Parasites of three-spined stickleback, *Gasterosteus aculeatus* L., from Faroe Islands

Sníkar í føroyskum kombikki, Gasterosteus aculeatus *L.*

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ABSTRACT

In the years 2002-2004 samples of three-spined stickleback, *Gasterosteus aculeatus* L**.,** from Faroe Islands were investigated from six different sites, three marine and three freshwater. Ten parasites or disease conditions were found in 1105 fish. The cestode *Schistocephalus solidus* appeared in freshwater samples only (prevalence of infection 6.4-62.8 %). The nematode *Hysterothylacium aduncum* (4.3 -5.7 %) and the copepod *Thersitina gasterostei* (7.4-100 %) were only recorded in marine samples. The ciliate *Trichodina* sp.(1.7-100 %) and the monogenean *Gyrodactylus* sp. (1.1–41.8 %) were found in all localities. Of digeneans *Diplostomum* sp.(5.0 %) was seen in one marine sample and an unidentified species was seen at two localities in freshwater. Diseased conditions found were "fin rot", "white spot disease" and "unidentified cysts." Statistical analyses suggest a significant difference in total parasitofauna between marine and freshwater localities ($p<0.01$).

ÚRTAK

Í tíðini 2002-2004 blivu sýni tikin av kombikki, *Gasterosteus aculeatus* L., á seks støðum í Føroyum: trý støð, har salt var í og trý har onki salt var í umhvørvinum. Tíggju sløg av sníkum ella sjúkrastøðu blivu funnin í tilsamans 1105 fiskum. Bendilormurin *Schistocephalus solidus* sást bert, har onki salt var (í 6.4 – 62.8 %). Rundormurin *Hysterothylacium aduncum* (4.3 -5.7 %) og krabbadýrið *Thersitina gasterostei* (7.4-100 %) vóru bert sædd, har salt er í umhvørvinum. Einkyknudýrið *Trichodina* sp.(1.7-100 %) and monogena iktan *Gyrodactylus* sp. (1.1–41.8 %) vóru funning á øllum støðum. Digena iktan, *Diplostomum* sp.(5.0 %), var bert funnin á einum stað og eitt ónavngreinað slag varð sætt á tveimum støðum, har onki salt var. Sæddar sjúkrastøður vóru: "fjaðra-rot", "hvít-bletta-sjúka" og "ónavnagreiddar bløðrur". Hagfrøðiligar kanningar vístu ein týðandi mun millum sølt og ósølt støð í tali og títtleika av sníkum ($p<0.01$).

INTRODUCTION

The three-spined stickleback, *Gasterosteus aculeatus* L. was first reported in the Faroe Islands during the journeys of J.C. Svabo in the years 1781-82 (Svabo, 1796). Its biology and life-history in the Faroes are treated in Joensen & Tåning (1970), who also reported

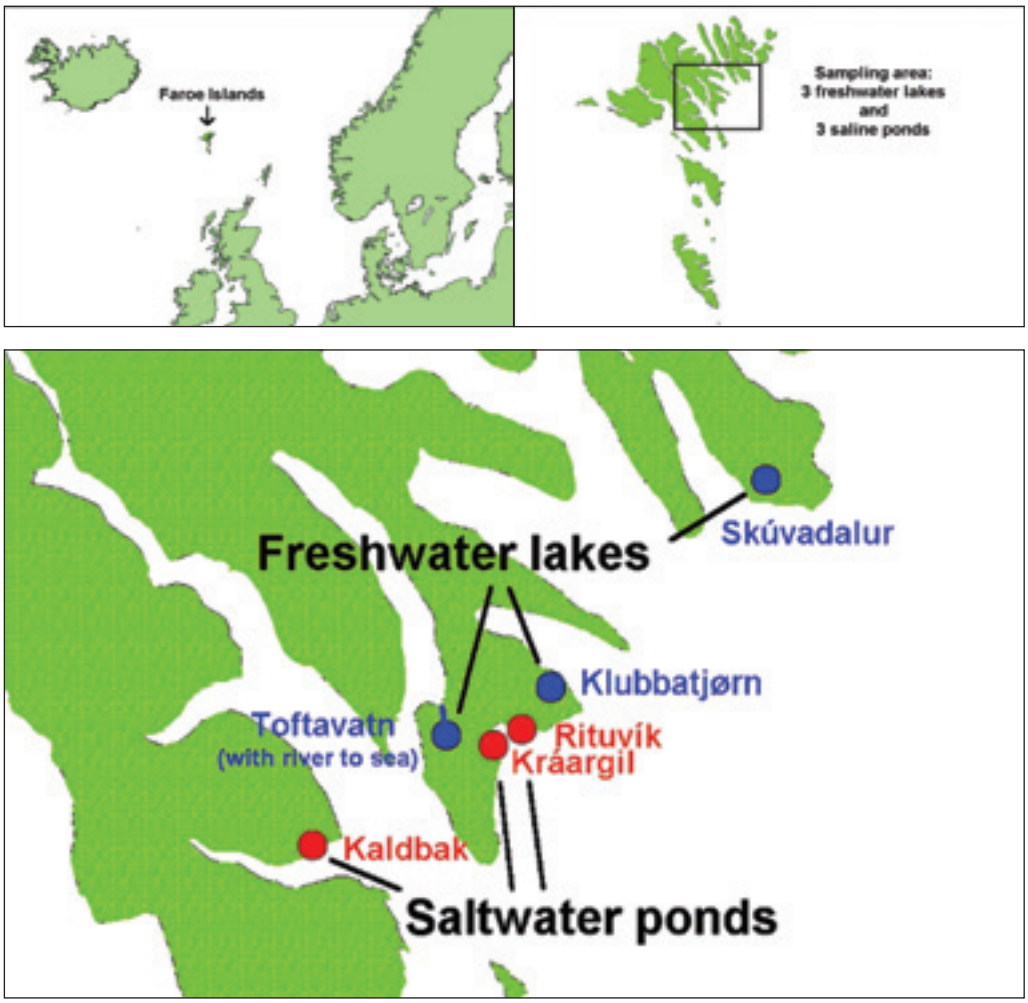

***Fig. 1A**. Maps of the sampling sites*

two parasites, *Thersitina gasterostei* (Pagenstecher, 1861) and *Schistocephalus solidus* Creplin, 1829. The former was first described in sticklebacks from the Faroes by Stephensen (1929). Apart from these records there seems no systematic parasitological work carried out on sticklebacks from Faroes.

The present work describes and discusses selected parasitofauna from six localities and compares the marine and freshwater environment of sticklebacks. The work was initiated by the IBOY-project (International Biodiversity Organisation Year), examining the use of parasite infection in sticklebacks as an indicator of the environment status in the northern hemisphere (Marcogliese and Cone, 1997**).** The most recent up-date on the use of sticklebacks as an indicator species

is that of Katsiadaki (2007) which includes a short section on including parasites in such monitoring.

The primary goal of this work is to provide baseline data on the parasites of sticklebacks in the Faroe Islands. This work may then be applied to both evolutionary and environmental questions in the future.

MATERIALS AND METHODS

Sampling sites (Fig. 1 A). Samples were obtained from the three islands of *Streymoy* (Kaldbak = KA), *Eysturoy* (Rituvík = RI, Kráargil = KR, Toftavatn = TO and Klubbatjørn = KL) and *Borðoy* (Skúvadalur = SK). The sampling design (randomized blocks, 3 x 2) is comprised of three marine (KA, RV and KR) and three freshwater environments (TO, KL and SK). The two salinity groups represent the variety in area and height found in each group on the Faroe Islands. Sticklebacks were caught in traps (large lakes) or with nets (shallow and small ponds). A total of 1105 fish were examined, ranging from 35-101 fish in each sampling. From each site a total of 130-249 were sampled in the time period 2002-2004.

Temperature was measured 10 cm below surface (range 11.5 – 21.8 degr.C). Salinity was determined by an "Autosal 8400A Guildline"– apparatus (range for saline sites was 0.222-34.954 psi, and 0.0605-0.1405 psi for freshwater sites).

Treatment of samples. Sticklebacks were killed in approx. 18 % formaldehyde, then immediately diluted to approx. 6% formaldehyde and stored for further examination.

Examination of fish. The sticklebacks were measured in mm (total length) and weighed to milligram, thus giving the possibility of condition factor studies. The status of the plates were recorded as "low-plated" (leiurus), "partially-plated"(semiarmatus) or "whole-plated" (trachurus), the second spine setting the limit between low- and partially plated. For a detailed explanation of plate morphology see Wootton (1976). Also the keel in the tail region was recorded, if present. External and internal inspection for parasites was carried out on a dissecting scope at 16x, followed by light microscopical examination when necessary. After dissection each fish and its parasites were stored in 70 % ethanol. *Thersitina gasterostei* and *Trichodina* sp. were occasionally stained in "Quick-stain" for microscopy or microphotographing.

Statistical tests used were: two-way ANOVA, Chi-square-test and paired sign test.

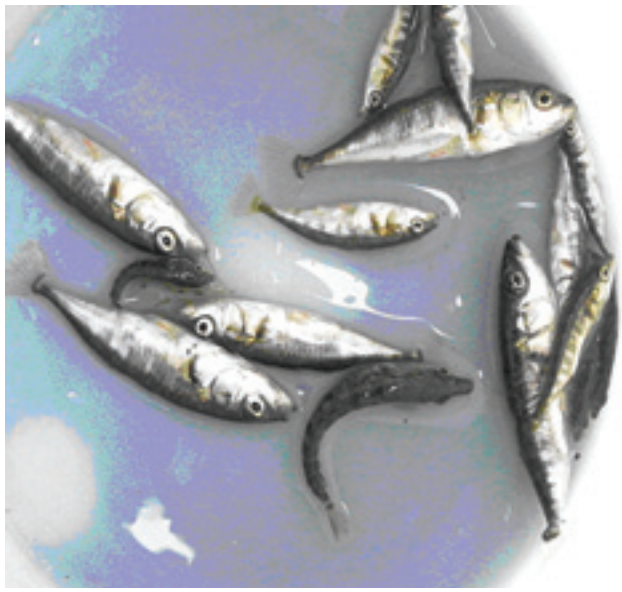

Fig. 1B*. Sticklebacks from Faroe Islands, Aug. 2003 (marine form, Rituvík).*

PLACE / YR	2002	2003	2004	Total
Toftavatn (TO)	54	51	47	152
Skúvadalur (SK)	94	58	58	210
Klubbatjørn (KL)	76	54	58	188
Kaldbak (KA)	35	47	48	130
Kráargil (KR)	81	101	67	249
Rituvík (RI)	78	51	47	176
Total	**418**	**362**	**325**	**1105**

Table 1. *List of stockleback-samples from the different localities.*

RESULTS

Sticklebacks

The number of hosts by location and year is given in Table 1. The sampling area is shown in Fig. 1A, and one example of Faroese sticklebacks is given in Fig. 1 B.

Protozoa

While counting the *Trichodina* on skin, gills and fins (Figs. 2 C and 2 D), they were regarded as one species. However, according to Ozer (2003), two species of *Trichodina* should be found: *T. domerguei* Wallengren, 1897 on the skin of the stickleback and *T. tenuidens* Fauré-Fremiet, 1944 on its gills. This work did not distinguish between these two species. One freshwater site (Klubbatjørn), and, one marine site (Kaldbak) may be classified as "low"-infection areas (prevalence of infection lower than approx. 30 %). All the other sites may for all three years be classified as "high"-infection areas (prevalence of infection is higher than some 70 %). Intensity of infection is following a similar pattern. The main impression is that a high prevalence of

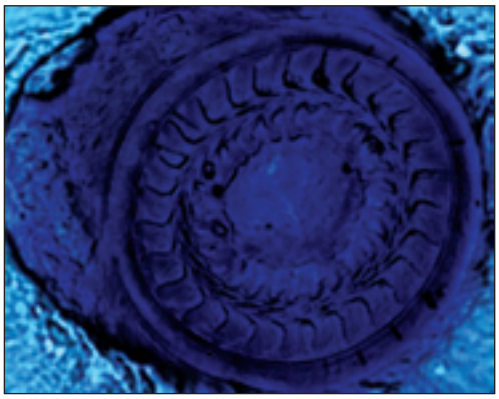

Fig. 2C. *Trichodina domerguei (quick-stain, x 1000)*

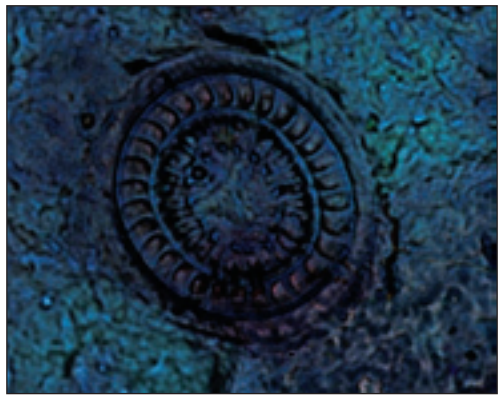

Fig. 2D. *Trichodina tenuidens (quick-stain, x 1000)*

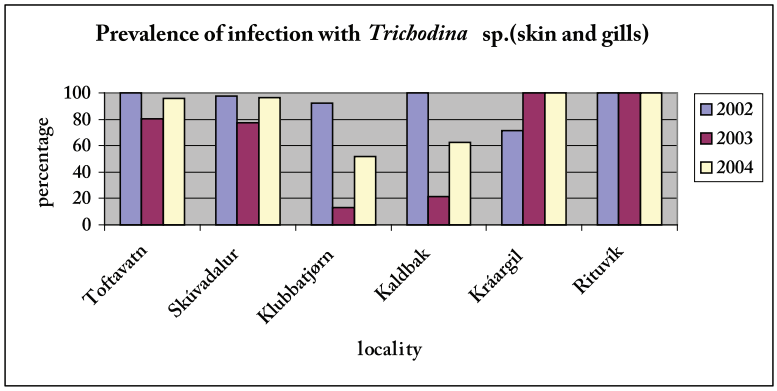

Fig. 2.A. Prevalence of infection with the ciliate Trichodina *sp.in sticklebacks from all stations.*

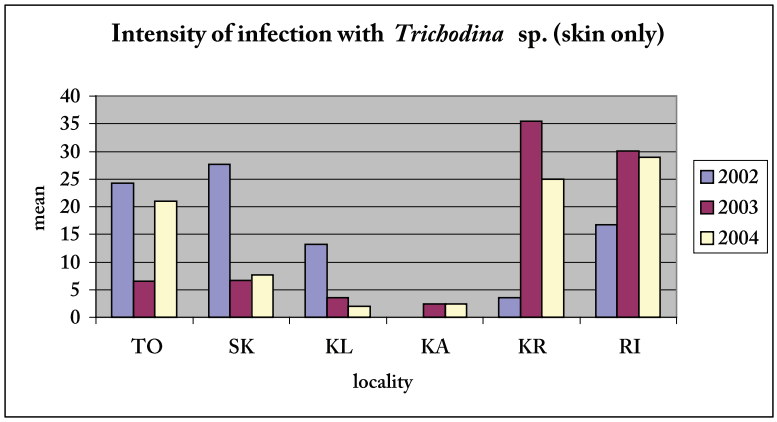

Fig. 2.B. Intensity of infection with Trichodina *sp., in sticklebacks from all stations.*

infection correlates with a high number of *Trichodina* sp. per fish (more than approx 15, see Figs. 2A and 2 B). Most fish were only infected on the "skin" (fins included). In 6 samples (out of 18) both "skin" and gills (inside the gill cavity) were infected. In one sample

PREVALENCE OF INFECTION TRICHODINA			
	2002	2003	2004
Toftavatn	100	80,4	95,8
Skúvadalur	97,9	77,6	96,6
Klubbatjørn	92,1	12,96	51,7
Kaldbak	100	21,3	62,5
Kráargil	71,6	100	100
Rituvík	100	100	100

Table 2A. *Prevalence of infection with* Trichodina *sp. (skin and gills).*

INTENSITY OF INFECTION TRICHODINA			
	2002	2003	2004
TO	24,3	6,5	21,0
SK	27,7	6,7	7,7
KL	13,1	3,6	1,9
KA	0	2,4	2,4
KR	3,6	35,5	24,98
RI	16,8	30,1	28,9

Table 2B. *Intensity of infection with* Trichodina *sp. (skin only).*

	2002	2003	2004
TO	S	S	S+G
SK	S	S+G	S+G
KL	S+G	S	S+G
KA	G	S	S+G
KR	S	S	S
RI	S	S	S

Table 2C. *Intensity of infection with* Trichodina *sp. (skin only).*
S=skin (fins included)
G=gills (inside gill cavity)

(Kaldbak, in 2002) only gills were infected (see Tables 2A, 2B and 2C). According to Ozer (2003) this should be *T. tenuidens.*

The highest number found of *Trichodina* (both species) on one fish was 305 (Kráargil, in 2003**).**

A two-way-ANOVA-test of total number *Trichodina* (both species) sp. for all 1105 fish (length and year included) showed that a significant difference was between localities ($p<0.001$). There was also a significant interaction between localites and years ($p<0.001$), indicating that special effects were observed for certain localities in certain years. A significant difference between years could be seen ($p=0.03$), but the interpretation is not straightforward, because of the interaction. Seven outlayers are included in the material, but the result was unchanged, if they were removed.

2. Metazoa

2.1 *Thersitina gasterostei* (Crustacea, Copepoda), Figs. 3 A-B and Figs. 4 A-C

The distribution of this external parasite was strictly marine, it was not found in the three freshwater localities and one marine (Kaldbak). There is a tendency of attaining more parasites per fish when prevalence of infection is low (Fig.3A and 3 B.). The difference in numbers of *Thersitina* between left and right side of fish is not significant, neither between years nor localities (paired sign-test and chi-square, $p>0.05$). Hence the total counts from the two sides are shown in Figs. 3 A and 3 B.

Highest number of *Thersitina gasterostei* on one fish was 52, which was found three times (in fish from Kráargil 2002 and 2004 and in one fish from Rituvík 2003).

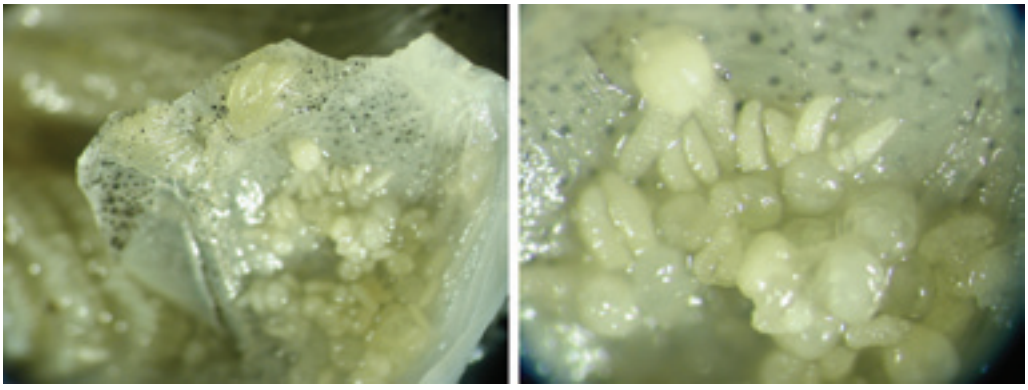

Fig. 4.A. *Photograps of* Thersitina gasterostei, *(below the operculum) at different magnifications.*

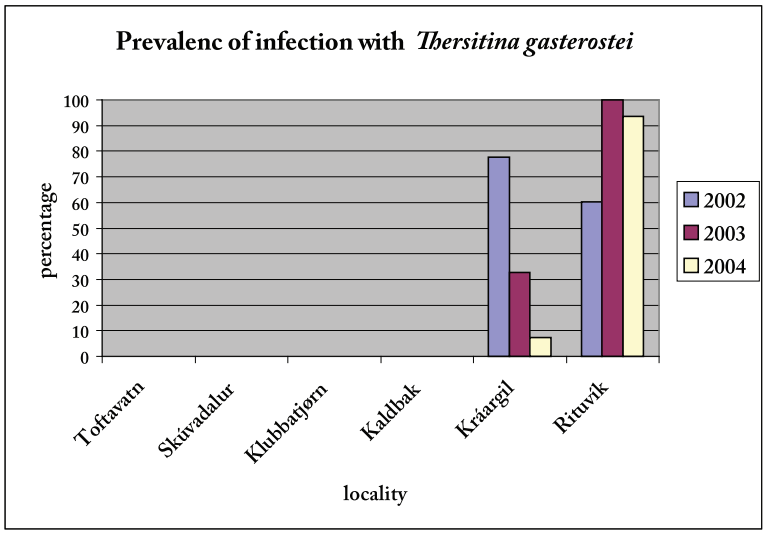

Fig. 3.A. *Prevalence of infection with the* Thersitina gasterostei, *in sticklebacks from all stations.*

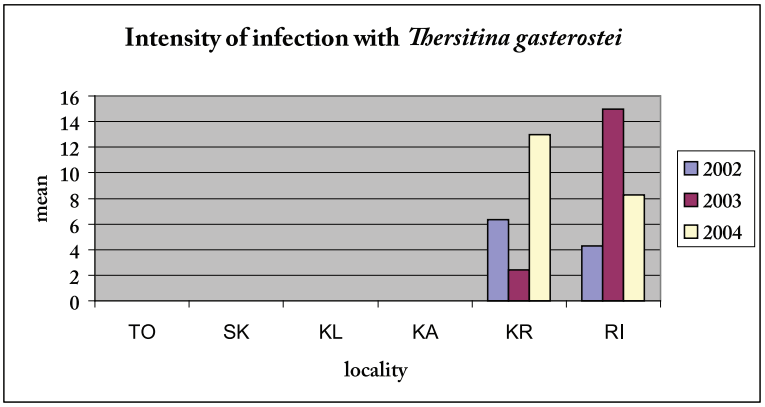

Fig. 3.B. *Intensity of infection with the* Thersitina gasterostei, *in sticklebacks from all stations.*

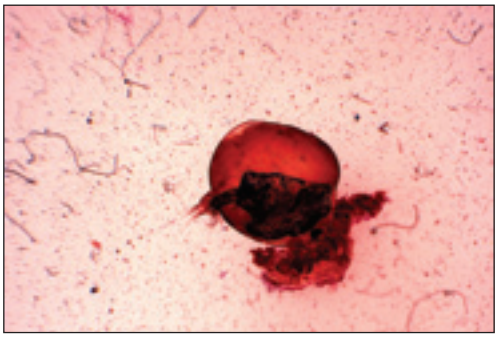

Fig. 4.B. *Micrographs of* Thersitina gasterostei *(male, quick-stain, x 50)*

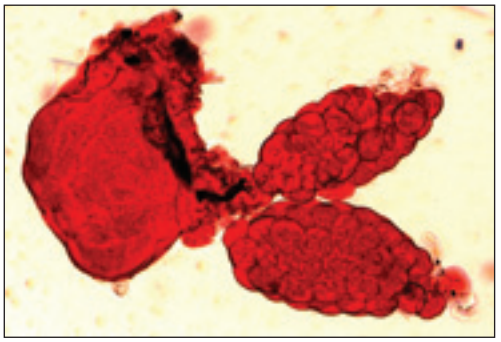

Fig. 4.C. *Micrograph of* Thersitina gasterostei *(female, quick-stain, x 100)*

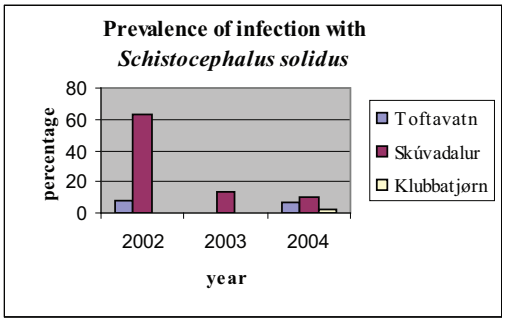

Fig. 5.A. Prevalence of infection with Schistocephalus solidus, *in sticklebacks from all stations.*

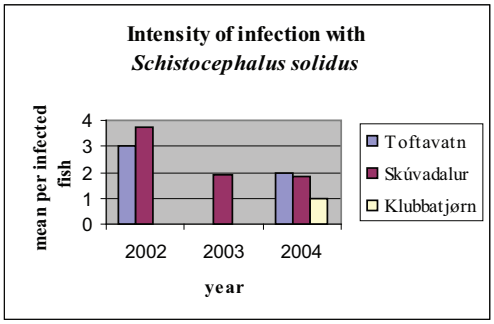

Fig. 5.B. Intensity of infection with Schistocephalus solidus, *in sticklebacks from all stations.*

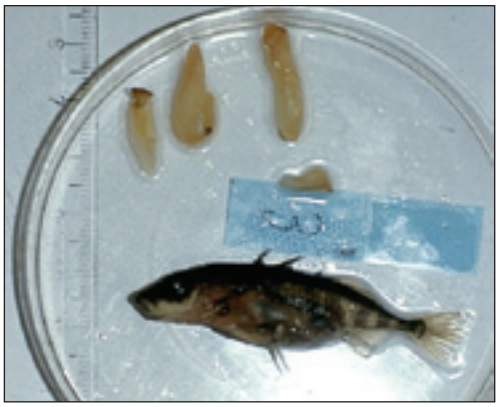

Fig. 5.D. Plerocercoids of Shistocephalus solidus, *dissected out from a stickleback*

2.2. *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda)

Kaldbak, which has the closest contact with the sea, was the only locality where nematodes were found. A single, third-stage larvae of *Hysterothylacium aduncum* was observed in the body cavity of two fish, filling the cavity totally (in 2002 and 2003). The lengths of the two sticklebacks infected in 2002, were 3.85 cm and 4.45 cm (mean length of sample 3.71 cm, standard deviation 0.76 cm); the condition factors were 0.8460 and 0.9459, respectively (mean of sample 0.9207, standard deviation 0.1022). The corresponding figures in 2003 were: lengths 3.5 and

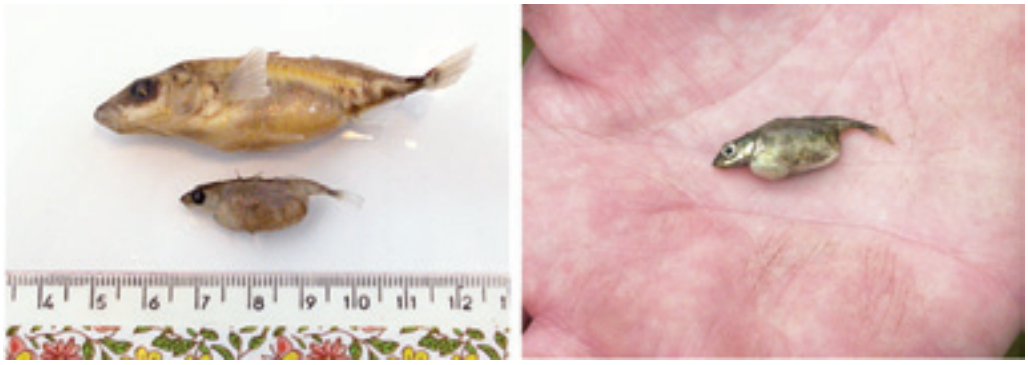

Fig. 5.C. Sticklebacks infected with Shistocephalus solidus *(remark: extended stomach).*

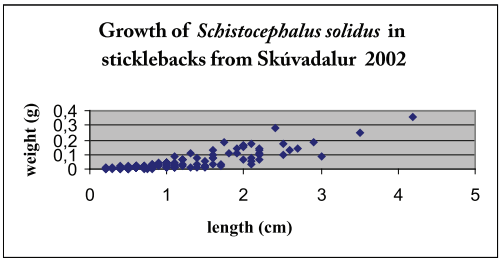

Fig. 6.A. "Growth" (weight versus length) of plerocercoids of Schistocephalus solidus *in sticklebacks from Skúvadalur, 2002.*

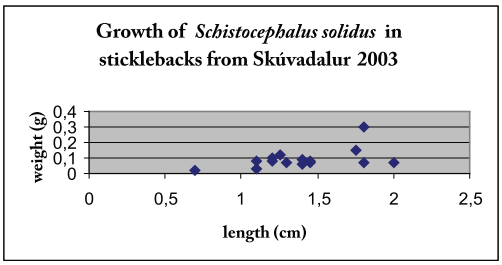

Fig. 6.B. "Growth" (weight versus length) of plerocercoids of Schistocephalus solidus *in sticklebacks from Skúvadalur, 2003.*

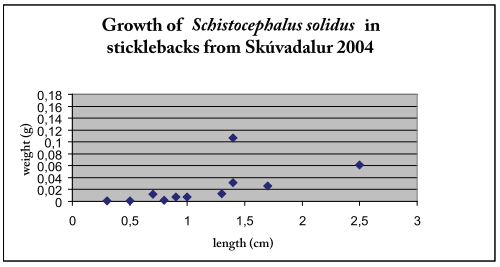

Fig. 6.C. "Growth" (weight versus length) of plerocercoids of Schistocephalus solidus *in sticklebacks from Skúvadalur, 2004.*

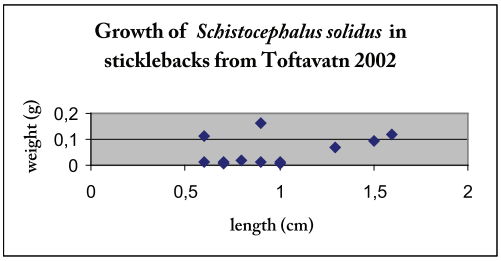

Fig. 6.D. "Growth" (weight versus length) of plerocercoids of Schistocephalus solidus *in sticklebacks from Toftavatn, 2002.*

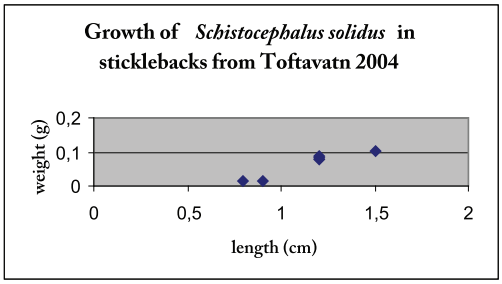

Fig. 6.E. "Growth" (weight versus length) of plerocercoids of Schistocephalus solidus *in sticklebacks from Toftavatn, 2004.*

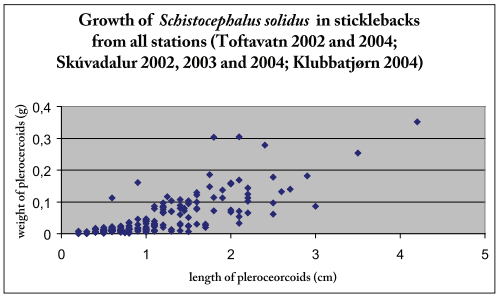

Fig. 6.F. "Growth" (weight versus length) of plerocercoids of Schistocephalus solidus *in sticklebacks from all stations (in Figs. 6 .A-E)*

3.4 cm (mean length 3.71 cm, SD 0.76), condition factors 1.0000 and 0.9101 (mean 0.8659, SD 0.0947).

2.3. *Schistocephalus solidus* (Cestoda); Figs. 5 A-D and Figs. 6 A-F.

Plerocercoids of the cestode were found in the body cavity, sometimes filling it completely. In extreme cases an en enormous

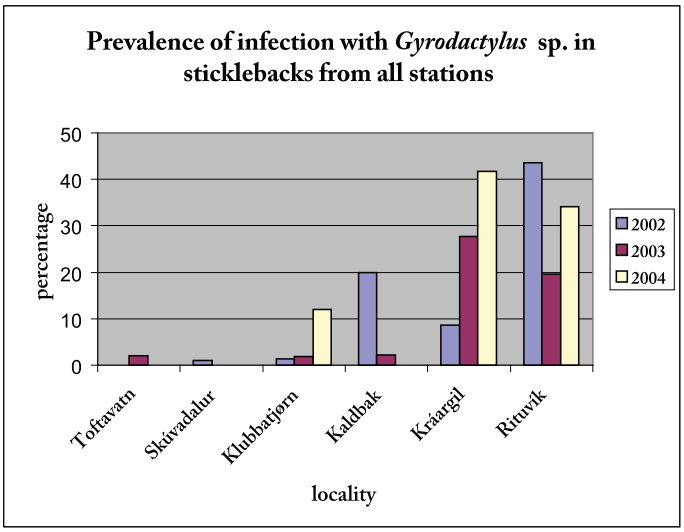

Fig. 7. Prevalence of infection with Gyrodactylus *sp. In sticklebacks from all stations..*

swelling of the abdomen was observed, due to large specimens of plerocercoids. This cestode was only seen in freshwater samples (Figs. 5A and 5 B). Prevalence of infection was 1.7-62.8 %. In Klubbatjørn only one fish was infected one year (2004). In the Toftavatn-2002-sample no fish was infected. There seems to be a tendency of diminishing values in the three-year period examined (Fig. 5 A). The growth of the plerocercoids in the fish is shown in Figs. 6 A-F. The largest plerocercoid was 4.5 cm.

Intensity of infection was 1.8-3.8 and seems to follow the prevalence of infection (Fig. 5B). The maximum number of plerocercoids in one fish was 82 (Skúvadalur, in 2002) where the different developmental stages of the small plerocercoids could be seen. The highest mean value per infected fish (3.8) was also found in this sample. Fish with an extended stomach (Fig. 5 C) were usually filled with a weight of 20-30 % cestodes, compared to body weight.

2.4. *Gyrodactylus* sp. (Monogenea). Fig. 7.
Sticklebacks from the marine localities have the far highest prevalence of infection (0-43.6 %), compared to freshwater localites (0-12.1 %) (Fig. 7). The intensity of infection is ranging from 1-31. The highest mean was 2.6, (Kráargil, 2003) and highest value was 31 specimen of *Gyrodactylus* sp.)

2.5. *Diplostoma* sp. and other digeneans (Digenea)
This parasite of the eye was only observed in 2004 (Kráargil, 2003, prevalence of infection 4.96%). Unidentified digeneans were seen in two samples (Toftavatn, 2004 and Klubbatjørn 2004; prevalence of infection 8.5 % and 3.5 %, respectively). All specimens were so small that they may have escaped attention the other years.

3. Disease conditions

Three types of diseased conditions could be observed: “fin rot”, “white spot disease” and “cysts”, all on the outside of the fish. No at-

tempt was made to cultivate or section the infected places, thus allowing further identification.

DISCUSSION

The Faroe Islands were a center of glaciation during the Pleistocene and were entirely glaciated (Johansen, 1975). Based upon sedimentation data deglaciation probably occurred prior to 10400-10000 BP (Wastegård *et al.*, 2001). Thus the stickleback populations and their parasites described above may have arrived as early as, or just prior to, this time period. Ongoing genetic and morphologic research (Makinen, 2007) with three-spined stickleback populations has revealed that the sample from Rituvik does not fit the pattern of other marine samples and is quite distinct from other Scandinavian populations (Baltic Sea, North Sea and Barents Sea). Faroe sticklebacks in freshwater, also show a high degree of isolation, perhaps sub-speciation or even speciation, according to Friðriksson (2005). This diversity in the host population has been labeled as a phylogenetic raceme model (Bell and Foster, 1994), in which a core of marine populations has diverged repeatedly into many freshwater populations.

One might expect that dramatic differentiation in host populations would be reflected in high parasite speciation due to the isolation of small populations with subsequent genetic drift accompanied by strong local selection. Such does not appear to be the case. None of the parasites found here are unique to the Faroe Island populations of *Gasterosteus aculeatus* (Barber, 2007). For those species utilizing a bird as a final host, such as *Schistocephalus solidus*, the ease with which the final hosts (in this case larid gulls) transverse long distances to nest and find food provides an explanation of why speciation has not occurred regularly in such groups.

However, *Trichodina domerguei*, *T. tenuidens*, *Gyrodactylus* sp. and *Thersitina gasterostei* all have direct life cycles. Also, with the possible exception of *T. gasterostei*, this group has a much shorter generation time than its host. Such situations have been cited as leading to speciation in other fish parasites including *Gyrocotyle* spp. (Bristow, 1992), Cestoda (Renaud *et al.*, 1990) and Digenea (Gibson and Køie, 1991). In each of the papers mentioned, isolation due to glaciation, was cited as the mechanism for generating a new parasite species, without host speciation occurring. In Gibson and Køie (1991) the host species was *Gasterosteus aculeatus*.

The difference between Faroese freshwater and marine biota seems very distinct. *Thersitina gasterorstei* is only found in marine samples and *Schistocephalus solidus* has been observed only in freshwater samples. *Thersitina* seems to be restriced to open sea localities (Rituvík and Kráargil) because the sticklebacks in the sheltered locality Kaldbak were not infected. *Schistocephalus solidus* also showed a somewhat varied pattern of infestation by locality, and there are clear/significant differences seen in different years ($p < 0.01$).

Overall, the biodiversity of the parasites of sticklebacks in the Faroes is low, with a maximum of 4 species per population. It is also quite even by species count with the minimum being 3. The total number of parasite species recorded world-wide for *Gas-*

terosteus aculeatus is approximately 150 (Barber, 2007). In the freshwater systems investigated *Trichodina* spp. and *Schistocephalus solidus* are the dominant species. In the marine environment the parasites appear to be very evenly distributed with the exception of the nematode *Hysterothylacium aduncum*, which was found twice at one marine locality. However, its low occurrence does not affect the overall picture of biodiversity.

In conclusion Faroese stickleback populations should offer good possibilites for future studies while trying to establish the effect on the host of single parasite species, like *Schistocephalus solidus* and *Thersitina gasterostei.* Other Faroese islands also remain to be investigated, especially from an evolutionary point of view.

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