2.8	13.0	3.7	31.5	7.4	13.0	16.7	1.9	11.2
16/07/93	27.8	20.4	96.3	114.8	0.0	20.4	7.4	53.7	42.6
07/09/93	137.0	46.3	46.3	1214.8	5.6	177.8	44.4	433.3	263.2
11/10/93	5.6	9.3	29.6	1088.9	13.0	127.8	33.3	583.3	236.3
09/11/93	11.1	3.7	118.5	622.2	27.8	207.4	44.4	851.9	235.9
10/01/94	42.6	1.9	66.7	301.9	5.6	585.2	329.6	281.5	201.9
02/02/94	40.7	18.5	14.8	205.6	829.6	200.0	1029.6	100.0	304.9
01/03/94	22.2	107.4	11.1	488.9	31.5	11.1	20.4	29.6	90.3
19/04/94	1.9	0.0	1.9	183.3	16.7	50.0	118.5	5.6	47.2
10/05/94	0.0	0.0	0.0	5.6	0.0	44.4	4.6	6.5	7.6
14/06/94	1.9	1.9	0.0	0.0	1.9	0.0	1.9	0.0	0.9
18/07/94	42.6	27.8	20.4	470.4	29.6	107.4	148.1	63.0	113.7

Female	Station								
date	OO5	OO10	BZ05	BZ10	BG5	BG10	SR5	SR10	mean
09/09/92	114.8	183.3	281.5	1155.6	29.6	163.0	75.9	385.2	298.6
01/10/92	33.3	20.4	16.7	277.8	57.4	585.2	101.9	251.9	168.1
30/10/92	94.4	96.3	161.1	150.0	5.6	103.7	118.5	437.0	145.8
08/12/92	107.4	33.3	22.2	48.1	100.0	246.3	105.6	85.2	93.5
09/02/93	20.4	7.4	1.9	118.5	31.5	157.4	416.7	170.4	115.5
11/03/93	13.0		0.0	33.3	7.4	1.9	55.6	33.3	20.6
08/04/93	0.0	7.4	0.0	22.2	3.7	28.7	61.1	33.3	19.6
11/05/93	0.0	1.9	0.0	187.0	1.9	103.7	257.4	7.4	69.9
08/06/93	2.8	11.1	5.6	87.0	11.1	51.9	13.0	3.7	23.3
16/07/93	11.1	20.4	59.3	55.6	3.7	5.6	5.6	44.4	25.7
07/09/93	85.2	38.9	37.0	622.2	1.9	222.2	29.6	283.3	165.0
11/10/93	7.4	11.1	22.2	629.6	14.8	96.3	14.8	377.8	146.8
09/11/93	14.8	1.9	96.3	525.9	18.5	259.3	55.6	681.5	206.7
10/01/94	61.1	13.0	55.6	277.8	7.4	725.9	364.8	192.6	212.3
02/02/94	33.3	14.8	16.7	240.7	807.4	348.1	1251.9	107.4	352.5
01/03/94	20.4	116.7	13.0	614.8	81.5	13.0	88.9	66.7	126.9
19/04/94	0.0	0.0	3.7	81.5	16.7	61.1	385.2	25.9	71.8
10/05/94	0.0	0.0	0.0	31.5	1.9	159.3	47.2	30.6	33.8
14/06/94	5.6	1.9	1.9	0.0	0.0	0.0	22.2	1.9	4.2
18/07/94	29.6	20.4	27.8	240.7	25.9	64.8	77.8	63.0	68.8

Addendum to chapter 2 (continued)

Densities (n / 1000 m^2) of *Pomatoschistus minutus* and *P. lozanoi*, per sampling station and sample date.

Pomatoschistus minutus

Juvenile	Station								
date	OO5	OO10	BZ05	BZ10	BG5	BG10	SR5	SR10	mean
09/09/92	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
01/10/92	0.0	0.0	1.9	0.0	1.9	0.0	0.0	0.0	0.5
30/10/92	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
08/12/92	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09/02/93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11/03/93	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
08/04/93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11/05/93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
08/06/93	1.9	0.0	0.0	22.2	0.0	7.4	11.1	0.0	5.3
16/07/93	0.0	0.0	18.5	11.1	0.0	0.0	0.0	0.0	3.7
07/09/93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11/10/93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09/11/93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10/01/94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
02/02/94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
01/03/94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19/04/94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10/05/94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14/06/94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18/07/94	1.9	0.0	0.0	14.8	1.9	0.0	0.0	0.0	2.3

Total	Station								
date	OO5	OO10	BZ05	BZ10	BG5	BG10	SR5	SR10	mean
09/09/92	281.5	392.6	575.9	2533.3	59.3	400.0	172.2	844.4	657.4
01/10/92	59.3	37.0	48.1	737.0	101.9	1303.7	288.9	1333.3	488.7
30/10/92	164.8	161.1	314.8	364.8	11.1	229.6	218.5	807.4	284.0
08/12/92	157.4	61.1	38.9	77.8	170.4	466.7	207.4	172.2	169.0
09/02/93	33.3	18.5	3.7	225.9	64.8	385.2	692.6	400.0	228.0
11/03/93	16.7		1.9	68.5	18.5	14.8	90.7	51.9	37.6
08/04/93	0.0	7.4	0.0	48.1	3.7	38.0	74.1	38.9	26.3
11/05/93	1.9	1.9	0.0	227.8	1.9	227.8	270.4	16.7	93.5
08/06/93	7.4	24.1	9.3	140.7	18.5	72.2	40.7	5.6	39.8
16/07/93	38.9	40.7	174.1	181.5	3.7	25.9	13.0	98.1	72.0
07/09/93	222.2	85.2	83.3	1837.0	7.4	400.0	74.1	716.7	428.2
11/10/93	13.0	20.4	51.9	1718.5	27.8	224.1	48.1	961.1	383.1
09/11/93	25.9	5.6	214.8	1148.1	46.3	466.7	100.0	1533.3	442.6
10/01/94	103.7	14.8	122.2	579.6	13.0	1311.1	694.4	474.1	414.1
02/02/94	74.1	33.3	31.5	446.3	1637.0	548.1	2281.5	207.4	657.4
01/03/94	42.6	224.1	24.1	1103.7	113.0	24.1	109.3	96.3	217.1
19/04/94	1.9	0.0	5.6	264.8	33.3	111.1	503.7	31.5	119.0
10/05/94	0.0	0.0	0.0	37.0	1.9	203.7	51.9	37.0	41.4
14/06/94	7.4	3.7	1.9	0.0	1.9	0.0	24.1	1.9	5.1
18/07/94	74.1	48.1	48.1	725.9	57.4	172.2	225.9	125.9	184.7

Addendum to chapter 2 (continued)
Densities (n / 1000 m²) of *Pomatoschistus minutus* and *P. lozanoi*, per sampling station and sample date.

Pomatoschistus lozanoi

Male	Station								
date	OO5	OO10	BZ05	BZ10	BG05	BG10	SR05	SR10	mean
09/09/92	14.81	177.78	118.52	88.89	0.00	0.00	12.96	0.00	51.62
01/10/92	31.48	29.63	55.56	118.52	85.19	22.22	31.48	14.81	48.61
30/10/92	24.07	20.37	35.19	25.93	0.00	7.41	12.96	0.00	15.74
08/12/92	20.37	20.37	3.70	12.96	12.96	12.96	9.26	14.81	13.43
09/02/93	16.67	5.56	0.00	18.52	14.81	35.19	188.89	27.78	38.43
11/03/93	20.37		3.70	16.67	1.85	0.00	3.70	3.70	7.14
08/04/93	7.41	42.59	5.56	5.56	3.70	27.78	22.22	27.78	17.82
11/05/93	9.26	9.26	14.81	120.37	11.11	51.85	40.74	29.63	35.88
08/06/93	19.44	24.07	5.56	29.63	0.00	9.26	53.70	7.41	18.63
16/07/93	0.00	0.00	0.00	1.85	0.00	0.00	0.00	0.00	0.23
07/09/93	90.74	25.93	40.74	44.44	1.85	0.00	0.00	3.70	25.93
11/10/93	5.56	27.78	46.30	192.59	12.96	11.11	9.26	20.37	40.74
09/11/93	9.26	0.00	146.30	370.37	25.93	37.04	68.52	251.85	113.66
10/01/94	59.26	25.93	18.52	40.74	1.85	96.30	72.22	118.52	54.17
02/02/94	25.93	1.85	11.11	57.41	96.30	107.41	148.15	22.22	58.80
01/03/94	18.52	25.93	7.41	51.85	16.67	0.00	0.00	14.81	16.90
19/04/94	22.22	11.11	24.07	14.81	155.56	55.56	1,318.52	22.22	203.01
10/05/94	46.30	25.93	11.11	16.67	1.85	162.96	18.52	81.48	45.60
14/06/94	25.93	9.26	22.22	3.70	11.11	1.85	16.67	3.70	11.81
18/07/94	3.70	0.00	0.00	14.81	0.00	0.00	5.56	1.85	3.24

Female	Station								
date	OO5	OO10	BZ05	BZ10	BG05	BG10	SR05	SR10	mean
09/09/92	24.07	105.56	90.74	29.63	1.85	0.00	3.70	0.00	31.94
01/10/92	18.52	7.41	11.11	51.85	72.22	22.22	24.07	0.00	25.93
30/10/92	11.11	12.96	16.67	14.81	0.00	0.00	9.26	0.00	8.10
08/12/92	25.93	22.22	9.26	11.11	1.85	11.11	1.85	11.11	11.81
09/02/93	12.96	0.00	3.70	1.85	12.96	37.04	127.78	25.93	27.78
11/03/93	14.81		3.70	16.67	1.85	3.70	0.00	0.00	5.82
08/04/93	3.70	20.37	9.26	7.41	3.70	4.63	18.52	25.93	11.69
11/05/93	5.56	16.67	22.22	118.52	14.81	57.41	50.00	24.07	38.66
08/06/93	16.67	14.81	7.41	59.26	9.26	12.96	66.67	1.85	23.61
16/07/93	0.00	0.00	1.85	1.85	0.00	0.00	0.00	0.00	0.46
07/09/93	62.96	20.37	35.19	14.81	0.00	0.00	0.00	3.70	17.13
11/10/93	3.70	14.81	33.33	111.11	12.96	12.96	5.56	11.11	25.69
09/11/93	1.85	0.00	64.81	155.56	3.70	29.63	16.67	66.67	42.36
10/01/94	29.63	12.96	20.37	22.22	0.00	66.67	31.48	88.89	34.03
02/02/94	12.96	5.56	9.26	27.78	66.67	72.22	111.11	14.81	40.05
01/03/94	3.70	27.78	3.70	44.44	3.70	0.00	14.81	7.41	13.19
19/04/94	18.52	3.70	20.37	7.41	96.30	14.81	1,022.22	11.11	149.31
10/05/94	57.41	12.96	7.41	11.11	0.00	98.15	26.85	20.37	29.28
14/06/94	14.81	7.41	18.52	3.70	3.70	5.56	14.81	1.85	8.80
18/07/94	3.70	0.00	0.00	1.85	0.00	0.00	1.85	0.00	0.93

Addendum to chapter 2 (continued)

Densities (n / 1000 m²) of *Pomatoschistus minutus* and *P. lozanoi*, per sampling station and sample date.

Pomatoschistus lozanoi

Juvenile	Station								
date	OO5	OO10	BZ05	BZ10	BG05	BG10	SR05	SR10	mean
09/09/92	20.37	94.44	35.19	44.44	0.00	0.00	0.00	0.00	24.31
01/10/92	0.00	0.00	0.00	7.41	11.11	0.00	12.96	0.00	3.94
30/10/92	0.00	0.00	1.85	3.70	0.00	0.00	1.85	0.00	0.93
08/12/92	1.85	0.00	0.00	0.00	1.85	0.00	0.00	0.00	0.46
09/02/93	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	1.39
11/03/93	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/04/93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/05/93	0.00	0.00	0.00	0.00	0.00	1.85	0.00	0.00	0.23
08/06/93	0.00	0.00	0.00	0.00	0.00	3.70	0.00	0.00	0.46
16/07/93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/09/93	35.19	18.52	14.81	14.81	0.00	0.00	0.00	3.70	10.88
11/10/93	0.00	0.00	18.52	44.44	0.00	1.85	0.00	0.00	8.10
09/11/93	0.00	0.00	7.41	7.41	0.00	0.00	0.00	14.81	3.70
10/01/94	9.26	0.00	1.85	0.00	0.00	22.22	11.11	0.00	5.56
02/02/94	0.00	0.00	0.00	0.00	0.00	5.56	0.00	0.00	0.69
01/03/94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19/04/94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10/05/94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14/06/94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18/07/94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Total	Station								
date	OO5	OO10	BZ05	BZ10	BG05	BG10	SR05	SR10	mean
09/09/92	59.26	377.78	244.44	162.96	1.85	0.00	16.67	0.00	107.87
01/10/92	50.00	37.04	66.67	177.78	168.52	44.44	68.52	14.81	78.47
30/10/92	35.19	33.33	53.70	44.44	0.00	7.41	24.07	0.00	24.77
08/12/92	48.15	42.59	12.96	24.07	16.67	24.07	11.11	25.93	25.69
09/02/93	29.63	5.56	3.70	20.37	27.78	72.22	327.78	53.70	67.59
11/03/93	35.19		7.41	33.33	3.70	3.70	3.70	3.70	12.96
08/04/93	11.11	62.96	14.81	12.96	7.41	32.41	40.74	53.70	29.51
11/05/93	14.81	25.93	37.04	238.89	25.93	111.11	90.74	53.70	74.77
08/06/93	36.11	38.89	12.96	88.89	9.26	25.93	120.37	9.26	42.71
16/07/93	0.00	0.00	1.85	3.70	0.00	0.00	0.00	0.00	0.69
07/09/93	188.89	64.81	90.74	74.07	1.85	0.00	0.00	11.11	53.94
11/10/93	9.26	42.59	98.15	348.15	25.93	25.93	14.81	31.48	74.54
09/11/93	11.11	0.00	218.52	533.33	29.63	66.67	85.19	333.33	159.72
10/01/94	98.15	38.89	40.74	62.96	1.85	185.19	114.81	207.41	93.75
02/02/94	38.89	7.41	20.37	85.19	162.96	185.19	259.26	37.04	99.54
01/03/94	22.22	53.70	11.11	96.30	20.37	0.00	14.81	22.22	30.09
19/04/94	40.74	14.81	44.44	22.22	251.85	70.37	2,340.74	33.33	352.31
10/05/94	103.70	38.89	18.52	27.78	1.85	261.11	45.37	101.85	74.88
14/06/94	40.74	16.67	40.74	7.41	14.81	7.41	31.48	5.56	20.60
18/07/94	7.41	0.00	0.00	16.67	0.00	0.00	7.41	1.85	4.17

Chapter 3

Gyrodactylus longidactylus n. sp., a monogenean from *Pomatoschistus lozanoi* (de Buen, 1923) (Gobiidae) from the North Sea.

Geets, A.[1], Malmberg, G.[2] and Ollevier, F.[1]

[1] *Laboratory for Ecology and Aquaculture, Catholic University Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*

[2] *Department of Zoology, University of Stockholm, S-106 91 Stockholm, Sweden*

This chapter is published in *Systematic Parasitology*, (1998) **41**, (1), 63-70.

Abstract

Gyrodactylus longidactylus n. sp., a gyrodactylid monogenean parasitising the gills of *Pomatoschistus lozanoi* (de Buen, 1923) in the North Sea is described. It is a species with rather small anchors which are only connected with a ventral bar which lacks a ventral bar membrane. The marginal hooks have long handles, which are always longer than the total length of the anchor. The pharynx has long pharyngeal processes. Measurements, drawings of the opisthaptoral hard parts, penis and pharynx and SEM micrographs of this monogenean are presented. *G. longidactylus* n. sp. is the first monogenean species described from *P. lozanoi*.

Introduction

Species of *Pomatoschistus* (Gobiidae) are the most abundant benthic fish in the North Sea (Fonds, 1971) and the Dutch Voordelta area (Hamerlynck *et al.*, 1993). During a general study on their parasite fauna, a previously undescribed *Gyrodactylus* species was found on the gill arches and gill filaments of Lozano's goby, *Pomatoschistus lozanoi* (de Buen, 1923). *P.*

lozanoi has only been quite recently accepted as a separate species (Fonds, 1973, Webb, 1980). The differences between *P. lozanoi* and the sympatric *Pomatoschistus minutus* might have been overlooked in previous studies (Doornbos & Twisk, 1987, Henderson, 1989). Nevertheless, the new *Gyrodactylus* species seems to be host specific, since none of the other three sympatric *Pomatoschistus* spp. in the research area, *P. minutus*, *P. microps* and *P. pictus*, harboured this monogenean. The present paper describes the new *Gyrodactylus* species from *P. lozanoi*.

Material and methods

Collection of the specimens

Specimens of *Pomatoschistus lozanoi* were caught near the Oostende Bank (51°17'30"N, 2°48'30"E) on the 21st of August 1996, transported in water from the locality and immediately screened in the laboratory for gyrodactylids. The parasites were collected *in vivo* from the gills and either mounted in ammonium picrate glycerine for light microscopy or fixed in glutaraldehyde for scanning electron microscopy.

The gyrodactylid was first found on the gill arches and gill filaments of formalin-fixed *P. lozanoi* of the Voordelta area (51°41'N, 3°45'E), (while screening an entire yearclass: June '93 till July '94 for a population dynamics study). Eighty two formaline-fixed specimens were collected (all from June and July 1993). These specimens were used for comparison with the live specimens collected at the Oostende Bank. All specimens were determined as a new species of *Gyrodactylus* Nordmann, 1832, which was found only on *P. lozanoi* and not on the closely related sympatric gobies *P. minutus, P. microps* or *P. pictus*.

Light Microscopy

Whole-mounts of 34 specimens were made by flattening and fixing live gyrodactylids in ammonium picrate glycerine, according to Malmberg (1970). Phase contrast microscopy was used for studying the hard parts of the opisthaptor. All specimens were studied, but for taxonomic purposes only measurements of the 11 best (most flattened) specimens were used.

Drawings were made with a drawing tube and a Leitz Wetzlar microscope (objectives: 40x and 90x oil immersion). Some specimens were subsequently mounted permanently following the method of Ergens (1969), but using DePeX (a synthetic resin) rather than Canada Balsam.

Scanning Electron Microscopy (SEM)

Live specimens collected from the gills of *P. lozanoi* were fixed in glutaraldehyde (2% solution in sodium cacodylate buffer), rinsed in sodium cacodylate buffer, dehydrated in acetone and dried in a Balzers union Critical Point Dryer. Some specimens were fixed and mounted individually (Figs 3.3 and 3.4), others were fixed attached to the gill filaments and gill arches and mounted *in situ* (Figs 3.5, 3.6 and 3.7). The specimens were sputter coated with gold in a Balzers Union Sputter Coater Device and scanned in a Philips-515 scanning electron microscope.

Measurements

Most measurements were taken directly from the drawings and were carried out according to Malmberg (1970). For the description of the species, measurements were taken from only the 11 best prepared specimens. Other specimens were drawn and measured for comparison. Figure 3.1 gives a schematic view of the measurements taken on the body, anchors, marginal hooks, pharynx and penis. In each specimen, both anchors were measured and the mean and standard deviation of all measurement was calculated. Since the anteriormost pair of marginal hooks in some species tend to be shorter than the posterior ones (Malmberg, 1970), at least one anterior and one posterior marginal hook was measured per specimen to cover the range of size variation. Marginal hooks were numbered according to Malmberg (1970). In some cases, the marginal hook could not be assigned a number or they were measured on an embryo; hence, in Table 3.1, the total number of marginal hooks measured may exceed the sum of the number of anterior and posterior marginal hooks. Opisthaptor length and width were estimated on the SEM micrographs since most of the specimens, when mounted on slides, do not exhibit a distinct opisthaptor. All measurements are given in micrometres.

Table. 3.1: Morphological measurements (in micrometres) of *Gyrodactylus longidactylus* n. sp. from *Pomatoschistus lozanoi*. Measurements were done on the holotype and paratypes and on additional specimens.

Measurement[1]	Number measured (Number of specimens)	Mean ± S.D.	Range (μm)
Total body length (TBODYL)	10 (10)	522.4 ± 160.0	342.5 - 770
Total body width (TBODYW)	10 (10)	137.6 ± 24.5	97 - 175.5
Opisthaptor length[2]	3 (3)	38.0 ± 2.3	36 - 40.5
Opisthaptor width[2]	3 (3)	47.9 ± 8.1	40 - 56
Pharynx total length (TPHARL)	4 (4)	78.0 ± 9.4	66 - 89
Pharynx total width (PHARW)	4 (4)	71.6 ± 3.3	67.5 - 75.5
Pharynx length (PHARL)	4 (4)	65.2 ± 3.7	61 - 69
Pharynx anterior width (ANTPW)	4 (4)	64.2 ± 3.6	59.5 - 67.5
Penis length (PENISL)	7 (7)	12.1 ± 1.2	11 - 14
Penis width (PENISW)	7 (7)	13.0 ± 1.3	11.5 - 15.5
Anchor length (LA)	22 (11)	26.8 ± 1.7	23 - 29.5
Anchor shaft length (LAS)	22 (11)	20.2 ± 1.5	16 - 22
Anchor point length (LAP)	20 (10)	16.9 ± 1.0	15.5 - 19
Anchor root length (LAR)	20 (10)	11.7 ± 1.3	9.5 - 14.5
Ventral bar length (LVB)	9 (9)	8.1 ± 0.8	7 - 9
Ventral bar median width (MWVB)	10 (10)	3.4 ± 0.6	3 - 4.5
Ventral bar basal width (BWVB)	8 (8)	3.8 ± 0.6	3.5 - 5
Marginal hook total length (LMH)	28 (10)	38.2 ± 1.1	35.5 - 40
Anterior[3] marginal hook total length	11 (6)	38.0 ± 1.3	35.5 - 40
Posterior[4] marginal hook total length	4 (4)	39.0 ± 0.3	39 - 39.5
Marginal hook sickle length (LSI)	29 (11)	6.3 ± 0.3	5.5 - 7
Anterior marginal hook sickle length	11 (7)	6.3 ± 0.4	5.5 - 6.5
Posterior marginal hook sickle length	4 (3)	6.7 ± 0.3	6.5 - 7
Marginal hook handle length (LH)	29 (11)	32.4 ± 1.2	28.5 - 34
Anterior marginal hook handle length	12 (8)	31.9 ± 1.6	28.5 - 34
Posterior marginal hook handle length	5 (4)	32.6 ± 0.6	31.5 - 33.5
Marginal hook filament loop (LOOP)	16 (8)	10.2 ± 1.0	9 - 13

---

[1] For a schematic drawing of the structures and measurements see figure 3.1.

[2] Measured on scanning electron microscope micrographs (not on holotype or paratypes).

[3] Marginal hooks no. 1,2 or 3

[4] Marginal hooks no. 6,7 or 8

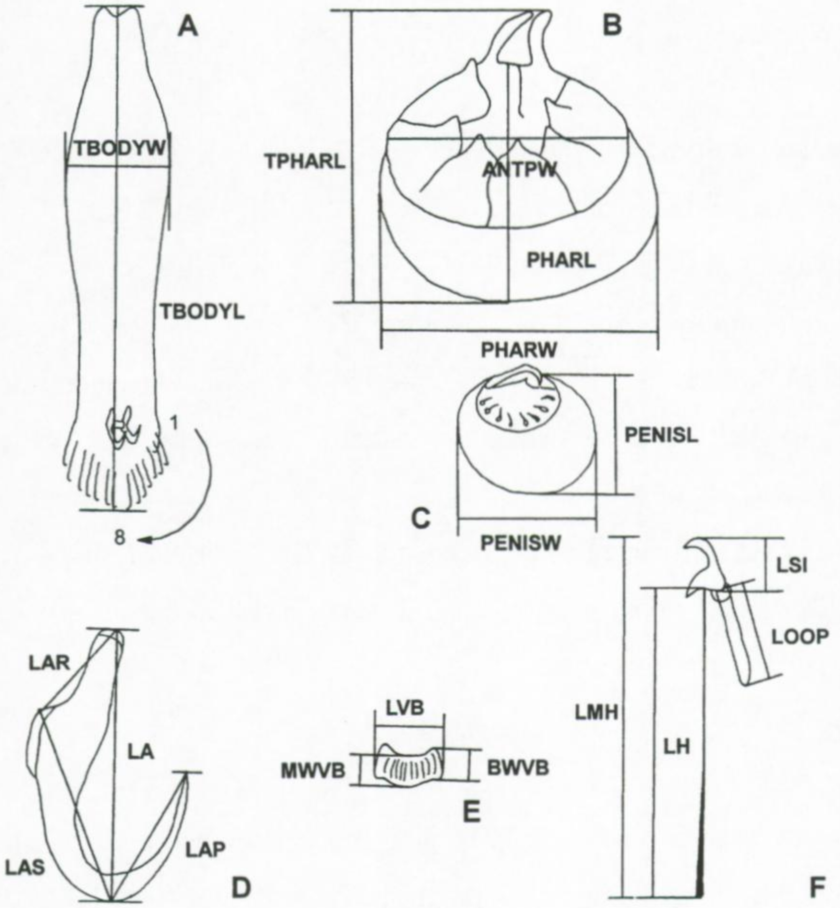


Figure 3.1: Method of measuring different parts of *Gyrodactylus longidactylus* n. sp.: (A) body, with marginal hooks numbered according to Malmberg (1970), (B) pharynx, (C) penis and opisthaptoral hard parts: (D) anchor, (E) ventral bar and (F) marginal hook. For abbreviations see table 3.1. (Drawings are at different scales.)

Family Gyrodactylidae Cobbold, 1864

Subfamily Gyrodactylinae Cobbold, 1864

Genus *Gyrodactylus* Nordmann, 1832

Gyrodactylus longidactylus n. sp.

Type-host: *Pomatoschistus lozanoi* (de Buen, 1923) (Gobiidae)

Site: Gill arches and gill filaments

Type-locality: Oostende Bank, Belgium (51°17'30"N, 2°48'30"E)

Water temperature and salinity: 18.5°C, 34.4 ppt (21/8/1996)

Specimens studied: 34 (from 7 *P. lozanoi*) collected live at the Oostende Bank; 82 formalin-fixed from the Voordelta area. Drawn: 20; measured for description: 11; additional specimens from the type locality were used for SEM.

The holotype and several paratypes are deposited in The National History Museum, London (holotype: 1997.12.22.1, paratypes: 1997.10.1.2-4 and 1997.12.22.2-6)

Description

Measurements are listed in Table 3.1. The largest specimen had a body length of 769.7 μm and 160.0 μm width. Body elongated with a bi-lobed prohaptor (Fig. 3.3). Opisthaptor small and readily visible in SEM micrographs (Figs 3.3 and 3.4), but not in whole mounts. Body covered with dense microvilli (Fig. 3.3). Anchors small, always smaller than marginal hook handles (Figs 3.2 and 3.4). When studying the "footprints" on SEM micrographs, clear imprint of anchors is lacking (Figs 3.6 and 3.7). Anchor points long; shafts short. Marginal hooks with long handles, longer than total length of anchors. Mean total length, length of sickle and length of handle of anterior marginal hooks tends to be slightly smaller than those of posterior marginal hooks (Table 3.1); this is in accordance with previous observations (Malmberg, 1970) on other gyrodactylid species. Dorsal bar reduced, only attachments to anchors remaining. No ventral bar membrane was observed. Ventral bar processes absent (Figs 3.2A and 3.2B). The pharynx large, with 8 long processes of which two ventral ones longer than remainder (Fig. 3.2D). Penis present in most specimens; its small spines are arranged in single arched row (Fig. 3.2C). In two specimens a clear connection (ductus) between the penis and the vesicula seminalis was observed. Excretory system with small bladders.

Diagnosis

Distinction between opisthaptor and body not clear in whole mounts. Anchors rather small. Shape of anchors similar to these of *G. emembranatus* Malmberg, 1970, but smaller. Ventral bar lacking a membrane. Dorsal bar reduced, except for the attachment to the anchors. Marginal hooks with long handles. Marginal hook sickle robust, similar in size and shape to those in *G. callariatis* Malmberg, 1957. Pharynx with 8 long pharyngeal processes, two ventral ones longer than the others. Penis with one large and 8 small spines in single arched row.

Etymology

The species name, *Gyrodactylus longidactylus* n. sp., refers to the long fingers on the opisthaptor associated with long marginal hook handles (Fig. 3.4).

Discussion

Gyrodactylus longidactylus n. sp. is the first monogenean described from *P. lozanoi*. Only very few species of *Gyrodactylus* are described from *Pomatoschistus* spp. Gläser (1974) described *Gyrodactylus rugiensis* and *G. micropsi* both from *P. minutus* and *P. microps* from an inlet of the Baltic Sea (Insel Rügen, Germany). Appleby (1996) studied the population dynamics of a *Gyrodactylus* sp. on the sand goby *P. minutus* in the Oslo Fjord. The latter species had opisthaptoral hard parts very similar to *G. proterorhini* Ergens, 1967 or *G. arcuatus* Bychowski *sensu* Bychowski and Poljanski, 1953. The opisthaptoral parts of the above three species are different from those of *Gyrodactylus longidactylus* n. sp., although the shape of the anchors of *G. longidactylus* n. sp. resembles that of *G. emembranatus* Malmberg, 1970 from *Gadus morhua* L. but are smaller. Furthermore, *G. emembranatus* has a different type of marginal hook sickle and shorter pharyngeal processes. These two species, however, have a similar opisthaptor shape.

Studies on the variation in size of the opisthaptoral hard parts of several *Gyrodactylus* spp. during periods of different temperature indicate that size generally increases with decreasing temperatures (Malmberg, 1970, Mo, 1991a,b, 1993, Appleby,1996). Since all the specimens studied were caught during the summer period (August '96 for the live specimens, and June, July '93 for the formalin-fixed specimens) a wider size range than presented in this description is likely.

Gyrodactylus longidactylus n. sp. appears to be host specific. On the four species of *Pomatoschistus* from the Voordelta area, this parasite was found only on *P. lozanoi*. It was not found on the sand goby, *P. minutus*, which lives sympatrically with *P. lozanoi* in the Belgian and Dutch coastal waters and which is phylogenetically closely related to *P. lozanoi* (Wallis & Beardmore, 1984), nor on *P. microps* and *P. pictus* of the same sampling area.

Acknowledgements

We sincerely thank Mr. O. Decock of RVZ (Fisheries Research Institute, Ostende, Belgium) for providing us with live gobies and Mr. J. Cillis of the Royal Belgian Institute of Natural Sciences (Brussels, Belgium) for making the superb scanning micrographs. The 'Vlaamse Leergangen vzw.' provided the first author with financial support for visiting the Department of Zoology at the Stockholm University and part of this study was financed by NFWO project G.2094.92.

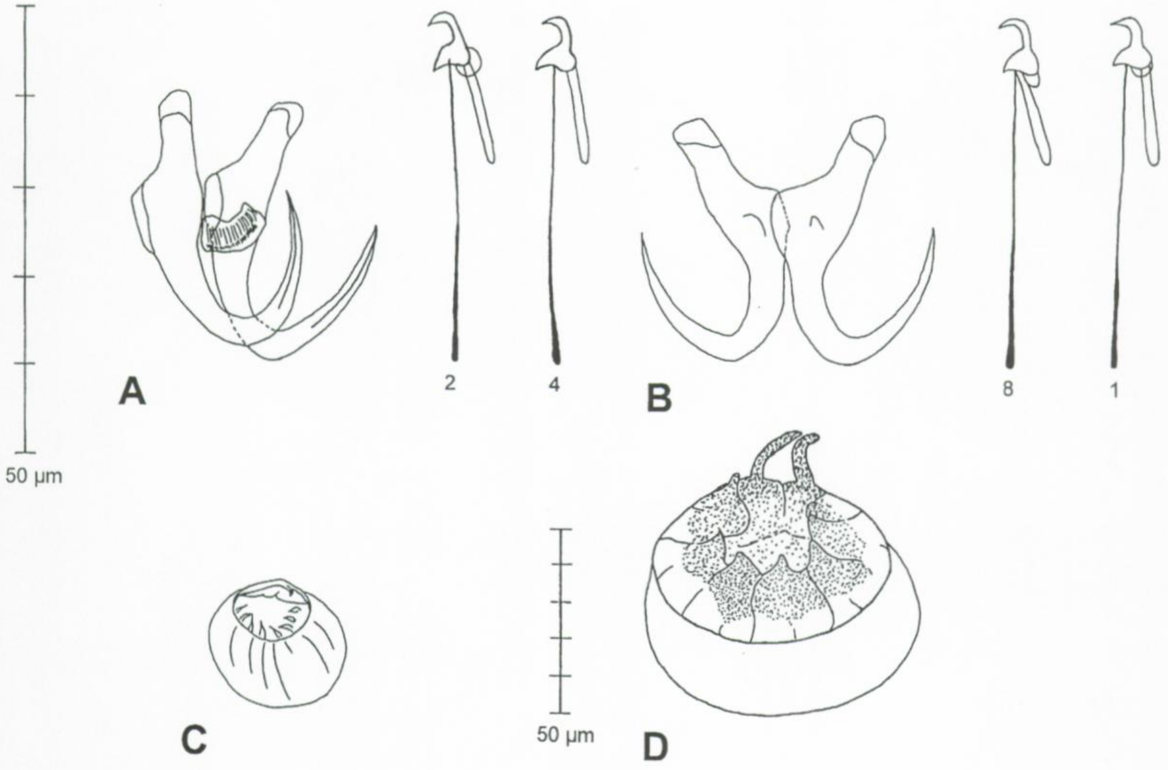


Figure 3.2: *Gyrodactylus longidactylus* n. sp. from *Pomatoschistus lozanoi* (Oostende Bank, North Sea, 21 Aug. 1996) (A) Anchors with ventral bar and marginal hooks from the holotype (number of marginal hook indicated). (B) Anchors and marginal hooks from a paratype (number of marginal hook indicated). (C) Penis from the holotype. (D) Pharynx with long pharyngeal processes from the holotype. Scale-bars: 50 µm (A, B and C, large scale-bar; D, small scale-bar)

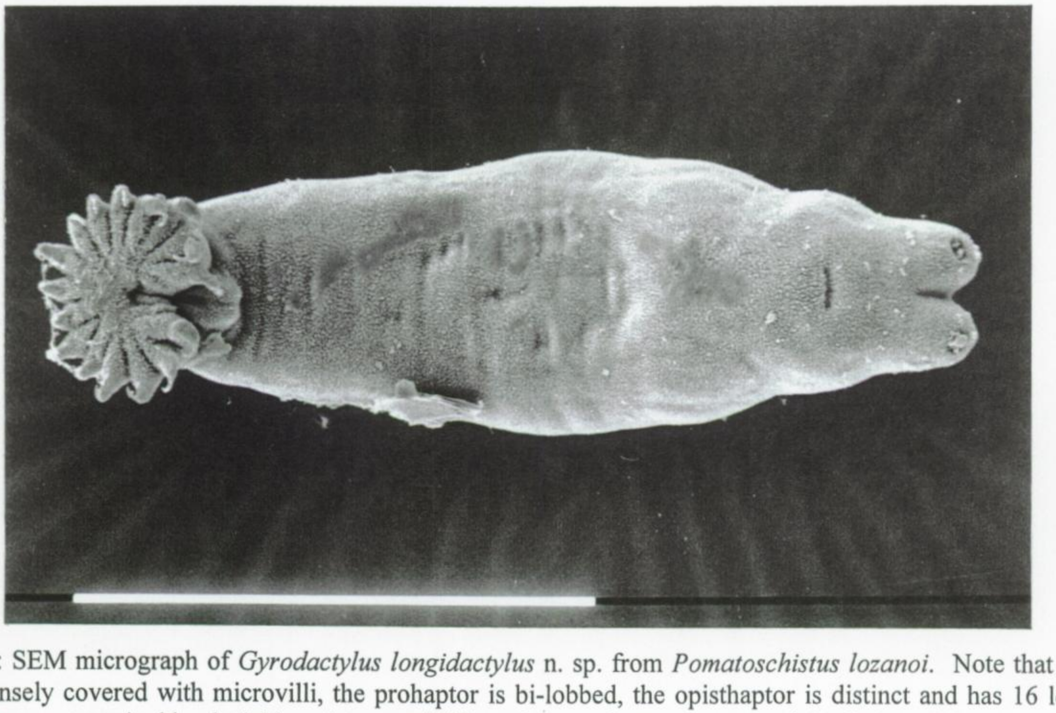


Figure 3.3: SEM micrograph of *Gyrodactylus longidactylus* n. sp. from *Pomatoschistus lozanoi*. Note that the body is densely covered with microvilli, the prohaptor is bi-lobbed, the opisthaptor is distinct and has 16 long marginal fingers, marginal hook sickle points are visible and the anchors are retracted. Scale-bar: 100 µm.

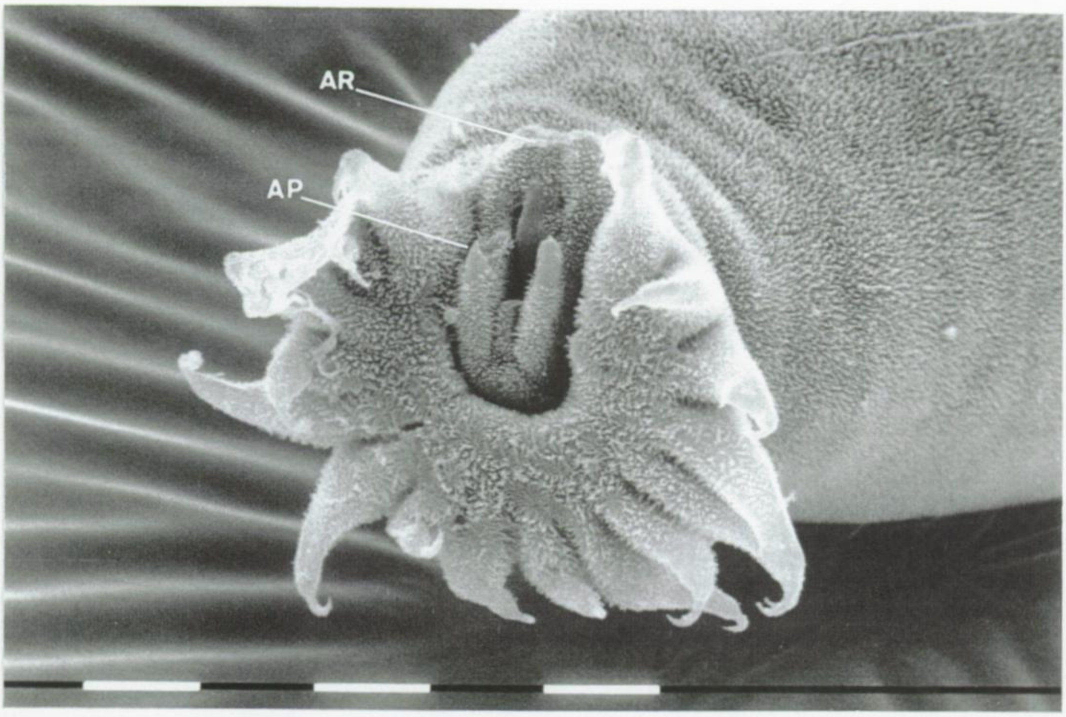


Figure 3.4: SEM micrograph of the opisthaptor of *Gyrodactylus longidactylus* n. sp. Note the long marginal fingers with gaffing hook sickles and that the anchor points of the retracted anchors are visible (AP = anchor point, AR = anchor root). Scale-bar: 10 µm.

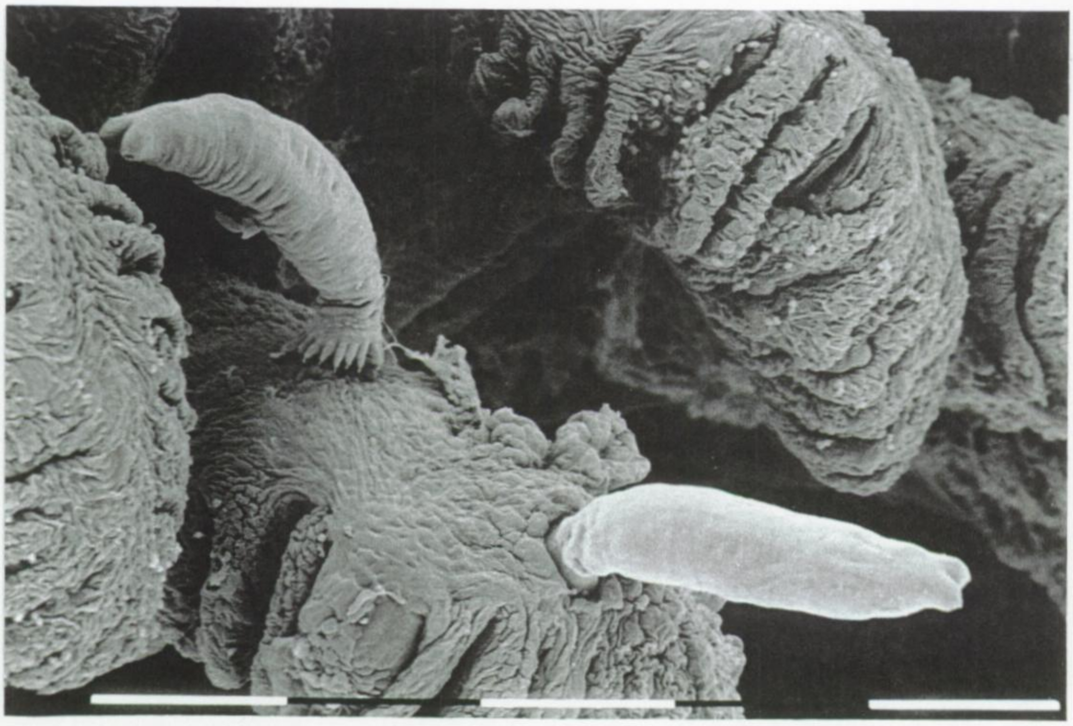


Figure 3.5: SEM micrograph of two *Gyrodactylus longidactylus* n. sp. specimens in situ on gill filaments of *Pomatoschistus lozanoi*. On the specimen to the left, the long fingers with the marginal hooks are visible. The opisthaptor of the specimen on the right is partly covered by the gill filament tissue. Scale-bar: 100 µm.

Figure 3.6: SEM micrograph of a *Gyrodactylus longidactylus* n. sp. on a gill arch of *Pomatoschistus lozanoi*. To the right, a "footprint" on the gill arch epithelium caused by *G. longidactylus* n. sp. specimen is present (arrow). No imprinting of the anchors are visible. Scale-bar: 100 μm.

Figure 3.7: SEM micrograph of a *Gyrodactylus longidactylus* n. sp. leaving a "footprint" on the gill arch epithelium of *Pomatoschistus lozanoi* after attachment. Note that the border between the opisthaptor and the body (= peduncle) is not as distinct in this specimen as in the specimens of figures 3.3 and 3.6. Scale-bar: 10 μm.

References

Appleby, C. (1996) Population dynamics of *Gyrodactylus* sp. (Monogenea) infecting the sand goby in the Oslo Fjord, Norway. *Journal of Fish Biology*, **49**, 402-410.

Appleby, C. (1996) Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology*, **33**, 199-207.

Doornbos, G. & Twisk, F. (1987) Density, growth and annual food consumption of gobiid fish of the saline lake Grevelingen, the Netherlands. *Netherlands Journal of Sea Research*, **21**, 45-74.

Ergens, R. (1969) The suitability of ammonium picate-glycerin in preparing slides of lower monogenoidea. *Folia Parasitologica (Praha)*, **16**, 320.

Fonds, M. (1971) The seasonal abundance and vertebral variation of *Pomatoschistus minutus minutus* and *lozanoi* (Gobiidae) in the Dutch Wadden Sea. *Vie et Milieu*, **22** (suppl.), 393-408.

Fonds, M. (1973) Sand gobies in the Dutch Wadden Sea (*Pomatoschistus*, Gobiidae, Pisces). *Netherlands Journal of Sea Research*, **6**, 417-478.

Gläser, H.-J. (1974) Eine neue Artgruppe des Subgenus *Gyrodactylus* (*Paranephrotus*) (Monogenea, Gyrodactylidae). *Zoologischer Anzeiger, Jena*, **192**, 271-278.

Hamerlynck, O., Hostens, K., Arellano, R.V., Mees, J. & Van Damme, P.A. (1993) The mobile epibentic fauna of soft bottoms in the Dutch delta (south-west Netherlands): spacial structure. *Netherlands Journal of Aquatic Ecology*, **27**,343-358.

Henderson, P.A. (1989) On the structure of the inshore fish community of England and Wales. *Journal of the Marine Biological Association U.K.*, **69**, 145-163.

Malmberg, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologi*, Serie 2, **23**, 235 pp.

Mo, T.A. (1991a) Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology*, **19**, 231-240.

Mo, T.A. (1991b) Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology*, **20**, 11-19.

Mo, T.A. (1993) Seasonal variation of opistaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *Salmo salar* L. parr in the River Sandviksela, Norway. *Systematic Parasitology*, **26**, 225-231.

Wallis, G. P. & Beardmore, J.A. (1984) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces: Gobiidae). *Biological Journal of the Linnean Society*, **22**, 107-123.

Webb, C.J. (1980) Systematics of the *Pomatoschistus minutus* complex (Teleostei: Gobiidae). *Philosophical Transactions of the Royal Society of London, Serie B: Biological Sciences*, **291**, 201-241.

Chapter 4

Species of Gyrodactylidae (Monogenea) from gobies (*Pomatoschistus* spp., Gobiidae, Pisces) of the Voordelta and surrounding areas: a description with notes on host-specificity.

Geets, A. and Ollevier, F.

Laboratory for Ecology and Aquaculture, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

This chapter has been submitted to *Systematic Parasitology*

Abstract

Five *Gyrodactylus* species (Monogenea) are presented from four sympatric goby species, *Pomatoschistus minutus*, *P. lozanoi*, *P. pictus* and *P. microps*, from the Voordelta (The Netherlands) and surrounding areas (Southern Bight of the North Sea, Belgium). A comparison was made with gyrodactylids collected from gobies from the Stockholm Archipelago (Sweden) and from the Oslo Fjord (Norway). In the Voordelta, *G. longidactylus* Geets, Malmberg and Ollevier, 1998 was found on *P. lozanoi* and *Gyrodactylus* sp. on *P. microps*. *P. microps* from the brackish Zeeschelde (Belgium) hosted *G. micropsi*. A single specimen of *G. rugiensis* was found on the skin of *P. lozanoi*. On *P. minutus*, *P. lozanoi* and *P. pictus* of the Voordelta, the most abundant gyrodactylids were *Gyrodactylus* cf. *arcuatus, Gyrodactylus arcuatus*-like specimens. The morphology of *G. arcuatus* collected from three-spined sticklebacks (*Gasterosteus aculeatus*) from marine, brackish and freshwater is compared with that of *G.* cf. *arcuatus* collected from gobies. Host-dependent morphological variations of *G.* cf. *arcuatus* were observed. Attention is drawn to the host specificity of the described species.

Introduction

Species of the genus *Pomatoschistus* Gill, 1864 have a wide distribution in marine and estuarine habitats (Miller, 1986). In the Voordelta (The Netherlands) and surrounding areas, four species are common: the sand goby, *P. minutus* (Pallas, 1770), a benthic euryhaline inshore species, Lozano's goby, *P. lozanoi* (de Buen, 1923), a more hyperbenthic, stenohaline marine species, the painted goby, *P. pictus* (Malm, 1865) which is a marine inshore species and the common goby, *P. microps* (Krøyer, 1838) which is an estuarine species. Although they are phylogenetically closely related (Webb, 1980, Wallis & Beardmore, 1984a, 1984b), they occupy distinct ecological niches (Hamerlynck & Cattrijsse, 1994, Edlund & Magnhagen, 1981, Magnhagen & Wiederholm, 1982). In spite of the sound overall knowledge on their biology, not much studies have paid attention to the role of *Pomatoschistus* spp. as hosts for monogenean parasites. Gläser (1974) described two *Gyrodactylus* species: *G. micropsi* and *G. rugiensis* from both *P. microps* and *P. minutus* from an inlet from the Baltic. Llewellyn *et al.* (1984) reported a *Gyrodactylus* species from gobies from England. The population dynamics of *Gyrodactylus* sp. on sand gobies from the Oslo Fjord, Norway was studied by Appleby (1996a).

Gyrodactylids are very closely associated with their host since they are holoxenic (one host in their life cycle) and their viviparous reproduction occurs on the host. Although some authors suggested that gyrodactylids are less host specific than commonly thought (Bakke *et al.*, 1992, Harris, 1993), other studies showed a narrow host specificity for certain species (Malmberg, 1970, Harris, 1985, Cone & Wiles, 1985). *Gyrodactylus* species are considered to be particulary suited for investigations of speciation and evolution of host specificity in parasitology (Harris, 1985). The high host specificity of monogeneans of the genus *Dactylogyrus* led to the identification of a new *Labeo* species (Cyprinidae, Pisces) (Guégan *et al.*, 1988). A study by Euzet *et al.* (1989) proved that monogeneans of the genus *Protoancylodiscoïdes* appeared to be as good an indicator for their hosts, *Chrysichthys* spp. (Bagridae, Pisces), as allozymes. A prerequisite for studies on host specificity and coevolution, however, is a sound knowledge of the different *Gyrodactylus* species present on particular host species, their morphological variation and host range. This study describes the *Gyrodactylus* species from gobies (*Pomatoschistus* spp.) of the Voordelta and surrounding areas. Special attention is given to the host-dependent morphological variation of *G.* cf. *arcuatus* from *P. minutus*, *P. lozanoi* and *P. pictus*, and to the host-specificity of the presented species.

Material and methods

Specimens of Gyrodactylidae were first noticed on the gills of formalin-fixed gobies, *Pomatoschistus minutus, P. lozanoi, P. microps* and *P. pictus* from the Voordelta area (51° 45' N, 3° 45' E, The Netherlands). These gobies were collected for the study on the population dynamics of their endo- and ectoparasites. For determination and description of the gyrodactylids, new samples of live gobies (*P. minutus* and *P. lozanoi*) were collected from the Voordelta Area and the Oostende Bank (51°17'N, 2°48'E, Belgium). A map of the sampling areas is given in Figure 4.1. Since no live specimens of *P. pictus* and *P. microps* could be collected from the Voordelta Area, the gyrodactylids from the formalin-fixed hosts are described here. Additionally, live *P. microps* were collected and checked for monogeneans from the brackish Zeeschelde at the nuclear power station in Doel (51°19'N, 4°16'E, Belgium).

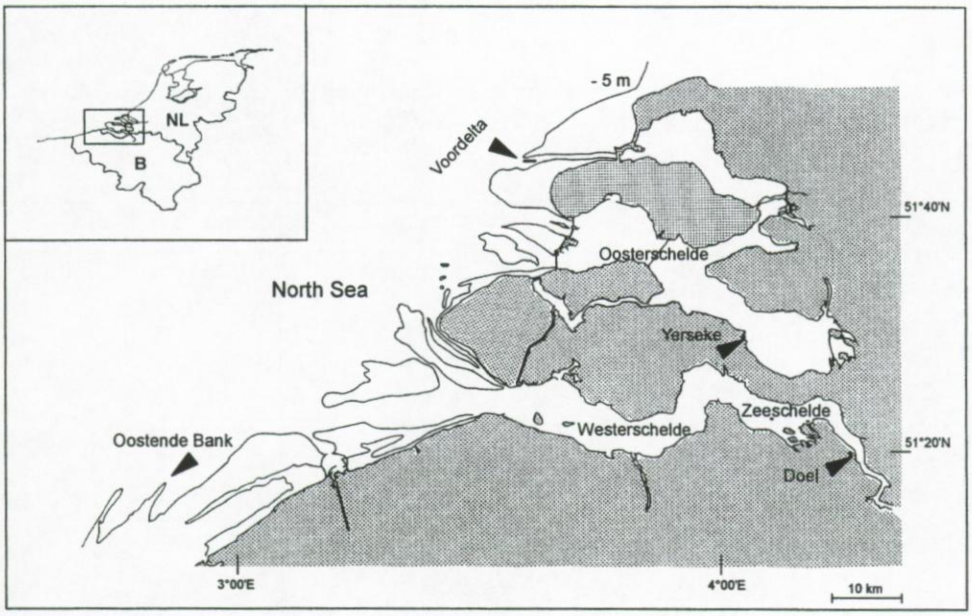


Figure 4.1: Map of the Voordelta and surrounding areas with indication (arrows) of the sampling points. (NL: The Netherlands, B: Belgium, -5 m: 5 m depth line).

Table 4.1: Overview of samples used for description of *Gyrodactylus* species from gobies (*Pomatoschistus* spp.) from the Voordelta (The Netherlands) and Oostende Bank (Belgium).

Host	Locality	Date	Fixation	Parasite species	Location	N studied	N drawn
P. minutus	Voordelta	11/10/93	APG	***G. cf. arcuatus***	G	19	5
			APG	”	S	17	-
		19/04/94	APG	”	G	37	1
			APG	”	S	3	-
		18/07/94	APG	”	G	2	-
	Oostende Bank	21/08/96	APG	”	G	5	3
P. lozanoi	Voordelta	11/10/93	APG	***G. cf. arcuatus***	G	2	2
			APG	”	S	7	1
		10/05/94	APG	”	G	10	-
			APG	”	S	2	2
		14/06/94	APG	”	G	1	1
		18/07/94	APG	”	G	1	-
	Oostende Bank	26/06/96	APG	”	G	22	6
		21/08/96	APG	”	G	8	2
			APG	***G. longidactylus***	G	34	20
	Voordelta	08/06/93	F	”	G	2	-
		16/07/93	F	”	G	80	2
	Oostende Bank	26/06/96	APG	***G. rugiensis***	S	1	1
P. pictus	Voordelta	08/06/93	F	***G. cf. arcuatus***	G	2	1
		16/07/93	F	”	G	62	4
P. microps	Voordelta	10/01/94	F	***Gyrodactylus sp.***	G	9	6
	Doel	06/09/94	APG	***G. micropsi***	G	15	4

G = Gills (both gill filaments and gill arches), S = Skin
APG: Ammonium picrate glycerine, F: Formaline

Table 4.2: Overview of comparative material used for determination and description of *Gyrodactylus* species of the Voordelta and surrounding areas

Host	Locality	Date	Fixation	Parasite species	Location	N studied	N drawn
P. minutus	Oslo	11/11/93	APG	***G. cf. arcuatus***	G	4	4
				”	M	1	1
		21/02/94	APG	”	M	5	4
		05/08/94	APG	”	M	1	1
	Stockholm	24/11/94	APG	***G. micropsi***	B	15	2
	Baltic Sea	20/06/73	CB	***G. rugiensis***	F	2	1
P. microps	Stockholm	24/11/94	APG	***G. micropsi***	B	6	2
	Baltic Sea	27/06/73	CB	***G. micropsi***	F	2	2
G. aculeatus	Lozen	07/11/97	APG	***G. arcuatus***	G	5	5
		04/12/97	APG	”	G	11	7
	Doel	29/09/97	APG	***G. arcuatus***	G	4	4
	Yerseke	16/09/97	APG	***G. arcuatus***	G	13	13

G = Gills (gill filaments and gill arches), M = Mouth, B = Body, F = Fins
APG: Ammonium picrate glycerine, F: Formaline, CB: Canada balsam

For comparison, *P. minutus* and *P. microps* from the Stockholm Archipelago (Sweden) were screened for Gyrodactylidae and slides of *Gyrodactylus* sp. from *P. minutus* from the Oslo fjord (Norway) were kindly provided by Dr. C. Appleby. Furthermore, live specimens of *Gyrodactylus arcuatus* were collected on samples of three-spined sticklebacks (*Gasterosteus aculeatus*) from a marine site (Yerseke, The Netherlands), a brackish area (Doel, Belgium) and one freshwater pond (Lozen, 51°12'N, 5°30'E, Belgium). Table 4.1 gives an overview of the material used from the goby species from the Voordelta and surrounding areas and Table 4.2 lists the material used for comparison.

The fish were transported to the lab immediately after landing and checked for gyrodactylids on the body surface and the gills using a stereo microscope. Live gyrodactylids were removed, mounted in a drop of water to flatten them and then fixed between slide and coverslip with ammonium picrate glycerine (Malmberg, 1970). The hard parts of the opisthaptor were studied using phase contrast microscopy. Drawings were made with a drawing tube on a Leitz Wezlar microscope (90x oil immersion objective) and on an Olympus microscope (100x oil immersion objective). Detailed drawings for determination were made from the best specimens (indicated by 'number drawn' in Table 4.1 and 4.2). All mounted specimens were studied. For measuring purposes ammonium picrate glycerine-fixed, nicely flattened specimens were preferably used, except for gyrodactylids from *P. pictus* and *P. microps*, of which only formaline-fixed specimens were available. Measurements were done according to Malmberg (1970) and Geets *et al.* (1998). Whenever possible, morphological comparisons were done between specimens caught in the same season to exclude seasonal variation.

Live gyrodactylids were collected from *P. lozanoi* of the Oostende Bank and fixed for scanning electron microscopy (SEM). The fixation method is described in Geets *et al.* (1998). The specimens were scanned in a Philips-515 scanning electron microscope.

Table 4.3: Morphological measurements (mean ± S.D. and range) on *Gyrodactylus* species from the common goby, *Pomatoschistus microps* and Lozano's goby, *P. lozanoi*.

Parasite species	***Gyrodactylus micropsi***	***Gyrodactylus* sp.**	***Gyrodactylus longidactylus***	***Gyrodactylus rugiensis***
Host species	*P. microps*	*P. microps*	*P. lozanoi*	*P. lozanoi*
Locality	Doel	Voordelta	Oostende Bank	Oostende Bank
measurement				
TBODYL	325.5 ± 51.0 257.1-431.6	319.5 ± 16.3 293.9-336.7	522.4 ± 160.0 342.4-769.7	413.2
TBODYW	75.9 ± 13.6 52.0-97.9	101.4 ± 27.8 58.2-131.6	137.6 ± 24.5 96.9-175.7	116.3
LAR	15.0 ± 1.0 13.3-15.5	8.5 ± 0.8 7.7-10.0	11.7 ± 1.3 9.4-14.4	19.4
LAS	30.1 ± 0.5 29.4-30.6	23.4 ± 0.6 19.4-21.7	20.2 ± 1.5 16.1-22.2	40.2
LA	43.0 ± 0.8 41.7-43.9	26.4 ± 0.6 25.6-27.2	26.8 ± 1.7 23.0-29.4	56.4
LAP	19.7 ± 0.9 18.9-21.1	13.8 ± 1.2 12.2-16.1	16.9 ± 1.0 15.5-18.9	30.8
LVB	18.3 ± 0.8 17.2-18.9	14.6 ± 0.7 13.9-15.3	8.1 ± 0.8 6.9-8.8	23.3
MDVBP	19.6 ± 1.2 17.8-20.6	18.4 ± 0.8 17.5-18.9	- -	25
MWVB	4.4 ± 0.5 3.9-5.0	3.7 ± 0.3 3.3-3.9	3.4 ± 0.6 2.8-4.4	5.5
VBM	11.1 ± 0.9 10.0-12.2	5.1 ± 1.0 4.4-5.8	3.8 ± 0.6 -	10.0
BWVB	5.3 ± 0.6 5.0-6.1	4.4 ± 0.0 4.4-4.4	- 3.3-5.0	5.5
TBWVB	16.9 ± 1.3 15.5-18.3	10.0 ± 0.8 9.4-10.6	- -	-
TWVB	- -	10.3 ± 0.4 10.0-10.6	- -	-
LH	22.0 ± 0.3 21.7-22.2	21.4 ± 1.0 20.6-22.8	32.4 ± 1.2 28.3-33.3	21.7
LSI	5.1 ± 0.3 5.0-5.5	4.7 ± 0.3 4.4-5.0	6.3 ± 0.3 5.5-7.2	5.6
LMH	27.2 ± 0.0 27.2-27.2	25.9 ± 1.0 25.0-27.2	38.2 ± 1.1 35.5-40.0	27.2
LOOP	8.5 ± 0.6 7.8-8.9	7.1 ± 0.2 6.9-7.2	10.2 ± 1.0 8.9-12.8	8.9
PWSI	3.4 ± 0.3 3.1-3.6	3.6 ± 0.3 3.3-3.9	- -	3.9
DWSI	3.1 ± 0.3 3.1-3.6	2.9 ± 0.2 2.8-3.3	- -	3.6
n measured	4⁽¹⁾	5⁽²⁾	11⁽¹⁾	1⁽¹⁾

(1) Specimens fixed live in ammonium picrate glycerine

(2) Specimens fixed in formalin

Abbreviations of measurements:

TBODYL: Total body length, **TBODYW**: Total body width, **LAR**: Length of anchor root, **LAS**: Length of anchor shaft, **LA**: Length of anchor, **LAP**: Length of anchor point, **LVB**: Length of ventral bar, **MDVBP**: Maximal distance of ventral bar processes, **MWVB**: Median width of ventral bar, **VBM**: Length of ventral bar membrane, **BWVB**: Basal width of ventral bar, **TBWVB**: Total basal width of ventral bar, **TWVB**: Total width of ventral bar, **LH**: Length of marginal hook handle, **LSI**: Length of marginal hook sickle, **LMH**: Length of marginal hook, **LOOP**: Length of filament loop, **PWSI**: Proximal width of sickle, **DWSI**: Distal width of sickle.

Results

In total, five different species of the genus *Gyrodactylus* Nordmann, 1832 were found on the four goby species studied, *Pomatoschistus minutus*, *P. lozanoi*, *P. pictus* and *P. microps*, of the Voordelta, Oostende Bank and Zeeschelde (Table 4.1). *Gyrodactylus longidactylus* Geets, Malmberg and Ollevier, 1998, a new species described in Chapter 3, was found on *P. lozanoi* (Voordelta and Oostende Bank) and an undescribed *Gyrodactylus* sp. was present on the gills of formalin-fixed *P. microps* of the Voordelta. The species status of the *Gyrodactylus* cf. *arcuatus* specimens from *P. minutus, P. lozanoi* and *P. pictus* is still unclear.

Gyrodactylus longidactylus seems to be host specific since it was not found on any of the other sympatric goby species (Table 4.5). Typical for *G. longidactylus* are the long marginal hook handles, which are always longer than the total length of the anchor (Figs 4.2 A and a).

Table 4.5: Overall infection level of three *Gyrodactylus* species on four *Pomatoschistus* species. In the Voordelta (VD), data from monthly samples from June 1993 till July 1994 were combined. Oostende Bank (O) samples were from August 1996.
n F = total number of fish investigated, n G = total number of *Gyrodactylus*, P % = prevalence (percentage of infected fish)

		P. minutus			*P. lozanoi*			*P. pictus*			*P. microps*		
		n F	n G	P %	n F	n G	P %	n F	n G	P %	n F	n G	P %
G. cf. *arcuatus*	VD	369	**2028**	**29.5**	318	**256**	**5.6**	23	**64**	**8.7**	15	0	0
	O	21	**5**	**9.5**	7	**8**	**57.1**	-	-	-	-	-	-
G. longidactylus	VD	369	0	0	318	**82**	**1.0**	23	0	0	15	0	0
	O	21	0	0	7	**34**	**71.0**	-	-	-	-	-	-
Gyrodactylus sp.	VD	369	0	0	318	0	0	23	0	0	15	**9**	**6.7**

Gyrodactylus sp. (Figs 4.2 C,c1 and c2) was only found on *P. microps* of the Voordelta. *P. minutus*, *P. lozanoi* and *P. pictus* from the same sample were not infected with this species. Live *P. microps* from the brackish Zeeschelde in Doel did not yield *Gyrodactylus* sp. Since no live *P. microps* were caught in later samples from the Voordelta or from the Oostende Bank, the description given here was based on the formalin-fixed material only. Both *G. longidactylus* and *Gyrodactylus* sp. have rather small anchors, but in contrast with *G. longidactylus*, the marginal hook handles of *Gyrodactylus* sp. have about the same size as the

Table 4.4: Morphological measurements (mean ± S.D. and range) on *Gyrodactylus* cf. *arcuatus* from sand goby, *Pomatoschistus minutus,* Lozano's goby, *P. lozanoi,* painted goby, *P. pictus* and *G. arcuatus* from three-spined stickleback, *Gasterosteus aculeatus*.

Parasite species	***Gyrodactylus* cf. *arcuatus***	***Gyrodactylus* cf. *arcuatus***	***Gyrodactylus* cf. *arcuatus***	***Gyrodactylus arcuatus***
Host species	*P. minutus*	*P. lozanoi*	*P. pictus*	*Gasterosteus aculeatus*
Locality	Voordelta, Oostende Bank	Voordelta, Oostende Bank	Voordelta	Yerseke, Doel, Lozan
measurement				
TBODYL	337.9 ± 62.9 220.4-440.8	374.1 ± 86.4 223.5-547.9	301.0 ± 32.7 235.7-352.0	495.4 ± 109.4 287.8-682.6
TBODYW	81.1 ± 13.4 55.1-104.1	86.0 ± 22.0 52.0-122.4	95.9 ± 12.2 79.6-113.3	108.3 ± 16.3 76.5-137.8
LAR	6.0 ± 1.0 3.1 - 10.1	7.7 ± 1.4 4.9-11.3	8.7 ± 0.8 6.1-10.1	9.7 ± 1.2 7.3-12.8
LAS	27.1 ± 1.4 23.7-31.1	30.1 ± 1.7 21.9-34.0	24.0 ± 1.1 21.3-26.8	29.9 ± 1.8 26.2-33.5
LA	32.8 ± 1.8 29.7-37.3	37.5 ± 2.3 26.2-44.0	32.2 ± 1.1 29.3-34.2	37.7 ± 2.4 34.2-42.7
LAP	16.5 ± 1.4 12.7-19.5	18.0 ± 1.7 12.8-21.7	14.3 ± 0.6 12.8-15.2	15.9 ± 1.2 12.8-17.7
LVB	15.0 ± 0.7 12.2-17.7	16.3 ± 1.3 11.6-20.0	14.4 ± 0.8 12.8-15.8	17.4 ± 1.4 14.6-20.1
MDVBP	18.5 ± 0.8 14.0-22.6	19.8 ± 1.7 14.6-24.3	18.2 ± 1.8 11.0-21.3	22.3 ± 1.9 18.3-25.6
MWVB	3.7 ± 0.4 2.6-5.5	3.5 ± 0.4 3.0-4.9	3.3 ± 0.4 2.4-4.3	4.1 ± 0.6 3.1-5.2
VBM	6.8 ± 1.4 4.7-11.6	7.5 ± 1.4 4.6-13.4	8.9 ± 0.7 7.3-10.4	10.4 ± 1.2 7.9-12.2
BWVB	6.1 ± 0.5 4.0-7.0	6.5 ± 0.4 3.1-9.2	5.3 ± 0.6 4.3-7.3	5.6 ± 0.6 4.3-6.7
TBWVB	12.9 ± 1.2 10.7-17.7	14.0 ± 1.7 12.0-18.3	14.2 ± 1.5 8.5-17.1	17.0 ± 1.6 13.4-20.1
TWVB	16.7 ± 1.1 14.7-21.3	18.8 ± 1.8 15.2-21.3	17.5 ± 1.0 15.8-19.5	22.5 ± 1.6 19.5-25.6
LH	17.6 ± 2.5 11.8-20.3	14.8 ± 1.0 12.2-19.5	17.0 ± 0.9 15.8-19.5	17.5 ± 1.0 15.8-19.2
LSI	4.2 ± 0.3 3.6-4.9	4.4 ± 0.3 3.3-4.9	1.2 ± 0.3 3.7-4.9	4.6 ± 0.3 4.0-4.9
LMH	19.0 ± 0.9 16.7-22.6	18.4 ± 1.1 15.8-23.8	20.7 ± 0.8 19.5-22.6	21.7 ± 1.1 19.5-23.8
LOOP	7.4 ± 0.4 5.5-9.2	7.4 ± 0.4 5.3-14.6	7.5 ± 0.8 6.4-9.8	6.6 ± 0.6 4.9-7.3
PWSI	3.6 ± 0.3 3.0-4.6	3.9 ± 0.3 2.7-4.3	3.6 ± 0.3 3.1-4.0	3.7 ± 0.3 3.1-4.3
DWSI	2.6 ± 0.4 1.0-3.1	2.8 ± 0.2 1.2-3.4	2.6 ± 0.2 2.4-3.3	2.5 ± 0.1 2.3-2.7
n measured	31(1)	14(1)	26(2)	26(1)

(1) Specimens fixed live in ammonium picrate glycerine

(2) Specimens fixed in formalin

Abbreviations of measurements: see Table 4. 3.

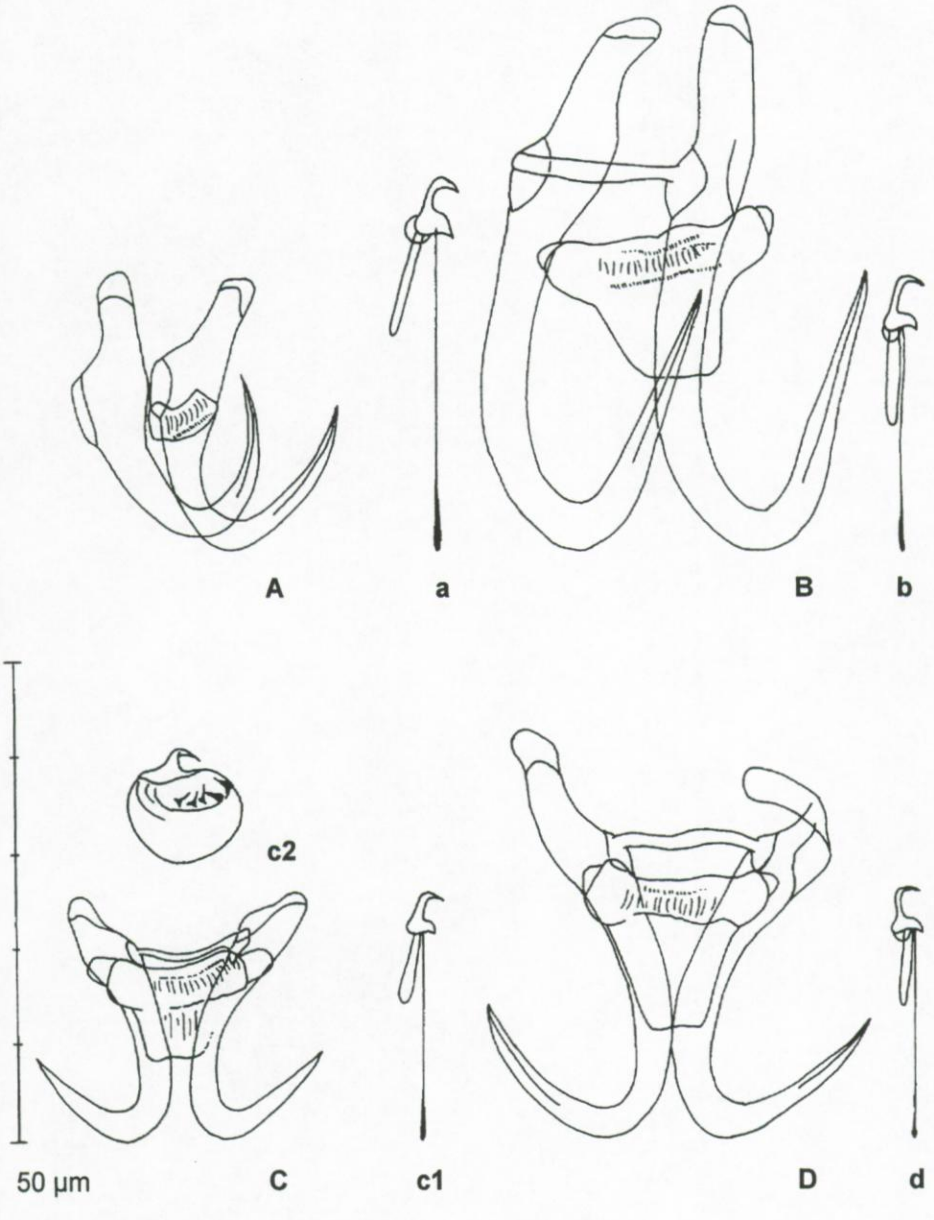


Figure 4.2: (A) Anchors and (a) marginal hook of *Gyrodactylus longidactylus* from the gills of *Pomatoschistus lozanoi*. (B) Anchor and (b) marginal hook of *Gyrodactylus rugiensis* from the skin of *P. lozanoi*. (C) Anchor, (c1) marginal hook and (c2) penis of *Gyrodactylus* sp. from the gills of *P. microps* from the Voordelta Area. (D) Anchor and (d) marginal hook from *Gyrodactylus micropsi* from *P. microps* from Doel.

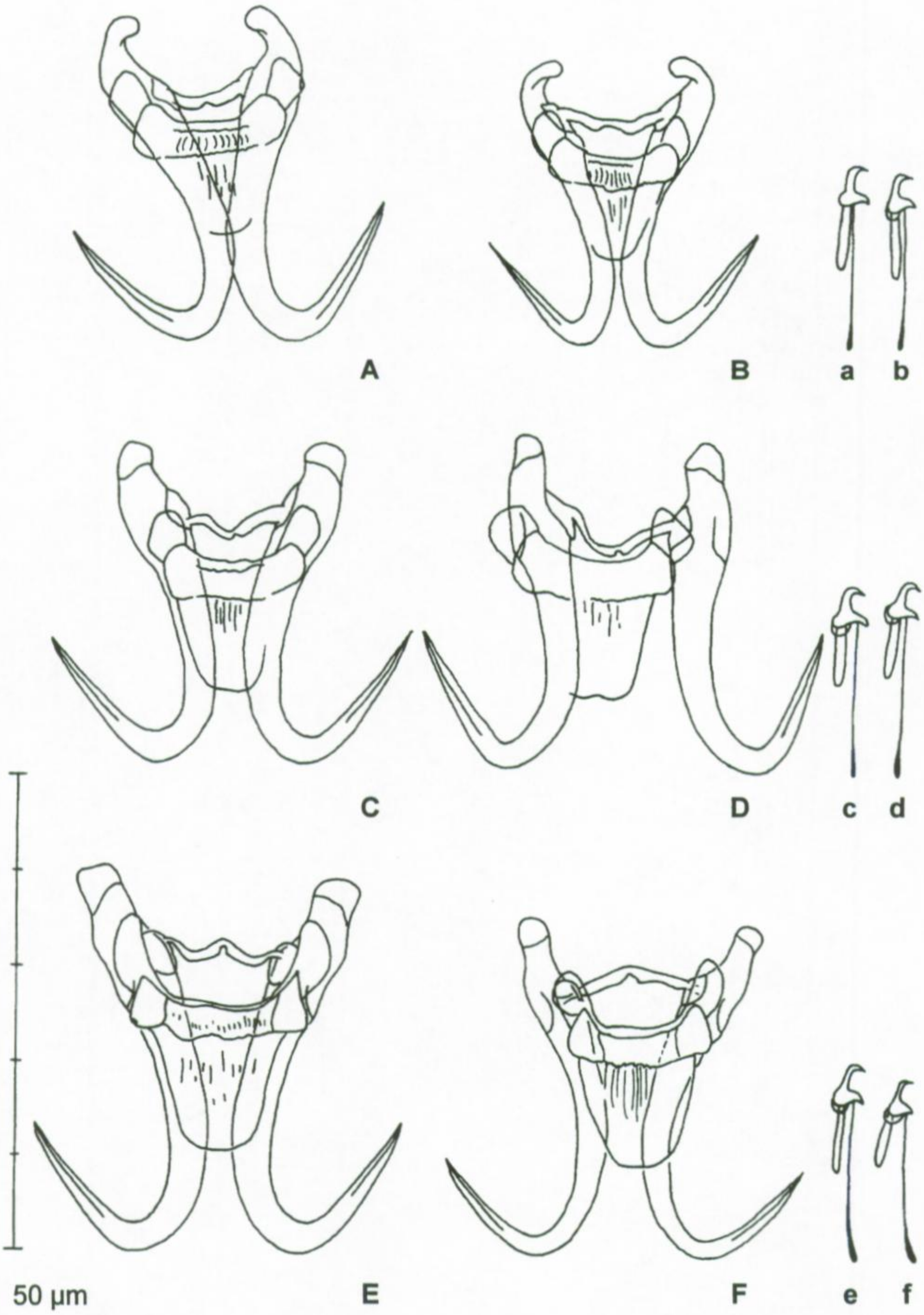


Figure 4.3: Anchors (indicated with capital letters) and marginal hook (small letters) of *Gyrodactylus* cf. *arcuatus* from *Pomatoschistus minutus* and *Gyrodactylus arcuatus* from *Gasterosteus aculeatus*:

A & a: from the gills of *Pomatoschistus minutus*, Voordelta, 11/10/93

B & b: from the gills of *P. minutus*, Oostende Bank, 21/8/96

C & c: from the gills of *P. minutus*, Oslo , 11/11/93 (drawn from material from the collection of Dr. C. Appleby)

D & d: from the mouth of *P. minutus*, Oslo, 11/11/93 (drawn from material from the collection of Dr. C. Appleby)

E & e: *Gyrodactylus arcuatus* from the fins from *Gasterosteus aculeatus* (total length of host 6 cm), Kieler hafen (Germany), 19/6/58 (redrawn after original drawings from Prof. G. Malmberg) (Malmberg, 1964)

F & f: *Gyrodactylus arcuatus* from the fins from *G. aculeatus* (total length of host 1.35 cm), Namdö (Sweden), 19/6/58 (redrawn after original drawings from Prof. G. Malmberg) (Malmberg, 1964)

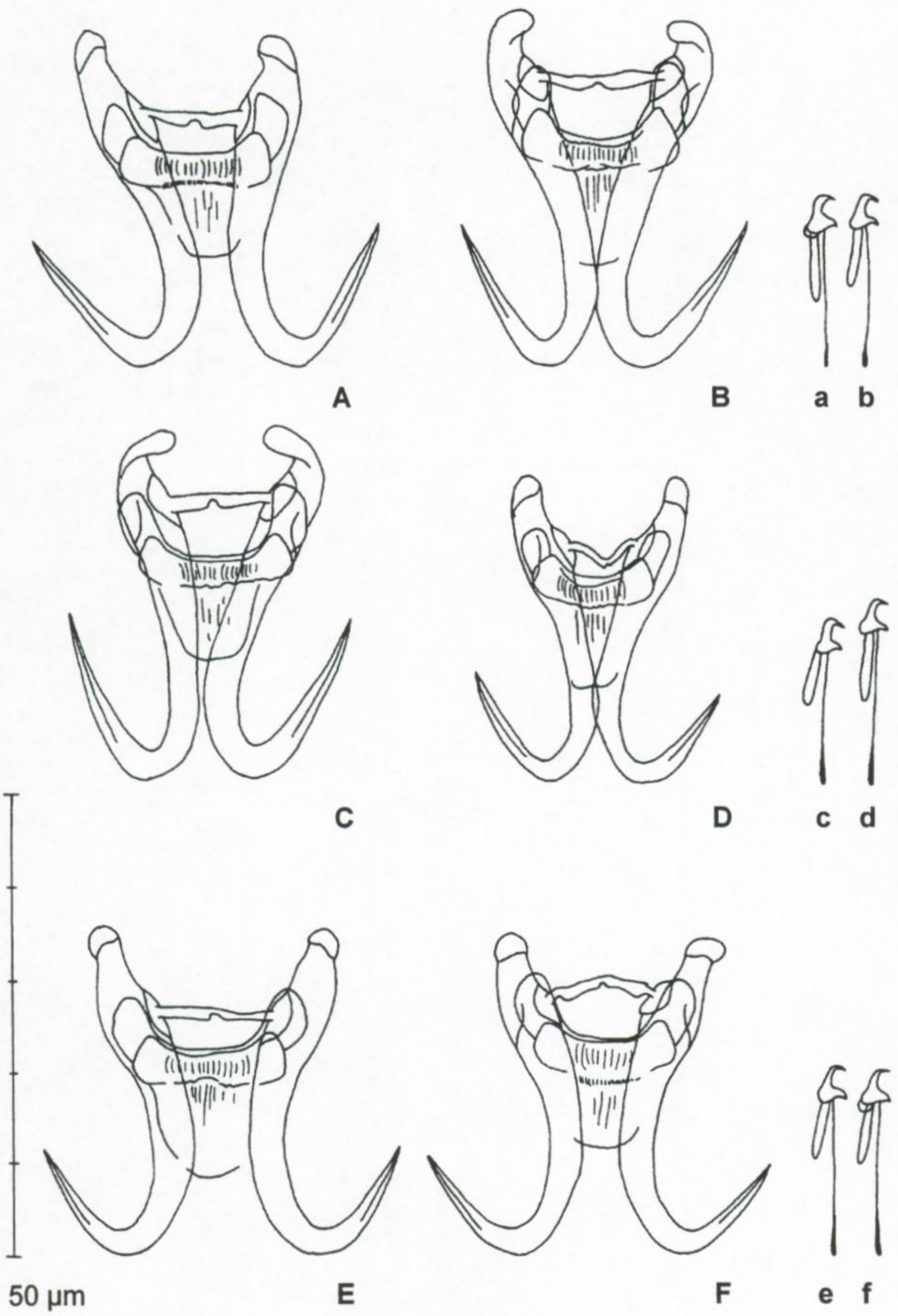


Figure 4.4: Anchors (indicated with capital letters) and marginal hook (small letters) of *Gyrodactylus* cf. *arcuatus* from *Pomatoschistus lozanoi* and *P. pictus* and *Gyrodactylus arcuatus* from *Gasterosteus aculeatus*:

A & a: from the gills of *Pomatoschistus lozanoi*, Voordelta, 10/5/94
B & b: from the gills of *P. lozanoi*, Oostende Bank, 21/8/96
C & c: from the skin of *P. lozanoi*, Voordelta, 10/5/94
D & d: from the gills of *P. pictus*, Voordelta, 8/6/93 (formaline fixed specimen)
E & e: *Gyrodactylus arcuatus* from the gills of *Gasterosteus aculeatus*, Yerseke (brackish-marine), 16/9/97
F & f: *Gyrodactylus arcuatus* from the gills of *G. aculeatus*, Lozen (freshwater), 7/11/97

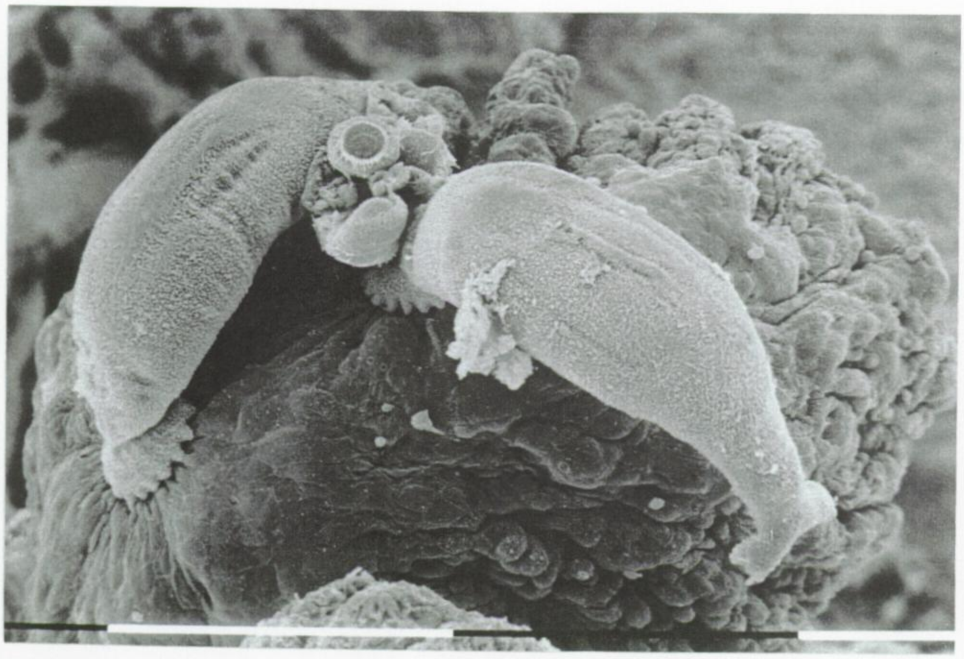


Figure 4.5: Two specimens of *Gyrodactylus* cf. *arcuatus* attached to the gill arches of *Pomatoschistus lozanoi* (Oostende Bank, 21/8/97). SEM micrograph, scale bar: 100 µm.

Figure 4.6: *Gyrodactylus* cf. *arcuatus* from *Pomatoschistus lozanoi*: ventral view SEM micrograph, scale bar: 100 µm.

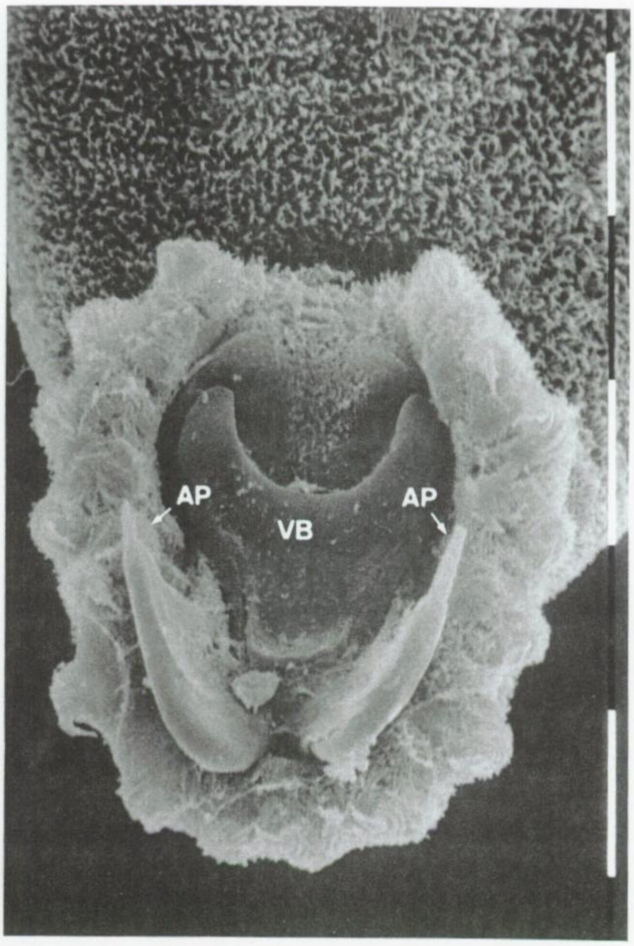


Figure 4.7: Opisthaptor of *Gyrodactylus* cf. *arcuatus* of *Pomatoschistus lozanoi*: ventral view. Note the ventral bar (VB) and the anchor points (AP) SEM micrograph, scale bar: 10 µm.

total anchor length. *Gyrodactylus* sp. has a ventral bar with small but distinct ventral bar processes and a ventral bar membrane. A dorsal bar is present. Bladders were noticed in three of nine specimens and the penis contains one row of spines (Fig. 4.2 c2). The pharynx probably has long processes but since this feature was only vaguely shown in one specimen, further confirmation is necessary. Measurements of the different opisthaptoral structures are given in Table 4.3.

P. microps from the brackish Zeeschelde (Doel) harboured *G. micropsi* Gläser, 1974 on their gills (Fig. 4.2 D and d). For determination purposes, a comparison was made with original slides of *G. micropsi* from the collection of Gläser. *G. micropsi* has larger anchors (29.7-37.3 µm) than all other species of *Gyrodactylus* present in this study, with the exception of *G. rugiensis*. The ventral bar carries a large ventral bar membrane and two small processes. *G. micropsi* was also found on the body surface of *P. minutus* and *P. microps* from the Stockholm Archipelago. Gläser (1974) described *G. micropsi* from *P. minutus* and *P. microps* both on gills and fins. *G. micropsi* from the gills of *P. microps* from the Zeeschelde are more conform to the 'gill-form' (Gläser, 1974), while specimens from the body surface of the gobies from the Stockholm Archipelago are more similar to the 'fin-form' described by Gläser (1974).

One specimen of *Gyrodactylus rugiensis* Gläser, 1974 was found on the skin of *P. lozanoi* of the Oostende Bank (Fig. 4.2 B and b). Of all five *Gyrodactylus* species described in this study, *G. rugiensis* has the largest anchors, even larger than those of *G. micropsi*. The specimen was compared with a paratype (Gläser, 1974) and the opisthaptoral hard parts were almost identical in shape and size.

The most frequently encountered gyrodactylids on the gills and skin of *P. minutus* and *P. lozanoi* and on the gills of *P. pictus* was a species here called *Gyrodactylus* cf. *arcuatus,* since the opisthaptoral hard parts were similar to *Gyrodactylus arcuatus* Bychowsky *sensu* Bychowski & Poljanski, 1953. On *P. pictus* and *P. minutus* of the Voordelta and surrounding areas, this was the only species found. Comparison of the *G.* cf. *arcuatus* specimens from the three different goby species with *G. arcuatus* specimens described by Malmberg (1970) from its type host the three-spined stickleback, *Gasterosteus aculeatus,* and with *G. arcuatus* specimens obtained from three-spined sticklebacks from Yerseke (The Netherlands), Doel and Lozen (Belgium), revealed some host-dependent morphological variations of the opisthaptoral hard parts. Table 4.4 gives an overview of the morphological measurements of *G.* cf. *arcuatus* from the different goby hosts, compared with measurements of *G. arcuatus* from the three-spined sticklebacks. Figures 4.3 and 4.4 present anchors and marginal hooks of *G.* cf. *arcuatus* from different hosts, localities and seasons as well as drawings from *G. arcuatus* from the three-spined stickleback. *G.* cf. *arcuatus* from *P. minutus* had slightly shorter anchor shafts than *G. arcuatus* from sticklebacks from Yerseke. Also the ventral bar processes were slightly shorter. On *P. lozanoi* (Voordelta, Oostende), *G.* cf. *arcuatus* from the gills had on average slightly longer anchor points than *G.* cf. *arcuatus* from *P. minutus* from the same season and same sampling place, but the range overlapped. The *G.* cf. *arcuatus* specimens found on the skin of *P. lozanoi* tend to have slightly bigger anchors than those on the gills. Appleby (1996b) also reported that some characters of the opisthaptoral hard parts of *G. callariatis* from Atlantic cod were significantly longer on specimens collected at external sites than on those from internal sites. The specimens collected from *P. minutus* from Oslo (material from Dr. C. Appleby) are very similar to those collected from *P. minutus* in our samples, except for one specimen from August 1994 which had much bigger anchors and was more comparable to *G.* cf. *arcuatus* from *P. lozanoi* found in our summer samples. Figures 4.5, 4.6 and 4.7 show SEM micrographs from *G.* cf. *arcuatus* from the gills of *P. lozanoi*. The anchors, with the anchor points sticking out, the ventral bar and marginal hooks are clearly visible.

Morphologically, *G.* cf. *arcuatus* of *P. pictus* and *P. minutus* of the same area and season are quite similar, although they differ in length of the anchor point which is shorter in *G.* cf. *arcuatus* from *P. pictus*. There was also a tendency in smaller shaft length in comparison with the specimens from *P. minutus* and *P. lozanoi*. The shaft length of *G.* cf. *arcuatus* from *P. pictus* was definitely shorter than of *G. arcuatus* from the three-spined sticklebacks.

Data presented in Table 4.5 indicate the host preferences of *G.* cf. *arcuatus*, *G. longidactylus* and *Gyrodactylus* sp. In this table, the percentage of infection (prevalence) of the four *Pomatoschistus* species are given for the Voordelta and Oostende samples. The Voordelta data are derived from a study on seasonal population dynamics of the gyrodactylids from gobies in the Voordelta (see Chapter 6). *G.* cf. *arcuatus* parasitizing *P. minutus*, *P. lozanoi* and *P. pictus*, exhibits the broadest host range, while *G. longidactylus* was exclusively found on *P. lozanoi* and *Gyrodactylus* sp. on *P. microps*.

Discussion

Gläser (1974) described *Gyrodactylus micropsi* and *G. rugiensis* from *Pomatoschistus minutus* and *P. microps*. Both Monogenea species were found in our samples. Gläser (1974) noted that *G. micropsi* occurred on gobies of brackish water. *G. micropsi* was only found on *P. microps* from brackish water and not in marine samples, which could be an indication for a brackish water niche of this species.

One specimen of *G. rugiensis* was found on the skin of *P. lozanoi*. *P. lozanoi* is a new host record for *G. rugiensis*. More observations are necessary to confirm the status of *P. lozanoi* as a host for this parasite. The rarity of the observation is probably due to the fact that in this study less attention was given to the skin as microhabitat for gyrodactylids than to the gills. Gläser (1974) reported that *G. rugiensis* was found more on the skin than on gills of *P. minutus*.

Gyrodactylus sp., collected on the gills of *P. microps* of the Voordelta area, did not fit any know species description. Since only nine specimens could be collected and all were from formalin-fixed hosts, the material seemed insufficient to give a detailed species description and therefore we preferred not to name the species.

G. longidactylus on *P. lozanoi* and *Gyrodactylus* sp. on *P. microps*, seem to be host-specific, which is indicated by the data represented in Table 4.5. Apart from *P. lozanoi*, none of the other gobies from the same area (and the same catches) harboured *G. longidactylus*. For *Gyrodactylus* sp. on *P. microps*, we should be careful in drawing definite conclusions on their host specificity, since only nine specimens could be collected. It was remarkable that *Gyrodactylus* sp. was only found on *P. microps* from seawater and not on the same goby species from brackish water. Screening of a larger sample is needed to conclude on the extend of host- and habitat specificity of this species.

In the present study, the most common gyrodactylid species was *G.* cf. *arcuatus*. Specimens were found on three out of four goby hosts screened. In a study on the population dynamics of *Gyrodactylus* sp. from *P. minutus* in the Oslo Fjord, Appleby (1996a) left the species status of this monogenean uncertain. He stated that the opisthaptoral hard parts were similar to those of *G. arcuatus* from the three-spined stickleback and to those of *G. proterorhini* Ergens, 1967 from the Eastern European goby, *Proterorhinus marmoratus*. However, *G. proterorhini* has anchor roots with caps and those are not found in our *Gyrodactylus* specimens from *P. minutus*, nor in the Appleby's *Gyrodactylus* specimens from the Oslo Fjord. Therefore, in both cases, *G. proterorhini* can be excluded.

The description of *G. arcuatus* Bychowski *sensu* Bychowsky and Poljanski, 1953 fits more the specimens of the gobiid hosts. Malmberg (1970) indicated a possible low degree of host-specificity of *G. arcuatus* since the species temporarily is able to spread to other fish species than *Gasterosteus aculeatus*. Although the ranges of the measurements of morphological features of *G.* cf. *arcuatus* specimens of gobies overlapped to a great extend with those of *G. arcuatus* from the three-spined stickleback, some consistent host-dependent morphological differences were observed. These are: shorter anchor shafts in *G.* cf. *arcuatus* from *P. minutus* and *P. pictus* than in *G. arcuatus* from the three-spined stickleback, shorter length of the ventral bar in specimens from the three goby hosts than from the sticklebacks, a tendency for shorter marginal hooks in specimens from *P. minutus* and *P. lozanoi* and shorter anchor points in specimens from *P. pictus* when compared to *G. arcuatus* from the sticklebacks. This suggests the presence of different forms (possibly different species) on the goby hosts.

Describing *Gyrodactylus* species purely on a morphological basis does not always lead to satisfactory differentiation between species. Harris (1993) notes considerable morphological differences between populations, even after allowance is made for the effect of water temperature. Morphological variation in *G. avalonia*, the North-American counterpart of *G. arcuatus*, led to the description of four species which subsequently were all synonymised by Cone & Wiles (1985) with *G. avalonia*. But, *G. avalonia* itself shows a striking morphological similarity with *G. arcuatus* and its species status is still questionable. Seasonal variation in size of the hooks , which is known for other *Gyrodactylus* species (Malmberg, 1970, Mo 1991a, 1991b, 1993, Appleby 1996b), must be taken into account when comparing specimens. It should be noted that all *G.* cf. *arcuatus* specimens from *P. pictus* were collected in summer (June and July '93) when watertemperature is high and anchors are possibly smaller than in winter samples. Therefore, the size ranges of the morphological features of *G.* cf. *arcuatus* from *P. pictus* given in this study are probably too narrow.

Our results urge for a more extensive morphometric study on more specimens from different goby hosts and different seasons to clarify the significance of these different forms of *G.* cf. *arcuatus* (see chapter 5). At present, we prefer to keep the name *G.* cf. *arcuatus* for the specimens found on *P. minutus*, *P. lozanoi* and *P. pictus*. Furthermore, genetic studies are in process to determine whether genetic or environmental (e.g. host-dependent) factors are responsible for the observed differences amongst *G.* cf. *arcuatus*.

Acknowledgements

We would like to thank Mr. O. Decock of RVZ (Fisheries Research Institute, Ostend, Belgium) for the help with collecting live gobies and Mr. J. Cillis of the Royal Belgian Institute of Natural Sciences for the superb scanning photographs. We are gratefull to Dr. G. Malmberg for his patience when explaining how to determine gyrodactylids. Dr. C. Appleby kindly provided us with *Gyrodactylus* specimens from Norwegian sand gobies. Dr. L. De Meester, Dr. F. Volckaert and Dr. C. Appleby supplied us with their usefull comments on the manuscript. Our thanks go also to Dr. L. De Meester, B. Geets and A. Vermeulen for their help during sampling. Part of this study was financed by NFWO-project G.2094.92 and 'Vlaamse Leergangen vzw' provided the first author with financial support for visiting the Department of Zoology at Stockholm University (Sweden).

References

Appleby, C. (1996a) Population dynamics of *Gyrodactylus* sp. (Monogenea) infecting the sand goby in the Oslo fjord, Norway. *Journal of Fish Biology*, **49**, 402-410.

Appleby, C. (1996b) Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea, Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology*, **33**, 199-207

Bakke, T. A., Harris, P. D., Jansen, P.A. & Hansen, L. P. (1992) Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms*, **13**, 63-74.

Cone, D. K. & Wiles, M. (1985) The systematics and zoogeography of *Gyrodactylus* species (Monogenea) parasitizing gasterosteid fishes in North America. *Canadian Journal of Zoo*logy, **63**, 956-960.

Euzet, L., Agnèse, J.-F. & Lambert, A. (1989) Valeur des parasites comme crière d'identification de l' espèce hôte. Démonstration convergente par l'étude parasitologique des Monogènes branchiaux et l' analyse génétique des Poissons hôtes. *Comptes rendus des Seances de l' Academie de Sciences, Paris,série III*, **308**, 385-388.

Edlund, A.-M. & Magnhagen, C. (1981) Food segregation and consumption suppression in two coexisting fishes, *Pomatoschistus minutus* and *P. microps* : an experimental demonstration of competition. *Oikos*, 36, 23-27.

Guégan, J.-F., Lambert, A. & Euzet, L. (1988) Etudes des Monogènes des Cyprinidae du genre Labeo en Afrique de Ouest. I. Genre *Dactylogyrus* Diesing, 1850. *Rev. Hydrobiol. trop.*, **21** (2), 135-151.

Geets, A., Malmberg, G. & Ollevier, F. (1998) *Gyrodactylus longidactylus* n. sp., a monogenean from *Pomatoschistus lozanoi* (de Buen, 1923) (Gobiidae) from the North Sea. *Systematic Parasitology,* **41**, (1), 63-70.

Gläser, H.-J. (1974) Eine neue Artgruppe des Subgenus *Gyrodactylus* (*Paranephrotus*) (Monogenea, Gyrodactylidae). *Zoologischer Anzeiger, Jena*, **192**, 3/4, 271-278.

Hamerlynck, O. & Cattrijsse, A. (1994) The food of *Pomatoschistus minutus* (Pisces, Gobiidae) in Belgian coastal waters, and a comparison with the food of its potential competitor *P. lozanoi. Journal of Fish Biology*, **44**, 753-771.

Harris, P. D. (1985) Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) from freshwater fishes in southern England, with a description of *Gyrodactylus rogatensis* sp. nov. from the bullhead *Cottus gobio* L. *Journal of Natural History*, **19**, 791-809.

Harris, P. D. (1993) Les interactions entre la reproduction et la biologie des populations chez les Monogènes Gyrodactylidae: revue. *Bulletin Français de la Pêche et de la Pisciculture*, **328**, 1, 47-65.

Llewellyn, J. Green, J. E. & Kearn, G. C. (1984) A check-list of monogenean (Platyheminth) parasites of Plymouth hosts. *Journal of the marine Biological Association of the United Kingdom*, **64**, 881-887.

Magnhagen, C. & Wiederholm, A.-M. (1982) Habitat and food preferences of *Pomatoschistus minutus* and *P. microps* (Gobiidae) when alone and together: an experimental study. *Oikos*, **39**, 152-156.

Malmberg, G. (1964) Taxonomical and ecological problems in *Gyrodactylus* (Trematoda, Monogenea). in: *Parasitic worms and aquatic conditions*. Ergens, R and Rysavy, B. (eds.), 29th October-2nd November 1962, Prague, Publishing House of the Czechoslovak Academi of Sciences, pp. 205-230.

Malmberg, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologi*, Serie 2, 23, 235pp

Miller, P. J. (1986) Gobiidae. in: Fishes of the North-eastern Atlantic and the Mediterranean. Eds. Whitehead, P. J. P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J. and Tortonese, E. Volume III, Paris UNESCO, 1019-1085.

Mo, T. A. (1991a) Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology*, **19**, 231-240.

Mo, T. A. (1991b) Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology*, **20**, 11-19.

Mo, T. A. (1993) Seasonal variation of opistaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *Salmo salar* L. parr in the River Sandviksela, Norway. *Systematic Parasitology*, **26**, 225-231.

Wallis, G. P. & Beardmore, J. A. (1984a) Genetic variation and environmental heterogeneity in some closely related goby species. Genetica, 62, 223-237.

Wallis, G. P. and Beardmore, J. A. (1984b) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces: Gobiidae). *Biological Journal of the Linnean Society*, **22**, 107-123.

Webb, C. J. (1980) Systematics of the *Pomatoschistus minutus* complex (Teleostei: Gobiidae). *Philosophical Transactions of the Royal Society of London, Serie B: Biological Sciences*, **291**, 201-241.

Chapter 5

Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach.

Geets, A.[1], Appleby, C.[2], and Ollevier, F.[1]

[1] *Laboratory for Ecology and Aquaculture, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*

[2] *National Veterinary Institute, P.O.Box 8156 Dep., N-0033 Oslo, Norway*

This chapter has been accepted in *Parasitology*

Abstract

Gyrodactylus arcuatus-like monogeneans collected from the gills from three goby species, *Pomatoschistus minutus*, *P. lozanoi* and *P. pictus,* from the Voordelta Area (SW Netherlands) were compared on a morphometric basis with *G. arcuatus* from its type host, the three-spined stickleback, *Gasterosteus aculeatus*. Univariate and multivariate analysis was carried out on measurements of 17 characters of the opisthaptoral hard parts of 268 specimens. The contribution of host-dependent and seasonal factors to the observed morphological variation is discussed. Univariate statistics (ANOVA) were used to detect features that were useful in separating the gyrodactylids from the different host groups. Subsequent factor analysis and discriminant analysis, combining all variables, led to the separation of three distinct forms (species) dependent on the host-species harbouring them. *G. arcuatus* (*sensu stricto*) was only present on *G. aculeatus*, while *P. minutus* and *P. lozanoi* carried a common species, *Gyrodactylus* sp. 1, and *P. pictus* hosted *Gyrodactylus* sp. 2. Seasonal variation in size of the sclerites was demonstrated on the *Gyrodactylus* species from *P. minutus* and *P. lozanoi*, and should be taken into consideration when specimens are compared. The findings are discussed

in view of the possibility of phylogenetic host-specificity of the gyrodactylids occurring on goby species that live sympatrically in the Voordelta area.

Introduction

The identification of monogeneans of the genus *Gyrodactylus* is mainly based on the dimensions and shapes of the hard parts of the opisthaptor. The knowledge of the excretory system can provide supplementary taxonomic information (Malmberg, 1970). However, ranges of commonly used measurements often overlap between similar species, which makes identification difficult on the bases of measurements only. Also, large variation in the shape and size of sclerites of specimens collected from the same host and season occur. On top of this, seasonal variation in size, linked to changing water temperature, has been observed in several *Gyrodactylus* species (Malmberg, 1970, Mo, 1991a, 1991b, 1991c, 1993, Appleby, 1996b).

In a previous study (Chapter 4) on the gyrodactylids from gobies from the Voordelta area, *Gyrodactylus arcuatus*-like specimens were found on three goby species, *Pomatoschistus minutus* (Pallas, 1770), *P. lozanoi* (de Buen, 1923) and *P. pictus* (Malm, 1865) (Geets & Ollevier, 1998). The type-host for *Gyrodactylus arcuatus* is the three-spined stickleback, *Gasterosteus aculeatus* L. Except for Appleby (1996a) who noted a *Gyrodactylus* sp. resembling *G. arcuatus* on sand gobies (*P. minutus*) from the Oslo Fjord, no other records of this species infecting gobies were found. This raised a question on the significance of the observed morphological variation between the *Gyrodactylus* cf. *arcuatus* specimens from the gobies and *G. arcuatus* from the three-spined stickleback. *Gyrodactylus arcuatus* is known to show considerable morphological variation. Harris (1993) reported that the variation of hamulus (anchor) dimensions is 5-15% of the mean. This was partly explained by the occasional sexual reproduction of *G. arcuatus*, which would imply higher genetic heterozygosity and hence higher morphological variation than in asexual species. Harris (1993) also noted considerable morphological differences between populations, even after allowance was made for the effect of water temperature. Although the ranges of the morphological features of the *G.* cf. *arcuatus* specimens partly overlapped with those of *G.*

arcuatus (Geets & Ollevier, 1998), significant morphological variation was observed. These observations urged an investigation of the species status of the *G. arcuatus*-like specimens from the three goby hosts and a more elaborate comparison with the *G. arcuatus* from its type-host, *Gasterosteus aculeatus*. To be able to assess host-dependent variation, we also set out to analyse the seasonal variation in the size of the hooks.

A multivariate approach seemed appropriate since it makes it possible to consider all morphological variables simultaneously. Silan & Maillard (1989) used morphometry and mathematical modelling to distinguish between two Monogenea, *Diplectanum aequans* and *D. laubieri* from the seabass, *Dicentrarchus labrax.* Bray & des Clers (1992) used principal component analysis (PCA) and discriminant analysis to analyse the multivariate differences between *Lepidapedon elongatum*-like worms from five gadiform fish hosts, and subsequently proposed to separate them as five oioxenic species. In taxonomical studies of digeneans, PCA has also been effective in discriminating two bucephalid digenean species from the genus *Rhipidocotyle,* with the description of a new species from the intestine of pike, *Esox lucius* (Gibson *et al.*, 1992). Höglund & Thulin (1992) applied PCA to identify two different *Diplostomum* species.

This study investigates the contribution of host-dependent and seasonal factors to the observed morphological variation of the opistaptoral hard parts of *Gyrodactylus arcuatus*-like specimens from three *Pomatoschistus* species and of *G. arcuatus* from *G. aculeatus*, in an attempt to provide a better description of the species present.

Monogeneans are known to have a high degree of host specificity (Rohde, 1978). Gyrodactylid monogeneans are holoxenous, hyperviviparous with embryos developing within each other inside the mothers uterus and asexual reproduction predominates in some *Gyrodactylus* species (e.g. *Gyrodactylus arcuatus*) (Harris, 1993). Their rapid reproduction in close relationship with the host will enhance the chance of co-evolution between hosts and parasites. Therefore, we will discuss our findings in view of possible phylogenetic host specificity and co-evolution between the Gyrodactylids and their goby hosts from the Voordelta Area.

Table 5.1: Overview of samples of *Gyrodactylus* cf. *arcuatus* studied from *Pomatoschistus minutus, P. lozanoi, P. pictus* and *Gyrodactylus arcuatus* from *Gasterosteus aculeatus* from different locations and seasons.

Host	Locality	Date (Season[1])	Water temperature (°C)	n Gyrodactylids measured	Method of fixation[3]
P. minutus (pm)	Voordelta	Jun. '93 (S1)	16.8	22	F
		Oct. '93 (A)	14.1	12	A
		Nov.'93 (A)	10.0	10	F
		Feb. '94 (W)	5.8	23	F
		Apr. '94 (SP)	8.2	14	A
		May '94 (SP)	12.1	21	F
		Jul. '94 (S2)	20.2	2	A
	Oostende Bank	Aug. '96 (S2)	18.5	5	A
				Total: 109	
P. minutus (pmo)	Oslo Fjord	Nov. '93 (A)	10.0	5	A
		Feb. '94 (W)	0.0	4	A
		Aug. '94 (S2)	19.5	1	A
				Total: 10	
P. lozanoi (pl)	Voordelta	Jun. '93 (S1)	16.8	23	F
		Oct. '93 (A)	14.1	18	F
				1	A
		Feb. '94 (W)	5.8	20	F
		May. '94 (SP)	12.1	8	F
				8	A
		Jun. '94 (SP)	14.5	2	F
				1	A
	Oostende Bank	Aug. '96 (S2)	18.5	4	A
				Total: 85	
P. pictus (pp)	Voordelta	Jun. '93 (S1)	16.8	1	F
		Jul. '93 (S1)	17.7	25	F
				Total: 26	
G. aculeatus (ga)	Stockholm	Jul. '56 (S1)	-	12[2]	A
	Doel	Sep. '97 (A)	15.8	4	A
	Yerseke	Oct '97 (A)	-	10	A
	Lozen	Nov. '97 (A)	-	5	A
				Total: 38	

[1] Season (range of water temperature): S1: summer 1 (16.8-17.7 °C), A: autumn (10-15.7 °C), W: winter (0-5.8 °C), SP: spring (8.2-14.5 °C), S2: summer 2 (18.5-20.2 °C)

[2] 2 specimens: all 17 features measured and 10 specimens: 10 features measured (LAR, LA, LAP, LVB, VBM, LH, LSI, LMH, LOOP, DWSI)

[3] F: Formaline fixed specimens, A: specimens fixed live in ammonium picrate glycerine

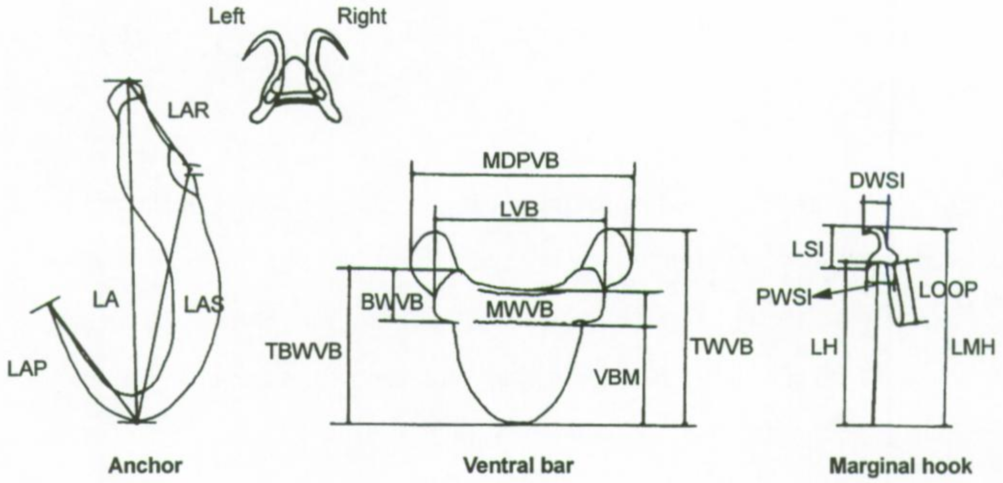
Material and methods

In total 268 specimens of *Gyrodactylus* cf. *arcuatus* were measured from four host species: three sympatric goby species, *Pomatoschistus minutus*, *P. lozanoi* and *P. pictus,* and the three-spined stickleback, *Gasterosteus aculeatus.* Table 5.1 gives details on the provenance of the specimens studied.

Most monogeneans were derived from the gills of formalin-fixed gobies, which were sampled monthly between June 1993 and July 1994 in the Voordelta Area (SW Netherlands, 51° 45'N, 3° 45'E). The gyrodactylids were mounted in ammonium picrate glycerine. Per season, approximately 20 nicely flattened specimens were used for measuring. In Table 5.1, these formalin-fixed specimens are indicated with 'F'. From *P. pictus*, only formalin-fixed specimens from the summer were available.

Additionally, live gyrodactylids from *P. minutus, P. lozanoi* and *G. aculeatus* were collected and mounted in ammonium picrate glycerine (fixation method A in Table 5.1). *Gyrodactylus* sp. from sand gobies from a bay near Oslo were compared to the specimens from *P. minutus* from the Voordelta and Oostend Bank. As a reference, specimens of *Gyrodactylus arcuatus* from its type-host *Gasterosteus aculeatus* were included in the study. Three-spined sticklebacks were obtained from three different habitats: a freshwater pond (Lozen, Belgium), a brackish (Doel, Belgium, ca. 10 ‰ salinity) and a marine station (Yerseke, The Netherlands, 30.1 ‰ salinity). The original measurements from *Gyrodactylus arcuatus* from *G. aculeatus* from the Stockholm region, provided by Dr. G. Malmberg, were also incorporated in the dataset. Throughout the introduction, material and methods and results, the gyrodactylids from gobies will be referred to as *Gyrodactylus* cf. *arcuatus*, since they clearly reminded of *G. arcuatus* from *G. aculeatus*, but their species status was still unclear because of the observed morphological variation.

Seventeen morphological features of the opisthaptoral hard parts were measured (Fig. 5.1) in accordance to the suggestions of Malmberg (1970), Harris (1985) and Shinn *et al*. (1996). Both left and right anchor were measured. All measurements were used for calculating the mean, variance and range. Since no significant difference between left and right anchor was



LAR: Length of anchor root
LAS: Length of anchor shaft
LA: Total length of anchor
LAP: Length of anchor point

LVB: Length of ventral bar
MDPVB: Maximal distance between ventral bar processes
MWVB: Median width of ventral bar
VBM: Length of ventral bar membrane
BWVB: Basal width of ventral bar
TBWVB: Total basal width of ventral bar
TWVB: Total width of ventral bar

LH: Length of handle
LSI: Length of sickle
LMH: Total length of marginal hook
LOOP: Length of filament loop
PWSI: Proximal width of sickle
DWSI: Distal width of sickle

Figure 5.1: Schematic overview of 17 morphological characters measured on the anchor, ventral bar and marginal hook of 268 *Gyrodactylus* cf. *arcuatus* specimens using light microscopy (phase contrast). Not drawn to scale.

Table 5.2: Mean in µm and coefficient of variation (C.V in % = 100 x the square root of the variance divided by the mean) for all 17 morphological features measured on *Gyrodactylus* cf. *arcuatus*, considered for all hosts pooled and for each host species separately. The range between brackets contains 95% of the measurements (-1.96 S.D, +1.96 S.D). (*feature measured for 28 out of 38 specimens of *Gyrodactylus arcuatus* from *Gasterosteus aculeatus*). For abbreviations see Figure 5.1.

Feature		All hosts	*P. minutus*	*P. minutus* Oslo	*P. lozanoi*	*P. pictus*	*G. aculeatus*
		n = 268	n = 109	n = 10	n = 85	n = 26	n = 38 (*= 28)
Anchor							
LAR	mean	7.3 (4.2-10.5)	6.3 (4.1-8.5)	9.3 (7.6-11.1)	6.9 (4.3-9.4)	8.7 (7.4-10.1)	9.8 (7.1-12.5)
	C.V.	23.3	18.0	9.4	18.9	7.9	13.9
LAS	mean	27.3 (23.4-31.2)	27.4 (25.0-29.9)	27.2 (24.1-30.3)	27.4 (23.2-31.5)	24.0 (21.9-26.0)	29.9 (26.3-33.6)*
	C.V.	7.7	4.6	5.7	7.7	4.4	6.3
LA	mean	34.3 (29.0-39.7)	33.5 (30.4-36.6)	34.9 (30.9-38.9)	33.9 (28.7-39.2)	32.2 (30.1-34.3)	38.9 (33.0-44.7)
	C.V.	8.4	4.7	5.9	7.8	3.3	7.7
LAP	mean	16.4 (13.1-19.6)	16.6 (13.9-19.2)	17.3 (15-4-19.3)	16.4 (12.8-20.1)	14.3 (13.2-15.3)	16.7 (13.3-20.1)
	C.V.	9.8	8.1	5.8	11.3	3.7	10.4
Ventral Bar							
LVB	mean	15.6 (11.3-20.1)	15.2 (13.2-17.1)	16.8 (15.2-18.4)	15.0 (12.3-17.7)	14.4 (12.9-15.9)	19.2 (12.5-26.0)
	C.V.	14.3	6.7	4.9	9.3	5.3	17.9
MDPVB	mean	19.3 (16.1-22.6)	19.0 (16.5-21.6)	20.3 (18.1-22.4)	18.9 (15.6-22.1)	18.2 (14.7-21.8)	22.3 (18.4-26.2)*
	C.V.	9.8	6.8	5.5	8.8	9.9	8.9
MWVB	mean	3.6 (2.6-4.7)	3.7 (2.6-4.8)	4.5 (3.6-5.4)	3.4 (2.6-4.2)	3.3 (2.5-4.1)	4.1 (3.0-5.3)*
	C.V.	15.8	14.6	10.1	12.1	12.0	14.1
VBM	mean	9.2 (5.8-12.6)	8.8 (5.6-11.9)	8.8 (6.9-10.7)	9.1 (5.9-12.4)	8.9 (7.5-10.4)	11.1 (7.7-14.4)
	C.V.	18.7	18.4	11.1	18.1	8.2	15.4
BWVB	mean	5.2 (3.4-7.1)	5.3 (3.8-6.7)	5.9 (4.4-7.4)	4.9 (2.6-7.2)	5.3 (4.1-6.6)	5.7 (4.5-6.9)*
	C.V.	17.7	13.9	12.7	23.9	12.1	10.6
TBWVB	mean	15.1 (12.0-18.2)	14.9 (11.7-18.2)	15.7 (14.4-17.1)	14.8 (12.0-17.7)	14.2 (11.4-17.1)	19.1 (13.9-20.2)*
	C.V.	11.2	11.0	4.4	9.8	10.2	9.3
TWVB	mean	18.7 (15.1-22.3)	18.3 (15.2-21.4)	19.7 (18.1-21.3)	18.0 (14.9-21.2)	17.5 (15.6-19.4)	22.7 (19.2-26.2)*
	C.V.	11.3	8.7	4.1	8.9	5.5	7.9
Marginal Hook							
LH	mean	16.2 (13.1-19.4)	16.2 (12.5-19.8)	16.7 (15.0-18.4)	15.4 (13.2-17.6)	17.0 (15.3-18.7)	17.7 (15.6-19.9)
	C.V.	10.1	11.6	5.3	7.4	5.2	6.2
LSI	mean	4.2 (3.4-4.9)	4.1 (3.5-4.7)	4.3 (3.7-4.9)	4.1 (3.4-4.8)	4.2 (3.7-4.8)	4.7 (3.8-5.5)
	C.V.	9.4	7.9	6.7	8.9	6.9	8.8
LMH	mean	19.8 (17.0-22.6)	19.3 (16.8-21.7)	20.5 (18.4-22.6)	19.1 (16.7-21.5)	20.7 (19.1-22.3)	21.8 (19.7-24.0)
	C.V.	7.7	6.4	5.2	6.4	3.9	5.0
LOOP	mean	7.1 (5.8-8.5)	7.2 (5.9-8.6)	6.6 (5.7-7.6)	7.2 (5.7-8.6)	7.5 (6.0-9.0)	6.7 (5.6-7.8)
	C.V.	10.1	9.4	7.2	10.3	10.4	8.5
PWSI	mean	3.6 (3.1-4.2)	3.6 (3.1-4.2)	3.4 (3.0-3.9)	3.6 (3.0-4.2)	3.6 (3.1-4.1)	3.7 (3.2-4.2)*
	C.V.	7.9	8.0	6.7	8.3	6.9	6.6
DWSI	mean	2.6 (1.9-3.4)	2.6 (2.1-3.2)	2.5 (2.3-2.7)	2.6 (2.0-3.2)	2.6 (2.1-3.1)	2.8 (1.5-4.2)
	C.V.	14.4	10.8	3.9	11.6	8.9	24.7

detected (T-test for dependent samples, $p>0.05$), the mean of the measurements of the left and right anchor from the same specimen was used in the multivariate dataset.

Two methods of measuring were tried out: using drawings made with a drawing mirror on an Olympus microscope (100 x, oil immersion objective, light microscopy and phase contrast) and using prints from a video print system on a Leitz microscope (90 x oil immersion objective, light microscopy and interference contrast). Both methods showed to be equally reliable for the live fixed gyrodactylids. For the formalin-fixed specimens, however, the drawing method gave more satisfying results because these specimens are not always completely flattened. There was no significant difference in size between formalin-fixed and ammonium picrate glycerine-fixed specimens from the same host and season (T-test for independent samples, $p>0.05$ for all measured features).

The morphological features are described by their means and their relative variability expressed by the coefficient of variation (C.V.= 100 x the square root of the variance divided by the mean). Correlation between features was analysed using Pearsons correlation coefficient.

A one-way analysis of variance (ANOVA) of each feature was used to test the hypothesis of significant differences of the means between the specimens of the distinct host groups, against the null hypothesis of a common form. Tukey's honest significant difference (HSD) test for unequal sample sizes was performed for *post hoc* multiple comparisons between the means of each group. The raw dataset was used as the assumptions for analysis of variance (normal distribution, homogeneity of variances and no correlation of means and variances) were fulfilled. Statistical analysis was carried out with STATISTICA 4.1 for Windows and p-levels were set on 0.05.

A factor analysis was run to detect the structure in the relationship between the variables, and to get an exploratory view of the relative contribution of each variable to the overall variability of the measurements. A forward stepwise discriminant analysis was used to determine the morphological features which contribute most to the discrimination between the gyrodactylids of the different hosts. If specimens from distinct hosts form separate groups, this may suggest that different species are involved.

Results

In Table 5.2, the mean, 95% confidence intervals and the coefficient of variation of each feature are given for all hosts combined and for each host species separately. The highest coefficients of variation were found when all host groups are pooled. The variability is reduced when each host group is considered separately. Seasonal variation could be involved, since the lowest coefficients of variation are noted in the *Pomatoschistus pictus* group, for which only summer samples were available.

The length of the anchor root (LAR), the length of the ventral bar membrane (VBM) and the distal width of the marginal hook sickle (DWSI) are the most variable. A first source of variability could be the measuring error. This certainly has to be taken into consideration for the smallest features (e.g. DWSI). Also, a curved feature (e.g. DWSI) will have a higher variability than a linear measurement of a feature of the same size (e.g. PWSI). Furthermore, the rigidity of a structure seems may play a role in the observed variability. The anchor root, for example, is the softest part of the anchor and it shows the highest coefficient of variation. In formalin-fixed specimens, the anchor root was generally more curved, making accurate measuring more difficult, than in live fixed specimens. The high variability in the ventral bar membrane measurements can at least partly be explained by the sometimes poor visibility of this structure in the slides.

Pearson's correlation coefficients between all variables are shown in Table 5.3. High correlations between variables indicate that these variables are likely to provide redundant information on the variability between the gyrodactylids of different host species. For example, the total length of the anchor (LA) and the length of the anchor shaft (LAS) are highly positively correlated ($r = 0.84$). They are both measured along the same direction of the anchor and they would be almost 100 % correlated if it were not that the measurements of the anchor roots were so variable (cf. supra measuring error).

Table 5.3: Pearson's correlation coefficients for 17 morphometric features, measured on *Gyrodactylus arcuatus*-like monogeneans on four host species. Correlations in bold are significant at $p < 0.05$. Correlations >.70 are underlined. For abbreviations see Figure 5.1.

n = 258	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 LAR	1.00																
2 LAS	**.13**	1.00															
3 LA	**.59**	**.84**	1.00														
4 LAP	**.13**	**.51**	**.50**	1.00													
5 LVB	**.42**	**.60**	**.65**	**.30**	1.00												
6 MDPVB	**.42**	**.52**	**.59**	**.25**	**.77**	1.00											
7 MWVB	**.26**	**.37**	**.40**	**.27**	**.50**	**.42**	1.00										
8 VBM	**.24**	**.18**	**.24**	-.03	**.20**	**.22**	.04	1.00									
9 BWVB	**.29**	**.27**	**.36**	**.27**	**.41**	**.32**	**.34**	**-.38**	1.00								
10 TBWVB	**.32**	**.38**	**.44**	.11	**.44**	**.43**	**.30**	**.79**	-.05	1.00							
11 TWVB	**.50**	**.57**	**.66**	**.19**	**.65**	**.58**	**.39**	**.62**	**.17**	**.80**	1.00						
12 LH	**.26**	.08	**.16**	.01	**.22**	**.22**	**.21**	-.08	**.31**	.04	**.16**	1.00					
13 LSI	**.38**	**.28**	**.41**	**.16**	**.39**	**.36**	**.20**	-.01	**.35**	**.13**	**.27**	**.34**	1.00				
14 LMH	**.47**	.12	**.29**	-.03	**.35**	**.39**	**.26**	**.19**	**.13**	**.30**	**.37**	**.71**	**.52**	1.00			
15 LOOP	-.11	-.09	-.07	.09	-.02	-.06	.03	-.11	.10	**-.13**	**-.17**	**.16**	.05	.04	1.00		
16 PWSI	**.14**	**.17**	**.24**	**.23**	**.27**	**.25**	.09	-.08	**.17**	.03	.08	.11	**.26**	**.16**	**.17**	1.00	
17 DWSI	**-.13**	.05	.01	**.13**	.05	.03	-.06	-.04	.00	.03	-.01	-.01	.04	-.03	**.16**	**.36**	1.00
	Anchor				Ventral Bar							Marginal Hook					

Table 5.4: Analysis of Variance testing for the effect of different host species on morphological traits of *Gyrodactylus* cf. *arcuatus*. (F-values for main effect). Large F-values are given in bold. Differences are significant at p<0.01 unless indicated n.s. (= not significant). For abbreviations see Table 5.1 and Figure 5.1.

Season:	All	Summer 1	Autumn
Host:	pm, pl, pp, pmo, ga	pm, pl, pp, ga	pm, pl, ga
N measured:	109, 85, 26 ,10 ,38	22, 23, 26, 12	22, 19, 26
Variable	df Effect: 4 df Error: 263	df Effect: 3 df Error: 79	df Effect: 2 df Error: 64
LAR	**92.21**	**52.63**	**88.88**
LAS	**47.77**	**37.85**	**42.96**
LA	**54.12**	**93.83**	**54.47**
LAP	15.52	30.77	0.66 n.s.
LVB	**54.51**	**117.93**	**49.18**
MDPVB	32.67	2.89	**59.74**
MWVB	25.20	9.17	17.43
VBM	16.00	30.64	13.02
BWVB	6.20	15.26	22.19
TBWVB	15.25	7.68	21.25
TWVB	**57.74**	28.9	**63.28**
LH	19.13	**49.66**	16.55
LSI	21.88	26.09	22.37
LMH	**46.76**	**52.37**	**42.45**
LOOP	7.41	7.44	7.98
PWSI	1.82 n.s.	0.20 n.s.	0.98 n.s.
DWSI	4.59	28.79	0.87 n.s.

Therefore, the length of the anchor shaft (LAS) may be a more reliable feature to measure than the total anchor length (LA) to reveal differences between closely related *Gyrodactylus* species, since the former does not include the anchor root.

The features measured on the same structure (anchor, ventral bar or marginal hook) show higher correlations than when measured on different structures. Some examples include: the total length of the anchor (LA) and shaft length (LAS) ($r = 0.84$), ventral bar length (LVB) and maximal distance between the processes of the ventral bar (MDPVB) ($r = 0.77$), total length of the marginal hook (LMH) and length of handle (LH) ($r = 0.71$). This implies that taking measurements on different structures (anchors, marginal hooks or ventral bar) yield complementary information.

A one-way analysis of variance (ANOVA) on each measured feature gives an indication of the significance of the host-dependent morphological variation of the parasites. Table 5.4 gives an overview of the F-values for the overall difference between the groups. The higher the F-value, the larger the overall difference between the groups. First, an ANOVA was conducted with all hosts of all seasons pooled. Subsequently, only specimens from summer or autumn were selected for the ANOVA, to exclude the possibility of seasonal variation causing separation of the groups. The measurements of the gyrodactylids from *P. minutus* from Oslo were not included in the analysis of the autumn sample, since it concerned only 5 specimens. Almost all variables showed a highly significant difference ($p < 0.01$) between the gyrodactylids from the different host groups. Five features (LAR, LAS, LA, LVB, LMH) separate the groups well (high F-values) in both seasons and in the overall dataset. LAP only separated the groups when the parasites from *P. pictus* were included (All and Summer 1). In Figure 5.2, the mean, standard error and standard deviation of these features are shown for each host group. *Gyrodactylus arcuatus* from its type-host *G. aculeatus* had significantly larger anchors (LA, LAR, LAS) than the specimens found on the three gobies (*P. minutus*, *P. lozanoi* and *P. pictus*) (Tukey's Honest Significant Difference Test for unequal sample size). Also the ventral bar (LVB) and the marginal hooks (LMH) were longer. The results were consistent for both seasons. The *Gyrodactylus* cf. *arcuatus* specimens of *P. pictus* also seemed to be rather different from those of the other hosts from the same season. The anchor shaft (LAS) and the anchor point (LAP) were significantly shorter.

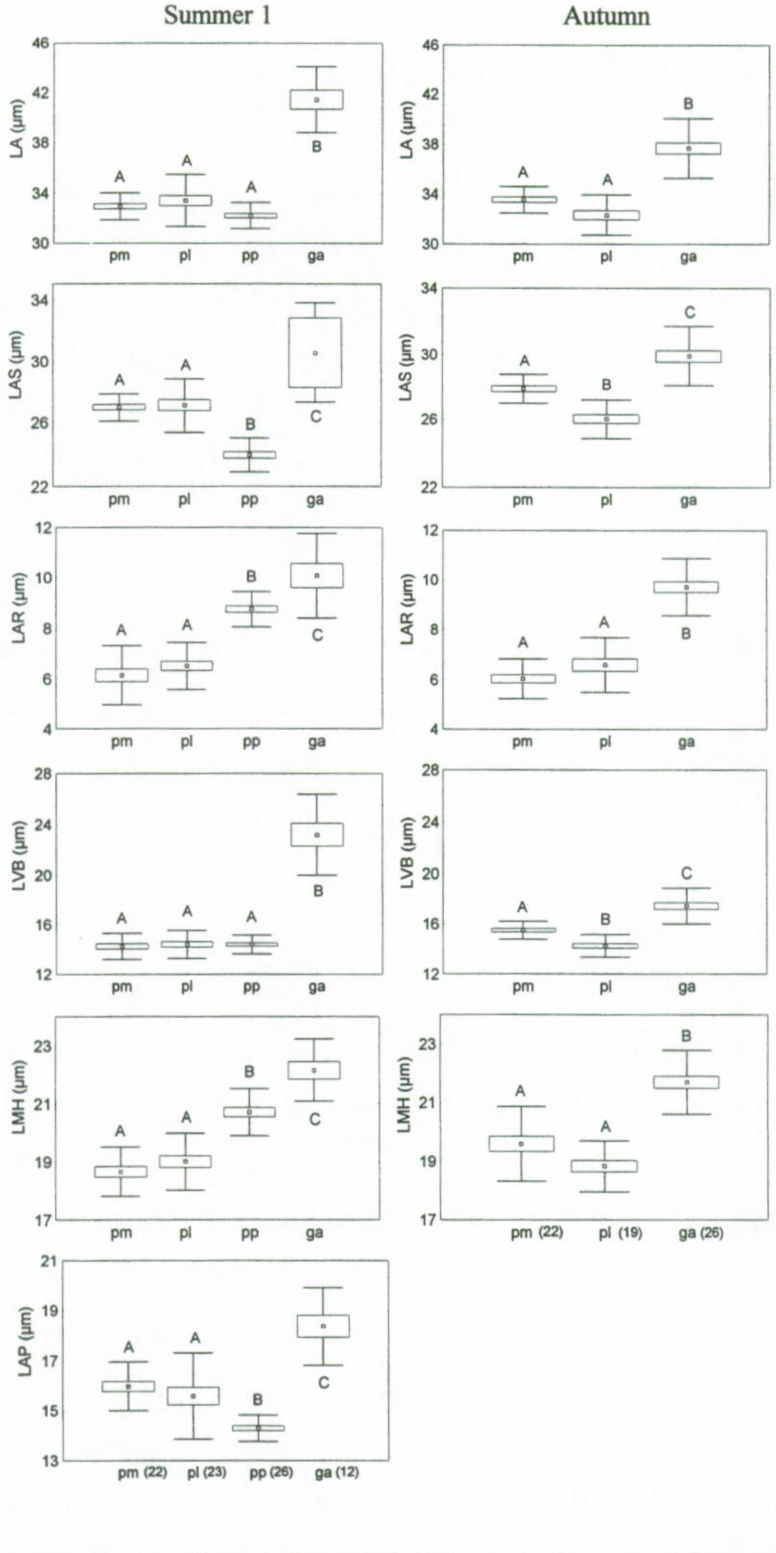


Figure 5.2: Mean, standard error (box) and standard deviation (whiskers) of characters measured on the opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* which give a good separation (ANOVA) between the host groups in two seasons: summer and autumn. For abbreviations see Figure 5.1 and Table 5.1. Letters indicate the significant different groups ($p<0.05$, Tukey's HSD test for unequal sample sizes). Number of gyrodactylids measured per host group are indicated in the lowermost graphs.

The marginal hooks (LMH) were longer than those of the monogeneans found on *P. minutus* and *P. lozanoi*, but significantly shorter than the ones of *G. arcuatus* from the sticklebacks. The *Gyrodactylus* specimens from *P. minutus* and *P. lozanoi* could not readily be separated from each other using the univariate approach.

Figure 5.3A presents a biplot of the first plane of a factor analysis (principal component analysis on the correlation matrix) of all 17 variables and all cases (all host groups and all seasons). Four factors have an eigenvalue exceeding 1.00. The first two factors account for 46.3 % of the total variance. Factor 1, representing the highest variability found in the sample, is determined mainly by measurements of the anchor (LAS, LA) and of the ventral bar (LVB, MDPVB and TWVB). Factor loadings (which are the correlation coefficients between the variable and the respective factor) for these variables on factor 1 are larger than 0.70. On the second factor, only the length of the ventral bar membrane (VBM) has a substantial factor loading (>0.70). Since the variability of the length of the ventral bar membrane could at least partly be caused by measuring errors (cfr. supra), a subsequent analysis, excluding this variable was carried out. In a factor analysis without VBM, the first factor is determined by LAS, LA, LVB, MDPVB and TWVB, while the second factor is determined by variables of the marginal hooks, LH and LMH. The overall conclusions about the grouping of the different host groups were the same as when VBM was included.

Plotting the variables in a biplot also gives insight in the relationships between them. The closer variables plot together, the more they are correlated. For example, the measurements on the anchor (LAR, LA, LAS) cluster all together, except for the length of the anchor point (LAP). This implies that measuring the anchor point provides additional information on the encountered variability of the *Gyrodactylus* specimens.

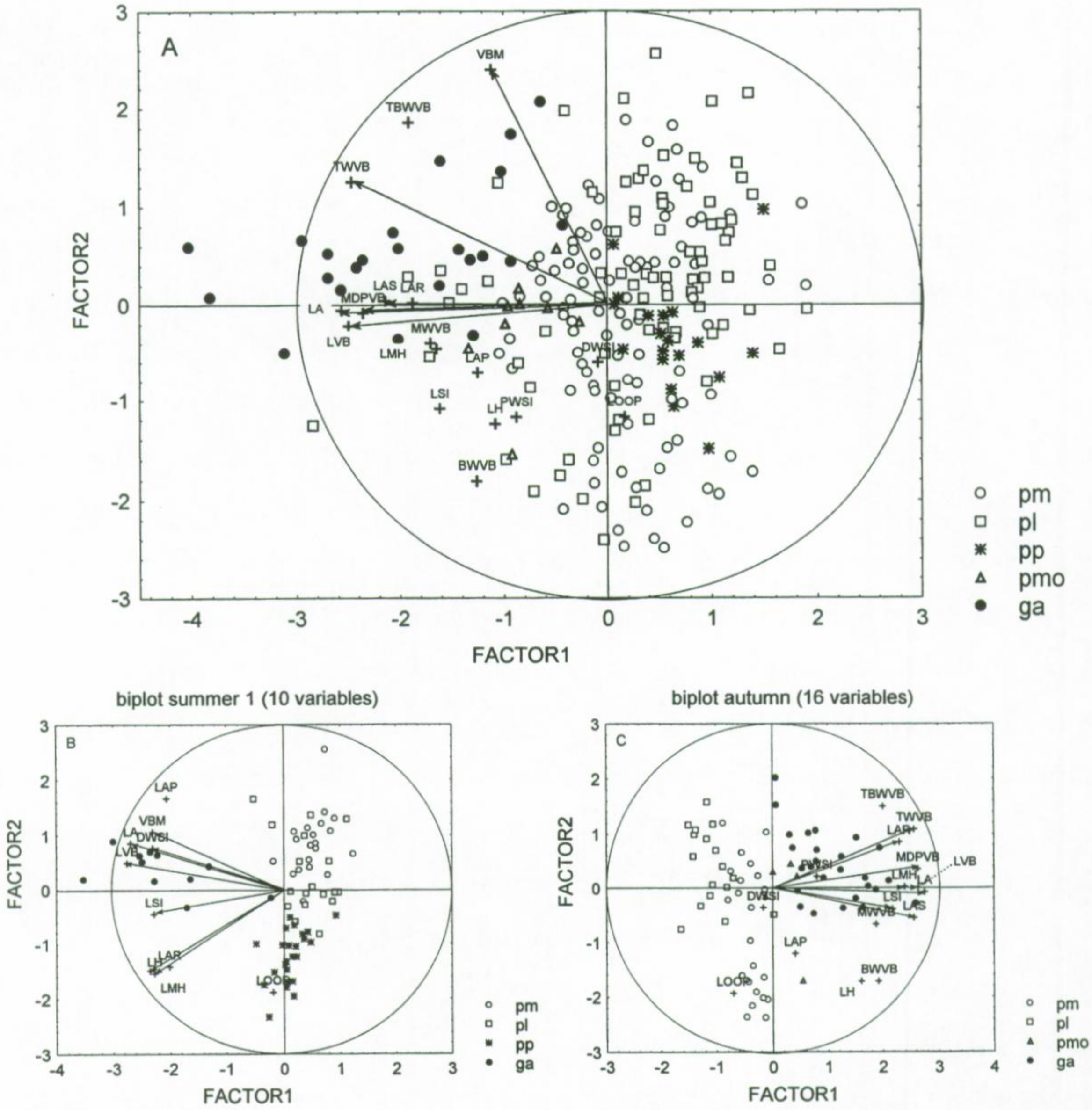


Figure 5.3: Factor analysis on measurements of opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from different host groups. Biplots: A: all gyrodactylids from all seasons, B: only gyrodactylids from summer 1 sample, C: only gyrodactylids from autumn samples. Circle indicates a factor loading of 1.0. Arrows indicate variables with factor loadings > 0.70. For abbreviations of features see Figure 5.1. Host species groups: pm= *Pomatoschistus minutus* from Voordelta and Oostend Banks, pl= *P. lozanoi*, pp= *P. pictus*, pmo= *P. minutus* from Oslo Fjord, ga= *Gasterosteus aculeatus.*

As shown in the biplot (Fig. 5.3A), the first factor partly separates the gyrodactylids of the different host groups. Specimens of *G. arcuatus* from sticklebacks (*Gasterosteus aculeatus*) are clearly separated from individuals from *P. pictus*. The gyrodactylids from the three gobies are not clearly separated from each other by this analysis. Furthermore, the clusters of specimens of *P. minutus*, *P. minutus* from Oslo and *P. lozanoi* seem to overlap partly with the stickleback cluster. But, one must take into account that seasonal variation within the host groups might cause some overlap of the groups. An analysis per season, on summer and autumn samples respectively, indeed gave a better separation according to the host groups (Figs 5.3B and 5.3C). In both graphs, the *Gyrodactylus arcuatus* specimens from the sticklebacks form a separate cluster from the gyrodactylids from gobies. The specimens found on *P. pictus* also seems to differ from the ones on the other two goby hosts. The autumn sample includes only five specimens from the sand gobies from Oslo and these cluster between the specimens from *P. minutus* and those from *Gasterosteus aculeatus*.

A forward stepwise discriminant analysis was carried out to look for the best possible discrimination between the gyrodactylids of the different host groups. A graphic presentation of the discriminant analysis on 17 variables for gyrodactylids from all host groups (regardless of season) is shown in Figure 5.4. Root 1 and root 2 describe 90 % of the total variability in the sample. The multivariate differences between the gyrodactylids agreed well with the hypothesis that different forms (species) of gyrodactylids occur on the least related host species. The discriminant analysis gave a better discrimination of the groups than the factor biplots.

Table 5.5: Summary of the forward stepwise discriminant function analysis. Number of variables in the model: 17 (for abbreviations see figure 5.1); grouping: 5 host groups (pm, pmo, pl, pp, ga; for abbreviations of features see Table 5.1) N = 258; *=p<0.05; F to enter: 1.0; F to remove: 0.0.

Feature	Wilks' Lambda	Partial Lambda	F-remove (4, 237)	Tolerance
LAR	0.065	0.670	29.16*	0.19
LAS	0.054	0.814	13.53*	0.08
LAP	0.065	0.671	29.05*	0.53
LA	0.052	0.844	10.91*	0.06
MWVB	0.051	0.858	9.76*	0.78
LMH	0.047	0.928	4.56*	0.40
TWVB	0.052	0.843	11.02*	0.28
TBWVB	0.048	0.909	5.93*	0.22
LOOP	0.047	0.931	4.35*	0.83
DWSI	0.047	0.936	4.02*	0.80
LH	0.046	0.959	2.47*	0.46
PWSI	0.046	0.946	3.33*	0.70
LVB	0.046	0.951	3.02*	0.40
MDPVB	0.046	0.949	3.17*	0.53
BWVB	0.046	0.954	2.81*	0.46
VBM	0.045	0.970	1.79	0.24
LSI	0.045	0.979	1.25	0.67

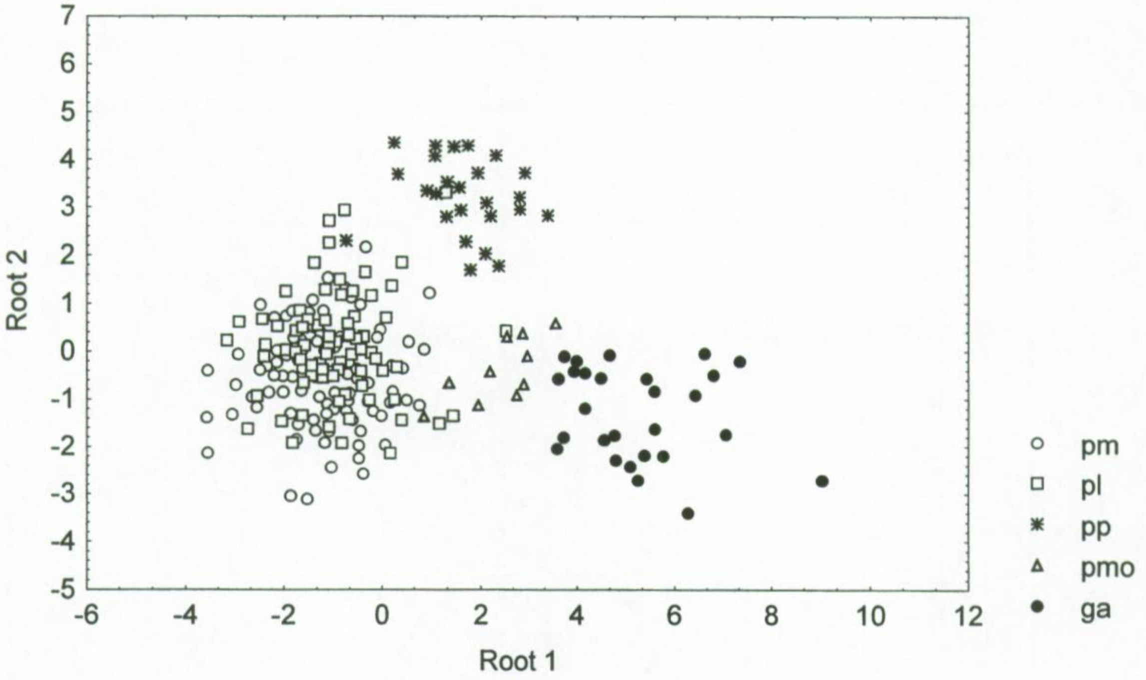


Figure 5.4: Plot of forward stepwise discriminant analysis (root 1 vs. root 2) of morphological measurements on 258 *Gyrodactylus* cf. *arcuatus* specimens from five host groups. Host species groups: pm= *Pomatoschistus minutus* from Voordelta and Oostend Banks, pl= *P. lozanoi*, pp= *P. pictus*, pmo= *P. minutus* from Oslo Fjord, ga= *Gasterosteus aculeatus*.

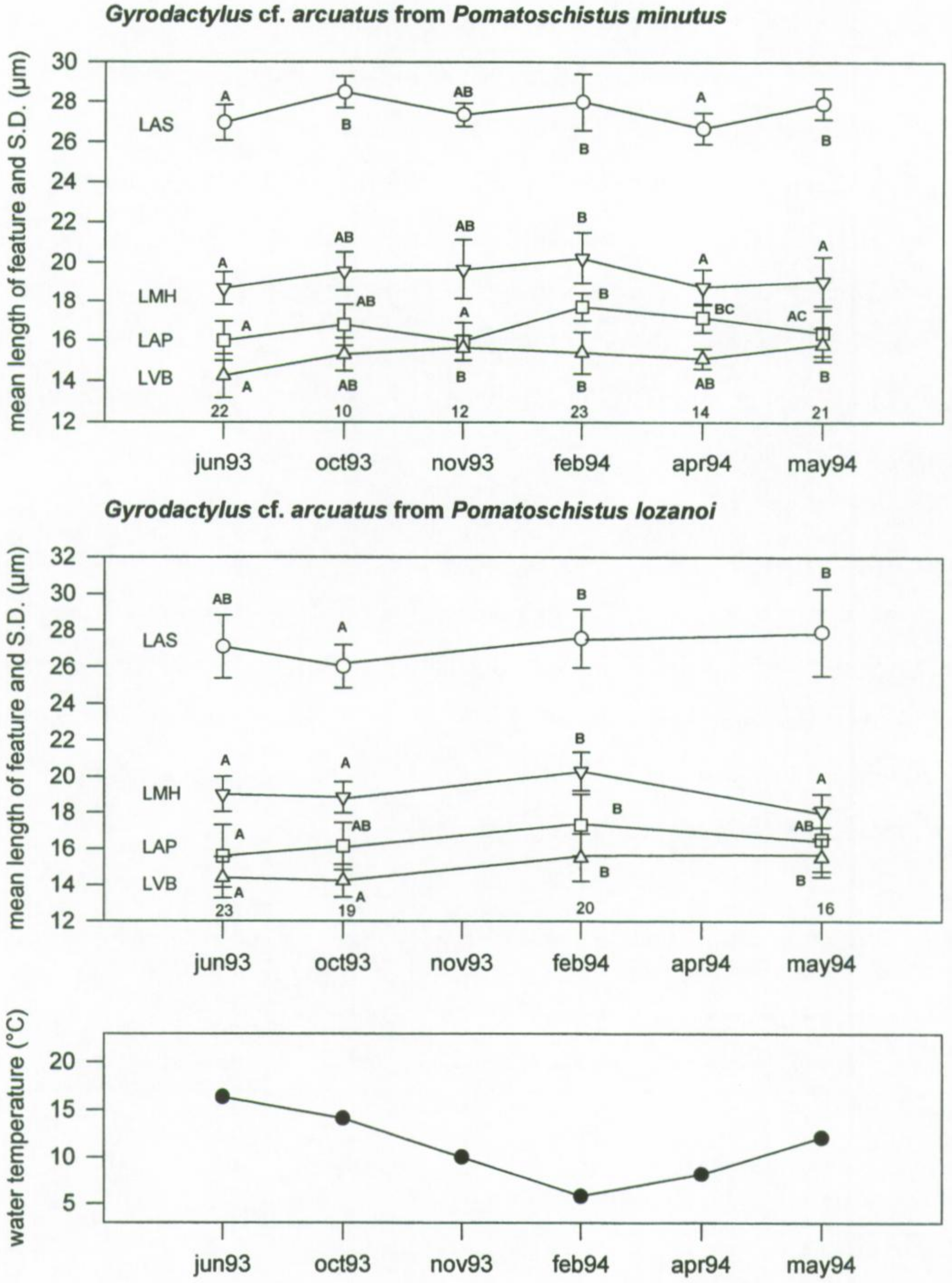
The gyrodactylids from *P. pictus* and the *G. arcuatus* specimens from *G. aculeatus* both separate clearly from each other and from the other host groups. Root 1 is responsible for the split off of the *Gyrodactylus arcuatus* specimens of *G. aculeatus* and root 2 for the *P. pictus* specimens. Again, this analysis can not clearly distinguish the monogeneans from *P. minutus* and *P. lozanoi* on a morphometric basis. The specimens of *P. minutus* from Oslo gain a position in between the gobies of the Voordelta and the sticklebacks. Table 5.5 summarizes the results of the stepwise discriminant analysis. The partial Wilk's lambda denotes the unique contribution of the respective variables to the discrimination of the *a priori* defined groups. The lower its value, the greater discriminatory power of the variable. In our analysis, the length of the anchor root (LAR) contributes most, followed by the length of anchor point LAP, LAS, TWVB, LA,...

The classification matrix (Table 5.6) gives an overview of how well the model classifies cases in the *a priori* defined groups. The gyrodactylids from *P. minutus* and *P. lozanoi* have the highest percentage of misclassification. But taking a closer look, it turns out that they are mostly misclassified amongst each other: 25 % of *P. minutus* specimens are classified as having *P. lozanoi* as a host and 20 % vice versa. This again suggests a common *Gyrodactylus* cf. *arcuatus* species on *P. minutus* and *P. lozanoi*.

Table 5.6: Classification matrix for the discriminant analysis on measurements of *Gyrodactylus* cf. *arcuatus* belonging to *a priori* defined host groups.

Group	percent correct	pm p=0.20	pl p=0.20	pp p=0.20	pmo p=0.20	ga p=0.20	observed total
P. minutus (pm)	70.6	77	28	1	3	0	109
P. lozanoi (pl)	71.8	17	61	4	3	0	85
P. pictus (pp)	100.0	0	0	26	0	0	26
P. minutus Oslo (pmo)	100.0	0	0	0	10	0	10
G. aculeatus (ga)	96.43	0	0	0	1	27	28
Total	77.91	94	89	31	17	27	258

The opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from *P. minutus* and *P. lozanoi* of the Voordelta Area vary seasonally in size (Fig. 5.5). Four characters (LAS, LAP, LVB and LMH) were selected for the analysis. These characters are easily measurable and represent the three main structures: anchors, ventral bar and marginal hooks. Significant differences between the means of each month were detected with the Tukey's HSD test for unequal



Gyrodactylus* cf. *arcuatus* from *Pomatoschistus minutus

Gyrodactylus* cf. *arcuatus* from *Pomatoschistus lozanoi

Figure 5.5: Seasonal variation in size of four morphological features of the opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from *P. minutus* and *P. lozanoi* of the Voordelta Area. Letters indicate significant differences in size between months ($p < 0.05$, Tukey HSD test for unequal sample sizes). Numbers of specimens measured per month are indicated at the bottom of the graphs. For abbreviations see Figure 5.1.

The opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from *P. minutus* and *P. lozanoi* of the Voordelta Area vary seasonally in size (Fig. 5.5). Four characters (LAS, LAP, LVB and LMH) were selected for the analysis. These characters are easily measurable and represent the three main structures: anchors, ventral bar and marginal hooks. Significant differences between the means of each month were detected with the Tukey's HSD test for unequal sample size. In general, the largest opisthaptoral hard parts were found in the months with the lowest water temperature (February 1994, 5.8 °C). In June 1993, when water temperature reached its maximum (16.8 °C), the hooks were significantly smaller than in other months (except for LAS of specimens from *P. lozanoi*). The observed seasonal variation was partly responsible for the overlap of the *Gyrodactylus* groups of the different hosts in the factor analysis biplot when all data were pooled.

Discussion

Morphometry on 17 features of the anchors, ventral bar and marginal hooks of *Gyrodactylus arcuatus*-like specimens from four host species was carried out to gain insight in the host-dependent and seasonal variations in size of the sclerites. Because of the overlap in the range of most variables, it is not easy to distinguish readily between the *Gyrodactylus* cf. *arcuatus* species from the goby hosts and *G. arcuatus* from its type-host, *Gasterosteus aculeatus*. The use of multivariate analysis, combining different morphological variables, results however in a seperation of distinct 'forms' dependent on the host species harbouring them. The question arises which status should be given to these forms. What at first was regarded as host-dependent variation of possibly the same *Gyrodactylus* species, in casu *G. arcuatus*, seems to be the variation between morphologically very similar but nevertheless distinct species of Gyrodactylidae. Preliminary analysis of the genetic variation between *G.* cf. *arcuatus* from *P. minutus* and *P. lozanoi* and *G. arcuatus* from *Gasterosteus aculeatus* seem to confirm the existence of a distinct species on the gobies, which is clearly different from *G. arcuatus* found on the three-spined stickleback (Marek Zietara, personal communication).

From our results, it can be concluded that *G. arcuatus* (*sensu stricto*) was only found on the three-spined stickleback, *G. aculeatus,* and not on the goby hosts. *G. arcuatus* had significantly longer anchors (LA, LAS, LAR), a longer ventral bar (LVB) and longer marginal hooks (LMH) than the *Gyrodactylus* specimens from gobies. Sticklebacks collected in the same seine hauls with sand gobies in the Oslo Fjord were never infected with anything resembling *G. arcuatus*, only *G. branchicus* was found (Chris Appleby, personal observation). This observation also provides strong evidence for a strict host specificity of the *G.* cf. *arcuatus* species towards gobies.

The monogeneans found on the painted goby, *P. pictus*, could clearly be distinguished from the specimens infecting the other goby hosts (*P. minutus* and *P. lozanoi*), in having much smaller anchor shafts (LAS) and anchor points (LAP) and longer marginal hooks (LMH). Since only formalin-fixed specimens from one season (June-July 1993) were available for this study, more specimens should be studied to formally describe the species. Untill then, the use of the name *Gyrodactylus* sp. 2 is proposed.

Pomatoschistus minutus and *P. lozanoi* harboured a common species, which will be indicated as *Gyrodactylus* sp. 1 until a complete discription of the species can be presented. Differneces observed between specimens of *Gyrodactylus* sp. 1 from the two hosts, were not large enough to seperate them on a morphometric basis. Preliminary genetic data seem to confirm that we are indeed dealing with one species parasitizing both hosts (Marek Zietara, personal communication). Phylogenetic analysis based on molecular markers could shed more light on the taxonomic basis of the observed variability. Molecular techniques are increasingly being used in parasitology as a tool to identify closely related species. Cunningham *et al.* (1995a, 1995b) employed DNA technology to distinguish *G. salaris* from other gyrodactylids found on salmonids in the UK.

When identifying gyrodactylid species on a morphological basis, variation in size caused by differences in water temperature should be taken into account. In our study too, the distinction between the different gyrodactylids of the respective host groups became more clear when only specimens from the same season (water temperature) were compared. Within *Gyrodactylus* sp. 1 a tendency for bigger hooks in colder periods and *vice versa* was observed, although this pattern was not as clear as reported in other studies. Mo (1991a, 1991b, 1991c) showed a significant relationship between the size of opisthaptoral hard parts of *G. salaris* on

Salmo salar and *Onchorhynchus mykiss* and water temperature. Decreasing length of the hard parts with increasing water temperature was also noted in *G. callariatis* from *Gadus morhua*. An age difference of these worms did not influence the size of the hard parts (Appleby, 1996b). Both authors pointed out that the shape of the hooks did not change with water temperature.

Morphological differences between gyrodactylids specimens from brackish water and freshwater populations were reported for *G. phoxini* (Malmberg, 1964) and *G. arcuatus* (Malmberg, 1970). The specimens from *P. minutus*, *P. lozanoi* and *P. pictus* and from *G. aculeatus* from Doel and Yerseke are all from marine or brackish habitats. We were unable to find clear differences between the *G. arcuatus* specimens from the freshwater sticklebacks (Lozen) and those from brackish (Doel) or marine (Yerseke) origin. Harris (1985) suggests a marine origin of *G. arcuatus*. *G. arcuatus* has a wide salinity tolerance and its closest relatives are found in species infecting gadids (Malmberg, 1970). It is striking that *P. minutus*, *P. lozanoi* and *P. pictus*, all preferring more marine habitats, harboured *G.* cf. *arcuatus*, while *P. microps*, which is the most euryhaline species, was not infected.

The present findings further constitute to the evidence for (phylogenetic) host specificity of gyrodactylids for their goby hosts. Host-specificity was already indicated in a previous study, describing other gyrodactylid species found on the sympatric gobies of the Voordelta and surrounding areas (Geets & Ollevier, 1998). Rohde (1978), studying differences in host-specificity of Monogenea and Digenea at different latitudes, concluded that monogenea have a high degree of host-specificity at all latitudes. The dependence of parasites on a particular host species led to the postulation of the existence of co-evolution between parasites and hosts (Llewellyn & Tully, 1969, Anderson & May, 1982, May & Anderson, 1983, Toft & Karter, 1990, Dobson & Merenlender, 1991). High phylogenetic host-specificity may enhance co-evolution between host and parasite and could lead to a hypothetically perfect agreement between host and parasite phylogenies (Guégan & Agnèse, 1991). In this context, Guégan & Agnèse (1991) studied the phylogenetic relationships between 5 *Labeo* species and 14 *Dactylogyrus* species. They concluded that phylogenetic evolution, known as the Farenholz' rule (Eichler, 1948), as well as non-phylogenetic evolution (through sequential colonization and host switching) were displayed.

We summarize our findings by showing the phylogenetic tree suggested by Wallis & Beardmore (1984) for the gobies together with the different species (forms) of gyrodactylids found (Fig. 5.6). *P. minutus* and *P. lozanoi*, both phylogenetically closely related, harbour the same (or a closely related) species, *Gyrodactylus* sp. 1. Preliminary data on the genetic variability of *Gyrodactylus* sp.1 from both *P. minutus* and *P. lozanoi* indeed confirms that we are dealing with one species (Marek Zietara, personal communication). The painted goby, *P. pictus*, which according to Wallis & Beardmore (1984) speciated much earlier, harbours a clearly distinct form, *Gyrodactylus* sp. 2. The common goby, *P. microps*, did not harbour any of the *G. arcuatus*-like species described here, but has its own, still undescribed, species, *Gyrodactylus sp.* in the marine samples of the Voordelta while *G. micropsi* parasitized the gills of the brackish water population of *P. microps* from Doel. Another recently described species, *Gyrodactylus longidactylus*, was oioxenic on *P. lozanoi* (Geets *et al.*, 1998). *Gyrodactylus rugiensis*, previously described from *P. minutus* (Gläser,1974), was found on *P. lozanoi* on one occasion (Geets & Ollevier, 1998). For the *Gyrodactylus arcuatus*-like species (*Gyrodactylus* sp. 1 and *Gyrodactylus* sp. 2) our data suggest that phylogenetic evolution may have played a role. The closer the phylogenetic relationship of the gobies, the more (morphologically) similar the gyrodactylids. Whether these morphological similarities are a reflection of their (phylo-) genetic similarities need further research using molecular tools. Further research is planned to investigate the genetic basis of this intriguing observation. This research could provide more insight into the phylogeny of the *Gyrodactylus* species found on gobies and verify if the Farenholtz' rule, 'parasite phylogeny mirrors host phylogeny', also stands for the *Pomatoschistus-Gyrodactylus* model.

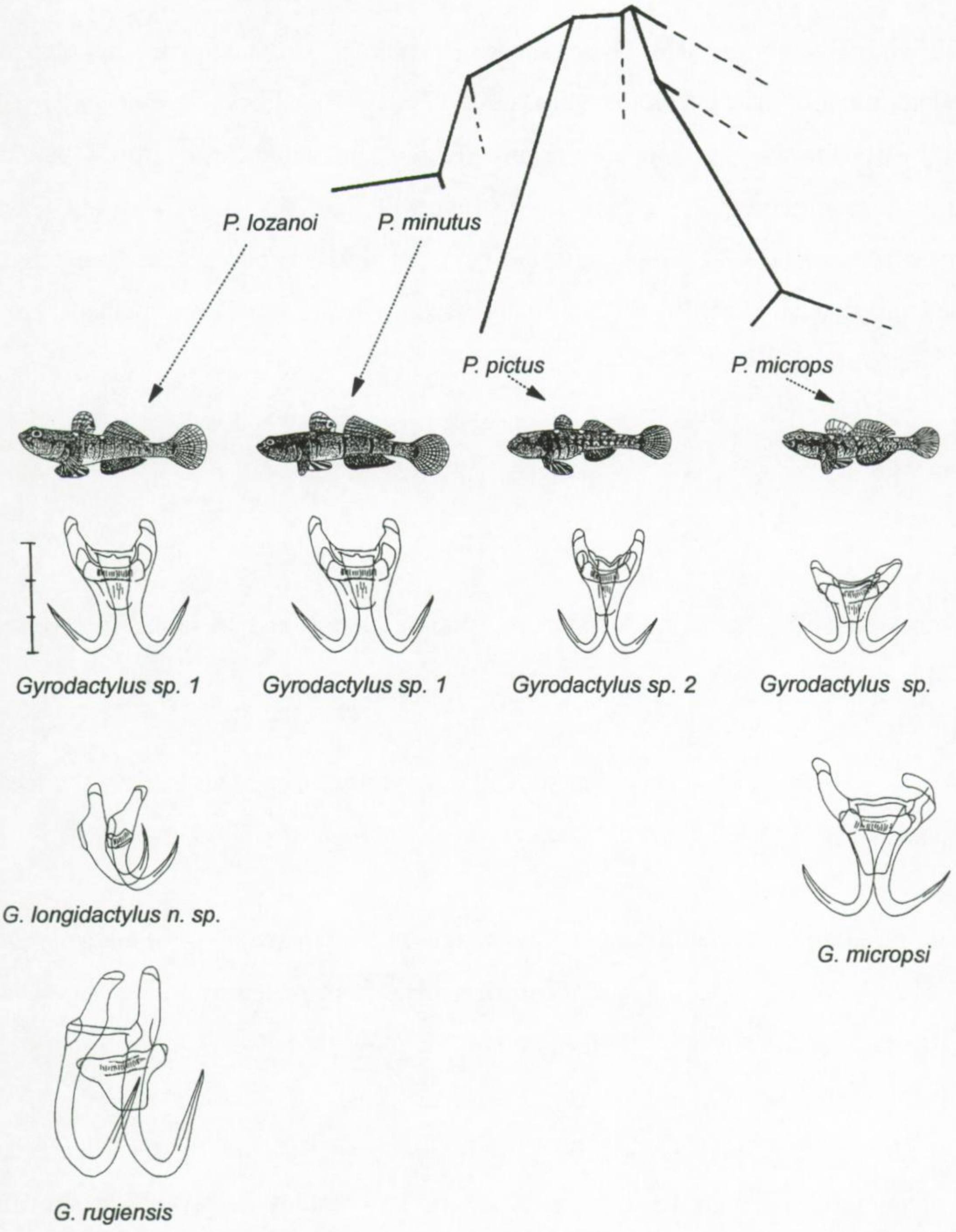


Figure 5.6: A schematic overview of the phylogenetic relationships of *Pomatoschistus* spp., according to Wallis & Beardmore (1984), and their respective *Gyrodactylus* spp. (Gobies are not drawn to scale (drawings after Fonds & Veldhuis, 1973), all sclerites are drawn to scale, scalebar = 30 µm).

Acknowledgements

The first author wants to thank G. Malmberg (Stockholm University) for his help with the initial determination of the gyrodactylids and B. Goddeeris and A. Vermeulen of the Royal Belgian Institute for Natural Sciences for providing lab space and for the use of the videoprint system. We are grateful to K. Cottenie for the help with the discriminant analysis. Part of this study was financed by FWO-Vlaanderen project G.2094.92. The 'Vlaamse Leergangen vzw.' provided the first author with financial support for visiting the Department of Zoology at Stockholm University.

References

Anderson, R. M. & May, R. M. (1982) Coevolution in hosts and parasites. *Parasitology*, **85**, 111-126.

Appleby, C. (1996a) Population dynamics of *Gyrodactylus* sp. (Monogenea) infecting the sand goby in the Oslo fjord, Norway. *Journal of Fish Biology*, **49**, 402-410.

Appleby, C. (1996b) Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea, Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology*, **33**, 199-207

Bray, R. A. & des Clers, S. A. (1992) Multivariate analyses of metrical features in the *Lepidapedon elongatum* (Lebour, 1908) species-complex (Digenea, Lepocreadiidae) in deep and shallow water gadiform fishes of the NE Atlantic. *Systematic Parasitology*, **21**, 223-232.

Cunningham, C. O., McGillivray, D. M., MacKenzie, K. & Melvin, W. T. (1995a) Identification of *Gyrodactylus* (Monogenea) species parasitizing salmonid fish using DNA probes. *Journal of Fish Diseases*, **18**, 539-544.

Cunningham, C. O., McGillivray, D. M., MacKenzie, K. & Melvin, W. T. (1995b) Discrimination between *Gyrodactylus salaris*, *G. derjavini* and *G. truttae* (Platyhelminthes: Monogenea) using restriction fragment length polymorphisms and an oligonucleotide probe within the small subunit ribosomal RNA gene. *Parasitology*, **11**, 87-94.

Dobson, A. P. & Merenlender, A. (1991) Coevolution of macroparasites and their hosts. in: *Parasite-Host Associations: coexistence or conflict*. Eds. Toft, C. A., Aeschlimann, A. and Bolis, L., 83-101

Eichler, W. (1948) Some rules in ectoparasitism. *Annals and magazine of Natural History, including Zoology, Botany and Geology*, **1**, 588-598.

Fonds, M., & Veldhuis, C. (1973) The oxygen consumption of four *Pomatoschistus* species (Pisces, Gobiidae) in relation to water temperature. *Netherlands Journal of Sea Research*, **7,** 376-386.

Geets, A. & Ollevier, F. (1998) Species of Gyrodactylidae (Trematoda: Monogenea) from gobies (*Pomatoschistus* sp., Gobiidae, Pisces) of the Voordelta and surrounding areas: a description with notes on host-specificity. *Systematic Parasitology*, (submitted).

Geets, A., Malmberg, G. & Ollevier, F. (1998) *Gyrodactylus longidactylus* n.sp., a monogenean from *Pomatoschistus lozanoi* (de Buen, 1923) (Gobiidae) from the North Sea. *Systematic Parasitology*, **41,** (1), 63-70.

Gibson, D. I., Taskinen, J. & Valtonen E. T. (1992) Studies on bucephalid digeneans parasitising molluscs and fishes in Finland. II. The description of *Rhipidocotyle fennica* n. sp. and its discrimination by principal component analysis. *Systematic Parasitology*, **23**, 67-79.

Gläser, H.-J. (1974) Eine neue Artgruppe des Subgenus *Gyrodactylus* (*Paranephrotus*) (Monogenea, Gyrodactylidae). *Zoologischer Anzeiger, Jena*, **192,** 271-278.

Guégan, J.-F. & Agnèse, J.-F. (1991) Parasite evolutionary events inferred from host phylogeny: the case of *Labeo* species (Teleostei, Cyprinidae) and their dactylogyrid parasites (Monogenea, Dactylogyridae). *Canadian Journal of Zoology*, **69**, 595-603.

Harris, P. D. (1985) Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) from freshwater fishes in southern England, with a description of *Gyrodactylus rogatensis* sp. nov. from the bullhead *Cottus gobio* L. *Journal of Natural History*, **19**, 791-809.

Harris, P. D. (1993) Les interactions entre la reproduction et la biologie des populations chez les Monogènes Gyrodactylidae: revue. *Bulletin Français de la Pêche et de la Pisciculture*, **328**, 1, 47-65.

Höglund, J. & Thulin, J. (1992) Identification of *Diplostomum* spp. in the retina of perch *Perca fluviatilis* and the lens of roach *Rutilus rutilus* from the Baltic Sea - an experimental study. *Systematic Parasitology*, **21**, 1-19.

Llewellyn, J. & Tully, C. M. (1969) A comparison of speciation in Diclidophorinean monogenean gill parasites and in their fish hosts. *Journal of the Fisheries Research Board of Canada*, **26**, 1063-1074

Malmberg, G. (1964) Taxonomical and ecological problems in *Gyrodactylus* (Trematoda, Monogenea). in: *Parasitic worms and aquatic conditions: Proceedings of a Symposium, Prague, October 29th- November 2nd, 1969*. Eds. Ergens, R. and Ryšavý, B. Publishing house of the Czechoslovak Academy of Sciences: 205-230.

Malmberg, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologi*, Serie 2, **23**, 235 pp.

May, R. M. & Anderson, R. M. (1983) Epidemiology and genetics in the coevolution of parasites and hosts. *Proceedings of the Royal Society of London*, Serie B, **219**, 281-313.

Mo, T. A. (1991a) Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. *Systematic Parasitology*, **19**, 231-240.

Mo, T. A. (1991b) Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea, Gyrodactylidae on rainbow trout *Onchorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in south-eastern Norway. *Systematic Parasitology*, **20**, 1-9.

Mo, T. A. (1991c) Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology*, **20**, 11-19.

Mo, T. A. (1993) Seasonal variation of opisthaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *Salmo salar* L. parr in the River Sandviksela, Norway. *Systematic Parasitology*, **26**, 225-231.

Rohde, K. (1978) Latitudinal differences in host-specificity of marine Monogenea and Digenea. *Marine Biology*, **47**: 125-134.

Rohde, K. (1982) *Ecology of Marine Parasites*. St. Lucia, University of Queensland Press, 245 pp.

Shinn, A. P., des Clers, S., Gibson, D. I. & Sommerville, C. (1996) Multivariate analyses of morphometrical features from *Gyrodactylus* spp. (Monogenea) parasitising British salmonids: Light microscope based studies. *Systematic Parasitology*, **33**, 115-125.

Silan, P. & Maillard, C. (1989) Biologie comparée du développement et discrimination des Diplectanidae ectoparasites du bar (Teleostei). *Annales des Sciences naturelles, Zoologie, Paris, 13e Série*, **10**, 31-45.

Toft, C. A. & Karter, A. J. (1990) Parasite-host coevolution. *Trends in Ecology and Evolution*, **5**, 326-329.

Wallis, G. P. & Beardmore, J. A. (1984) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces: Gobiidae). *Biological Journal of the Linnean Society*, **22**, 107-123.

Chapter 6

Population dynamics of *Gyrodactylus* sp. 1 (Monogenea) from two sympatric goby species, *Pomatoschistus minutus* and *P. lozanoi* (Gobiidae: Pisces), in the Grevelingen Voordelta Area (SW Netherlands).

Geets, A.[1], Beuls, I.[1,2] & Ollevier, F.[1]

[1] *Laboratory for Ecology and Aquaculture, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*

[2] *Koninklijk Museum voor Midden-Afrika, Leuvensesteenweg, 11, B-3080 Tervuren, Belgium.*

This chapter has been submitted to *Parasitology*

Abstract

The seasonal dynamics of the population of the ectoparasite *Gyrodactylus* sp. 1 on two sympatric goby species of the Grevelingen Voordelta area were studied from June 1993 till July 1994. Prevalence, mean intensity and abundance of the monogenean showed clear fluctuations throughout the year. The overlap of two generations of *Pomatoschistus minutus* in summer enhanced the transmission from highly infected spawning and post-spawning sand gobies, and possibly also from adult *P. lozanoi*, to juvenile *P. minutus*. Young *P. minutus* became infected shortly after they adapted to benthic life. In autumn, the *Gyrodactylus* population on the new cohort slowly built up. When water temperature dropped during winter time, the *Gyrodactylus* population size also declined. A clear increase in prevalence and mean intensity of *Gyrodactylus* sp. 1 from March onwards coincided with an increase in gonadosomatic index of *P. minutus*, indicating the start of the spawning season. In both goby species, males were more infected than females in the beginning of the spawning season, but later on females carried a higher parasite load. The infection with *Gyrodactylus* sp. 1 was generally much lower on *P. lozanoi* than on *P. minutus*. Our data suggest that differences in

ecological niche of the two goby species influenced their parasite load, *P. minutus* apparently being a more suitable host for *Gyrodactylus* sp. 1 than *P. lozanoi*.

Introduction

Studies on the seasonal fluctuations of the abundance of *Gyrodactylus* species are largely confined to a limited range of hosts, mostly fish of economic value (e.g. salmonids). Although gobies of the genus *Pomatoschistus* are an important component of the fish fauna of the shallow waters along the European coasts, they have largely been overlooked as hosts for monogenean parasites. Gläser (1974) described two *Gyrodactylus* species from gobies: *G. micropsi* and *G. rugiensis*, both occurring on the sand goby *P. minutus* and on the common goby *P. microps*. The seasonal dynamics and topographical distribution of a *Gyrodactylus* sp. on sand goby was described in the Oslo Fjord by Appleby (1996a). A first study of the *Gyrodactylus* species of four *Pomatoschistus* species, *P. minutus*, *P. lozanoi*, *P. pictus* and *P. microps* of the Belgian and Dutch coastal waters revealed the presence of at least 5 species (Geets & Ollevier, 1998). Subsequent research on host-dependent morphological variation of *Gyrodactylus arcuatus*-like specimens found on *P. minutus, P. lozanoi* and *P. pictus,* led to their separation into two species: *Gyrodactylus* sp. 1 on *P. minutus* and *P. lozanoi* and *Gyrodactylus* sp. 2 on *P. pictus* (Geets *et al.,* 1998a). Preliminary results on genetic variation of *Gyrodactylus* sp. 1 confirm that the gyrodactylids occurring on *P. minutus* and *P. lozanoi* belong to the same species (Marek Zietara, personal communication). Besides *Gyrodactylus* sp. 1, *P. lozanoi* also harboured a new species, *Gyrodactylus longidactylus,* on its gills (Geets *et al.,* 1998b).

The present study investigates the seasonal dynamics of *Gyrodactylus* sp. 1 on the sympatric gobies *P. minutus* and *P. lozanoi* of the Grevelingen Voordelta Area. In addition to fluctuations in water temperatures, differences in host behaviour are often cited as a major factor causing seasonal changes in abundance of *Gyrodactylus* species (Scott & Nokes, 1984, Bauer & Karimov, 1990, Nie & Kennedy, 1991, Mo, 1992). *P. minutus* and *P. lozanoi* are the most abundant goby species of the Grevelingen Voordelta. Although they are phylogenetically closely related (Wallis & Beardmore, 1984) and share the same habitats, they show distinct ecological niches. In Belgian coastal waters, *P. minutus* spawns two months earlier (March to June) than *P. lozanoi* (June to August) (Hamerlynck, 1993). Besides differences in reproduction period, the two goby species also differ in food niche. *P. minutus* is regarded as a generalist, feeding on a variety of benthic and epibenthic prey, while *P. lozanoi* is more of a specialist, feeding predominantly on hyperbenthic and pelagic food items (Hamerlynck & Cattrijsse, 1994). Hence, *P. minutus* will spend more time feeding on or near to the bottom than *P. lozanoi.* We want to assess the impact of differences in ecological niche of *P. minutus* and *P. lozanoi* on the infection dynamics of *Gyrodactylus* sp. 1.

Material and methods

Monthly samples of gobies were collected from June 1993 till July 1994 at four sampling stations in the Grevelingen Voordelta Area: Schaar van Renesse (51°45'N, 3°46'E), Brouwershavense Gat (51°45'N, 3°43'E), Bollen van het Zand (51°45'N, 3°39'E) and Ooster (51°45'30"N, 3°38'E). The Grevelingen Voordelta Area is a shallow coastal area. Gobies were caught with a 2.7 m beam trawl with one trickling chain and a chain in the groundrope and a 6 m long shrimp net with 5x5 mm mesh size in the cod end. The beam trawl was operated from R.V. Luctor (34m, 500 hp). Hauls were 1000 m long. Immediately after capture, gobies were anaesthetised in a saturated solution of Benzocaïne (ethyl aminobenzoaat) in seawater and preserved in 4% formaldehyde solution.

The highest densities of gobies were caught in 'Schaar van Renesse' and 'Bollen van het Zand' (Chapter 2). For parasitological research, gobies from these two stations were preferably used. In case not enough gobies were present in the catches of those two stations, the samples were supplemented with hosts from the other two stations, 'Brouwers-havense Gat' and 'Ooster'. An Analysis of Variance (ANOVA) on standard length and wet weight of the gobies and a Kruskal-Wallis ANOVA on number of gyrodactylids per host showed no significant differences between the sampling stations ($p>0.05$). Therefore, data derived from different stations were pooled. Per sampling station, salinity (‰), water temperature (°C), oxygen saturation (%), pH, conductivity (µS/cm), Secchi depth (cm) and sampling depth (m) were recorded. Monthly averages can be found in chapter 2, Table 2.1. Ranges are given in

Table 6.1. Most environmental variables were relatively stable throughout the year, except for water temperature. Water temperature is cited as a major factor involved in the seasonal occurrence of *Gyrodactylus* species (Appleby, 1996b). The seasonal fluctuation of water temperature in the Grevelingen Voordelta Area is shown in Table 6.2 and in Figures 6.4 and 6.5.

Table 6.1: Environmental variables recorded in the Grevelingen Voordelta Area measured monthly between 8/6/93 and 14/6/94. Ranges are given with indication of the month in which the minimum and maximum was measured between brackets. Monthly measurements of water temperature are given in Table 6.2.

Environmental variable	Range	
Water temperature (°C)	4.4 - 17.7	(March '94 - July '93)
Salinity (‰)	30.1 - 33.7	(June '94 - October '93)
Oxygen (%)	89.5 - 122.7	(November '93 - May '94)
Oxygen (mg/l)	7.2 - 10.9	(July '93 - May '94)
pH	7.7 - 8.5	(October '93 - May '94)
Conductivity (µS/cm)	46.3 - 51.2	(June'94 - October '93)
Secchi depth (cm)	38 - 270	(Februari '94 - October '93)

Determination of the gobies was based on their colour patterns and on the patterns of the sensory papillae on the head (Edlund *et al*., 1980, Hamerlynck, 1990). The different shape of the genital papilla was used for sexing the gobies (Fonds, 1973). In very young specimens (<25mm) sex determination was not always possible and they were classified as 'juveniles'. Standard length to the nearest mm and wet weight as well as ungutted or 'net weight' (without gonads and gasterointestinal tract) to 0.01 g were measured. Gobies were placed on a Whatman filter paper for 5 minutes to dry, before weighing. Overlapping yearclasses (e.g. in June and July 1993 for *P. minutus*) were separated using length frequency distributions (chapter 2). When necessary, otoliths, scales and the development of the gonads were checked. To be able to follow the gonadal development and to link this with the spawning activity of the gobies, gonads of 10 males and 10 females per goby species and per month were weighted up to 0.001 g. Before weighing, the gonads were placed on Whatman filter paper for 5 minutes which absorbed most of the water and formalin. Gonadal development was screened for the 1993 cohort from November 1994 onwards for *P. minutus* and from February 1994 for *P. lozanoi*. In younger gobies, gonads were hardly developed and weighing would not have been accurate. The gonadosomatic index (GSI) is expressed as a percentage of the net body weight, which is the somatic weight excluding the intestinal tract, liver and gonads:

$$\text{GSI} = \text{gonad wet weight} * 100 / \text{net body weight}$$

Per monthly sample, about 30 randomly chosen *P. minutus* and 30 *P. lozanoi* (per species 15 males and 15 females) were screened for *Gyrodactylus* infection. In samples where two cohorts overlapped, gobies from each yearclass were screened. Gobies were dissected, and left and right gills, mouth and gill chambers were checked for gyrodactylids under a dissecting microscope. External sites of the host (body, fins) were not screened. All gyrodactylids were counted and mounted on microscopical slides using ammonium picrate glycerine (Malmberg, 1970), for determination. Determination was done with a 100x oil phase contrast objective on an Olympus microscope.

Prevalence, mean intensity and abundance were used according to the recommendations of Margolis *et al*. (1982). To quantify the proportion of gyrodactylids on female and male hosts contributing to the total mean intensity of *Gyrodactylus* infection, the following equation was used:

$$\text{MI} \times \text{MIm} / (\text{MIm} + \text{MIf}) = \text{proportion of mean intensity derived from male hosts}$$

(weighed according to number of infected males in the sample)

$$\text{MI} \times \text{MIf} / (\text{MIm} + \text{MIf}) = \text{proportion of mean intensity derived from female hosts}$$

(weighed according to number of infected females in the sample)

with MI = mean intensity as number of gyrodactylids on all infected hosts, MIm = mean intensity as number of gyrodactylids on infected male hosts and MIf = mean intensity as number of gyrodactylids on infected female hosts.

As an indication for overdispersion, the variance-to-mean ratio (s^2/mean) of the abundance was calculated.

Table 6.2: Summary of samples investigated for seasonal abundance of *Gyrodactylus* sp. 1 on *P. minutus* and *P. lozanoi* of the Grevelingen Voordelta Area.

Pomatoschistus minutus

Date	Temp. (°C)	Year-class	N fish	m-f-j	Standard length ± S.D. (mm)	Standard length: range (mm)	Wet weight ± S.D. (g)	N infected fish (m-f-j[1])	Total N *Gyrodactylus* sp.1 [2] (range [3])
08/06/93	16.8	1992	42	7-35-0	51 ±5	44-64	1.92 ±0.59	4-31-0	1109 (1-201)
16/07/93	17.7	1992	2	0-2-0	58 ±1	57-59	2.72 ±0.15	0-2-0	39 (1-38)
08/06/93	16.8	1993	21	0-0-21	27 ±2	21-30	0.23 ±0.06	0-0-0	0
16/07/93	17.7	1993	51	31-10-10	38 ±7	25-53	0.75 ±0.50	3-2-0	84 (1-40)
07/09/93	16.4	1993	30	15-15-0	51 ±4	44-60	1.70 ±0.43	3-1-0	98 (2-37)
11/10/93	14.1	1993	30	15-15-0	55 ±6	41-69	2.36 ±0.78	0-0-0	0
09/11/93	10.3	1993	30	15-15-0	59 ±5	47-67	3.00 ±0.94	3-4-0	30 (1-23)
10/01/94	5.1	1993	30	15-15-0	57 ±6	44-68	2.92 ±1.02	2-5-0	44 (1-13)
02/02/94	5.8	1993	29	14-15-0	48 ±8	34-68	1.57 ±1.03	2-5-0	51 (1-21)
01/03/94	4.4	1993	30	15-15-0	53 ±6	41-67	2.24 ±0.91	4-4-0	191 (4-71)
19/04/94	8.2	1993	30	15-15-0	54 ±7	42-73	2.43 ±1.14	4-7-0	172 (1-108)
10/05/94	12.1	1993	26	11-15-0	51 ±5	40-60	2.14 ±0.72	4-12-0	126 (1-31)
14/06/94	14.5	1993	8	1-7-0	50 ±2	48-52	1.87 ±0.19	1-5-0	84 (9-16)
18/07/94	-	1994	30	15-15-0	47 ±4	41-60	1.45 ±0.44	0-0-0	0
Total			389	169-189-31				109	2028

[1] m: male, f: female, j: juvenile

[2] total number of gyrodactylids from all fish in the sample

[3] range = minimum and maximum number gyrodactylids found on the infected fishes

Pomatoschistus lozanoi

Date	Temp. (°C)	Year-class	N fish	m-f-j	Standard length ± S.D. (mm)	Standard length: range (mm)	Wet weight ± S.D. (g)	N infected fish (m-f-j[1])	Total N *Gyrodactylus* sp.1 [2] (range [3])
08/06/93	16.8	1992	27	14-13-0	47 ±4	40-55	1.47 ±0.41	1-4-0	88 (1-52)
16/07/93	17.7	1992	2	1-1-0	47 ±4	44-50	1.22 ±0.23	0-1-0	46 (46)
07/09/93	16.4	1993	31	10-11-10	35 ±4	28-46	0.44 ±0.21	0-0-0	0
11/10/93	14.1	1993	30	9-5-16	34 ±4	24-39	0.46 ±0.15	1-1-2	24 (1-16)
09/11/93	10.3	1993	30	15-15-0	43 ±4	34-49	0.93 ±0.34	2-0-0	4 (1-3)
10/01/94	5.1	1993	30	16-13-1	42 ±6	32-52	0.86 ±0.48	0-1-0	4 (4)
02/02/94	5.8	1993	51	28-23-0	46 ±5	35-55	1.01 ±0.35	1-1-0	25 (4-21)
01/03/94	4.4	1993	31	17-14-0	47 ±7	32-55	1.26 ±0.56	1-0-0	44 (44)
19/04/94	8.2	1993	30	15-15-0	42 ±5	33-52	0.88 ±0.34	0-0-0	0
10/05/94	12.1	1993	30	15-15-0	46 ±4	38-54	1.51 ±0.44	1-0-0	15 (15)
14/06/94	14.5	1993	11	7-4-0	43 ±5	35-49	1.13 ±0.39	1-0-0	6 (6)
18/07/94	-	1993	15	14-1-0	51 ±5	46-64	2.09 ±0.64	0-0-0	0
Total			318	160-131-27				18	256

[1] m: male, f: female, j: juvenile

[2] total number of gyrodactylids from all fish in the sample

[3] range = minimum and maximum number gyrodactylids found on the infected fishes

To test overall differences in gonadosomatic index, mean intensity and abundance of gyrodactylids over different months in a yearclass of gobies, a nonparametric Kruskal-Wallis ANOVA was used in most cases, since data were not normally distributed, means and variances were correlated and no satisfying transformation could be found to comply to the assumptions of a parametric ANOVA. Multiple comparisons were carried out according to Siegel & Castellan (1988). In the figures, differences between means are indicated with small characters, when nonparametric tests are used and with caps for parametric tests. An arcsin (square root (GSI/100)) transformation of gonadosomatic index normalised the data and homogenised the variances of GSI for female *P. minutus* and male *P. lozanoi*. To compare the means in these two datasets, a parametric ANOVA was used and *post hoc* comparisons between the different months were carried out using Tukey HSD test for unequal sample sizes.

A Mann-Whitney U test was used to test for significant differences in parasite load (abundance, mean intensity) between male and female hosts, and between parasite load on *P. minutus* and *P. lozanoi* in specific months. The overall (yearly) differences in prevalence, abundance between the two species were compared using Wilcoxon matched pairs test (July 1993 was excluded since only 2 *P. lozanoi* were present.). The occurrence of a correlation between standard length, gonadosomatic index and parasite load, and between temperature and mean intensity or abundance was checked using a Spearman Rank Order correlation. In all analyses, the level of significance was set at 5 %. Statistical analysis was carried out with STATISTICA 4.1 for Windows.

Results

During the sampling period, from June 1993 till July 1994, three yearclasses of *P. minutus* and two of *P. lozanoi* occurred (Table 6.2.). The analysed size range was 21 to 73 mm for *P. minutus* and 24 to 64 mm for *P. lozanoi*, the latter being a smaller species at adult size than *P. minutus*. Standard length (SL) of the analysed sand gobies increased from June to November for both sexes, stabilized during winter and showed a marked decline in February. Lozano's goby did not reach maximal size until February (Figs. 6.1 and 6.2).

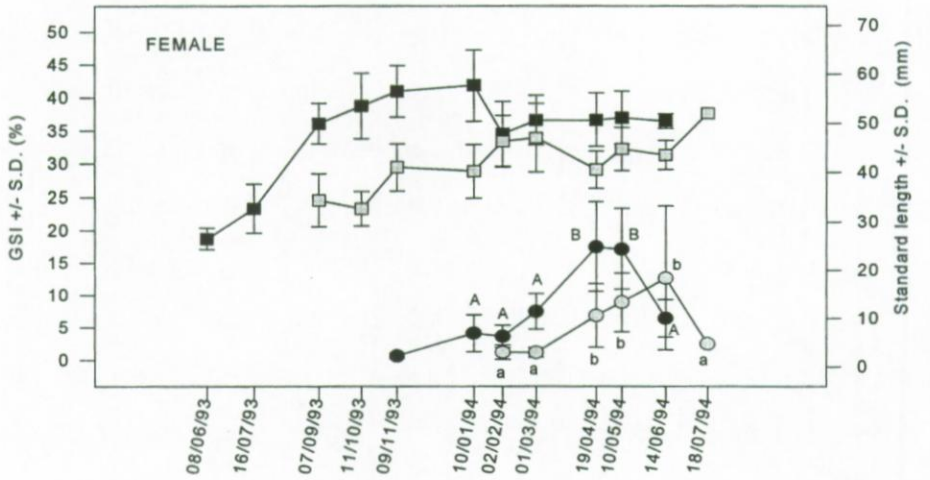


Figure 6.1: Changes in gonadosomatic index (GSI) and standard length of female *Pomatoschistus minutus* and *P. lozanoi* of the Grevelingen Voordelta area. Means of GSI sharing the same characters are not significantly different (within-species comparison). Small characters are used for nonparametric Kruskal-Wallis ANOVA and capitals for parametric ANOVA. (Legend see figure 6.2)

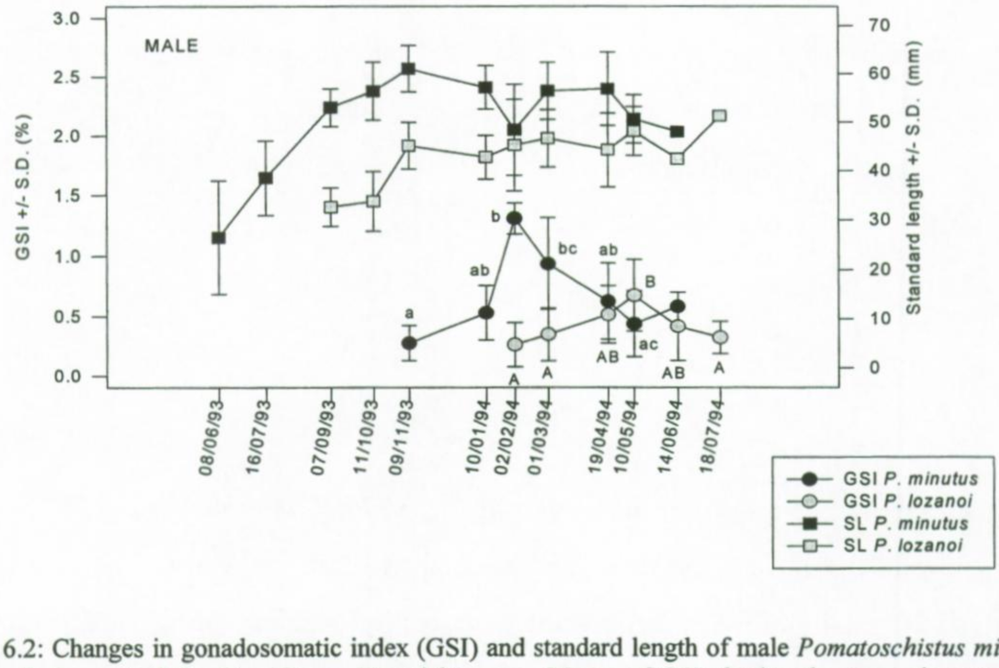
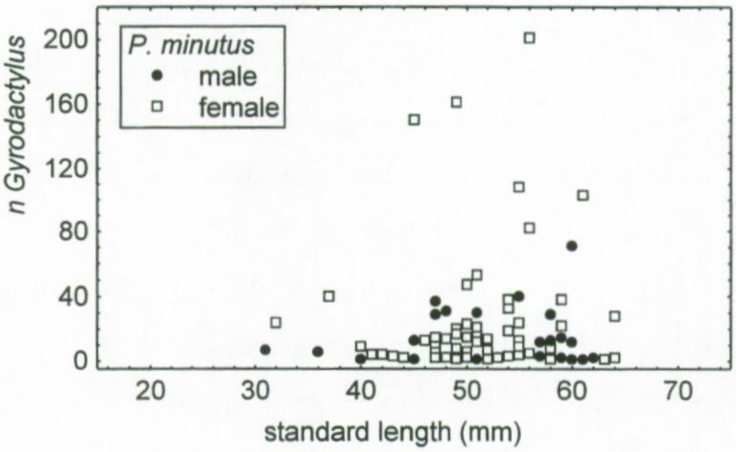


Figure 6.2: Changes in gonadosomatic index (GSI) and standard length of male *Pomatoschistus minutus* and *P. lozanoi* of the Grevelingen Voordelta area. Means of GSI sharing the same characters are not significantly different (within-species comparison). Small characters are used for nonparametric Kruskal-Wallis ANOVA and capitals for parametric ANOVA.

An overall significant difference in female gonadosomatic index (GSI) was shown for *P. minutus* (ANOVA, N=76, d.f.=6, p<0.01) over an 8 month period (November '93-June '94) and for *P. lozanoi* (Kruskal-Wallis ANOVA, N=44, d.f.=5, p<0.01) from January to July '94. The time trajectory of the gonadosomatic index of female *P. minutus* clearly shows ripening of the ovaries from February, March onwards. The GSI reaches its maximum in April (17.4%) and May (17.1%) to decline in June (Fig. 6.1). Ovary development in *P. lozanoi* females started in April and peaked two months later, in June (GSI=12.6%), followed by a decrease in July (Fig. 6.1). GSI in male gobies also differed significantly over the sampling period (Kruskal-Wallis ANOVA, *P. minutus*, N=65, d.f.=6, p<0.01; ANOVA, *P. lozanoi*, N=58, d.f.=5, p<0.01). In male *P. minutus*, the GSI increases from January onwards with a maximum in February (1.3%), decreasing steadily towards summer. Male *P. lozanoi* become sexually mature from March onwards and reaches a maximal GSI of 0.7% in May (Fig. 6.2). By then, nuptial colouring of male *P. lozanoi* is very pronounced.

In total, 389 sand gobies, *P. minutus*, and 318 Lozano's gobies, *P. lozanoi,* were analysed for their infection with *Gyrodactylus* species (Table 6.2). On *P. minutus*, only *Gyrodactylus* sp. 1 occurred. In June and July 1993, *P. lozanoi* of yearclass 1992 had a mixed infection of *Gyrodactylus* sp. 1 with *Gyrodactylus longidactylus* on the gills, earlier described as a new species for this host (Geets *et al.,* 1998b). Eighty two *G. longidactylus* were found on three adult *P. lozanoi*, two ripe females and a male. Since this *Gyrodactylus* species was not found on Lozano's gobies of the 1993 yearclass, no further analysis of its seasonal distribution could be carried out.

A total of 2028 *Gyrodactylus* sp. 1 from *P. minutus* and 256 from *P. lozanoi* were present in the samples. About half of them were recovered from hosts from the 1992 yearclass (caught in June and July 1993). The highest number of gyrodactylids on an individual host was 201, on a sexually mature female sand goby from the 1992 yearclass. In the 1993 cohort, as many as 108 worms were recovered from one female sand goby in April 1994. The maximum record on Lozano's gobies was 52 gyrodactylids on a ripe female from the 1992 cohort and 44 on a ripe male in March 1993. In general, *P. minutus* harboured more *Gyrodactylus* sp. 1 than *P. lozanoi*.



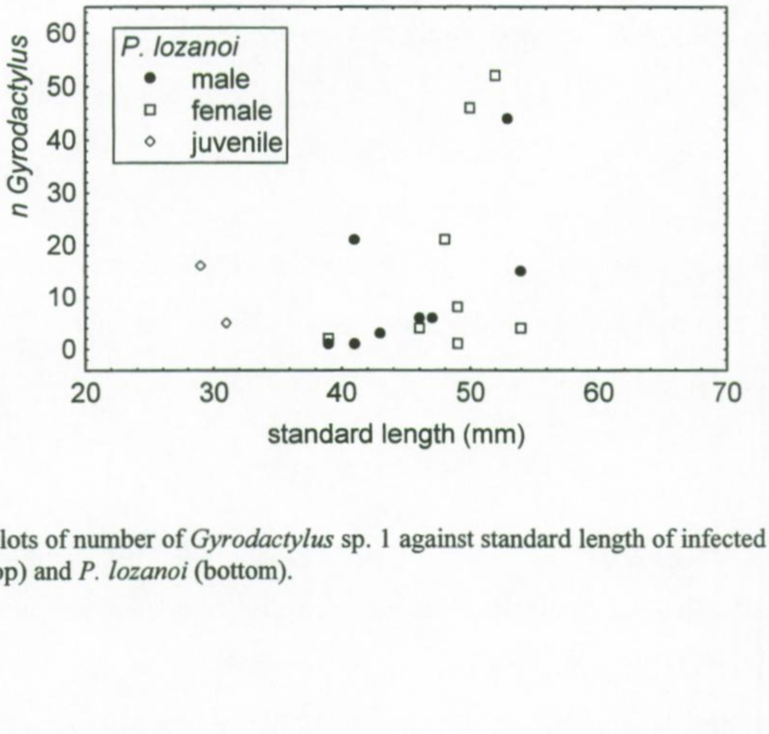


Figure 6.3: Scatterplots of number of *Gyrodactylus* sp. 1 against standard length of infected *Pomatoschistus minutus* (top) and *P. lozanoi* (bottom).

Wilcoxon Matched pairs test showed a significantly higher prevalence and abundance of gyrodactylids on *P. minutus* than on *P. lozanoi* over the year (N=11, $p<0.01$ in both cases, data of July 1993 were omitted because of too small sample size for *P. lozanoi*).

On both host species the highest infections were found in larger sizeclasses of fish (Fig. 6.3). However, the correlation between standard length and parasite load was not significant (Spearman Rank Order Correlation, *P. minutus*: r=0.06, N=109, $p>0.05$; *P. lozanoi*: r=0.39, N=18, $p>0.05$).

Clear seasonal changes in prevalence, mean intensity (Figs 6.4 and 6.5) and abundance (Figs 6.6 and 6.7) of *Gyrodactylus* sp. 1 were present. Although more gyrodactylids were recovered from hosts during the summer, temperature was not significantly correlated with prevalence, mean intensity or abundance (*P. minutus*: Spearman Rank Order Correlation, N=11, prevalence: r=-0.1, abundance: r=0.2, mean intensity: r=0.3, all $p>0.05$). In June and July 1993, adult *P. minutus* of the 1992 yearclass were present together with young sand gobies of the new 1993 generation (Fig. 6.4). The difference in prevalence and mean intensity of infection was remarkable. In June 1993, none of the juvenile sand gobies hosted gyrodactylids, while 83.3 % of the adults (mainly female) sand gobies were infected with an average of 31.7 worms. The first infections of juvenile sand gobies of the 1993 yearclass were recorded in July. The mean intensity of infection then increased in September to 24.5 worms per infected fish. After a remarkable absence of gyrodactylids on sand gobies in the October sample, the prevalence increased to about 23 % in November, staying at this level throughout the winter. Although the prevalence was constant, mean intensity of infection decreased significantly from September to November (Kruskal-Wallis ANOVA, multiple comparisons $p<0.05$) and remained low during the winter months. From spring onwards, a clear increase in prevalence (Fig. 6.4) and abundance (Fig. 6.6) of *Gyrodactylus* sp. 1 was observed. Mean intensity (MI) of infection on the sand gobies rose from 7.2 in February to a peak of 23.8 in March (Kruskal-Wallis ANOVA, multiple comparisons $p<0.05$).

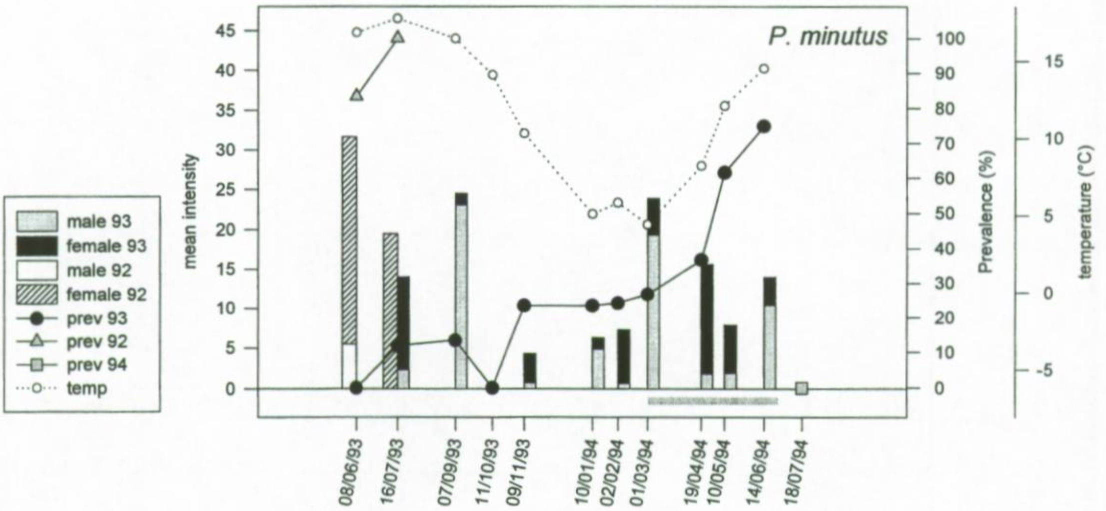


Figure 6.4: Seasonal changes in prevalence and mean intensity (stacked bars) of *Gyrodactylus* sp. 1 on *Pomatoschistus minutus* from the Grevelingen Voordelta Area. The horizontal shaded bar indicates the spawning season of *P. minutus*. Water temperature is given by small open circles. The years in the legend correspond to the respective yearclass of the goby hosts. Stacked bars give the proportional number of gyrodactylids recovered from male and female hosts.

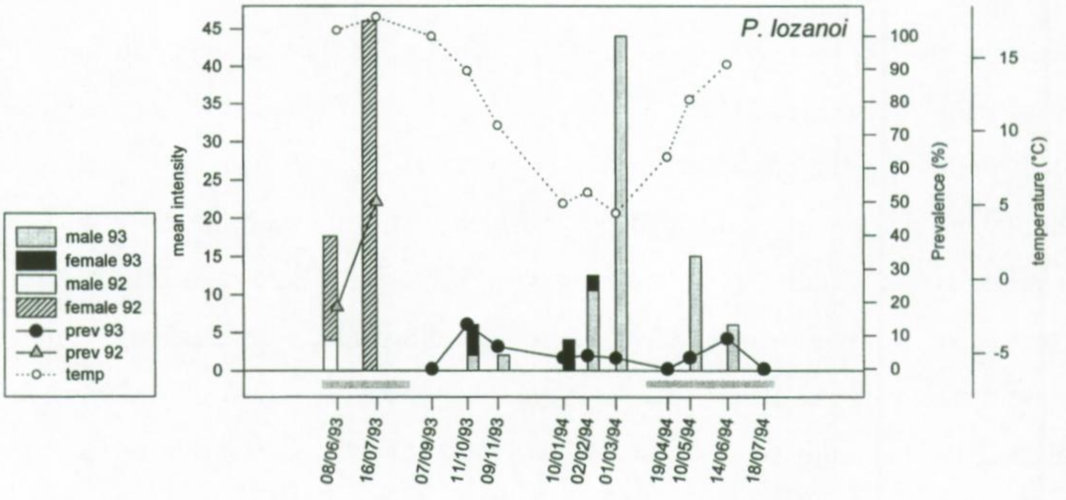


Figure 6.5: Seasonal changes in prevalence and mean intensity (stacked bars) of *Gyrodactylus* sp. 1 on *Pomatoschistus lozanoi* from the Grevelingen Voordelta Area. The horizontal shaded bars indicate the spawning season of *P. lozanoi*. Water temperature is given by small open circles. The years in the legend correspond to the respective yearclass of the goby hosts. Stacked bars give the proportional number of gyrodactylids recovered from male and female hosts.

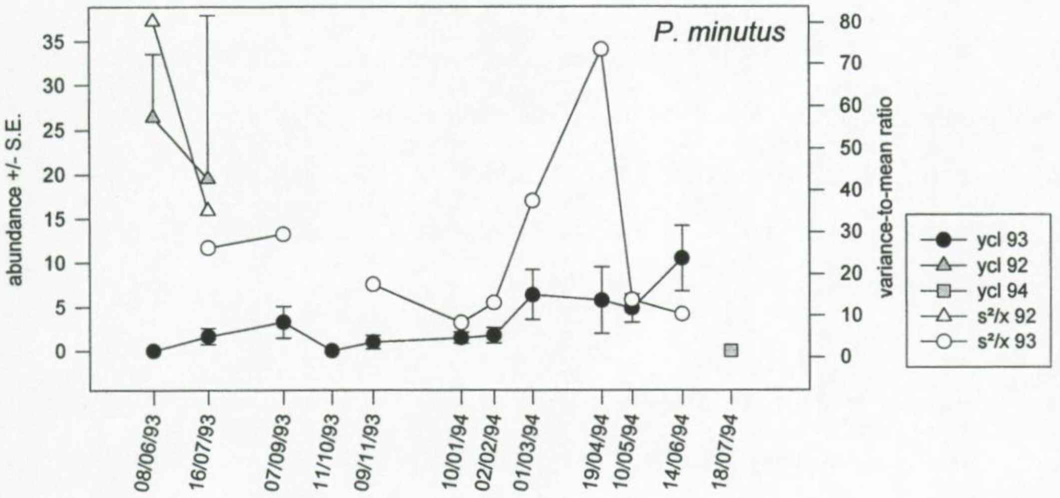


Figure 6.6: Seasonal changes in the abundance and the variance-to-mean ratio (s²/m) of *Gyrodactylus* sp. 1 on *Pomatoschistus minutus*. (Legend: ycl = yearclass of gobies)

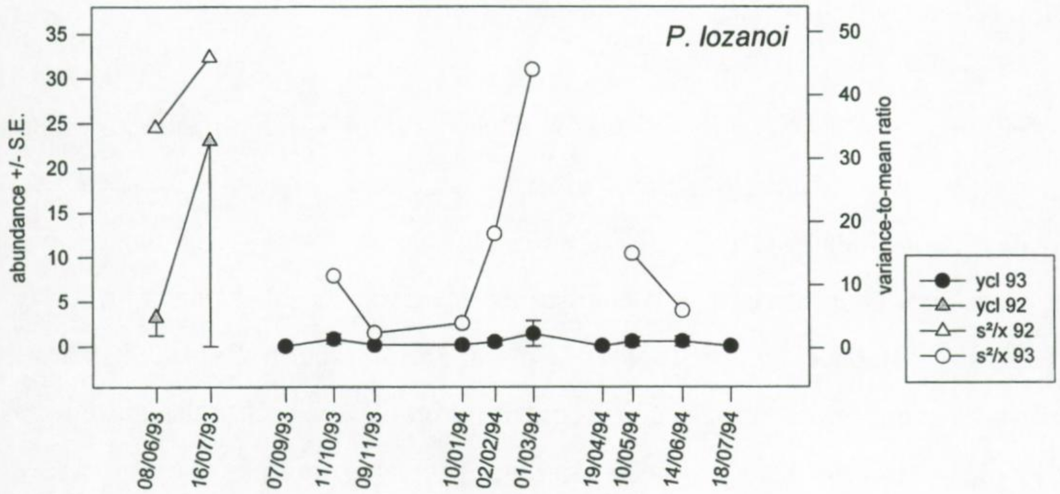


Figure 6.7: Seasonal changes in the abundance and the variance-to-mean ratio (s²/m) of *Gyrodactylus* sp. 1 on *Pomatoschistus lozanoi*. (Legend: ycl = yearclass of gobies)

In March, infected male sand gobies carried on average significantly more gyrodactylids (MI=38.7) than infected females (MI=9.0) (Mann-Whitney U test, $p<0.05$), while the opposite was true in April (male MI=3.0, female MI=22.8) (Mann-Whitney U test, $p<0.05$). While prevalence was equal for both sexes in March (26.6 %), in April and May it was clearly higher for females than for males (April: 46.6 % (f) vs. 26.6 % (m), May: 80.0 % (f) vs. 36.6 % (m). In female sand gobies, mean intensity of infection was positively correlated with their increasing gonadosomatic index (Spearman Rank Order correlation, N=7 November '93 to June '94, r=0.8, $p<0.05$).

In March and April, a high variance-to-mean ratio (Fig. 6.6) indicated an overdispersed distribution of the gyrodactylids in the host population, meaning that high parasite loads were present on a limited number of fish in the host population. In the following months, prevalence increased, abundance stayed at the same level and the infection spread out more evenly over the host population, as indicated by a lower variance-to-mean ratio. The highest prevalence (75%) and abundance (10.5) in the 1993 cohort of *P. minutus* was reached in June 1994. The juvenile sand gobies of the 1994 cohort, caught in July 1994, were totally free of infection with *Gyrodactylus* sp.1.

The infection with *Gyrodactylus* sp. 1 was much lower on *P. lozanoi* than on *P. minutus* (Fig. 6.4 and 6.5). Significant differences in prevalence and abundance were observed with Wilcoxon Matched Pairs test (N=11, $p<0.01$, cfr. supra). Because of these low infection levels, it was not always possible to confirm the observed seasonal trends on the *P. lozanoi* host population statistically. In June and July 1993, only adult *P. lozanoi* from the 1992 cohort were caught in the Grevelingen Voordelta (Fig. 6.5). The females hosted most of the gyrodactylids (81 %, weighed to the proportion of females present). In June, 18.5 % of the adult Lozano's gobies were infected, with a mean intensity of 17.6, which was significantly lower (Mann-Whitney U, $p<0.01$) than in the sand goby population (83.3 % infected adults, carrying on average 31.6 worms per infected fish). The elevated prevalence and mean intensity seen in July 1993 should be taken with caution, as only two adult female *P. lozanoi* were available for screening. One of them was infected (leading to a 50 % prevalence) with 46 worms. About a quarter (12) of these gyrodactylids were recovered from the mouth and gill cavity and were possibly migrating to external sites. Juvenile *P. lozanoi* from the 1993 yearclass were present from September onwards. None of them were infected, although 13.3 % juvenile *P. minutus* carried on average 24.5 worms per infected fish at that time. The first gyrodactylids on *P. lozanoi* young were recovered in October (Fig. 6.5). Compared to the infection on the same host during the rest of the year, the prevalence (13.3 %) and mean intensity (6.0) on these small gobies (mean standard length 34 mm) in October was rather high. Identical to the seasonal infection dynamics on *P. minutus*, a decrease in parasite load was noticed in winter. From February onwards, mean intensity increased again, but only one individual per month was found infected and the increase was therefore not statistically significant. Remarkably, from March onwards, all infected *P. lozanoi* were males. Abundance did not exceed 1.4 (March 1994) in the 1993 cohort and in April, May and June 1994 gyrodactylid abundance on *P. lozanoi* was significantly lower than in the sympatric *P. minutus* population (Mann-Whitney U, $p<0.01$, corrected according to Bonferroni method, Sokal & Rohlf, 1995) (Fig. 6.7).

Discussion

Seasonal changes in abundance of *Gyrodactylus* spp. have been reported in several studies. Major factors affecting the seasonal occurrence of gyrodactylids on a variety of host species include abiotic factors, of which water temperature is the most important in temperate areas, and biotic host-related factors, such as host behaviour, host sex, resistance and mortality (Scott & Nokes, 1984, Bauer & Karimov, 1990, Nie & Kennedy, 1991, Mo, 1992). The short life span of *P. minutus* and *P. lozanoi*, surviving only about one year (Miller, 1986, Arellano, 1995), certainly influences the population dynamics of their *Gyrodactylus* species. Present study revealed the population dynamics of *Gyrodactylus* sp. 1 on two sympatric, closely related hosts.

In June and July 1993, the population of *Gyrodactylus* sp. 1 mainly survived on adult (female) sand gobies and to a lesser extend on adult Lozano's gobies. After a crash in the *Gyrodactylus* population in late summer, most probably due to mortality in the host population of the post-spawners, the *Gyrodactylus* population partly recovered by infecting the susceptible new sand goby generation. Juvenile sand gobies became infected shortly after they adapted to benthic life. The overlap of the first born juveniles of the 1993 cohort with the highly infected adults of both *P. minutus* and *P. lozanoi*, enabled the transmission of the gyrodactylids to the new generation. Appleby (1996a) studied the population dynamics of *Gyrodactylus* sp. on the sand gobies in the Oslo Fjord (Norway). Morphological comparison of specimens of *Gyrodactylus* sp. from the Norwegian sand gobies with *Gyrodactylus* sp. 1 from our study showed there close resemblance (Geets *et al.,* 1998a). The population dynamics of the Norwegian gyrodactylids followed the same pattern as observed by us, with high infection levels on the spawning adult sand gobies in summer (maximal prevalence 100% and abundance 23.3 in June, water temperature 13°C), a decline on the post-spawners, followed by the transmission to the young host generation and a decrease of infection during winter due to low water temperature (Appleby, 1996a). The overall infection level found on the Norwegian sand gobies was higher than in our samples. It should be remarked that Appleby (1996a) also examined the external sites (head, body, fins) of the hosts for the presence of *Gyrodactylus* sp., and these were not included in our study, since we suspected too many parasites might have been detached by the packing in one batch and transport of the hosts. *Gyrodactylus* sp. showed a strong preference for the gill filaments on the Norwegian gobies during most of the year, except at high infection intensities in May and June, which coincides with the breeding period of the host (Appleby, 1996a). Therefore, the *Gyrodactylus* abundance on the gobies in the Grevelingen Voordelta is probably underestimated during the breeding season.

A characteristic autumn peak in *Gyrodactylus* abundance has been observed in several studies (Mo, 1992, Koskivaara *et al*., 1991, Appleby, 1996a, 1996b). The *Gyrodactylus* population size on the 1993 yearclass of gobies of the Grevelingen Voordelta also increased in mean intensity in September. Under laboratory conditions, it is known that the introduction of naive (non immune) hosts is important to maintain a population of *Gyrodactylus* spp. (Scott & Anderson, 1984). The appearance of a new young susceptible host population in the Grevelingen Voordelta in autumn probably enhanced the population growth of *Gyrodactylus* sp. 1. Furthermore, the water temperature in September was still quite high (16.4°C) which may have positively influenced the *Gyrodactylus* reproduction on their newly infected hosts.

The remarkable absence of gyrodactylids on sand gobies in October, while they were present on the *P. lozanoi* population, needs further explanation. Immunological responses of fish hosts on gyrodactylids have been considered as a factor for controlling parasite population densities. Immunity of fish to *Gyrodactylus* does not seem to last longer than 4 to 6 weeks, indicating that it is mediated by unspecific mechanisms (Lester & Adams, 1974, Scott & Robinson, 1984). The infection of the young sand gobies in summer might have invoked an immune response of which the effect is seen in October and lost later in the season. Together with declining water temperature, immune response in fish will decrease because, at variance with warm-blooded vertebrates, antibody formation in fishes is temperature dependent. (Corbel, 1978, Kinne, 1984). However, it is difficult to assess the impact of host immune response on the *Gyrodactylus* population in a field study. Therefore, no definitive reason for the absence of *Gyrodactylus* on sand gobies in October can be given at the moment.

The abrupt decline in mean intensity of *Gyrodactylus* sp. 1 during the winter coincides with reduced water temperatures. Several studies have indicated a decrease in growth rate of *Gyrodactylus* populations with lowering water temperature (Scott & Nokes, 1984, Gelnar, 1987, Jansen & Bakke, 1991, Appleby, 1996b, Soleng & Bakke, 1997). Bauer & Karimov (1990) compared the pattern of population dynamics of *Gyrodactylus vicinus* on *Schizothorax intermedius* in two water bodies, one with a constant water temperature and one with fluctuating temperatures throughout the year. When water temperature was constant fluctuations in occurrence of the parasites were insignificant. In the Grevelingen Voordelta, temperature seemed not to be the only major factor affecting *Gyrodactylus* abundance during spring. A marked increase in mean intensity of infection of *P. minutus* was clearly seen in March, when water temperature was still very low (4.4° C). The increasing GSI in that period indicated the start of the spawning season of *P. minutus*. Male GSI reached already a maximum in February. It is known that gobies change their behaviour during the spawning period (Nyman, 1953, Fonds, 1973, Nellbring, 1993). Males start to build nests under shells and will attract several females to deposit batches of eggs to the roof of the shell. Males will take care of the brood and will exhibit territorial behaviour. Females can deposit eggs in different nests (Lindström, 1992). The most important route for transmission of gyrodactylids of benthic hosts is through contact between fishes and detached parasites on the substrate (Bakke *et al.,* 1992). Second most important dispersal strategy is by direct transfer through host-host contact. Although, the importance of host-host contact for transmission is sometimes limited. For instance, dissemination of *G. salaris* within a salmon parr population was not dependent on physical contact between hosts, but was mainly mediated through contact between the hosts and parasites on the river bottom (Mo, 1992). Transmission from dead fish and by detached parasites in the planktonic drift could also occur but would be of limited importance (Bakke *et al.,* 1992). Both dispersal strategies, infecting hosts from the sediment or through close contacts between different host individuals, may be very effective during spawning, considering the bottom dwelling and schooling behaviour of the gobies. Male gobies, building nests and spending considerable time under the shells to take care of the eggs, may be expected to be more vulnerable to infection than females during the earlier phase of the spawning season. The fact that males indeed were more infected than females in the beginning of the spawning season (March) supports this idea. The transmission to spawning females is already seen in April, when a higher percentage of females were infected than males and they also carried higher parasite loads. Similar sex-dependent differences in infection were present on Norwegian sand gobies (Appleby, 1996a). Male *Salmo trutta* L. were more infected than females (Pickering & Christie, 1980). The positive correlation between GSI and mean intensity of infection in female *P. minutus* may indicate that females with more eggs, visiting more nests, would have a higher risk of infection. Alternatively, females with high GSI may channel more energy to egg production and possibly less to immune defence. Furthermore, Thomas (1964) indicated that the female hormone oestrogen depressed parasite levels in fish except during spawning. This might also be the case in the sand goby population, since females carried a higher proportion of the *Gyrodactylus* population than males throughout most of the spawning season, while the opposite was true during the rest of the year.

Throughout summer, an augmenting percentage of sand gobies became infected. The distribution of the parasite population changed from highly aggregated (high variance-to-mean ratio) in April to a more regular distribution in the subsequent months. The slight decrease in mean intensity from April onwards might indicate a loss of the most heavily infected hosts from the population. Lester (1984) stated that a rapid decline of overdispersion of a parasite population may be due to a high mortality of the most infected host individuals.

Throughout the sampling period, *P. lozanoi* was generally less infected with *Gyrodactylus* sp. 1 than *P. minutus*. *P. lozanoi* is described as a more hyperbenthic predator, spending more time in the water column than *P. minutus,* which prefers epibenthic and benthic prey (Hamerlynck, 1993, Hamerlynck & Cattrijsse, 1994, Hamerlynck *et al*., 1993). Given the dispersal strategy of gyrodactylids relying mainly on host-sediment or host-host contact, *P. minutus* will be more vulnerable to infection than *P. lozanoi*.

Furthermore, the difference in reproductive niche of *P. lozanoi*, spawning two months later than *P. minutus,* may also stongly influence the extent of the *Gyrodactylus* infection. The delay in spawning of *P. lozanoi* in the Grevelingen Voordelta is confirmed by the time trajectory of the GSI, that reaches its maximal values in May (male) and June (female), approximately two months later than in the sand gobies. However, in the Belgian coastal waters, Hamerlynck & Cattrijsse (1994) reported the maximal GSI to be reached at the same time for both goby species (April-May), and they suggested a possible delayed spawning of *P. lozanoi* to be due to competition for nest sites. This seems not to be the case in the Voordelta area. The new *P. lozanoi* generation was recruited from September onwards. The population of young Lozano's gobies overlapped only with young, only slightly infected *P. minutus* and with a declining population of post-spawning *P. lozanoi*. This might partly explain the relatively low parasitic load on young individuals of *P. lozanoi* in September and October, when compared to *P. minutus* of the same age and size in July.

On top of this, changes in water temperature also influences population growth of the gyrodactilids. From September onwards, water temperature starts to decline quickly. So, the *Gyrodactylus* population growth on *P. lozanoi* will not be boosted as much as it was the case on the young *P. minutus* generation which appeared in early summer.

Because of the low infection levels, seasonal patterns in gyrodactylid population dynamics on *P. lozanoi* were less obvious than for the parasite population on the sand gobies. Still, an infection pattern comparable to that of its sympatric relative *P. minutus* is seen during the spawning season. Male *P. lozanoi* also acquired *Gyrodactylus* infection before females, probably when building nests. Although in our samples, no infected mature females of the

1993 cohort were caught, a transmission from males to females is likely to occur, given our data on the high infection levels of females of the previous yearclass.

In conclusion, the ecological niche of *P. minutus* apparently makes it a more suitable host for *Gyrodactylus* sp. 1 than the sympatric, closely related *P. lozanoi*. The transmission of the parasites and their optimal population growth are facilitated by the sand gobies benthic life style and earlier reproductive period. The observed seasonal population dynamics were mainly caused by three factors. A first factor is the influx of a young susceptible population of gobies in summer, which become infected through contact with the adult gobies carrying the *Gyrodactylus* population, and on which the *Gyrodactylus* population can still grow to a considerable size before the onset of winter. A second factor is the lowering water temperature which will have a negative effect on the *Gyrodactylus* population growth in winter. A third factor is the crowding of the gobies and their frequent contact with the sediment because of their nesting behaviour during the spawning season, which in turn is a major factor that positively influences the abundance of the *Gyrodactylus* population. Additionally, the increasing water temperature in spring boosts the population growth.

Acknowledgements

We thank the crew of the R.V. Luctor (Centre for Estuarine and Coastal Ecology, Yerseke, The Netherlands) Wim Röber, Piet de Koeyer, Co van Sprundel and Hans Francke for their assistance during the sampling campaign. The help of Kris Hostens, Jan Mees, Renato Arellano and several volunteers from the lab of Ecology and Aquaculture (Katholieke Universiteit Leuven) and the section of Marine Biology (Universiteit Gent) was indispensable and is here acknowledged. Part of this study was financed by project G.2094.92 of the Fund for Scientific Research - Flanders (FWO).

References

Appleby, C. (1996a) Population dynamics of *Gyrodactylus* sp. (Monogenea) infecting the sand goby in the Oslo Fjord, Norway. *Journal of Fish Biology*, **49,** 402-410.

Appleby, C. (1996b) *Taxonomy and epidemiology of Gyrodactylus Nordmann, 1832 (Platyhelminthes: Monogenea): Field studies of the three species in Norway.* PhD thesis, Central Veterinary Laboratory, Oslo, Norway, 91 pp.

Arellano, R. V. (1995) *Age and growth of two sympatric goby species Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) using daily growth increments in the otoliths*. PhD thesis, University Gent, 178 pp.

Bakke, T. A., Harris, P. D., Jansen, P. A. & Hansen, L. P. (1992) Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Dis. Aquat. Org.*, **13,** 63-74.

Bauer, O. N. & Karimov, S. B. (1990) Patterns of parasitic infections of fishes in a water body with constant temperature. *Journal of Fish Biology*, **36,** 1-8.

Corbel, M. J. (1975) The immune response in fish: a review. *Journal of Fish Biology*, **7,** 539-563.

Edlund, A.-M., Sundmark, G. & Thorman, S. (1980) The identification of *Pomatoschistus pictus*, *P. microps* and *P. minutus* (Gobiidae, Pisces). *Sarsia*, **65,** 239-242.

Fonds, M. (1973) Sand gobies in the Dutch Wadden Sea (*Pomatoschistus*, Gobiidae, Pisces). *Neth. J. Sea Res.*, **6,** 4, 417-478.

Geets, A., Appleby, C. & Ollevier, F. (1998a) Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp.

and *G. arcuatus* from *Gasterosteus aculeatus:* a multivariate approach. *Parasitology*, submitted.

Geets, A., Malmberg, G. & Ollevier, F. (1998b) *Gyrodactylus longidactylus* n.sp., a monogenean from *Pomatoschistus lozanoi* (de Buen, 1923) from the North Sea. *Systematic Parasitology*, **41**, 1, 63-70.

Geets, A. & Ollevier, F. (1998) Species of Gyrodactylidae (Monogenea) from gobies *(Pomatoschistus* spp., Gobiidae, Pisces) of the Voordelta and surrounding areas: a description with notes on host-specificity. *Systematic Parasitology*, submitted.

Gelnar, M. (1987) Experimental verification of the effect of water temperature on micropopulation growth of *Gyrodactylus katharineri* Malmberg, 1964 (Monogenea) paraziting carp fry (*Cyprinus carpio* L.). *Folia Parasitol.*, **34,** 19-23.

Gläser, H.-J. (1974) Eine neue Artengruppe des Subgenus *Gyrodactylus* (*Paranephrotus*) (Monogenea, Gyrodactylidae). *Zool. Anz*, **192,** 271-278.

Hamerlynck, O. (1990) The identification of *Pomatoschistus minutus* (Pallas) and *Pomatoschistus lozanoi* (de Buen) (Pisces, Gobiidae). *J. Fish Biol.*, **37,** 723-728.

Hamerlynck, O. (1993) *The ecology of Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) in the shallow coastal waters and estuaries of the Southern Bight of the North Sea*. PhD thesis, University Gent, 187 pp.

Hamerlynck, O. & Cattrijsse, A. (1994) The food of *Pomatoschistus minutus* (Pisces, Gobiidae) in Belgian coastal waters, and a comparison with the food of its potential competitor *P. lozanoi*. *Journal of Fish Biology*, **44,** 753-771.

Hamerlynck, O., Cattrijsse, A. & Arellano, R. V. (1993) Daily ration of juvenile *Pomatoschistus lozanoi* de Buen (Pisces: Gobiidae). *ICES J. Mar. Sci.*, **50,** 471-480.

Jansen, P. A. & Bakke, T. A. (1991) Temperature-dependent reproduction and survival of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: monogenea) on Atlantic salmon (*Salmo salar* L.). *Parasitology*, **102,** 105-112.

Kinne, O. (1984) *Diseases of marine animals: Introduction, Pisces*. Hamburg, Biologische Anstalt Helgoland, 541 pp.

Koskivaara, M., Valtonen, E. T. & Prost, M. (1991) Seasonal occurrence of gyrodactylid monogeneans on the roach (*Rutilus rutilus*) and variation between four lakes of differing water quality in Finland. *Aqua Fennica*, **21,** 47-55.

Lester, R. J. G. (1984) A review of methods for estimating mortality due to parasites in wild fish populations. *Helgoländer Meeresunters.*, **37,** 53-64.

Lester, R. J. G. & Adams, J. R. (1974) *Gyrodactylus alexanderi*: reproduction, mortality and effect on its host *Gasterosteus aculeatus*. *Canadian Journal of Zoology*, **52,** 827-833.

Lindström, K. (1992) Female spawning patterns and male mating success in the sand goby *Pomatoschistus minutus*. *Marine Biology*, **113,** 475-480.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. & Schad, G. A. (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *J. Parasitol.*, **68,** 1, 131-133.

Miller, P. J. (1986) Gobiidae. in: P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (Eds) *Fishes of the North-eastern Atlantic and the Mediterranean*. pp. 1019-1085.

Mo, T. A. (1992) Seasonal variation in the prevalence and infestation intensity of *Gyrodactylus salaris* Malmberg, 1957 (Monogenean: Gyrodactylidae) on Atlantic salmon parr, *Salmo salar* L., in the River Batnfjordselva, Norway. *Journal of Fish Biology*, **41,** 697-707.

Nellbring, S. (1993) Spawning of two *Pomatoschistus* species (Gobiidae) in relation to nest availability and depth: a field experiment. *Netherlands Journal of Sea Research*, **31,** 2, 173-179.

Nie, P. & Kennedy, C. R. (1991) Occurrence and seasonal dynamics of *Pseudodactylogyrus anguillae* (Yin and Sproston) (Monogenea) in eel, *Anguilla anguilla* (L.), in England. *J. Fish Biol.*, **39,** 897-900.

Nyman, K.-J. (1953) Observations on the behaviour of *Gobius microps*. *Acta Soc. Pro Fauna Et Flora Fenn.* , **69,** 5, 1-11.

Pickering, A. D. & Christie, P. (1980) Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, *Salmo trutta* L. *Journal of Fish Biology*, **16,** 669-683.

Scott, M. E. & Anderson, R. M. (1984) The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology*, **89,** 159-194.

Scott, M. E. & Nokes, D. J. (1984) Temperature-dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies *(Poecilia reticulata)*. *Parasitology*, **89,** 221-227.

Scott, M. E. & Robinson, M. A. (1984) Challenge infections of *Gyrodactylus bullatarudis* (Monogenea) on guppies, *Poecilia reticulata* (Peters), following treatment. *Journal of Fish Biology*, **24,** 581-586.

Siegel, S. & Castellan, N. J. (1988) *Nonparametric Statistics for the Behavioural Sciences* (2nd edition).McGraw-Hill Book Company, 399 pp.

Sokal, R.R. & Rohlf, F.J. (1995) Biometry. (3th edition) W.H. Freeman and Company (eds), New York, 887 pp

Soleng, A. & Bakke, T. A. (1997) Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. *Can. J. Fish. Aquat. Sci.*, **54,** 1837-1845.

Thomas, J. D. (1964) A comparison between the helminth burdens of male and female brown trout, *Salmo trutta* L., from a natural population in the river Teify, West Wales. Parasitology, 54: 263-274.

Wallis, G. P. & Beardmore, J. A. (1984) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces, Gobiidae). *Biological Journal of the Linnean Society*, **22,** 107-123.

Chapter 7

Seasonal infection patterns of two sympatric goby species, *Pomatoschistus minutus* and *P. lozanoi,* with some endoparasitic helminths in relation to the ecological niche of the hosts

Geets, A.[1], Boden, G.[1,2], Hostens, K.[3], Hamerlynck, O.[3,4], & Ollevier, F.[1]

[1] *Laboratory for Ecology and Aquaculture, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*

[2] *Koninklijk Museum voor Midden-Afrika, Leuvensesteenweg, 11, B-3080 Tervuren, Belgium.*

[3] *Marine biology Section, Zoology Institute, Universiteit Gent, K. L. Ledeganckstraat, 35, B-9000 Gent, Belgium*

[4] *MDRE, PND, PB 3935 Nouakchott, Mauretania*

This chapter is in preparation for *Journal of Fish Biology*

Abstract

The seasonal dynamics of five endoparasitic helminths from *Pomatoschistus minutus* and *P. lozanoi* in the Grevelingen Voordelta Area (SW Netherlands) are studied: *Asymphylodora demeli* and *Derogenes varicus* (Trematoda, Digenea), plerocercoid larva of *Bothriocephalus* sp. (Cestoda), L3- and L4- stage *Hysterothylacium aduncum* and *Cucullanus* sp. (Nematoda). *Cucullanus* sp. occurred only occasionally in *P. minutus*. The component communities in both goby hosts were dominated by trematodes: *A. demeli* and *D. varicus* in *P. minutus* and *D. varicus* in *P. lozanoi*. *A. demeli* showed a marked preference for *P. minutus* over *P. lozanoi* as a final host, and was abundant in a limited infection period which ran from July to November. The defined seasonal occurrence of *A. demeli* combined with known dietary differences between *P. minutus* and *P. lozanoi*, led to the hypothesis that sedentary polychaetes could play an important role in transmitting this trematode to gobies. *Derogenes varicus* parasitized both *P. minutus* and *P. lozanoi* and a remarkable parallel trend in the time course of the infection was seen in both host. No differences in parasite load with *D. varicus* were found between overlapping age classes of a given host species. The main infection occurred in spring and early summer. During the spawning period, female *P. minutus* had a higher abundance of *D. varicus* than males, which was linked to differences in their diet. Larval stages of *H. aduncum* and *Bothriocephalus* sp. infected both gobies throughout the year. An elevated mean intensity of *H. aduncum* infection in May coincided with the spring bloom of one of the intermediate hosts, the calanoid copepod *Temora longicornis*, which is a preferred food item for both gobies in that period. The increased prevalence of *H. aduncum* in the *P. lozanoi* population from March onwards coincides with a spring bloom of mysids, *Schistomysis* spp., in the Voordelta. Interspecific differences in food niche between the two sympatric gobies studied were clearly reflected in differences in their endoparasitic fauna.

Introduction

Pomatoschistus minutus and *P. lozanoi* are two very common goby species in the shallow coastal areas of the North Sea. They are important components in the food web of these areas, taking an intermediate position: as predators of an array of invertebrates and as prey of a number of piscivorous fish (Fonds, 1973, Creutzberg & Witte, 1989, Hamerlynck & Hostens, 1993) and birds (Doornbos & Twisk, 1987, Zander & Döring, 1989). Many endoparasites have a complex life cycle that involves different hosts, and often depend on trophic interactions for their transmission (Marcogliese & Cone, 1997). The abundance and position in the food web makes *Pomatoschistus* species suitable hosts for a number of endoparasites (Zander, 1988, Zander & Döring, 1989).

P. minutus and *P. lozanoi* are phylogenetically very closely related species (Wallis & Beardmore, 1984) and occur sympatrically in the Grevelingen Voordelta Area. In some studies, both species were lumped together as *P. minutus* because of identification problems (Doornbos & Twisk, 1987, Henderson, 1989), which is unfortunate since they seem to have a distinct ecology. In the Belgian and Dutch coastal areas, a temporal segregation in the reproduction period is observed, *P. minutus* spawning about two months earlier (from March till July) than *P. lozanoi* (from May to September) (Fonds, 1973, Hamerlynck & Cattrijsse, 1994, Chapter 6 in this study). Less than 5% of the adults of both species survive their first spawning period (Doornbos & Twisk, 1987, Arellano, 1995). Both goby species also show a distinct partitioning in food resources: *Pomatoschistus minutus* feeding on a variety of benthic and epibenthic animals while *P. lozanoi* is more of a specialist, preying predominantly on hyperbenthic mysids (Hamerlynck *et al.,* 1986, Hamerlynck & Cattrijsse, 1994). This difference in food niche implies a spatial segregation, *P. minutus* spending more time near the bottom while *P. lozanoi* would be found more in the water column. The otoliths of *P. lozanoi* have a higher ratio of the sulcus acusticus to the sagitta area than *P. minutus*, which has been linked to the more frequent vertical migrations during pelagic feeding of *P. lozanoi* (Arellano *et al.*, 1995).

Marcogliese & Cone (1997) pleaded for including parasites in research of food webs to confirm possible trophic interactions, to reveal niche shifts in diet which may result from competitive interactions, or to indicate differences in feeding specialisation among individuals or closely related species. Food web structure, and particularly the extent to which the hosts concentrates on temporally or locally dominant food items, is very important in determining the component community of marine endoparasitic helminths (Holmes, 1990). Therefore, seasonal changes in endoparasite fauna are expected to be a reflection of temporal changes in feeding habits of the host. Furthermore, a difference in food niche between *P. minutus* and *P. lozanoi* may be mirrored in differences in the abundance or species richness of their respective parasite fauna.

In this paper, the seasonal dynamics of five endoparasitic helminths from *Pomatoschistus minutus* and *P. lozanoi* in the Grevelingen Voordelta Area are studied: *Asymphylodora demeli* and *Derogenes varicus* (Trematoda, Digenea), plerocercoid larva of *Bothriocephalus* sp. (Cestoda), *Hysterothylacium aduncum* and *Cucullanus* sp. (Nematoda). The aim is to investigate the possible link between differences in ecological niche of two closely related, sympatric gobies *P. minutus* and *P. lozanoi* (Hamerlynck & Cattrijsse, 1994) and observed seasonal and host-related differences in parasite fauna and parasite load.

Materials and Methods

Gobies were caught monthly, from June 1993 till July 1994, at four sampling stations in the shallow coastal waters of the Grevelingen Voordelta Area (SW Netherlands): Schaar van Renesse (51°45'N, 3°46'E), Brouwershavense Gat (51°45'N, 3°43'E), Bollen van het Zand (51°45'N, 3°39'E), Ooster (51°45'30"N, 3°38'E). A beam trawl with one trickling chain in the groundrope and a 6 m long shrimp net with 5x5 mm mesh size in the cod end was used for 1000 m hauls. Per sampling station, salinity (‰), water temperature (°C), oxygen saturation (%), pH, conductivity (µS/cm), Secchi depth (cm) and sampling depth (m) were recorded. Details on these environmental variables are given in Chapter 2. Immediately after capture, gobies were anaesthetised in a saturated solution of Benzocaïne (ethyl amino-benzoaat) in seawater to prevent regurgitation and then preserved in 4% formaldehyde solution.

Determination of gobies was based on Edlund *et al.* (1980) and Hamerlynck (1990). The different shape of the genital papilla was used for sexing the gobies (Fonds, 1973). In very young specimens (<25mm), sex determination was not always possible and these were classified as 'juveniles'. Standard length to the nearest mm was measured. Gobies of different yearclasses were separated using length frequency distributions and when necessary, otoliths, scales and the development of the gonads were checked.

Per monthly sample, approximately 60 *P. minutus* and 60 *P. lozanoi* (30 males and 30 females per species) were examined for endoparasites (Table 7.1). The majority (78%) of the examined gobies came from 'Schaar van Renesse' and 'Bollen van het Zand', since these stations had the highest densities of gobies (see Chapter 2). Only when not enough gobies were present, gobies from the other two sampling stations were used to supplement. Since no geographical differences in abundance with endoparasites could be detected (tested per parasite species and with host sexes both pooled and separate, Kruskal-Wallis Anova or a Mann-Whitney U test, $p>0.05$), data from gobies from different stations were pooled.

The gasterointestinal tract and the body cavity were examined for endoparasites. Our investigation was limited to the five most abundant helminths: *Asymphylodora demeli* and

Derogenes varicus (Trematoda, Digenea), plerocercoid larva of *Bothriocephalus* sp. (Cestoda), *Hysterothylacium aduncum* and *Cucullanus* sp. (Nematoda).

All parasites were counted under a dissecting microscope. For the determination of *Asymphylodora demeli*, *Derogenes varicus* and *Hysterothylacium aduncum*, most specimens were mounted on microscopical slides in glycerol jelly (Pritchard & Kruse, 1982) and studied with the light microscope (objectives 10x - 40x). *Bothriocephalus* sp. plerocercoid larva were divided in two developmental stages: the small stage, with less then five segments after the scolex, and the large stage with 5 or more segments. Larval stages of *Hysterothylacium aduncum* were determined according to Berland (1989). The terms prevalence, mean intensity and abundance were used according to the recommendations of Margolis *et al*. (1982).

The data on abundance and mean intensity of the parasite species were not normally distributed and no satisfying transformations could be found. Therefore, nonparametric statistics were used to test for significant differences in abundance and mean intensity between groups. A Mann-Whitney U test was used to test for differences in parasite load (abundance, mean intensity) between male and female hosts, and between parasite load on *P. minutus* and *P. lozanoi* in specific months. A Wilcoxon matched pairs test tested the overall yearly difference in percentage infection (prevalence) between *P. minutus* and *P. lozanoi* from yearclass 1993 (data used from September 1993 till June 1994). A Spearman Rank Order correlation was used to test for a possible correlation between standard length of the fish and parasite load. Significance levels were set on 5 % for all tests. Statistical analysis was carried out with STATISTICA 4.1 for Windows.

Table 7.1: Overview of samples of *Pomatoschistus minutus* and *P. lozanoi* from the Grevelingen Voordelta Area examined for endoparasitic helminths. (continued)

Pomatoschistus lozanoi

Date	Water-temperature (°C)	N fish m-f-j	Standard length ± S.D. (mm)	Yearclass	N inf. fish[1]	N Asym. (N inf. fish[2])		N Dero. (N inf. fish[2])		N Both. (N inf. fish[2])		N Hyst. (N inf. fish[2])	
08/06/93	16.8	14-13-0	47 ±4	1992	27	0	(0)	175	(27)	2	(2)	9	(8)
16/07/93	17.7	1-1-0	47 ±4	1992	2	0	(0)	9	(2)	1	(1)	0	(0)
07/09/93	16.4	21-10-19	35 ±4	1993	9	0	(0)	6	(5)	2	(2)	2	(2)
11/10/93	14.1	16-8-17	34 ±4	1993	15	4	(1)	8	(7)	7	(6)	1	(1)
09/11/93	10.3	56-35-6	43 ±4	1993	16	0	(0)	15	(13)	3	(3)	0	(0)
10/01/94	5.1	31-25-12	42 ±6	1993	22	24	(1)	12	(12)	7	(7)	4	(4)
02/02/94	5.8	44-35-0	46 ±5	1993	51	0	(0)	97	(45)	14	(10)	5	(5)
01/03/94	4.4	17-14-0	47 ±7	1993	30	0	(0)	163	(29)	3	(3)	6	(6)
19/04/94	8.2	34-34-0	42 ±5	1993	67	0	(0)	655	(67)	0	(0)	4	(4)
10/05/94	12.1	34-39-0	64 ±4	1993	71	0	(0)	456	(71)	4	(4)	20	(12)
14/06/94	14.5	7-4-0	43 ±5	1993	9	0	(0)	23	(9)	1	(1)	2	(2)
18/07/94	-	13-2-0	51 ±5	1993	7	0	(0)	21	(7)	0	(0)	3	(2)
Total		562			326	28	(2)	1640	(294)	44	(39)	56	(46)

---

[1] Number of fish infected with any of the five endoparasites

[2] Number of fish infected with a specific parasite

m-f-j: number of males-females and juvenile fish

Asym.: *Asymphylodora demeli*

Dero.: *Derogenes varicus*

Both.: *Bothriocephalus* sp.

Hyst.: *Hysterothylacium aduncum*

Table 7.1: Overview of samples of *Pomatoschistus minutus* and *P. lozanoi* from the Grevelingen Voordelta Area examined for endoparasitic helminths.

Pomatoschistus minutus

Date	Water-temperature (°C)	N fish m-f-j	Standard length ± S.D. (mm)	Yearclass	N inf. fish[1]	N Asym. (N inf. fish[2])		N Dero. (N inf. fish[2])		N Both. (N inf. fish[2])		N Hyst. (N inf. fish[2])		N Cucu. (N inf. fish[2])	
08/06/93	16.8	7-35-0	51 ±5	1992	40	0	(0)	151	(39)	4	(3)	7	(7)	1	(1)
16/07/93	17.7	0-2-0	58 ±1	1992	1	0	(0)	1	(1)	0	(0)	1	(1)	0	(0)
08/06/93	16.8	0-0-21	27 ±2	1993	19	0	(0)	45	(19)	0	(0)	0	(0)	0	(0)
16/07/93	17.7	31-10-10	38 ±7	1993	24	114	(5)	26	(18)	1	(1)	0	(0)	0	(0)
07/09/93	16.4	40-40-0	52 ±5	1993	45	2407	(32)	19	(16)	4	(3)	0	(0)	0	(0)
11/10/93	14.1	35-35-0	57 ±7	1993	31	1313	(21)	11	(11)	1	(1)	2	(2)	1	(0)
09/11/93	10.3	35-35-0	55 ±7	1993	25	334	(13)	8	(8)	2	(1)	2	(2)	2	(1)
10/01/94	5.1	29-28-0	54 ±7	1993	18	1	(1)	9	(9)	9	(7)	2	(2)	1	(2)
02/02/94	5.8	29-30-0	50 ±7	1993	24	48	(2)	20	(14)	14	(10)	1	(1)	0	(1)
01/03/94	4.4	36-45-0	52 ±7	1993	56	0	(0)	147	(49)	42	(12)	1	(1)	1	(0)
19/04/94	8.2	32-27-0	53 ±7	1993	57	0	(0)	845	(57)	3	(3)	2	(2)	3	(1)
10/05/94	12.1	11-45-0	53 ±5	1993	56	0	(0)	675	(55)	4	(4)	4	(3)	1	(2)
14/06/94	14.5	1-7-0	50 ±2	1993	8	0	(0)	57	(8)	0	(0)	0	(0)	0	(1)
18/07/94	-	20-20-0	47 ±4	1994	26	1744	(18)	20	(13)	0	(0)	0	(0)	0	(0)
Total		696			430	5961	(92)	2034	(317)	84	(45)	22	(21)	10	(9)

[1] Number of fish infected with any of the five endoparasites
[2] Number of fish infected with a specific parasite
m-f-j: number of males-females and juvenile fish
Asym.: *Asymphylodora demeli*
Dero.: *Derogenes varicus*
Both.: *Bothriocephalus* sp.
Hyst.: *Hysterothylacium aduncum*
Cucu.: *Cucullanus* sp.

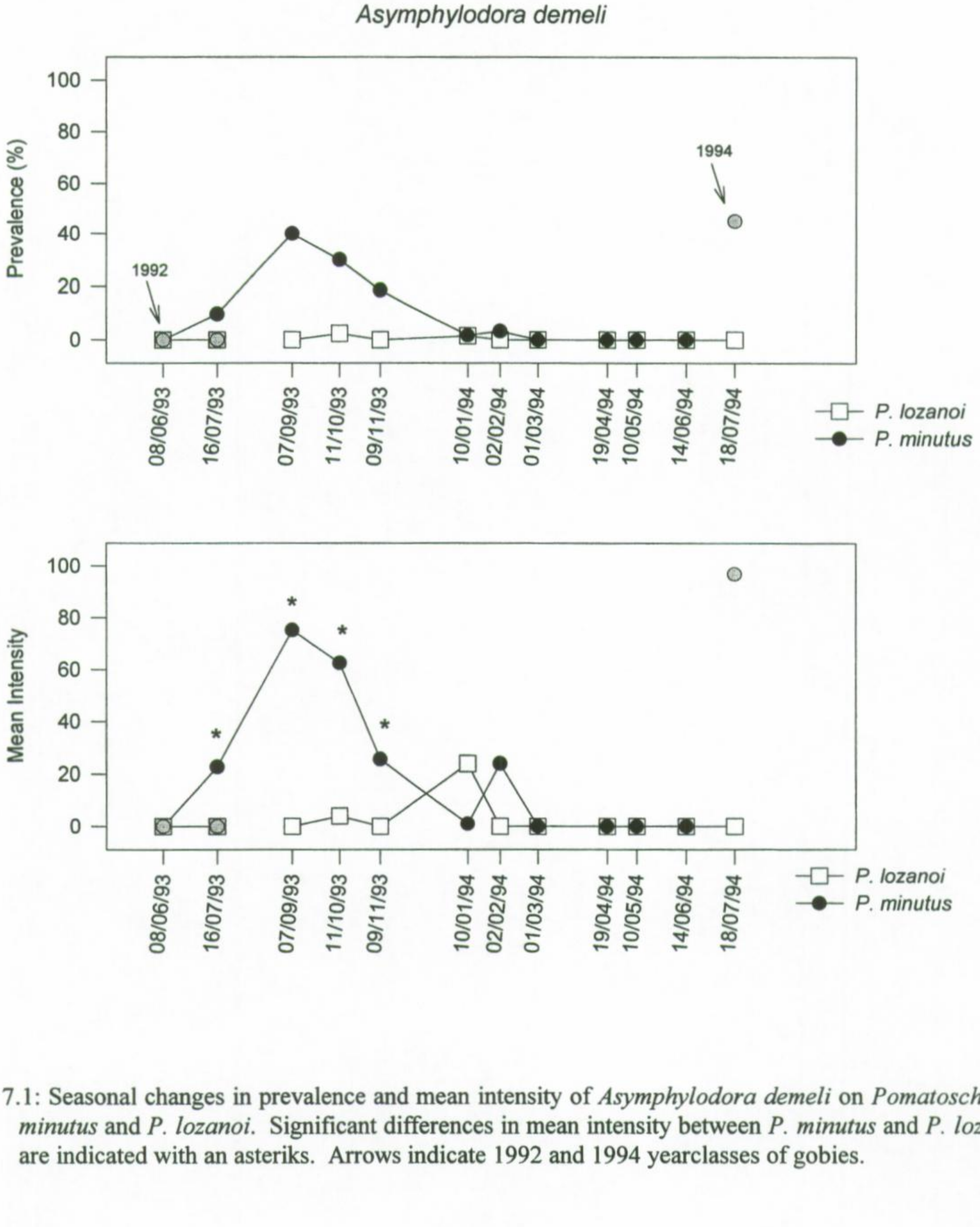


Figure 7.1: Seasonal changes in prevalence and mean intensity of *Asymphylodora demeli* on *Pomatoschistus minutus* and *P. lozanoi*. Significant differences in mean intensity between *P. minutus* and *P. lozanoi* are indicated with an asteriks. Arrows indicate 1992 and 1994 yearclasses of gobies.

Results

A total of 1258 gobies were examined for endoparasites: 696 *Pomatoschistus minutus* (Pallas, 1770) (55.3 %) and 562 *P. lozanoi* (de Buen, 1923) (44.7 %) (Table 7.1). A slightly higher proportion of *P. minutus* (61.8 %) were parasitized with endoparasitic helminths than *P. lozanoi* (58.0 %). Infected gobies hosted mostly only one endoparasite species. Only 12.6 % of the infected *P. minutus* and 15.6 % of the infected *P. lozanoi* carried two endoparasite species. One single *P. lozanoi* was found with three parasite species. The component communities of both goby host were numerically dominated by digenean trematodes: *Asymphylodora demeli* and *Derogenes varicus* in *P. minutus* and *D. varicus* in *P. lozanoi*. The nematode, *Cucullanus* sp. occurred occasionally in the intestine of *P. minutus*. Because of the low abundance of this nematode (a total of 10 worms were retrieved), no further analysis of the seasonal fluctuations were carried out. The gobies were final hosts for *Asymphylodora demeli* and *Derogenes varicus*. Larval stages of *Hysterothylacium aduncum* (third and fourth stage) and *Bothriocephalus* sp. (plerocercoid) used both gobies as intermediate and paratenic hosts, respectively. Seasonal changes in prevalence and mean intensity were observed for all four helminth species and will be discussed for each species separately.

* *Asymphylodora demeli*, Markowski, 1935 (Trematoda, Monorchiidae)

Asymphylodora demeli was almost exclusively found in the intestines of *P. minutus.* Only two *P. lozanoi* contained this parasite, one in October carrying 4 parasites and one in January with 24 specimens. In the 1993 yearclass, a significantly higher percentage *P. minutus* (12.0%) were infected than *P. lozanoi* (0.4%) (Wilcoxon matched pairs test, N=9, $p<0.05$). In June 1993, none of the adult *P. minutus* hosted *A. demeli*. Juvenile *P. minutus* became infected in July and prevalence and mean intensity increased rapidly (Fig. 7.1). A maximal mean intensity of 75.2 *A. demeli* was reached in September with 40 % of *P. minutus* population being infected. In October, *A. demeli* was still very common (62.5 per infected fish), decreasing towards November (mean intensity 25.7). From January onwards, hardly any *P. minutus* of the 1993 yearclass carried *A. demeli*. The parasite reappeared in great numbers in the juveniles of the 1994 yearclass: 45 % of the *P. minutus* juveniles were infected with an average of 96.8 digeneans per infected fish.

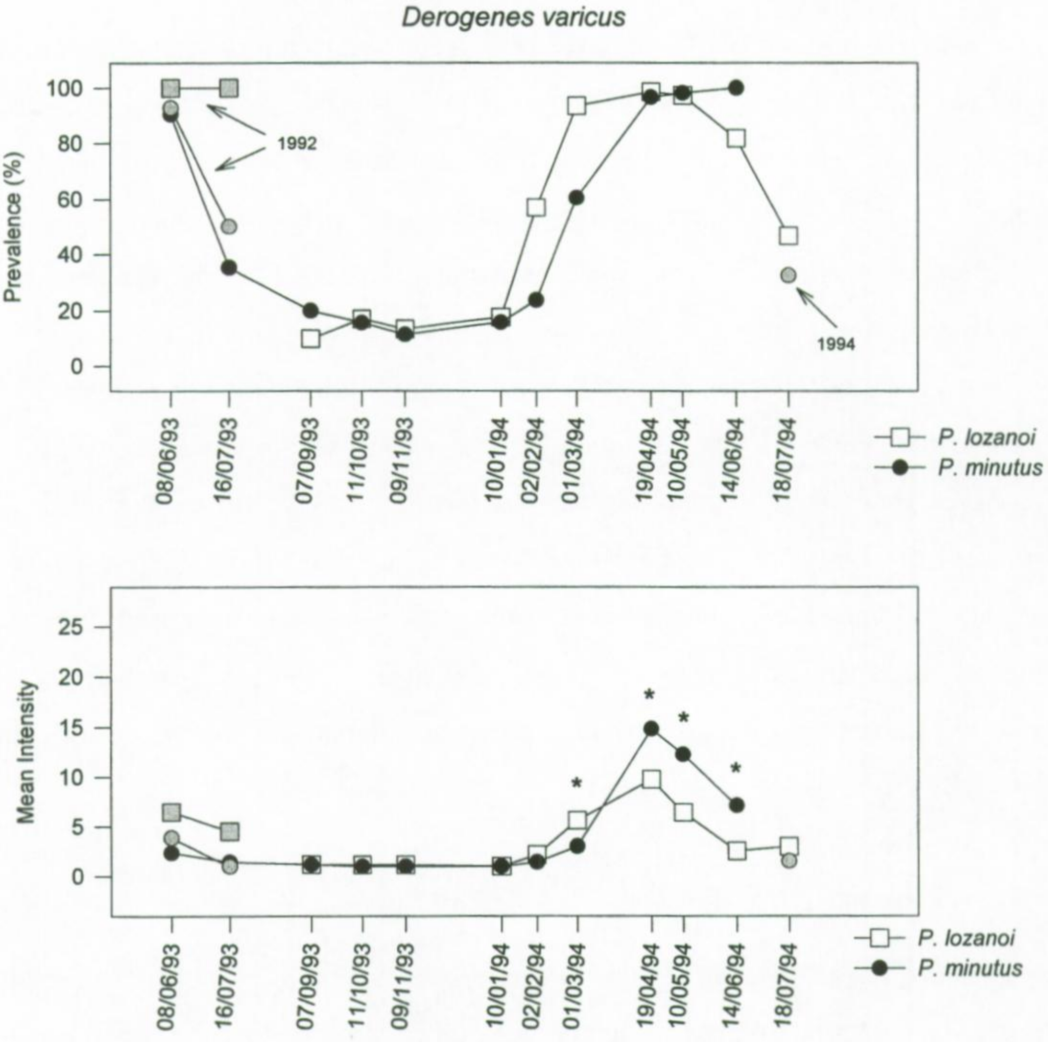


Figure 7.2: Seasonal changes in prevalence and mean intensity of *Derogenes varicus* on *Pomatoschistus minutus* and *P. lozanoi*. Significant differences in mean intensity between *P. minutus* and *P. lozanoi* are indicated with an asteriks. Arrows indicate 1992 and 1994 yearclasses of gobies.

Transmission of parasites from adults to juveniles did not seem to take place. Most of the time, *A. demeli* was equally abundant in male and female, except in July 1994, when young *P. minutus* females carried significantly more digeneans than the males (abundance: 62.9 and 24.3, respectively) (Fig. 7.5).

* *Derogenes varicus* (Müller, 1784) Looss, 1901 (Trematoda, Derogenidae)

Derogenes varicus parasitized both *P. minutus* and *P. lozanoi* and a remarkable parallel trend in the time course of the infection was seen in both host (Fig. 7.2). For the 1993 yearclass, a Wilcoxon matched pairs test did not show differences in prevalence with *D. varicus* in both goby hosts (average prevalence in 1993 yearclass: *P. minutus*: 49.0 %, *P. lozanoi*: 53.9 %, N=9, $p<0.05$). When age classes of gobies overlapped (June 1993 and July 1994), they carried about the same parasite load. The most important infection period ran from February till June. Maximal infection was reached in both goby species in April: 96.6 % of *P. minutus* were infected with an average of 14.3 parasites and 98.5 % of the *P. lozanoi* hosts carrying a mean of 9.6 *D. varicus*. Mean intensity and abundance was significantly higher in *P. minutus* from April to June when compared to *P. lozanoi*, but the opposite was true in February and March (Mann-Whitney U test, $p<0.05$). In June still a high prevalence of hosts with *D. varicus* occurred, but less parasites per infected fish were noted. In autumn and winter, almost no *D. varicus* were present. About equal percentages of male and female gobies were infected throughout most of the year, but *P. minutus* females carried significantly higher parasite loads (abundance) in April and May and in *P. lozanoi,* males had a higher abundance of *D. varicus* in February (Mann-Whitney U test, $p<0.01$) (Fig. 7.5).

A Spearman rank order correlation showed a significant negative correlation between the occurrence of *Asymphylodora demeli* and *Derogenes varicus* in *P. minutus* (N=696, r=0.25, $p<0.001$) and a positive correlation between *D. varicus* and *Hysterothylacium aduncum* in *P. lozanoi* (n=562, r=0.18, $p<0.01$).

* *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Anisakidae)

P. minutus and *P. lozanoi* were both intermediate hosts for the nematode *Hysterothylacium aduncum*. The majority of the nematodes were L3 stage larva (87.5% in *P. lozanoi* and 72.7 % in *P. minutus*) found in the body cavity, often on the liver.

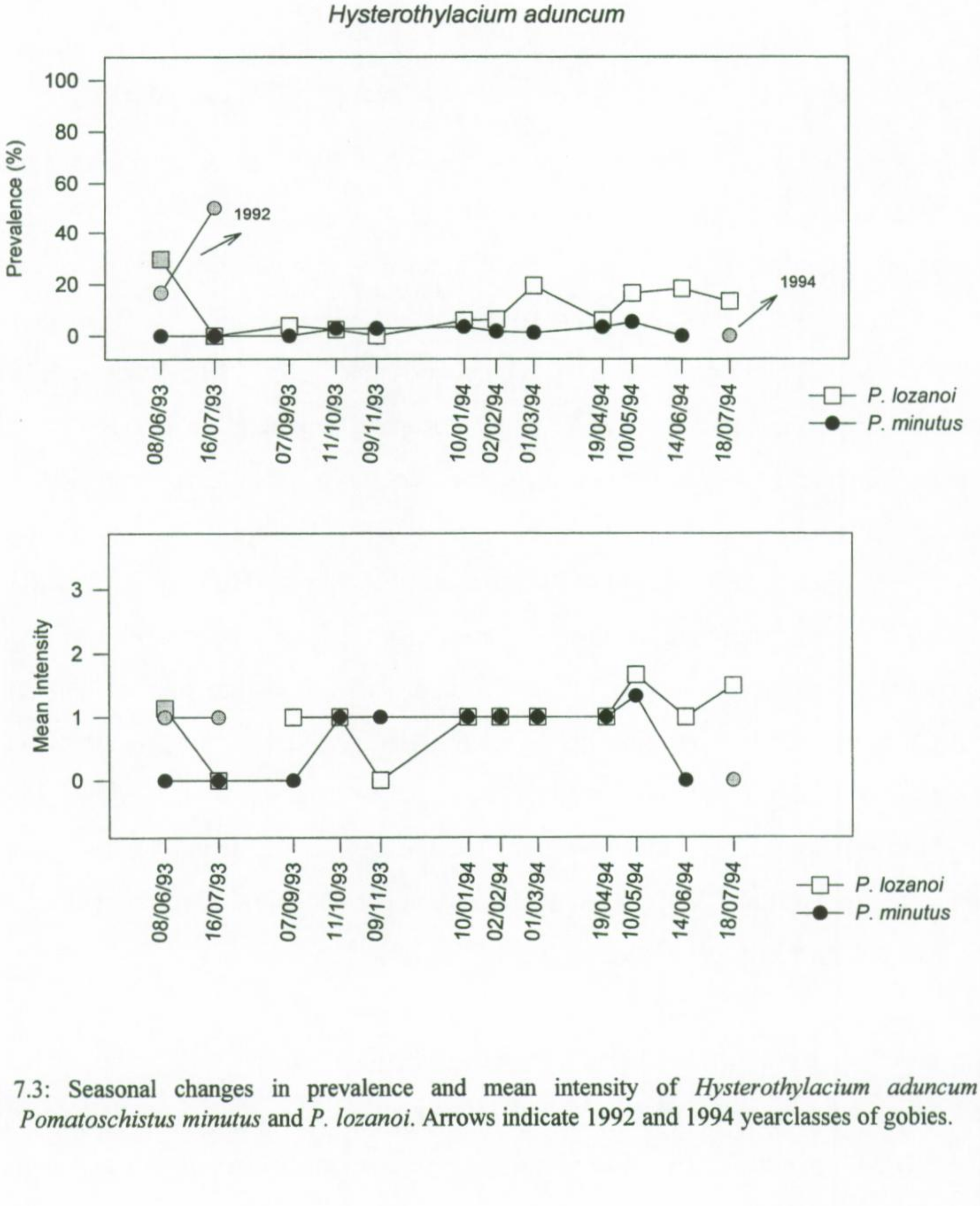


Figure 7.3: Seasonal changes in prevalence and mean intensity of *Hysterothylacium aduncum* on *Pomatoschistus minutus* and *P. lozanoi*. Arrows indicate 1992 and 1994 yearclasses of gobies.

Only once an L3 larva was recovered from the intestine of *P. lozanoi*. Occasionally, L4-stage larva were present in the gasterointestinal tract (Table 7.2). It was remarkable that most L4 stage larva from the intestine were smaller then the L3 stage larva found in the body cavity.

Table 7.2: Occurence of L3- and L4- stages of *Hysterothylacium aduncum* and small (<5 segments after scolex) and large (>5 segments) *Bothriocephalus* plerocercoids in *Pomatoschistus minutus* and *P. lozanoi.*.

	Hysterothylacium aduncum				*Bothriocephalus* plerocercoids			
	P. minutus		*P. lozanoi*		*P. minutus*		*P. lozanoi*	
date	L3	L4	L3	L4	small	large	small	large
08/06/93	6	1	9	-	-	4	-	2
16/07/93	1	-	-	-	-	1	-	1
07/09/93	-	-	2	-	3	1	2	-
11/10/93	2	-	-	1	1	-	2	5
09/11/93	2	-	-	-	1	1	2	1
10/01/94	1	1	4	-	3	6	3	4
02/02/94	-	1	5	-	3	11	8	6
01/03/94	1	-	3	3	8	34	2	1
19/04/94	1	1	1	3	-	3	-	-
10/05/94	2	2	20	-	2	2	-	4
14/06/94	-	-	2	-	-	-	-	1
18/07/94	-	-	3	-	-	-	-	-
Total n	16	6	49	7	21	63	19	25
%	72.7	27.3	87.5	12.5	25	75	43.2	56.2

The overall prevalence was higher for *P. lozanoi* (8.2 %) than for *P. minutus* (3.0%) (Wilcoxon matched pairs test on gobies of the 1993 yearclass, $N=9$, $p<0.05$). Most fish were infected with one nematode. A maximum of 7 nematodes were found in one female *P. lozanoi* of 41 mm in May. The gobies were infected throughout the year, but prevalence increased in *P. lozanoi* from late spring onwards (March till July) (Fig. 7.3). In May, a peak in mean intensity was noted in both goby species (1.3 for *P. minutus* and 1.6 for *P. lozanoi*).

Although mean intensity and abundance of infection was not significantly different between the sexes, the percentage infected females was markedly higher than the percentage infected males for both goby species: 85.7 % of the infected *P. minutus* were females and 63.0 % of the infected *P. lozanoi* (Fig. 7.5).

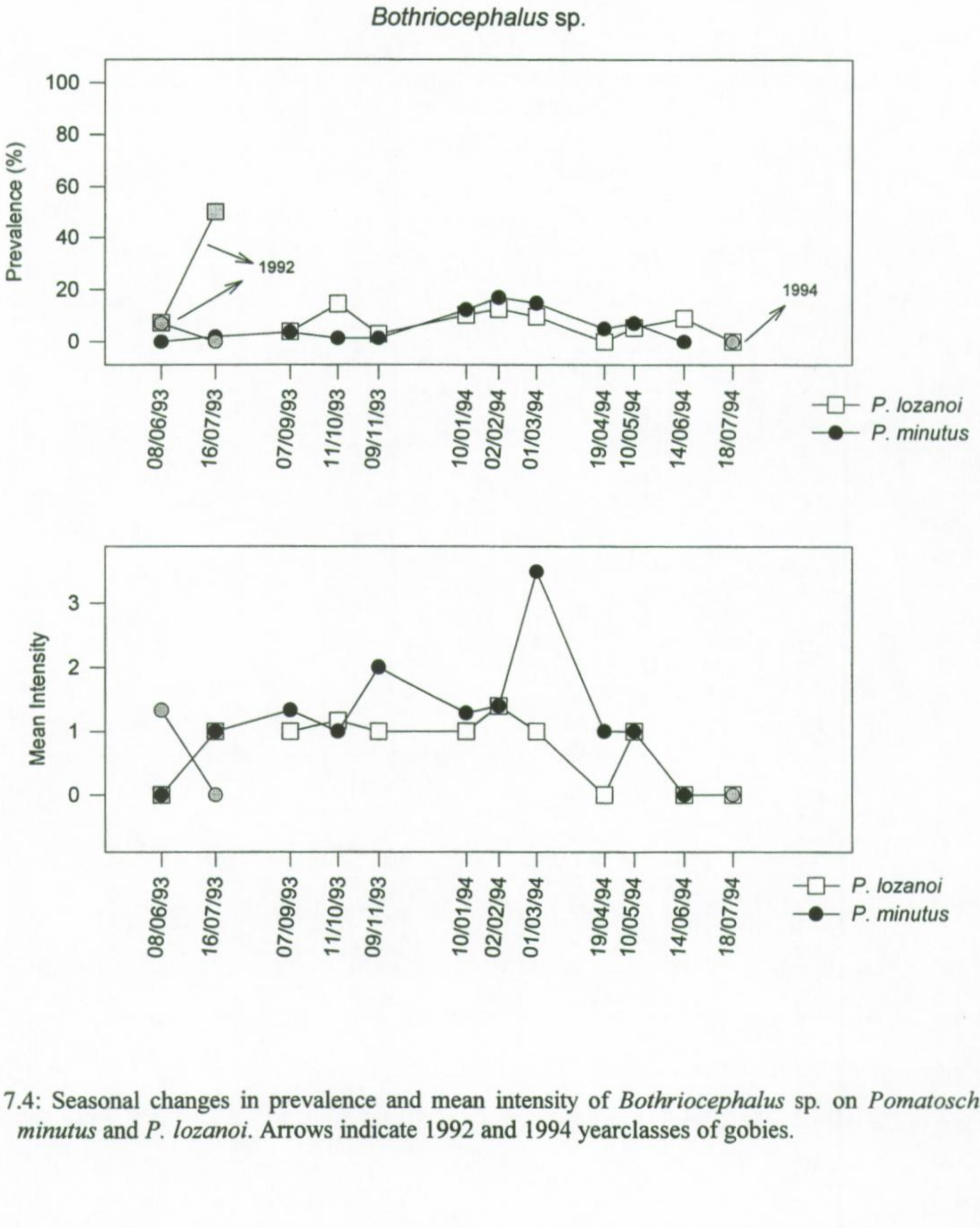


Figure 7.4: Seasonal changes in prevalence and mean intensity of *Bothriocephalus* sp. on *Pomatoschistus minutus* and *P. lozanoi*. Arrows indicate 1992 and 1994 yearclasses of gobies.

* *Bothriocephalus* sp. (Müller, 1776) (Cestoda, Pseudophyllidae)

Plerocercoid stages of *Bothriocephalus* sp. appeared throughout the year in both goby species (Fig. 7.4). The prevalence of *Bothriocephalus* sp. did not differ significantly between both hosts (Wilcoxon matched pairs test on 1993 yearclass, N=9, $p<0.05$). The average prevalence in 1993 yearclass of gobies was 6.9 % in *P. minutus* and 7.6% in *P. lozanoi*. They were always found in the first part of the gasterointestinal tract. The large larvae (with 5 or more segments) were most abundant (Table 7.2). These large plerocercoids were often coiled in a bag-like structure of thin layer of epithelium of the host's intestine, on the outside of the first part of the gasterointestinal tract. The scolex protruded through an opening into the gobies 'stomach'. The smaller stages were found in the lumen attached to the wall of the anterior part of the intestine. The small stage was found the whole year round, so infection seems continuous. Proportionally, the large stage became more frequent from January onwards in *P. minutus*. Highest infection levels were recorded in winter and early spring (January to March). Mean intensity in *P. minutus* peaked in March, when one male *P. minutus* was found infected with 14 plerocercoid larvae. During this period, male gobies were generally more infected than females, but this trend was not statistically significant (Fig. 7.5).

A Spearman Rank Order Correlation between parasite load and standard lengths of the fish did not show any significant correlation for the four helminth endoparasites.

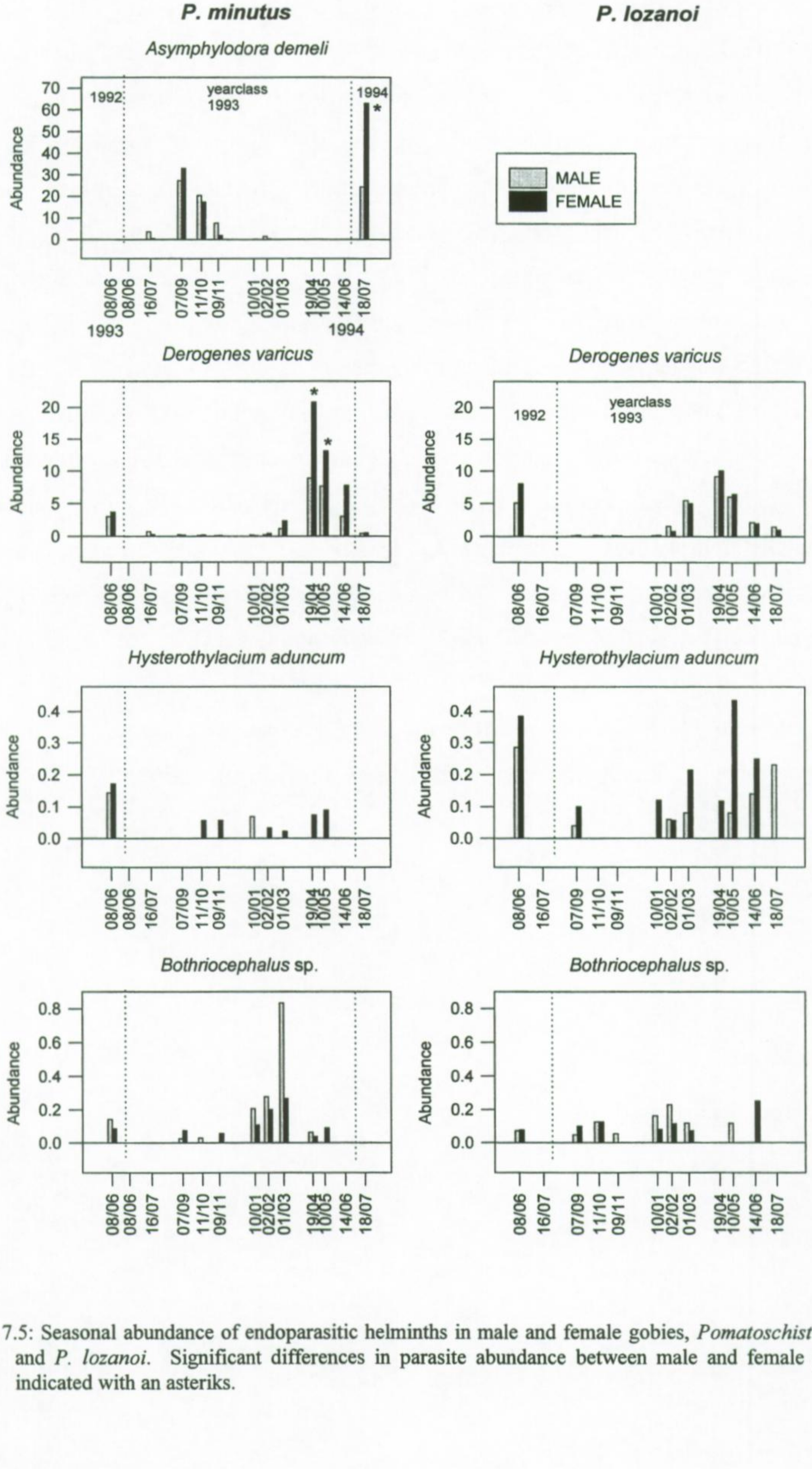


Figure 7.5: Seasonal abundance of endoparasitic helminths in male and female gobies, *Pomatoschistus minutus* and *P. lozanoi*. Significant differences in parasite abundance between male and female gobies are indicated with an asteriks.

Discussion

Clear differences between parasite loads and prevalence with four endoparasitic helminths between the sympatric *P. minutus* and *P. lozanoi* were observed. Since all of these parasites are transmitted through intermediate hosts, differences in the food preferences of both gobies could have a major effect on their respective parasite component communities. A detailed study on the food niche of *P. minutus* and *P. lozanoi* in the Belgian coastal waters was conducted by Hamerlynck *et al.* (1986, 1990) and Hamerlynck & Cattrijsse (1994). Clear seasonal and host-dependent differences in diet were observed. Comparison of their results with stomach analysis on both goby species from the Grevelingen Voordelta Area showed no considerable differences between the areas (Hamerlynck *et al.*, 1989). The interpretation of the differences in endoparasitic fauna reported in the current study will be based on the results of the above mentioned research.

* *Asymphylodora demeli*

Asymphylodora demeli showed a marked preference for *P. minutus* over *P. lozanoi* as a final host, and was abundant in a limited infection period which ran from July to November. Table 7.3 reports the different hosts which were described for *A. demeli*. The life cycle of *A. demeli* is not completely resolved but several authors described different stages of *A. demeli* in a range of hosts. *Hydrobia* species (Gastropoda) were reported as the first intermediate hosts carrying rediae and cercariae in the Baltic and the German Ostsee (Markowski, 1936, Reimer, 1973). *Asymphylodora* species have tailless cercarial stages that usually encyst as metacercaria in gastropods (Van Den Broek & De Jong, 1979). A variety of mollusc species, gastropods and a sedentary polychaete (*Alcmaria romijni*) acted as second intermediate hosts for the metacercaria in the Baltic and North Sea (Markowski, 1936, Lauckner, 1971, Margolis, 1971, Reimer, 1973, Vaes, 1974). As final hosts, *Pomatoschistus minutus*, *P. microps, P. pictus, Gobiusculus flavescens* and *Gobius niger* (Markowski, 1935a, Koter, 1962, Zander & Kesting, 1998) have been reported from the brackish Baltic Sea, although infection levels were generally very low. Reimer (1973) described an alternative life cycle for *A. demeli* from the Baltic sea, where the main definitive host for this species was roach (*Rutilus rutilus*), but mature specimens were also present in the polychaete *Nereis diversicolor*. Vaes (1974) also collected gravid specimens of *A. demeli* from the gut of *N. diversicolor* from the Belgian coast. In vitro infections of *Nereis* with metacercaria derived from *Hydrobia stagnalis* and *Cerastoderma glaucum* yielded gravid worms after a minimum of 8 days (Vaes, 1974). Direct infection with the cercariae was unsuccessful. Therefore, *Nereis diversicolor* was regarded as a final host and not as a second intermediate host with progenetic metacercariae. Progenesis, where sexual maturation of the metacercaria occurs, has been reported for *A. tincae* (Zietse *et al.*, 1981) and for *A. kubanicum* (Evans, 1978). Vaes (1974) stated that *N. diversicolor* could be regarded as the principal, and maybe the only, final host in the area. In the Grevelingen Voordelta, however, *P. minutus* carried high loads of gravid *A. demeli*, which underlined the importance of this goby species as a final host in this area. Although, the two infected *P. lozanoi* carried gravid *A. demeli*, the infection seemed accidental and *P. lozanoi* should not be regarded as an important host for *A. demeli* in the Grevelingen Voordelta Area.

The most probable infection route of gobies could be by eating the second intermediate hosts which contain metacercaria of *A. demeli*. When comparing the list of second intermediate hosts (Table 7.3) with the prey organisms of *P. minutus* and *P. lozanoi* from the Belgian coastal waters (Hamerlynck *et al.*, 1986), the bivalve *Cerastoderma edule* (=*Cardium edule*) is found in both lists. It was reported as a prey organism for *P. minutus* but not for *P. lozanoi* (Hamerlynck *et al.*, 1986). Bivalve siphons were a main food component in both hosts. Although the sedentary polychaete *Alcmaria romijni,* mentioned as second intermediate host (Margolis, 1971), was not recorded as prey item for the gobies of the Dutch coastal waters, several other sedentary polychaetes were reported as food for *P. minutus* (Hamerlynck *et al.*, 1986, Zander & Westphal, 1991). *Laniche conchilega* and *Pectinaria koreni* were numerically and gravimetrically most important (Hamerlynck & Cattrijsse, 1994). Only one species of sedentary polychaetes, *Lanice conchilega,* was prey for *P. lozanoi* (Hamerlynck *et al.*, 1986, Hamerlynck *et al.,* 1990)

Seasonal changes in food composition are more apparent in *P. minutus* than in *P. lozanoi*. In July, juvenile *P. minutus* mainly fed on epibenthic caprellid amphipods and hyperbenthic mysids, but *Lanice conchilega* was of subsidiary importance. *L. conchilega* was numerically important in July, August, October and December, whereas *Pectinaria koreni* and bivalve siphons were important prey items in September and in male *P. minutus* in April, when they are confined to the nest sites (Hamerlynck & Cattrijsse, 1994). The occurrence of *A. demeli* in

P. minutus was clearly limited to the late summer and autumn (July to November), and this coincides most with the dominance of *Lanice conchilega* in the food. Hence, this polychaete could possibly be a good candidate as second intermediate host for *A. demeli*, transmitting this digenean to *P. minutus* in the Grevelingen Voordelta Area. During parasitological analysis, it was noted that several *P. minutus* had remains of polychaetes in their stomach in July and October (Geets, unpublished observation), while this was less conspicuous in the other months. High densities and biomass of *Lanice conchilega* aulophorus larvae were present in the hyperbenthic community of the Voordelta area from May to September, indicating a build-up of the population of these sedentary polychaetes during that period (Hamerlynck & Mees, 1991). *L. conchilega* was only found in the food spectrum of *P. lozanoi* in August during spawning. During the rest of the year, mysids dominate the food spectrum. In early summer a sudden decrease of the mysid populations was noticed in the Voordelta Area (Mees *et al.*, 1993). Unfortunately, no samples of *P. lozanoi* were available from the Grevelingen Voordelta in August to investigate the possible presence of *A. demeli*.

A second possible pathway of infection of *P. minutus* could be through transmission of adult *A. demeli* from *Nereis diversicolor* to *P. minutus*. Hamerlynck *et al.* (1986) reported *Nereis* sp. as a food item for *P. minutus* and not for *P. lozanoi*. No data on horizontal transmission between final hosts in other *Asymphylodora* species are available, but have been reported for other digeneans (Køie, 1979, 1984, Maillard & Aussel, 1988). Our data also suggest that *A. demeli* and *D. varicus* probably use different intermediate hosts, since their infection periods did not show any overlap in the final host. Since most of the intermediate hosts of *D. varicus* are known, this already limits the possible candidates of intermediate hosts for *A. demeli*. Because no infection of *P. minutus* with *A. demeli* was recorded in spring and summer, the question arises how the *A. demeli* population is sustained during this period. Since the errant polychaete *Nereis diversicolor* was already mentioned in literature as an alternative final host, it would be worthwhile to investigate its importance as a host for *A. demeli* in the Grevelingen Voordelta area.

The differences in food niche of *P. minutus* and *P. lozanoi* are probably the primary cause for the observed difference in infection with *A. demeli*. Host specificity of *A. demeli* for *P. minutus* should not be ruled out. *A. tincae*, a freshwater relative of *A. demeli*, was host specific for tench (*Tinca tinca*) (Zietse *et al.*, 1981). Eight other species of cyprinids from the same lake were not infected. In vitro, metacercarial cysts of *A. tincae* opened within 15 seconds when in contact with tench bile, while it took at least 60 seconds or longer to excyst using bile of other cyprinids.

Seasonal differences in abundance of *A. demeli* are probably linked to changes in food composition of *P. minutus* and not to fluctuating availability of infected second intermediate hosts. Metacercaria of *A. tincae* were available in the snail population throughout the year, and seasonal dynamics with high infections in spring and summer were related to the higher feeding activity of the final fish host in that period (Zietse *et al.*, 1981). Evans (1978) found no infection of roach (*Rutilus rutilus*) with *A. kubanicum* until they were two years old when the snail *Bithynia tentaculata* (second intermediate host for *A. kubanicum*) became a prominent food item. In accordance to our study, Evans (1978) did not find any major differences in infection with *A. kubanicum* between the sexes of roach. The drop in infection levels in *P. minutus* from November onwards could be linked to a change in the hosts' feeding habits, excluding the second intermediate host. Furthermore, the death of the adult parasites after egg laying, the slower development and excystment of metacercaria and a lower process of maturation of the adults with lower temperature (Zietse *et al.*, 1971, Evans, 1978, Van Den Broek & De Jong, 1979) probably added to the observed decrease in infection levels. *Asymphylodora* species are reported to hibernate predominantly as larval stages in the first and second intermediate hosts (Evans,1978), although some specimens were also found in the final host (Zietse *et al.*, 1981).

* *Derogenes varicus*

The similarity in the seasonal fluctuation of the population densities of *Derogenes varicus* between both goby species implies largely equal chances of infection. *D. varicus* is regarded as one of the most widely distributed digenetic trematodes, found in temperate, subarctic and subantarctic regions and in the colder waters underlying the warm surface waters of the tropics (Manter, 1955, Køie, 1979). It is recorded from more than a hundred fish hosts. The life cycle of *D. varicus* was described by Køie (1979) (Table 7.3). As a first intermediate hosts, the snails *Natica* spp. carry rediae which develop in free-swimming cercariae. These free-swimming stages will develop into metacercaria when eaten by calanoid and harpacticoid copepods which are second intermediate hosts. Hermit crabs (*Pagurus* sp.) and chaetognaths (*Sagitta* sp.) also carry metacercaria. Køie (1979) presumed that *Sagitta* spp. became infected by eating copepods and acts as transport hosts. *Temora longicornis* and *Centropages hamatus* were experimentally infected next to four other calanoid copepods and small harpacticoid copepods (Køie, 1979). Amphipods (*Gammarus* spp.), isopods (*Idotea* sp.), mysids (*Praunus* sp.), decapods (*Cragnon cragnon*, *Pagurus bernhardus*) and cirripedians did not get infected when placed several months together with infested *Natica spp.*

Hamerlynck *et al*. (1990) reported *Temora longicornis* as a dominant food item for *P. lozanoi* in April and May, later on being replaced by other calanoid copepods (*Centropages* sp.) and harpacticoids. From September onwards, mysids and amphipods, which are not suited as intermediate hosts for *D. varicus* (Køie, 1979), replaced these food items. In *P. minutus*, *Temora longicornis* was the dominant prey for females during spawning in April and May. Male *P. minutus* have a different diet in that period, mainly feeding on bivalve siphons, *Pomatoschistus* eggs and *Pectinaria* sp. These feeding patterns coincided nicely with the increase in prevalence of *D. varicus* in both hosts from March onwards. The significantly higher parasite load in *P. minutus* females compared to males in April and May is possibly related to the higher amount of *T. longicornis* in their food. A higher mean intensity of *D. varicus* in *P. minutus* could be due to the fact that *P. minutus* is more of a generalist than *P. lozanoi*. For example, two times more species of harpacticoid copepods are found on the menu of *P. minutus* than on *P. lozanoi* (Hamerlynck *et al*., 1986), thus increasing the chance of infection of *P. minutus* with *D. varicus*.

Køie (1979) suggested in her search for the life cycle of *D. varicus* that gobies may only function as transport hosts since *D. varicus* did not occur naturally in gobies of the Danish waters. However, our data for the Grevelingen Voordelta Area point out that both goby species are important final hosts for *D. varicus*. *Derogenes* specimens found in the intestinal tract of the gobies were mostly gravid. Gobies, being an important food item for cod (*Gadus morhua*) (Arntz, 1978, Salvanes & Nordeide, 1993) could be an ideal host for transmitting the parasite to this predator. Køie (1979, 1984) cited *D. varicus* as a common cod parasite and the adults may be transferred from one fish to the other. In cod, it is most abundant from

April to July (Meskal, 1967), which coincides with the high abundance found in gobies during that period.

The positive correlation between the occurrence of *D. varicus* and the nematode *Hysterothylacium aduncum* in *P. lozanoi*, is probably due to the dominance of *Temora longicornis* in the food of *P. lozanoi* in April and May and the fact that both parasites use this invertebrate as an important intermediate host. The bloom of *Temora longicornis* reported in April-May (Daro & van Gijsegem, 1984, Hamerlynck *et al.*, 1989) is synchronized with the increase of both parasites in the gobies.

* *Hysterothylacium aduncum*

Hysterothylacium aduncum third and fourth stage larvae were found in both gobies. The life cycle of *Hysterothylacium aduncum* is quite complex. The morphology of the larval stages and all possible pathways of the life cycle were described by Køie (1993) (Table 7.3). The first and second moult of the larva occurs in the egg, which by then contains a third stage larva. At least one intermediate host, a crustacean, is obligatory in the transmission of *H. aduncum*. A large range of calanoid and harpacticoid copepods, mysids (*Neomysis integer*), amphipods (*Gammarus* spp.), isopods (*Idotea* sp.) became easily experimentally infected by feeding them eggs with third stage larva. Mysids and chaetognaths (*Sagitta* sp.) were also recorded as important intermediate and transport hosts for *H. aduncum* in the North West Atlantic (Jackson *et al.*, 1997). Marcogliese (1996) updated the intermediate host list with several marine crustaceans. Third stage larva are only infective to fish after growth in one or more intermediate hosts. Køie (1993) showed that larval development in the fish depended on the length of the third stage larva when it entered the fish host. Larva smaller than 2 mm died after a few days, larva between 2 and 3 mm were able to penetrate the intestinal wall and enter the body cavity where they grew. Larva longer than about 3 mm remained in the intestinal lumen and moulted into a fourth stage larva in a few days. This explains why we found L4 stage larva in the intestine of our gobies which were smaller than the L3 stages in the body cavity.

P. lozanoi has a rather specialized diet, with the calanoid copepod *Temora longicornis* and mysids *Schistomysis* spp. providing most of its energy throughout the year (Hamerlynck *et al.*, 1990). This could maybe explain the higher overall prevalence of *H. aduncum* in *P. lozanoi* in comparison to *P. minutus*, which is a generalist and feeds more on benthic species. Furthermore, *Hysterothylacium* third stage larva can survive for at least two months (Køie, 1993), so a concentration effect could play a role.

The observed increase of the prevalence of *H. aduncum* in the *P. lozanoi* population from March onwards coincides with the spring peak in abundance of *Schistomysis spiritus* (a possible intermediate host) in the Voordelta area (Mees *et al.,* 1993). The increase in mean intensity in both goby species in May coincided with a marked dominance of the copepod *Temora longicornis* in the diet of *P. minutus* females and of *P. lozanoi* in April and May (Hamerlynck *et al*., 1990, Hamerlynck & Cattrijsse, 1994). Petersen (1992) also observed increased abundances of *H. aduncum* in *P. minutus* of the German Wadden Sea in May and June. More females than males were infected in both goby species. *P. minutus* males were not infected in the spawning season. Differences in food preference of male and female *P. lozanoi* are not studied in detail. In accordance to what is known from its close relative *P. minutus*, however, it can be assumed that during the spawning period, *P. lozanoi* males who have to guard the eggs might switch to a more benthic diet, while females could go on feeding on more hyperbenthic prey, and thus be more vulnerable to infection with *H. aduncum*.

* *Bothriocephalus* sp.

Gobies have often been cited as intermediate hosts for plerocercoids of *Bothriocephalus scorpii* (Markowski, 1935b, 1936, Robert *et al*., 1988, Zander & Döring, 1989, Leducq *et al*., 1990). The life cycle of *Bothriocephalus scorpii* was described from the Baltic by Markowski (1935b). In an infection experiment, Markowski (1935b) used the copepod *Eurytemora hirundo* as the first intermediate host. Markowski (1935b) mentioned that other copepods could probably also be infected, but up till now further research on other invertebrate hosts is lacking. *P. minutus* would be the second intermediate host, and turbot, *Scophthalmus maximus*, brill, *Scophthalmus rhombus* and bull-rout, *Myoxocephalus scorpius*, are cited as final hosts. Since no major differences in infection between *P. minutus* and *P. lozanoi* were observed, the second intermediate host should be a food organism which both gobies eat in equal amounts.

In the North Sea, Davey & Peachey (1968), De Groot (1971) and Braber & De Groot (1973) reported differences in the distribution of *B. scorpii* in turbot and brill. They attributed this observation to the difference in structure of the intestinal tract of the hosts. But, a study of enzymatic polymorphism revealed that *Bothriocephalus scorpii* was a complex of different species. The cestodes of turbot were named *B. gregarius*, those of brill, *B. barbatus* (Renaud *et al*., 1983) and *Myoxocephalus scorpius* would harbour *B. scorpii* (Renaud & Gabrion, 1984). Subsequent research on the plerocercoids found in *Pomatoschistus marmoratus* and *P. minutus* along the French coast confirmed they belong to the species *B. gregarius* (Robert *et al*., 1988). The plerocercoid stages found in our gobies of the Dutch coastal waters are therefore most probably *B. gregarius*, but electrophoresis could give a conclusive answer. Although the complete life cycle is not solved for *B. gregarius*, the first intermediate host is most probably a copepod (Table 7.3).

The occurrence of the plerocercoid stage in young turbot which do not yet feed on gobies, opened the discussion on the host status (intermediate or paratenic) of the gobies (Jarecka, 1964, Davey & Peachey, 1968, Robert *et al*., 1988). Robert *et al*. (1988) proposed two types of life cycles for *B. gregarius*: (i) a short cycle with a copepod as obligatory intermediate host and mainly young turbot as a final host, and (ii) a long cycle in which the goby is an paratenic host acting between the copepod first intermediate host and the final host, when turbot starts to eat gobies. The gobies would play a fundamental role in the dynamics of *B. gregarius* by favouring the access of the parasite to its final host, and by concentrating the larvae at a higher trophic level, expressed by the high mean intensities in the definitive hosts.

In the Grevelingen Voordelta Area, the gobies were continuously infested throughout the year. The prevalence and mean intensity of *Bothriocephalus* plerocercoids slightly built up towards springtime. The increase in mean intensity could be explained by a high life expectancy of the plerocercoid, together with an ongoing infestation. Robert *et al*. (1988) estimated the life-span to be at least 80 days. The increased proportion of large larvae from January confirms this idea. Robert *et al*. (1988) observed an increase in size and segmentation of the plerocercoids with longer captivity of infected gobies. The drop in intensity of infection in June and July 1994 is probably due to the lower number of adult gobies investigated, the appearance of young uninfected *P. minutus* in July, and possibly a higher mortality of infected gobies due to increased predation or to lowered condition of the heavily infected fish.

* *General conclusion*

In the current study, it was shown that the seasonal and host-related differences in endoparasitic helminth fauna of both gobies from the same area are primarily linked to their difference in food niche, and are less affected by differences in reproductive niche. The seasonal dynamics and the differences in abundance of the monoxenous monogenean *Gyrodactylus* sp. 1 on *P. minutus* and *P. lozanoi* of the Grevelingen Voordelta Area were predominantly determined by the temporal differences in reproductive niche and in spatial distribution (benthic versus hyperbenthic) of both hosts (Chapter 6). The seasonal changes in feeding habits of *P. minutus* and *P. lozanoi* clearly played a role in the population dynamics of the trematodes *A. demeli* and *D. varicus*. Interspecific differences in food niche between *P. minutus* and *P. lozanoi*, with *P. minutus* being a generalist and *P. lozanoi* a specialised feeder, were reflected in their respective parasite fauna. A higher percentage of *P. minutus* carried endoparasites and this goby hosted five parasite species, while only four (of which *A. demeli* occurred only occasionally) were found in *P. lozanoi*.

Ecological host specificity (Rohde, 1982) is demonstrated in the case of *A. demeli*. This trematode was already reported from several (gobiid) hosts, but always in very low numbers. The ecology of *P. minutus* in the Grevelingen Voordelta seems to favour this parasite. Even, *D. varicus* and *H. aduncum*, which have a very broad host range (both intermediate and final) showed differences in abundance and prevalence between *P. minutus* and *P. lozanoi*.

The importance of the host's feeding habits for the determination of its parasite community was also observed in the sex-related differences of infection during the spawning period of the gobies, when male and female have a distinctly different diet. Generally , this seemed to act in the disadvantage of the females. By feeding more than males during spawning and by feeding on a broader and different food spectrum, females became more heavily infected by *D. varicus* and *H. aduncum*.

Table 7.3: Summary of host species reported for *Asymphylodora demeli*, *Derogenes varicus*, *Hysterothylacium aduncum* and *Bothriocephalus* sp. Numbers between brackets refer to references.

Asymphylodora demeli

1st intermediate host	**2nd intermediate host**	**final host**
Gastropoda	Gastropoda	Polychaeta: Errantia
Hydrobia stagnalis (2)	*Hydrobia ulvae* (1,2)	*Nereis diversicolor*(2,6)
Hydrobia ulvae (1,2)	*Hydrobia stagnalis (=ventrosa)* (1,2,6)	Pisces
	Theodoxus fluviatilis (1)	*Rutilus rutilus* (2,8)
	Limnaea ovata forma baltica (1,4)	*Pomatoschistus minutus* (7,8,9)
	Bivalvia	*Pomatoschistus microps* (7)
	Cardium edule (1)	*Pomatoschistus pictus* (7,8)
	Cardium lamarcki (2,3)	*Gobius niger* (7)
	Cerastoderma (=Cardium) glaucum (6)	*Gobiusculus flavescens* (8)
	Polychaeta: Sedentaria	
	Alcmaria romijni (5)	

Derogenes varicus

1st intermediate host	**2nd intermediate host**	**transport host**	**final host**
Gastropoda	Copepoda: Calanoidea	Chaetognata	Pisces
Natica catena (10)	*Temora longicornis* (10)	*Sagitta elegans* (10)	Several teleosts
Natica alderi (10)	*Paracalanus parvus* (10)		*Gadus morhua* (11,13)
Natica pallida (10)	*Pseudocalanus elongatus* (10)		flatfish (15)
	Acartia sp. (10)		gobies (12)
	Centropages hamatus (10)		*Anguilla anguilla* (14)
	Calanus finmarchicus (10)		
	Copepoda: Harpactoidea (10)		
	Anomura		
	Pagurus pubescens larva (10)		
	Polychaeta: Errantia		
	Hamothoe imbricata (5)		

Hysterothylacium aduncum

1st intermediate host	**2nd intermediate host or transport host**	**final host**
Copepoda: Calanoidea	Chaetognata	Pisces
Acartia tonsa (17)	*Sagitta* sp. (16,17,20)	several Teleosts (17,19)
Eurytemora affinis (17)	Polychaeta: Errantia	*Zoarches viviparus* (17,19)
Pseudocalanus elongatus (17)	*Harmotoe imbricata* (17)	*Gadus morhua* (11,20)
Temora longicornis (18)	*Gattyana cirrosa* (17)	
Copepoda harpacticoidea (17)	*Lepidonotus squamatus* (17)	
Mysidacea	*Nereis pelagica* (17)	
Neomysis integer (16,17)	Echinodermata	
Mysis stenolepis (16,20)	*Ophiura* spp. (17)	
Amphipoda	*Ophiopholis albida* (17)	
Gammarus spp. (16,18)	*Ophiopholis aculeatus* (17)	
Calliopius laeviuscullus (18)	Ctenophora	
Isopoda	*Pleurobranchia pileus* (17)	
Idotea sp. (17)	Pisces	
	Pomatoschistus spp. (12,29)	
	Gasterosteus aculeatus (17)	
	Pungitius pungitius (17)	
	Anguilla anguilla (14)	

→ this study?

Bothriocephalus (gregarius)

1st intermediate host	**transport host**	**final host**
Copepoda (21)	*Pomatoschistus* spp. (12,21,25,27,28)	*Scophthalmus maximus* (22,23,24)

Table 3: continued (references)

(1) Markowski (1936)
(2) Reimer (1973)
(3) Lauckner (1971)
(4) Szidat (1943)
(5) Margolis (1971)
(6) Vaes (1974)
(7) Koter (1962)
(8) Zander & Kesting (1998)
(9) Markowski (1935a)
(10) Køie (1979)
(11) Køie (1984)
(12) Zander et al. (1993)
(13) Meskal (1967)
(14) Køie (1988)
(15) Køie (1983)
(16) Marcogliese (1996)
(17) Køie (1993)
(18) Zander et al. (1994)
(19) Berland (1989)
(20) Jackson et al. (1997)
(21) Markowski (1935b)
(22) Davey & Peachey (1968)
(23) de Groot (1971)
(24) Braber & de groot (1973)
(25) Zander & Döring (1989)
(26) Renaud et al. (1983)
(27) Robert et al. (1988)
(28) Petersen (1992)

Acknowledgements

We thank the crew of R.V. Luctor, Wim Röber, Piet de Koeyer, Co van Sprundel, and Hans Francke (Centre for Estuarine and Coastal Ecology, Yerseke, The Netherlands) and several volunteers for their help on board. M. Køie, and B. Berland are acknowledged for their help with the determination of digeneans and nematodes. Part of this study was financed by NFWO project G.2094.92.

References

Arellano, R. V. (1995) *Age and growth of two sympatric goby species Pomatoschistus minutus Pallas and Pomatoschistus lozanoi de Buen (Pisces, Gobiidae) using daily growth increments in the otoliths*. PhD thesis, University Gent, 178 pp.

Arellano, R. V., Hamerlynck, O., Vinckx, M., Mees, J., Hostens, K. & Gijselinck, W. (1995) Changes in the ratio of the sulcus acusticus area to the sagitta area of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). *Marine Biology*, **122**, 355-360.

Arntz, W. E. (1978) The food of adult cod (*Gadus morhua* L.) in the western Baltic. *Meeresforsch.*, **26,** 60-69.

Berland, B. (1989) Identification of larval nematodes from fish. in Möller, H. (Ed.): *Nematode problems in North Atlantic fish.* 3-4 April 1989, Kiel, *Int. Counc. Explor. Sea.* C.M., 16-22.

Braber, L. & de Groot, S. J. (1973) The food of five flatfish species (Pleuronectiformes) in the southern North Sea. *Netherlands Journal of Sea Research*, **6** (1-2): 163-172

Creutzberg, F. & Witte, J. I. (1989) An attempt to estimate the predatory pressure exerted by the lesser weever, *Trachinus vipera* Cuvier, in the southern North Sea. *J. Fish Biol.*, **34,** 429-449.

Daro, M. H. & van Gijsegem, B. (1984) Ecological factors affecting weight feeding and production of five dominant copepods in the Southern Bight of the North Sea. *Rapports et Procès-Verbaux du Conseil International pour L' Exploration de la Mer*, **183,** 226-233.

Davey, J. T. & Peachey, J. E. (1968) *Bothriocephalus scorpii* (Cestoda: Pseudophyllidea) in turbot and brill from British coastal waters. *J. Mar. Biol. Ass. U.K.*, **48,** 335-340.

de Groot, S. J. (1971) *Bothriocephalus scorpii* (Müller) (Cestoda: Pseudophyllidea) in turbot *Scophthalmus maximus* (L.) and brill *S. rhombus* (L.) from the southern North Sea. *J. Fish Biol.*, **3,** 147-149.

Doornbos, G. & Twisk, F. (1987) Density, growth and annual food consumption of gobiid fish in the saline lake Grevelingen, the Netherlands. *Netherlands Journal of Sea Research*, **21,** 1, 45-74.

Edlund, A.-M., Sundmark, G. & Thorman, S. (1980) The identification of *Pomatoschistus pictus*, *P. microps* and *P. minutus* (Gobiidae, Pisces). *Sarsia*, **65,** 239-242.

Evans, N. A. (1978) The occurence and life history of *Asymphylodora kubanicum* (Platyhelminthes: Digenea: Monorchiidae) in the Worcester-Birmingham canal: with special reference to the feeding habits of the definitive host, *Rutilus rutilus*. *J. Zool. Lond.*, **184,** 143-153.

Fonds, M. (1973) Sand gobies in the Dutch Wadden Sea (*Pomatoschistus*, Gobiidae, Pisces). *Neth. J. Sea Res.*, **6,** 4, 417-478.

Hamerlynck, O. (1990) The identification of *Pomatoschistus minutus* (Pallas) and *Pomatoschistus lozanoi* (de Buen) (Pisces, Gobiidae). *J. Fish Biol.*, **37,** 723-728.

Hamerlynck, O. & Cattrijsse, A. (1994) The food of *Pomatoschistus minutus* (Pisces, Gobiidae) in Belgian coastal waters, and a comparison with the food of its potential competitor *P. lozanoi*. *Journal of Fish Biology*, **44,** 753-771.

Hamerlynck, O., Heip, C. & Redant, F. (1986) Life history, food consumption and resource partitioning in two sympatric gobies *Pomatoschistus minutus* and *P. lozanoi* in the Belgian coastal waters. *Int. Counc. Explor. Sea*, **14**, 17 pp.

Hamerlynck, O. & Hostens, K. (1993) Growth, production and consumption in 0-group bib *Trisopterus luscus* and whiting *Merlangius merlangus* in a shallow coastal area of the SW Netherlands. *ICES Journal of Marine Science*, **50,** 81-93.

Hamerlynck, O. & Mees, J. (1991) Temporal and spatial structure in the hyperbenthic community of a shallow coastal area and its relation to environmental variables. *Oceanologica Acta*, vol.sp. **11**, 205-212.

Hamerlynck, O., Mees, J., Van de Vijver, P., Van Landtschoote, E., Hostens, K. & Cattrijsse, A. (1989) *Eindverslag Hyperbenthosonderzoek Voordelta*. Intern report Rijkswaterstaat, dienst Getijdewateren, *University Gent*, 102 pp.

Hamerlynck, O., Van de Vyver, P. & Janssen, C. R. (1990) The trophic position of *Pomatoschistus lozanoi* (Pisces: Gobiidae) in the Southern Bight. *Proc. 24th Europ. Mar. Biol. Symp.: Trophic Relationships in the Marine Environment.* Barnes, M and Gibson, R. N. 1990, Aberdeen University press, 183-190

Henderson, P. A. (1989) On the structure of the inshore fish community of England and Wales. *J. Mar. Biol. Ass. U.K.*, **69,** 145-163.

Holmes, J. C. (1990) Helminth communities in marine fishes. in: G. Esch, A. Bush & J. Aho *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 5, pp. 101-130.

Jackson, C. J., Marcogliese, D. J. & Burt, M. D. B. (1997) Role of hyperbenthic crustacean in the transmission of marine helminth parasites. *Can. J. Fish. Aquat. Sci.* **54,** 815-820.

Jarecka, L. (1964) Cycle évolutif à un seul hôte intermédiaire chez *Bothriocephalus claviceps* (1). (Goeze, 1782), cestode de *Anguilla anguilla* L. *Annales De Parasitologie*, **39,** 2, 149-156.

Køie, M. (1979) On the morphology and life-history of *Derogenes varicus* (Müller, 1784) Looss, 1901 (Trematoda, Hemiuridae). *Z. Parasitenkd.*, **59,** 67-78.

Køie, M. (1983) Digenetic trematodes from *Limanda limanda* (L.) (Osteichthyes, Pleuronectidae) from Danish and adjacent waters with special reference to their life-histories. *Ophelia*, **22**, 2, 201-228.

Køie, M. (1984) Digenetic trematodes from *Gadus morhua* L. (Osteichthyes, Gadidae) from Danish and adjacent waters with special reference to their life-histories. *Ophelia*, **23,** 2, 195-222.

Køie, M. (1988) Parasites in European eel *Anguilla anguilla* (L.) from Danish freshwater, brackish and marine localities. *Ophelia*, 29,2, 93-118.

Køie, M. (1993) Aspects of the life-cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). *Can. J. Zool.*, **71,** 1289-1296.

Koter, M. (1962) Helminth parasites in *Gobiidae* of the Puck Bay. *Acta Parasitologica Polonica*, **10,** 1-13.

Lauckner, G. (1971) Zur Trematodenfauna der Herzmuscheln *Cardium edule* und *Cardium lamarcki*. *Helgoländer Wiss. Meeresunters.*, **22,** 377-400.

Maillard, C. & Aussel, J.-P. (1988) Host specificity of fish trematodes investigated by experimental ichthyophagy. *International Journal for Parasitology*, **18,** 4, 493-498.

Manter, H. W. (1955) The zoogeography of trematodes of marine fishes. *Exp. Parasitol.*, **4,** 62-86.

Marcogliese, D. J. (1996) Larval parasitic nematodes infecting marine crustaceans in Eastern Canada. 3. *Hysterothylacium aduncum. J. Helminth. Soc. Wash.*, **63,** 1, 12-18.

Margolis, L. (1971) Polychaetes as intermediate hosts of helminth parasites of Vertebrates: a review. *J. Fish. Res. Bd. Canada*, **28,** 1385-1392.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. & Schad, G. A. (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *J. Parasitol.*, **68,** 1, 131-133.

Markowski, St. (1935a) Die parasitischen Würmer von *Gobius minutus* pall. des Polnischen Balticums. *Sciences Naturelles*, **2,** 251-260.

Markowski, St. (1935b) Über den Entwicklungszyklus von *Bothriocephalus scorpii* (Müller 1776). *Bulletin International de l'Académie Polonaise des Sciences et des Lettres*, Série B: Sciences Naturelles (II), 1-17.

Markowski, St. (1936) Über die Trematodenfauna der baltischen Mollusken aus der Umgebung der Halbinsel Hel. *Bulletin International de l'Académie Polonaise des Sciences et des Lettres*, Série B: Sciences Naturelles (II), 285-317.

Mees, J., Cattrijsse, A. Hamerlynck, O. (1993) Distribution and abundance of shallow-water hyperbenthic mysids (Crustacea, Mysidacea) and euphausiids (Crustacea, Euphausiacea) in the Voordelta and the Westerschelde, Southwest Netherlands. *Cah. Biol. Mar.*, **34**, 165-186.

Meskal, F. H. (1967) Seasonal fluctuation in the population of two common trematode species from the stomach of the cod. *Sarsia* , **26,** 13-20.

Petersen, F. (1992) Nutritional condition and parasites of the sand goby *Pomatoschistus minutus* (Pallas) from the German Wadden Sea. *Ophelia*, **35,** 3, 197-207.

Pritchard, M. H. & Kruse, G. O. W. (1982) *The collection and preservation of animal parasites*. Lincoln and London, University of Nebraska Press, 141 pp.

Reimer, L. W. (1973) Das Auftreten eines Fischtrematoden der Gattung *Asymphylodora* Looss, 1899, bei *Nereis diversicolor* O. F. Müller als Beispiel für einen Alternativzyklus. *Zool. Anz. , Leipzig*, **191,** 3/4, 187-196.

Renaud, F. & Gabrion, C. (1984) Polymorphisme enzymatique de populations de groupe *Bothriocephalus scorpii* (Müller, 1776) (Cestoda, Pseudophyllidea). Etude des parasites de divers téléostéens des côtes de Finistère. *Bulletin de la Société Française de Parasitologie*, **2,** 95-98.

Renaud, F., Gabrion, C. & Pasteur, N. (1983) Le complexe *Bothriocephalus scorpii* (Mueller, 1776): différenciation par électrophorèse enzymatique des espèces parasites de Turbot (*Psetta maxima*) et de la Barbue (*Scophthalmus rhombus*). *Comptes Rendus de l' Académie Des Sciences, Paris*, **296,** 3, 127-129.

Robert, F., Renaud, F., Mathieu, E. & Gabrion, C. (1988) Importance of the paratenic host in the biology of *Bothriocephalus gregarius* (*Cestoda, Pseudophyllidea*), a parasite of the turbot. *International Journal for Parasitology*, **18,** 5, 611-621.

Rohde, K. (1982) *Ecology of Marine Parasites*. St. Lucia, University of Queensland Press, 245 pp.

Salvanes, A. G. V. & Nordeide, J. T. (1993) Dominating sublittoral fish species in a west Norwegian fjord and their trophic links to cod (*Gadus morhua* L.). *Sarsia*, **78,** 221-234.

Vaes, M. (1978) Infection of the common goby, *Pomatoschistus microps,* with *Aphalloides coelomicola* (Trematoda Digenea). *Vlaams Dierengeneeskundig Tijdschrift*, **47,** 3, 274-278.

Van Den Broek, E. & De Jong, N. (1979) Studies on the life cycle of *Asymphylodora tincae* (Modeer, 1790) (Trematoda: Monorchiidae) in a small lake near Amsterdam. Part 1. The morphology of various stages. *Journal of Helminthology*, **53,** 79-89.

Wallis, G. P. & Beardmore, J. A. (1984) An electrophoretic study of the systematic relationships of some closely related goby species (Pisces, Gobiidae). *Biological Journal of the Linnean Society*, **22,** 107-123.

Zander, C. D. (1988) Zur Bedeutung von Kleinfischen in Økosystemen der Ostsee. *Seevögel*, **9,** 4, 51-55.

Zander, C. D. & Döring, W. (1989) The role of gobies (Gobiidae, Teleostei) in the food web of shallow habitats of the Baltic Sea. *Proceedings of the 21st EMBS.* 14-19 September, 1986, Gdansk, Polish Academy of Sciences - Institute of Oceanology, pp. 499-508.

Zander, C. D. & Kesting, V. (1998) Colonization and seasonality of goby (Gobiidae, Teleostei) parasites from the Southwestern Baltic Sea. *Parasitol. Res.*, **84,** 459-466.

Zander, C.D., Groenewold, S. & Strohbach, U. (1994) Parasite transfer from crustacean to fish hosts in the Lübeck Bight, SW Baltic Sea. *Helgoländer Meeresuntersuchungen*, **48**, 89-105

Zander, C. D., Strohbach, U. & Groenewold, S. (1993) The importance of gobies (Gobiidae, Teleostei) as hosts and transmitters of parasites in the SW Baltic. *Helgoländer Meeresunters.*, **47,** 81-111.

Zander, C. D. & Westphal, D. (1991) Kleinfischparasiten der Ostseeförde Schlei und ihre Einbindung in die Nahrungskette. *Seevögel*, **12,** 1, 4-8.

Zietse, M. A., Van Den Broek, E. & Erwteman-Ooms, E. E. A. (1981) Studies on the life-cycle of *Asymphylodora tincae* (Modeer, 1790) (Trematoda: Monorchiidae) in a small lake near Amsterdam. Part 2: The relations between *Asymphylodora tincae* and its definitive host, *Tinca tinca*. *Journal of Helmintholgy*, **55,** 239-246.

General discussion

Parasites are important components in ecosystems, and in the last decades it is realized that their role in regulating host abundance is comparable to the role of predators in prey-predator interactions. Recently, parasites have received considerable attention in evolutionary ecology (Sheldon & Verhulst, 1996) and are regarded as important causal factors in maintaining sexual reproduction (Hamilton, 1980, Ladle, 1992, Ebert & Hamilton, 1996). The study of host-parasite communities is an important area of community ecology. An understanding of the population dynamics and coevolution of simple host-parasite systems is fundamental to the understanding of the structure of more complex parasite communities (Dobson, 1990). Marcogliese & Cone (1997) pleaded for the incorporating of parasites in studies of food webs since they provide complementary views of food web structure and dynamics.

In this thesis, we focused on two abundant Gobiidae, *Pomatoschistus minutus* and *P. lozanoi,* of the Grevelingen Voordelta area as hosts for helminth parasites. Although they are closely related and share the same habitat, it was shown that clear differences in parasite load and to a lesser extent in helminth species richness between the two hosts were present. Our research was carried out on the component community level of gill- and endoparasitic helminths. Holmes (1990) hypothesized that the component community of gasterointestinal helminths in marine teleosts occupy a broad band along the continuum from restricted (predictive) subsets to stochastic samples of the compound community. He furthermore stated that the extent to which component communities are real (and not merely aggregates of infracommunities, without much ecological significance) is a function of the importance of specificity of the parasites.

Our data seem to support the view that, at least for the host-parasite system studied, component communities are not merely the result of stochastic factors or individual differences between hosts, but the result of the specificity of host-parasite interactions. This specificity itself is function of the phylogenetic specificity of the parasites and of ecological or physiological aspects, largely determined by behavioural and physiological features of the host. The main question underlying our research was to what extent phylogenetic and ecological aspects contribute to the host specificity and to the structuring of the observed infection patterns and population dynamics of the parasites. We will here discuss in short the patterns emerging from our research.

How important is the contribution of phylogenetic host specificity in determining the component community in the *Pomatoschistus*-helminth association? The observation of at least three, possibly four, previously undescribed species, *Gyrodactylus longidactylus* on *P. lozanoi*, *Gyrodactylus* sp. on *P. microps*, *Gyrodactylus* sp. 1 on *P. minutus* and *P. lozanoi* and *Gyrodactylus* sp. 2 on *P. pictus,* each with an apparent limited host range, supports the idea that (phylogenetic) host specificity indeed may be very important in the Gyrodactylidae. This is in agreement with the observations of Rohde (1978). All other non-gyrodactylid helminth species from the gobies were already described parasitizing fish from different genera or invertebrate hosts, suggesting that host specificity in these species is low. Two other *Gyrodactylus* species which were encountered, *G. micropsi* and *G. rugiensis*. So far, they have only been reported from *Pomatoschistus* species, despite the fact that extensive research has been done on the *Gyrodactylus* species of other fish species which share the same habitats (e. g. flatfish species in marine habitats and three-spined sticklebacks in brackish waters). In the case of *Gyrodactylus* sp. 1 and *Gyrodactylus* sp. 2, it was remarkable that these morphological very similar species were found on *Pomatoschistus* species which were phylogenetically most closely related. In our initial identifications, *Gyrodactylus* sp. 1 and *Gyrodactylus* sp. 2 were called *Gyrodactylus* cf. *arcuatus* because of their resemblance with *G. arcuatus* parasitizing *Gasterosteus aculeatus*. More elaborate morphometric analysis, however, separated *Gyrodactylus* sp. 1 and *Gyrodactylus* sp. 2 on gobies from G; arcuatus on sticklebacks. In principle, the variability of morphological features observed by us could be host-induced, as it was the case in *Lernaeocera lusci* (Copepoda) from gobies (Van Damme, 1995). However, a preliminary genetical analysis of *G. arcuatus* from *Gasterosteus aculeatus* and *Gyrodactylus* sp. 1 from *P. minutus* and *P. lozanoi* confirms that these are separate species (Marek Zietara, personal communication) and that the *Gyrodactylus* sp. 1 specimens on *P. minutus* and *P. lozanoi* belong to the same species. The observation by Chris Appleby (personal communication) that in the Oslo Fjord, *Gyrodactylus* sp. 1 from *P. minutus* was never found on the three-spined stickleback, even though these fish are sympatric in that area and both are nest builders and use the same breeding grounds, is in agreement with the idea that two different species are involved. In the Oslo Fjord, the probability of the three-spined stickleback to encounter *Gyrodactylus* species from the gobies is probably quite high. Since infection was not observed, this again suggests the high host specificity of this *Gyrodactylus* species. All these observations together strongly suggest that the host specificity exhibited by the gyrodactylids has some phylogenetic basis. Nevertheless, the role of ecological factors can not be ruled out from field surveys only. Experimental infections of the different *Pomatoschistus* species with specific *Gyrodactylus* species may in the end be necessary to provide more conclusive results. In principle, such studies are possible, as is illustrated by the experimental work of Bakke *et al*. (1991a, 1991b, 1996) and Harris (1988).

Even though our results suggest that phylogeny is important in determining host-parasite interactions in the *Pomatoschisitus-Gyrodactylus* system, ecological factors may still play a role in determining the infection dynamics and the parasite load of *Gyrodactylus* species, as was demonstrated by our study on *Gyrodactylus* sp. 1 from *P. minutus* and *P. lozanoi*. Although both gobies hosted the parasite, the overall infection level of *P. lozanoi* was much lower than of *P. minutus*. The differences in ecological niche between *P. minutus* and *P. lozanoi* mainly concern three factors: diet, spatial habitat utilisation and timing of reproductive activities. Each factor may influence the probability of infection of certain parasite species, depending on their respective infection strategies. We observed a higher parasite load of *Gyrodactylus* sp. 1 on *P. minutus* compared to *P. lozanoi,* which was probably caused by differences between the hosts with respect to their occurrence in time and space. The temporal difference in reproductive niche, with *P. minutus* spawning two months earlier than *P. lozanoi*, resulted in a better transmission of the parasite from highly infected adult hosts to young in *P. minutus,* than was the case in *P. lozanoi*. In addition, the *Gyrodactylus* population on young *P. minutus* benefited from the relatively high prevailing temperatures in that period to boost its growth. It might be argued that the difference in time of spawning between the two gobiid species may be the result of different strategies in the face of a trade-off involving costs and benefits of an early versus late reproduction. *P. lozanoi* suffers a loss of potential growth by late oviposition, but may gain in having a lower parasite load than *P. minutus*. Although this is a plausible idea, it is difficult to test, because the two species most often coexist. In general, the data suggest that the main factor resulting in a higher parasite load in *P. minutus* compared to *P. lozanoi* is the more benthic lifestyle of the former species.

P. minutus will on average be more exposed to *Gyrodactylus* specimens, given the dispersal strategy of gyrodactylids which mainly involves host-sediment or host-host contact. This difference in spatial niche is of course linked with the specific feeding habits of *P. minutus,* which prey more on benthic organisms than *P. lozanoi*. But it is the place of feeding (the degree of association with the sediment) and not the diet itself which plays a role in the probability of getting infected with gyrodactylids.

Our results on the endoparasites of *Pomatoschistus* species suggest that in this parasite community, the food niche is the most important factor explaining the differences in infection level between *P. minutus* and *P. lozanoi*. In addition, changes in diet may also explain seasonal changes of the parasite population within each of the hosts and differences in parasite load between males and females. The infection with the trematode *Derogenes varicus* was least of all endoparasites determined by the differences in food niche between the gobies. This trematode is known to be a generalist and probability to get infected was largely the same for both gobies. *P. minutus* clearly was a more suitable host for *Asymphylodora demeli* (Trematoda) than *P. lozanoi*, which was only occasionally infected. The dietary differences between the two goby species and the seasonal changes in infection level of *P. minutus* is in agreement with the idea that sedentary polychaetes could be important intermediate hosts, since *P. minutus* feed heavily on polychaetes. A first approach to elucidate the life cycle of *A. demeli* could be a screening of sedentary polychaetes for this parasite and conducting experimental infections using different intermediate hosts.

We conclude that *P. minutus,* with his more benthic lifestyle and being more of a food generalist than *P. lozanoi*, has a higher chance of infection with helminth parasites and thus carries on average higher parasite loads. Combined with the fact that *P. minutus* is more abundant in the Grevelingen Voordelta area than *P. lozanoi* throughout the year, it makes *P. minutus* a more important transmitter or definitive host for parasites in the ecosystem.

References

Bakke, T. A., Jansen, P. A. & Hansen, L. P. (1991) Experimental transmission of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes, Monogenea) from the Atlantic salmon (*Salmo salar*) to the European eel (*Anguilla anguilla*). *Can. J. Zool.*, **69,** 733-737.

Bakke, T. A., Jansen, P. A. & Harris, P. D. (1996) Differences in susceptibility of anadromous and resident stocks of Arctic charr to infections of *Gyrodactylus salaris* under experimental conditions. *Journal of Fish Biology*, **49,** 341-351.

Bakke, T. A., Jansen, P. A. & Kennedy, C. R. (1991) The host specificity of *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea):susceptibility of *Oncorhynchus mykiss* (Walbaum) under experimental conditions. *Journal of Fish Biology*, **39,** 45-57.

Dobson, A. P. (1990) Models for multi-species parasite-host communities. in: G. Esch, A. Z. Bush & J. Aho (editors) *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 10, pp. 261-288.

Ebert, D. & Hamilton, W. D. (1996) Sex against virulence: the coevolution of parasitic diseases. *Trends in Evolution and Ecology*, **11,** 2, 79-82.

Hamilton, W. D. (1980) Sex versus non-sex versus parasite. *Oikos*, **35,** 282-290.

Harris, P. D. (1988) Changes in the site specificity of *Gyrodactylus turnbulli* Harris, 1986 (Monogenea) during infections of individual guppies (*Poecilia reticulata* Peters, 1859). *Can. J. Zool.*, **66,** 2854-2857.

Holmes, J. C. (1990) Helminth communities in marine fishes. in: G. Esch, A. Bush & J. Aho *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 5, pp. 101-130.

Ladle, R. J. (1992) Parasites and sex: catching the Red Queen. *Trends in Evolution and Ecology*, **7,** 12, 405-408.

Marcogliese, D. J. & Cone, D. K. (1997) Food webs: a plea for parasites. *Trends in Evolution and Ecology*, **12,** 8, 320-325.

Rohde, K. (1978) Latitudinal differences in host-specificity of marine Monogenea and Digenea. *Marine Biology*, **47,** 125-134.

Sheldon, B. C. & Verhulst, S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trend in Evolution and Ecology*, **11,** 8, 317-321.

Van Damme, P. A. & Ollevier, F. (1995) Morphological and morphometric study of crustacean parasites within the genus *Lernaeocera*. *International Journal for Parasitology*, **25,** 12, 1401-1411.

Summary

The host-parasite interactions between the sympatric goby species of the genus *Pomatoschistus* and some of their helminth parasites in the shallow coastal waters of the Grevelingen Voordelta Area are subject of the present thesis. The study focuses on *Pomatoschistus minutus* and *P. lozanoi*, numerically the most dominant species in the area. The influence of ecological and phylogenetic aspects on the infection patterns and the distribution of the parasites in the host population is examined.

In a first part, the hosts, *Pomatoschistus* spp., are the focus of study. Chapter 1 gives a short review of the literature on the life histories of *Pomatoschistus* species and their role as hosts for parasites. Chapter 2 deals with the population dynamics of *Pomatoschistus* species in the Grevelingen Voordelta area.

The second part mainly concerns the identification of the monogenean parasites of the genus *Gyrodactylus* and discusses their possible phylogenetic host-specificity. Chapter 3 describes a new *Gyrodactylus* species from *P. lozanoi*. In Chapter 4, all gyrodactylids from four *Pomatoschistus* species from the Voordelta and surrounding coastal areas are identified with notes on their host specificity. Chapter 5 focuses on the host-dependent and seasonal morphological variation of *Gyrodactylus arcuatus*-like monogeneans from three *Pomatoschistus* species, with a discussion of phylogenetic host-specificity and coevolution.

In a third and final part, the seasonal population dynamics of a *Gyrodactylus* species (Chapter 6) and of five endoparasitic helminths (Chapter 7) of *P. minutus* and *P. lozanoi* are presented. The influence of ecological factors, such as temperature and ecological niche of the hosts, on differences in abundance and prevalence of the parasites is discussed.

Chapter 1 provides a overview of the literature on the life histories of *Pomatoschistus* species with special emphasis on the aspects which could be important for their function as hosts for parasites in the marine ecosystem. It serves as a general introduction to the research presented in the thesis. A section on host-parasite relationships in marine fish briefly discusses the

patterns and processes which structure parasite component communities and the importance of host specificity. A final section reviews the existing literature on host-parasite relationships in gobies and formulates some predictions with respect to the importance of *Pomatoschistus* species as hosts for parasites, considering different characteristics of the gobies.

Chapter 2 focuses on the population dynamics of four *Pomatoschistus* species, *P. minutus*, *P. lozanoi, P. pictus* and *P. microps*, in four sampling stations in the Grevelingen Voordelta Area. The data were analysed in view of the choice of samples for parasitological research and as a basis for later interpretation of observed differences in the parasite fauna of the gobies. Attention is given to seasonal and geographical differences in goby densities, their growth, age and sex ratio. *P. minutus* proved to be numerically the most dominant species in the area, followed by *P. lozanoi*. *P. pictus* and *P. microps* occurred only occasionally. Generally, the highest densities of gobies were caught in two of the sampling stations, Schaar van Renesse and Bollen van het Zand. Therefore, gobies from these stations were preferably used for the study on seasonal dynamics of their parasite fauna (Chapter 6 and Chapter 7). Distinct seasonal fluctuations in densities of *P. minutus* and *P. lozanoi* were observed. Peak densities occurred in autumn and in late winter. The autumn peak could be related to the recruitment of the new cohorts, whereas the peak at the end of the winter indicates the return of the gobies from deeper waters. Length frequency distributions showed that specimens from three cohorts of *P. minutus* and two of *P. lozanoi* were present in the samples from May 1993 till July 1994. In summer, cohorts sometimes overlapped during one or two months. Most gobies survived only one year. Sex ratios of both goby species indicated that males were less easily caught than females during the spawning season, which is probably related to their nest-building behaviour. Growth curves indicated a fast growth of *P. minutus* from June till October. They reached adult size before winter, which was not the case for *P. lozanoi*. Temporal segregation of spawning of the two species together with their annual life cycle resulted in the presence of adult *P. lozanoi* together with juveniles of *P. minutus* in July in the study area.

With respect to the identification of the parasites of *P. minutus* and *P. lozanoi*, we soon realized that several determination problems had to be tackled before any ecological research could be carried out. Since one of our aims was to use monogenean parasites of the genus

Gyrodactylus to study the importance of phylogenetic host specificity, a proper distinction of the different species present was essential. This lead us to describe a new *Gyrodactylus* species in **Chapter 3.** *Gyrodactylus longidactylus* Geets, Malmberg and Ollevier, 1998 is a parasite of the gills of *Pomatoschistus lozanoi*. The marginal hooks of this species have remarkable long handles, leading to its species name. Measurements and drawings of the opisthaptoral hard parts, the penis and the pharynx as well as scanning electron microscopy micrographs of this species are presented. *G. longidactylus* n. sp. is the first monogenean species described from *P. lozanoi*.

In **Chapter 4**, the *Gyrodactylus* species from four sympatric goby species, *Pomatoschistus minutus*, *P. lozanoi*, *P. pictus* and *P. microps*, from the Voordelta and adjacent areas (Oostende Bank) are described and compared to type material and material from other geographical regions. For determination and description purposes, samples of live gobies (*P. minutus* and *P. lozanoi*) from the Voordelta Area and the Oostende Bank were collected. The description of the gyrodactylid species of *P. pictus* and *P. microps* from the marine areas (Voordelta) were based on formalin-fixed specimens. Next to *G. longidactylus* n. sp. from *P. lozanoi*, which was described in chapter 3, another previously undescribed species was found in the Voordelta area: *Gyrodactylus* n. sp. from *P. microps*. A single observation of *G. rugiensis* was made from the skin of *P. lozanoi* from the Oostende Bank. On *P. minutus*, *P. lozanoi* and *P. pictus* of the Voordelta, the most abundant monogeneans were *Gyrodactylus arcuatus*-like specimens. Comparison of these specimens with *G. arcuatus* from its type host *Gasterosteus aculeatus* (three-spined stickleback) revealed a partial overlap of the measurements of the morphological features. Despite of this overlap, consistent host-dependent morphological differences were observed. This suggests the presence of different forms (possibly different species) of *Gyrodactylus* cf. *arcuatus* on the goby hosts.

The findings on the host-dependent morphological variations observed in *Gyrodactylus arcuatus*-like monogeneans from *P. minutus*, *P. lozanoi* and *P. pictus* urged for a more elaborate comparison with *G. arcuatus* from the three-spined stickleback *Gasterosteus aculeatus*. The identification of monogeneans of the genus *Gyrodactylus* is mainly based on the dimensions and shapes of the hard parts of the opisthaptor. Since seasonal variation in size of the sclerities have been reported for other *Gyrodactylus* species, we also incorporated

seasonality as a factor in our analysis. **Chapter 5** reports the result of a morphometric study carried out on measurements of 17 features of the opisthaptoral parts of 268 specimens from *G. arcuatus*-like monogeneans isolated from four hosts and from different seasons. Multivariate analysis (factor- and discriminant analysis) proved to be a powerful tool in discriminating between the different forms found on different hosts, and provided information on which morphological features contribute most to the discrimination between these forms. Three distinct forms (species) of *Gyrodactylus* cf. *arcuatus* were present. *G. arcuatus* (*sensu stricto*) was only present on *G. aculeatus*. *P. minutus* and *P. lozanoi* carried a common species, *Gyrodactylus* sp. 1, whereas *P. pictus* hosted *Gyrodactylus* sp. 2. The combination of large anchor shaft, anchor points and ventral bar discriminated the specimens from the sticklebacks from those on the three goby species. *Gyrodactylus* sp. 2 had shorter anchor shafts and anchor points and longer marginal hooks than *Gyrodactylus* sp. 1. The size of the opisthaptoral hard parts of *Gyrodactylus* sp. 1 varied seasonally. A summarizing overview is given showing the different *Gyrodactylus* species described *on P. minutus, P. lozanoi, P. pictus* and *P. microps* in relation to the phylogeny of the goby hosts, as established from the literature. For *Gyrodactylus* sp. 1 and *Gyrodactylus* sp. 2, our data suggest that phylogenetic evolution may have played a role. In general, the closer the phylogenetic relationship between the gobies, the more (morphologically) similar their gyrodactylids were.

Chapter 6 and 7 focus on the role of the differences in ecological niche between *P. minutus* and *P. lozanoi* in determining their respective parasite fauna. In both chapters, data are presented on the helminths found on monthly samples of gobies from the Grevelingen Voordelta Area. Since the gobies came from the same area, even from the same catches, they were subject to the same seasonal environmental changes (abiotic and biotic). Unless species-specific differences in ecology have an impact, both goby species are thus expected to have equal opportunities to get infected with the prevailing parasites of the ecosystem. As former research had indicated that *Gyrodactylus* sp. 1 is common on both *P. minutus* and *P. lozanoi*, the seasonal abundance or prevalence of the parasite on the two hosts was compared in **chapter 6**. *P. lozanoi* was less infected than *P. minutus* throughout the sampling period. Prevalence, mean intensity and abundance of the monogenean showed clear fluctuations throughout the year. On the *P. minutus* population, two peaks of infection were seen. The first peak appears in autumn, when the *Gyrodactylus* population on the newly infected

juveniles of *P. minutus* built up. Lowering water temperatures in winter cause a decline of the monogenean population. The second peak of infection coincides with the spawning period of *P. minutus* in spring. Since the most important transmission of gyrodactylids of benthic hosts is via the sediment or through host-host contact, the nesting and schooling behaviour of the gobies may strongly promote transmission. The increasing water temperature in spring also favours the population growth of the Gyrodactylids.

Differences in ecological niche of the two goby species clearly influenced their parasite load. The difference in reproductive period between the two host species favoured the transmission and population growth of *Gyrodactylus* sp. 1 on *P. minutus*. Young *P. minutus* were already available from July, while juvenile *P. lozanoi* only appeared from September onwards. Therefore, juvenile *P. minutus* still overlapped with highly infected, (post-) spawning adult *P. minutus* and adult *P. lozanoi.* Young *P. lozanoi*, however, only overlapped with young, slightly infected *P. minutus* and a declining population of adult *P. lozanoi*. Furthermore, the water temperature was still high in July, but declined quickly from September onwards, which negatively influenced the population growth of *Gyrodactylus* sp. 1 on *P. lozanoi*. The difference in spatial niche also makes the benthic *P. minutus* more vulnerable to infection than the more pelagic *P. lozanoi*, given the dispersal of Gyrodactylids which goes mainly via the sediment. We conclude that, even though both *Pomatoschistus* species were infected with *Gyrodactylus* sp. 1, ecological aspects clearly influenced the host specificity of the gyrodactylid.

Besides differences in reproductive and spatial niche, *P. minutus* and *P. lozanoi* also differ in a third niche dimension, namely their food preference. Since many heteroxenous parasites enter the host through the food web via intermediate or transport hosts, host-dependent or temporal differences in diet tend to be reflected in the endoparasite fauna. **Chapter 7** deals with the seasonal dynamics in infection of some endoparasitic helminths of *P. minutus* and *P. lozanoi*. The study was limited to the most abundant helminths: *Asymphylodora demeli* (Trematoda), *Derogenes varicus* (Trematoda), *Hysterothylacium aduncum* (Nematoda), *Cucullanus* sp. (Nematoda) and *Bothriocephalus* sp. plerocercoid larva (Cestoda). Interspecific differences in food niche between both sympatric gobies were clearly reflected in differences of their endoparasitic fauna. *Cucullanus* sp. was only present in the intestine of *P.*

minutus and with a very low abundance. *A. demeli* showed a marked preference for *P. minutus* over *P. lozanoi* as a final host. The limited seasonal occurrence of *A. demeli* (from July to November) combined with known dietary differences between *P. minutus* and *P. lozanoi*, led to the hypothesis that sedentary polychaetes could play an important role in transmitting this trematode. *Derogenes varicus*, known to be a true generalist, did not show any host specificity. A remarkable parallel trend in the time course of the infection was seen in both host. Also, no differences in parasite load with *D. varicus* were found between overlapping age classes. Only during the spawning season, female *P. minutus* hosted on average more *D. varicus* than males, which was linked to their different food niche, with a dominance of the calanoid copepods (*Temora longicornis*) (an intermediate host for *D. varicus*) in the female diet. *Hysterothylacium aduncum* third and fourth stage larva infected both gobies throughout the year, but in general *P. lozanoi* showed a higher prevalence. *H. aduncum* uses predominantly pelagic copepods and also mysids as intermediate hosts. Since they are the bulk of the food of *P. lozanoi*, and less preferred by *P. minutus*, these dietary differences again explained the observed differences in parasite load. *Bothriocephalus* plerocercoid larva were equally abundant in both gobies.

Finally, the **general discussion** focuses on the relative importance of phylogenetic and ecological aspects on the infection patterns of the helminths of both gobies dealt with in this thesis.

Samenvatting

De gastheer-parasiet interacties tussen de sympatrische grondelsoorten van het genus *Pomatoschistus* en enkele van hun parasitaire helminthen in de ondiepe kustwateren van de Grevelingen Voordelta zijn het onderwerp van de hier voorgestelde thesis. Het onderzoek spitst zich toe op de twee grondelsoorten die in het gebied het talrijkst zijn: *Pomatoschistus minutus* en *P. lozanoi*. De invloed van fylogenetische en ecologische factoren op de infectiepatronen en de verdeling van de parasieten in de gastheerpopulaties wordt onderzocht.

In een eerste deel staan de gastheren, *Pomatoschistus* spp., centraal. Hoofdstuk 1 geeft een kort literatuur overzicht over de biologie van de *Pomatoschistus* soorten en belicht hun rol als gastheren voor parasieten. Hoofdstuk 2 behandelt de populatie dynamica van *Pomatoschistus* soorten van de Grevelingen Voordelta.

Het tweede deel behandelt hoofdzakelijk de identificatie van de Monogenea behorende tot het genus *Gyrodactylus* en bediscussieert hun mogelijke fylogenetische gastheerspecificiteit. In hoofdstuk 3 wordt een nieuwe *Gyrodactylus* soort van *P. lozanoi* beschreven. In hoofdstuk 4 komen dan alle gyrodactyliden van de vier belangrijkste *Pomatoschistus* soorten van de Voordelta en omgeving aan bod, met bemerkingen in verband met hun gastheerspecificiteit. Hoofdstuk 5 belicht de seizoenale en gastheer-afhankelijke morfologische variatie van *Gyrodactylus* cf. *arcuatus* van drie *Pomatoschistus* soorten en plaatst de resultaten in het kader van co-evolutie en fylogenetische gastheerspecificiteit.

In een derde en laatste deel wordt de seizoenale populatie dynamica van een *Gyrodactylus* soort (hoofdstuk 6) en van vijf endoparasitaire helminthen (hoofdstuk 7) van *P. minutus* en *P. lozanoi* behandeld. De invloed van ecologische factoren, zoals temperatuur en ecologische niche van de gastheren, op de verschillen in abundantie en prevalentie van de parasieten wordt onderzocht.

Hoofdstuk 1 omvat een overzicht van de literuur in verband met de biologie van de *Pomatoschistus* soorten met speciale aandacht voor de aspecten die belangrijk kunnen zijn in hun functie als gastheren voor parasieten in het mariene ecosysteem. Dit hoofdstuk doet dienst als algemene inleiding voor het onderzoek dat in de thesis naar voor wordt gebracht. Het deel over gastheer-parasiet relaties behandelt in het kort de processen die de parasietengemeenschap structureren en gaat in op het belang van gastheerspecificiteit. In een laatste deel wordt een overzicht gegeven van de bestaande literatuur in verband met parasiet-gastheer relaties in grondels, en worden een aantal voorspellingen geformuleerd over het mogelijke belang van *Pomatoschistus* soorten als gastheren, rekening houdend met bepaalde kenmerken van de grondels.

Hoofdstuk 2 behandelt de populatie dynamica van de vier meest voorkomende *Pomatoschistus* soorten, *P. minutus, P. lozanoi, P. pictus* en *P. microps*, in vier staalname stations in het Grevelingen Voordelta gebied. De gegevens werden geanalyseerd om de keuze van de stalen voor parasitologisch onderzoek te onderbouwen en als basis voor het interpreteren van de geobserveerde verschillen in parasitaire fauna van de grondelsoorten. De analyses werden toegespitst op de seizoenale en geografische verschillen in densiteiten van grondels, hun groei, leeftijd en sex-ratio. *P. minutus* bleek numerisch de meest dominante soort te zijn, gevolgd door *P. lozanoi*. *P. pictus* en *P. microps* waren eerder zeldzaam. De hoogste densiteiten van grondels werden gevangen in de volgende twee staalname stations: Schaar van Renesse en Bollen van het Zand. Vandaar dat grondels uit deze twee stations preferentieel werden gebruikt in het onderzoek naar de seizoenaliteit van hun parasitaire fauna (hoofdstukken 6 en 7). Duidelijke seizoenale fluctuaties in densiteiten van *P. minutus* en *P. lozanoi* werden vastgesteld. In de herfst en in de late winter werden de hoogste densiteiten genoteerd. De herfstpiek hing samen met de recrutering van de juvenielen van de nieuwe cohortes, terwijl de piek op het einde van de winter vooral een aanduiding zou zijn voor de terugkeer van grondels uit diepere wateren. De lengte-frequentie distributies duiden op het voorkomen van drie cohortes *P. minutus* en twee *P. lozanoi* in de periode van mei 1993 tot juli 1994. In de zomer kwamen de grondels van verschillende jaarklassen gedurende een paar maanden samen voor. De meeste grondels overleven slechts een jaar. De sex-ratio's van beide grondelsoorten geven aan dat de mannetjes minder makkelijk gevangen werden dan de wijfjes tijdens het paaiseizoen. Dit is waarschijnlijk gerelateerd aan hun broedgedrag en het verblijf van de mannetjes in nesten. De groeicurves toonden een zeer snelle groei van *P. minutus* van juni tot oktober. Ze bereikten de adulte lengte voor het aanbreken van de winter, wat niet het geval was voor *P. lozanoi*. De temporele segregatie van de reproductie van de beide grondelsoorten, in combinatie met het feit dat ze eenjarig zijn, resulteerde in het gelijktijdig voorkomen van adulte *P. lozanoi* met juveniele *P. minutus* in juli in het bestudeerde gebied.

Tijdens het identificeren van de parasieten van *P. minutus* en *P. lozanoi* groeide al snel het besef dat verscheidene determinatieproblemen zouden moeten opgelost worden voordat er van enig ecologisch onderzoek sprake kon zijn. Daar een van de doelstellingen van het onderzoek was om Monogenea van het genus *Gyrodactylus* te gebruiken bij het bestuderen van mogelijke fylogenetische gastheerspecificiteit, was het noodzakelijk om de verschillende aanwezige soorten duidelijk te kunnen onderscheiden. Dit leidde ons tot het beschrijven van een nieuwe *Gyrodactylus* soort in **hoofdstuk 3**. *Gyrodactylus longidactylus* Geets, Malmberg and Ollevier, 1998 parasiteert de kieuwen van *Pomatoschistus lozanoi*. De randhaken van de opisthaptor van deze soort hebben opmerkelijk lange staven, wat aanleiding gaf tot de soortnaam. Metingen en tekeningen van de gesclerotiseerde delen van de opisthaptor, de penis en de farynx en scanning-electronenmicroscopische foto's van deze soort worden weergegeven. *G. longidactylus* is de eerste Monogenea soort beschreven van *P. lozanoi*.

In **hoofdstuk 4**, beschrijven we de *Gyrodactylus* soorten die voorkomen bij de vier sympatrische grondelsoorten, *P. minutus, P. lozanoi, P. pictus* en *P. microps*, uit de Voordelta en aanpalende gebieden. De gyrodactyliden worden vergeleken met type materiaal en met materiaal van andere geografische regio's. Voor de determinatie en beschrijving werden levende grondels (*P. minutus* en *P. lozanoi*) verzameld van de Voordelta en van de Oostende bank. De beschrijving van de gyrodactyliden van *P. pictus* en *P. microps* daarentegen zijn gebaseerd op formaline-gefixeerde specimens. Naast *G. longidactylus* n. sp. van *P. lozanoi*, uitgebreid beschreven in het vorige hoofdstuk, werd een tot nog toe onbeschreven soort aangetroffen op de kieuwen van *P. microps* van de Voordelta: *Gyrodactylus* n. sp. Een enkel exemplaar van *G. rugiensis* werd aangetroffen op de huid van *P. lozanoi* van de Oostende bank. De meest abundante gyrodactyliden van *P. minutus, P. lozanoi* en *P. pictus* waren *Gyrodactylus arcuatus*-achtige Monogenea. De metingen van de morfologische kenmerken van *Gyrodactylus* cf. *arcuatus* van de grondels overlappen gedeeltelijk met deze van *Gyrodactylus arcuatus* van de type-gastheer *Gasterosteus aculeatus* (driedoornige stekelbaars). Er werden echter consistente gastheer-afhankelijke verschillen vastgesteld. Deze observaties suggereerden de aanwezigheid van verschillende vormen, of mogelijk zelfs verschillende soorten, van *Gyrodactylus* cf. *arcuatus* op de grondel gastheren.

De bevindingen in verband met de gastheer-afhankelijke morfologische variatie van *Gyrodactylus* cf. *arcuatus* van *P. minutus, P. lozanoi en P. pictus* vereiste een verder doorgedreven vergelijking met *G. arcuatus* van de driedoornige stekelbaars (*Gasterosteus aculeatus*). De identificatie van de Monogenea van het genus *Gyrodactylus* is hoofdzakelijk gebaseerd op de afmetingen en de vorm van de gesclerotiseerde delen van de opisthaptor. Vermits seizoenale variatie in grootte van de haken reeds werd gerapporteerd voor andere *Gyrodactylus* soorten, hebben we de factor seizoenaliteit ook in onze analyse opgenomen. **Hoofdstuk 5** beschrijft de resultaten van een morfometrische studie uitgevoerd op 17 afmetingen van de harde delen van de opisthaptor van 268 *Gyrodactylus arcuatus*-achtige specimens geïsoleerd van vier gastheren en uit verschillende seizoenen. Multivariate analyse (factor- en discriminant analyse) bleek het aangewezen middel te zijn om de verschillende vormen voorkomend op de verschillende gastheren te onderscheiden. Deze analyse duidde ook de morfologische kenmerken aan die bijdroegen tot dit onderscheid. Drie verschillende vormen (mogelijk soorten) van *Gyrodactylus* cf. *arcuatus* waren aanwezig in de stalen. *Gyrodactylus arcuatus* (*sensu stricto*) kwam enkel voor op *Gasterosteus aculeatus*. *P. minutus* en *P. lozanoi* droegen een gemeenschappelijke soort, *Gyrodactylus* sp. 1. *P. pictus* was gastheer voor *Gyrodactylus* sp. 2. De combinatie van een lange haakschacht, een lange haakpunt en een grote ventrale staaf onderscheidt de specimens gevonden op de driedoornige stekelbaars van deze van de grondels. *Gyrodactylus* sp. 2 had kortere haakschachten en haakpunten en grotere randhaken dan *Gyrodactylus* sp. 1. De afmetingen van de harde delen van de opisthaptor van *Gyrodactylus* sp. 1 vertoonden seizoenale variatie. Dit hoofdstuk wordt afgerond met een overzicht waarin de *Gyrodactylus* soorten van grondels van de Grevelingen Voordelta in relatie gebracht worden met de uit de literatuur beschreven fylogenetie van de grondels. Onze gegevens suggereren dat fylogenetische co-evolutie een rol zou kunnen gespeeld hebben voor *Gyrodactylus* sp. 1 en *Gyrodactylus* sp. 2. Algemeen stelden we vast dat hoe nauwer de fylogenetische verwantschap tussen de grondels was, hoe meer morfologisch gelijkend de gyrodactyliden waren.

Hoofdstukken 6 en 7 behandelen de rol die de verschillen in ecologische niche van *P. minutus* en *P. lozanoi* spelen in het bepalen van hun respectievelijke parasitaire fauna. In beide hoofdstukken worden de gegevens voorgesteld over het voorkomen van bepaalde parasieten in maandelijkse stalen van grondels uit het Grevelingen Voordelta gebied. Vermits de onderzochte grondels uit eenzelfde gebied kwamen en uit dezelfde vangsten, kunnen we veronderstellen dat ze aan dezelfde veranderingen in de omgeving (biotische en abiotische factoren) onderhevig waren. We kunnen verwachten dat beide grondelsoorten dezelfde kans hebben om geïnfecteerd te worden met de aanwezige parasieten uit het ecosysteem, tenzij soort-specifieke verschillen in ecologie van de grondels een invloed zouden hebben.

Daar uit voorgaand onderzoek gebleken was dat *Gyrodactylus* sp. 1 zowel op *P. minutus* als op *P. lozanoi* voorkwam, werd de seizoenale abundantie en prevalentie van deze parasiet op beide gastheersoorten vergeleken. Over de ganse staalnameperiode was *P. lozanoi* duidelijk minder geïnfecteerd dan *P. minutus*. Duidelijke seizoenale fluctuaties in prevalentie, gemiddelde intensiteit van infectie en abundantie waren aanwezig. Twee infectiepieken van *Gyrodactylus* sp. 1 werden waargenomen in de *P. minutus* populatie. De eerste piek deed zich voor in de herfst wanneer de *Gyrodactylus* populatie opbouwde op de pas geïnfecteerde juveniele *P. minutus* populatie. Vervolgens veroorzaakte een daling van de watertemperatuur in de winter een afname van de Monogenea populatie. Een tweede piek van infectie viel samen met de paaiperiode van *P. minutus* in de lente. Daar de belangrijkste overdracht van gyrodactyliden gebeurt via het sediment of via contact tussen de gastheren, zal de transmissie sterk bevorderd worden door het nest- en schoolgedrag van de grondels tijdens de paaiperiode. De stijging van de watertemperatuur in de lente bevorderde de populatie groei van de gyrodactyliden.

De verschillen in ecologische niche tussen de beide grondelsoorten beïnvloedt duidelijk hun parasitaire lading. De verschillen in voortplantingsperiode tussen de beide gastheersoorten bevorderde de transmissie en populatiegroei van *Gyrodactylus* sp. 1 op *P. minutus*. Juveniele *P. minutus* waren reeds aanwezig vanaf juli, terwijl de eerst juveniele *P. lozanoi* pas in september voorkwamen. De jonge *P. minutus* populatie overlapte met de sterk geïnfecteerde adulten van *P. minutus* en *P. lozanoi,* wat de mogelijkheden tot transmissie bevorderde. Jonge *P. lozanoi* daarentegen komen enkel samen voor met jonge, weinig geïnfecteerde, *P.*

minutus en met een dalende populatie van *P. lozanoi* adulten. Daarenboven was de watertemperatuur nog betrekkelijk hoog in juli, maar daalde ze snel vanaf september, wat een negatieve invloed uitoefende op de populatiegroei van *Gyrodactylus* sp. 1 op *P. lozanoi*. De verschillen in spatiële niche maakt dat de benthische *P. minutus* meer kans heeft om geïnfecteerd te worden dan de meer pelagische *P. lozanoi*, daar de dispersie van Gyrodactyliden hoofdzakelijk via het sediment gebeurt. We kunnen besluiten dat, niettegenstaande beide *Pomatoschistus* soorten gastheer zijn voor de hier besproken *Gyrodactylus* soort, ecologische aspecten een duidelijke invloed hebben op de gastheerspecificiteit van deze soort.

Naast het verschil in reproductieve en spatiële niche, verschillen *P. minutus* en *P. lozanoi* ook in een derde niche dimensie, namelijk in hun voedsel voorkeur. Gezien verscheidene heteroxene parasieten de gastheer via het voedselweb infecteren, door gebruik te maken van intermediaire en transportgastheren, kunnen gastheerafhankelijke of temporele verschillen in dieet gereflecteerd worden in de endoparasitaire fauna. **Hoofdstuk 7** behandelt de seizoenale verschillen in voorkomen van enkele endoparasitaire helminthen van *P. minutus* en *P. lozanoi*. De studie beperkte zich tot de meest abundante endoparasieten: *Asymphylodora demeli* (Trematoda), *Derogenes varicus* (Trematoda), *Hysterothylacium aduncum* (Nematoda), *Cucullanus* sp. (Nematoda) en plerocercoide larvestadia van *Bothriocephalus* sp. (Cestoda). Interspecifieke verschillen in voedselniche tussen beide grondelsoorten werden duidelijk gereflecteerd in verschillen in hun endoparasitaire fauna. *Cucullanus* sp. was enkel in zeer lage aantallen aanwezig in *P. minutus*. *A. demeli* vertoonde een duidelijke preferentie voor *P. minutus* als definitieve gastheer. Het beperkt seizoenale voorkomen van *A. demeli* (van juli tot november) gecombineerd met de gekende verschillen in dieet tussen *P. minutus* en *P. lozanoi* leidde tot de hypothese dat sedentaire polychaeten een belangrijke rol zouden kunnen vervullen als intermediaire gastheren van deze trematode. *Derogenes varicus*, die als generalist gekend staat, vertoonde geen gastheerspecificiteit. Een opvallend parallel temporeel verloop van de infectie bij beide gastheren werd waargenomen. Eveneens werden er geen verschillen in intensiteit van infectie met *D. varicus* tussen juveniele en adulte grondels vastgesteld. Enkel in het paaiseizoen bevatten *P. minutus* wijfjes meer *D. varicus* specimens dan de mannetjes. Dit werd verklaard door hun verschil in voedselniche in die periode, met een dominantie van calanoide copepoden (a.o. *Temora longicornis*) (tussengastheren voor *D. varicus*) in het dieet van de wijfjes. Derde en vierde larvale stadia van de nematode *Hysterothylacium aduncum* infecteerden beide grondels gans het jaar door, maar globaal was er een hoger percentage *P. lozanoi* geïnfecteerd dan *P. minutus*. *H. aduncum* gebruikt hoofdzakelijk pelagische copepoden en Mysidacea als intermediaire gastheren. Vermits deze organismen de hoofdbrok van het voedsel van *P. lozanoi* uitmaken, en minder geprefereerd zijn door *P. minutus*, kunnen deze verschillen in dieet weerom als verklaring dienen voor de geobserveerde verschillen in parasitaire lading. *Bothriocephalus* plerocercoide larven waren in gelijke mate aanwezig in beide grondelsoorten.

Een **algemene discussie** over het relatieve belang van fylogenetische of ecologische factoren in het bepalen van de infectiepatronen van de helminthen van beide grondelsoorten rondt de thesis af.

Glossary

- Parasitism: A close association between two organisms, one of which, the parasite, depends on the other, the host, deriving some benefit from it without necessarily damaging it. A parasite is usually smaller than its host (Rohde, 1982).

An ecological relationship between the populations of two different species of organisms: one of these is referred to as the parasite the other the host species. The features of this ecological relationship are:
(a) the parasite is physiologically dependent on the host
(b) the infection process produces or tends to produce an overdispersed distribution of parasites within the host population
(c) the parasite kills heavily infected hosts
(d) the parasite species has a higher reproductive potential than the host species (Crofton, 1971).

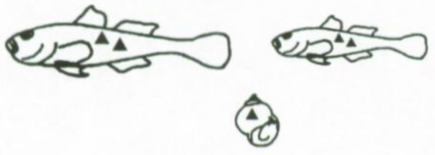
- Ectoparasites: live on the surface of the host (Rohde, 1982, Möller & Anders, 1986).
- Endoparasites: live in the host's interior (Rohde, 1982, Möller & Anders, 1986).
- Intermediate host: harbours the developing but sexually immature or larval stage of a parasite (Rohde, 1982, Möller & Anders, 1986).
- Definitive or final host: host which harbours the sexually mature parasite (Rohde, 1982, Möller & Anders, 1986).
- Paratenic or transport host: host which serves for dispersing the parasite species, but in which there is no development of the parasite (Rohde, 1982, Möller & Anders, 1986).
- Vector: is a very wide term which applies to hosts which carry an infective stage of a parasite (Rohde, 1982).
- Infrapopulation: all members of a given species of parasite in a single host (Esch *et al.*, 1990)

- Infracommunity: all infrapopulations of parasites on a single host (Esch *et al.*, 1990)

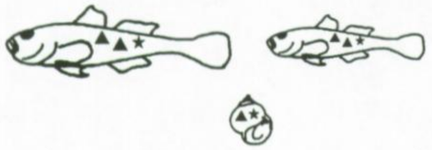
- Metapopulation: all infrapopulations from a given host species (adult and juveniles) in an ecosystem (Esch *et al.*, 1990)

- Component community: all infracommunities within a given host population (Esch *et al.*, 1990)

- Suprapopulation: all individuals (of all life cycle stages) of a given parasite in an ecosystem (Esch *et al.*, 1990)



- Compound community: all parasite communities in an ecosystem (Esch *et al.*, 1990)



- Prevalence: number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined (usually expressed as a percentage) (Margolis *et al.*, 1982)
- Abundance: mean number of individuals of a particular parasite species per host examined (Margolis *et al.*, 1982).
- Mean intensity: mean number of individuals of a particular parasite species per infected host in a sample (Margolis *et al.*, 1982).
- Site or location: Cell tissue or organ of the host where the parasite lives (Margolis *et al.*, 1982, Möller & Anders, 1986).
- Holoxenic parasites: parasites with a direct life cycle including only one host species (Connell, 1980).
- Heteroxenic parasite: parasite with an indirect life cycle including more than one host species (Connell, 1980).
- Monoxenic parasite or oioxenic parasite: a parasite which has only one host species (in a certain stage of its life cycle) (Odening, 1976) or which is host specific towards a single host species (Euzet & Combes, 1980).

- Oligoxenic parasite: parasite which infects only a few host species (Odening, 1976).
- Stenoxenic parasite: parasite which is host specific towards different but phylogenetically related hosts (Euzet & Combes, 1980).
- Polyxenic parasite: parasite which can infect many host species (Odening, 1976).
- Euryxenic parasite: parasite found in numerous hosts of unrelated taxa (Euzet & Combes, 1980).
- Cercaria: free-living (tailed) larva of a trematode (Rohde, 1982).
- Metacercaria: encysted larval stage of a trematode (Rohde, 1982).
- Opisthaptor: the posterior attachment organ of a monogenean (Rohde, 1982).
- Plerocercoid: a larval stage of a cestode (Rohde, 1982).

References

Connell, J. H. (1980) Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos*, **35,** 131-138.

Crofton, H. D. (1971) A quantitative approach to parasitism. *Parasitology*, **62,** 179-193.

Esch, G. W., Shostak, A. W., Marcogliese, D. J. & Goater, T. M. (1990) Patterns and processes in helminth parasite communities: an overview. in: G. Esch, A. Bush & J. Aho (editors) *Parasite communities: patterns and processes*. London, New York, Chapman and Hall Vol. 1, pp. 1-19.

Euzet, L. & Combes, C. (1980) Les problèmes de l'espèce chez les animaux parasites. in: Sociéte Zoologique de France (editor) *Les problèmes de l'espèce dans le regne animal*. Paris, Sociéte Zoologique de France Vol. 6, pp. 239-285.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. & Schad, G. A. (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *J. Parasitol.*, **68,** 1, 131-133.

Möller, H. & Anders, K. (1986) *Diseases and parasites of marine fishes*. Kiel, Verlag Möller, 365 pp.

Odening, K. (1976) Conception and terminology of hosts in parasitology. *Advances in Parasitology*, **14,** 1-93.

Rohde, K. (1982) *Ecology of Marine Parasites*. St. Lucia, University of Queensland Press, 245 pp.

Appendix

Publications which are part of this thesis:

Geets, A., Boden, G. & Ollevier, F. (1995): Population dynamics of the endoparasitic fauna of *Pomatoschistus minutus* (Gobiidae) of the Grevelingen Voordelta Area (SW-Netherlands). Proceedings of the XVII Symposium of the Scandinavian Society for Parasitology, Finland, 15-17 June 1995. *Bulletin of the Scandinavian Society for Parasitology*, **5** (2): 41

Geets, A., Malmberg, G. & Ollevier, F. (1998) *Gyrodactylus longidactylus* n. sp., a monogenean from *Pomatoschistus lozanoi* (de Buen, 1923) (Gobiidae) from the North Sea. *Systematic Parasitology*, **41**, (1), 63-70

Geets, A. & Ollevier, F. Species of Gyrodactylidae (Monogenea) from gobies (*Pomatoschistus* spp. Gobiidae, Pisces) of the Voordelta and surrounding areas: a discription with notes on host-specificity. *Systematic Parasitology*, submitted

Geets, A., Appleby, C. & Ollevier, F. Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology*, accepted

Geets, A., Beuls, I. & Ollevier, F. Population dynamics of *Gyrodactylus* sp. 1 (Monogenea) from two sympatric goby species, *Pomatoschistus minutus* and *P. lozanoi* (Gobiidae: Pisces) in the Grevelingen Voordelta Area (SW Netherlands) *Parasitology*, submitted

Geets, A., Boden, G., Hostens, K., Hamerlynck, O. & Ollevier, F. Seasonal infection patterns of two sympatric goby species *Pomatoschistus minutus* and *P. lozanoi* with some endoparasitic helminths in relation to the ecological niche of the hosts. *Journal of Fish Biology*, in preparation

Other publications:

Geets, A., Liewes, E.W. & Ollevier, F. (1992) The efficacy of some anthelmintics against the swimbladder nematode of eel *Anguillicola crassus* under salt water conditions . *Diseases of Aquatic Organisms*, **13**, 123-128

Geets, A. & Ollevier, F. (1996) Endoparasitic helminths of the whitespotted rabbitfish (*Siganus sutor* (Valenciennes, 1835)) of the Kenyan Coast: Distribution within the host population and microhabitat use. *Belgian Journal of Zoology*, **126** (1): 21-36

Van Damme, P.A., Geets, A., Hamerlynck, O. & Ollevier, F. (1997) The suprapopulation dynamics of *Lernaeocera branchialis* and *L. lusci* in the Oosterschelde: seasonal abundance on three definitive host species. *ICES Journal of Marine Sciences*, **54** (1): 24-31

Geets, A., Coene, C. & Ollevier, F. (1997): Ectoparasites of the whitespotted rabbitfish, *Siganus sutor* (Valenciennes, 1835) of the Kenyan Coast: distribution within the host population and site selection on the gills. *Parasitology*, **115**: 69-79