ICELANDIC RESEARCH FUND
FINAL REPORT

A signed copy of the final report shall be submitted to Rannís by email: (rannsoknasjodur@rannis.is). Titled: Icelandic research Fund-final report.

PRINCIPAL INVESTIGATOR (PI): **Bettina Scholz**

PI ID NUMBER: **2311662549**

EXPERT PANEL: **NATURAL SCIENCE AND ENVIRONMENTAL SCIENCE**

PROJECT TITLE: **Host-pathogen interactions between brackish and marine microphytobenthic diatom taxa and representatives of the Chytridiomycota, Oomycota and Labyrinthulomycota**

GRANT NUMBER: **141423-051**

TYPE OF GRANT: **Post Doc**

GRANT PERIOD: **June 2014-June 2017**

Signatures
This is to certify that all information in the final report is correct and that the report includes all relevant information.

Date and place 18. July 2017 Skagaströnd
Principal investigator
Person responsible for research facilities

To be filled out by Rannís

Final report received (date)	
Grant previously paid the final grant year (1st, 2nd and 3rd payments of the year)	
Grant paid upon approval of the report (4th payment of the year)	
Final report approved (date and signature)	

1. **Total cost and financing of the project. See Appendix**

2. **Cost and financing of the project in thousand ISK, according to the attached budget overview.**

Item	Awarded funding from IRF	Other financing	Total cost
Wages and wage related expenses	17.070	4.753	21.823
Operating expenses	2.003	2.538	4.541
Contracted services	0	422	422
Travel expenses	1.927	75	2.002
Overhead	0	0	0
Total	**21.000**	**7.788**	**28.788**

3. **PI's final report (2 – 4 pages)**

Active participants of the project: Dr. Bettina Scholz (B.S.); Prof. Dr. Frithjof C. Küpper (F.C.K); Prof. Dr. Wim Vyverman (W.V.); Prof. Dr. Ulf Karsten (U.K.); Halldór G. Ólafsson (H.G.Ó); Prof. Dr. Hjörleifur Einarsson (H.E.)
Role in the project: B.S., F.C.K., W.V., and U.K. conceived and designed the experiments; B.S. designed and performed the experiments, conducted the species, chemical and statistical analysis; wrote the manuscripts and reports; H.G.Ó. conducted parts of the field work in Iceland. H.E. provided advice regarding benthic sampling opportunities in the north of Iceland. In addition, F.C.K and U.K. provided samples from the Ythan estuary (Aberdeenshire, Scotland, UK) and Bodden Chain (Baltic Sea, Germany), respectively.

The following three hypotheses were addressed during the project:
I. Pathogen infection rates increase with increasing physiological stress of the diatom hosts, driven by variations in environmental conditions.
II. Several eukaryotic pathogens are highly selective in their choice of diatom host species. The specificity of such parasites relies on the biochemical characteristics and, in particular, metabolic exudates of the potential host cells (e.g. carbohydrate composition).

III. Benthic diatoms are able to develop chemical defense mechanisms against the attack of eukaryotic pathogens by production of volatile compounds.

In order to address these hypothesis a total of 291 environmental samples (209 microphytobenthic, 62 planktonic, 20 epiphyte samples from seaweeds) were screened and analysed, four chytrid-diatom host pairs isolated, 128 monoclonal cultures of diatom-hosts established and 87 experimental series were conducted with 30 monoclonal cultures of each host species. The results and findings are/will be comprised in eight publications in peer reviewed journals and two book chapters.

<u>To hypothesis I</u>: During the long-term monitoring of two transects in the Skagaströnd area from May 2014 to May 2017 and comparative occasional sampling events at seven different sites in north-west Iceland in the first year (including epiphytes on seaweeds), a total of 221 diatom taxa were recorded. Due to the high bentho-pelagic coupling in all tested areas in north Iceland, phytoplankton samples from the Húnaflói near Skagaströnd were also collected and screened from 2014-2016 (May-September). The long-term monitoring became necessary due to the high seasonal fluctuations between the years and will be continued in 2017. The results of the monitorings will be reported in four separate publications (Scholz et al. in preparation a, b, c, d). Additional samplings conducted in the Ythan estuary (Scotland, Aberdeen shire) and the Balic Sea (Germany) showed the presence of infections by chytrids and oomycetes on cyanobacteria and diatoms.

During the first year of the monitoring it became evident that the marine diatom taxa were infected by several different zoosporic parasites in relation to seasonal conditions (Scholz et al. 2016; Scholz et al. in preparation a, b, c, d). Overall 39 diatom species were found to be infected by different zoosporic parasites, in which the highest overall infection rate was observed in June 2015 (98% of the total planktonic diatom community). The infections were mainly caused by oomycetes, chytrids, aphelids and *Pirsonia*, while Thraustochytrids were only observed as saprophytes (in no case intact diatom cells were attacked during the conducted laboratory studies). The highest infection rate for example by chytrids in the microphytobenthos was observed at transect II in the Skagaströnd area in September 2016 (19.3% of the entire diatom community, Scholz et al. in preparation a), whereas in the phytoplankton infection rates of 48.7% were recorded in May 2015 (Scholz et al. in preparation b). Although it was planned to identify the parasites through molecular-taxonomic characterisation and DNA barcodes for terrestrial oomycetes are available (e.g. Scholz et al. 2016), DNA barcodes for most other zoosporic parasites are missing or are not conclusive and the existing ones rarely allow identification even to the genus level. In all cases, further difficulties of DNA-based methods are primer biases within mixed samples, going hand in hand with the troublesome establishment and maintenance of ‘pure’ dual cultures of host and parasite (Scholz et al. 2016). Furthermore, even though molecular methods are becoming widely available, there is still a scarce number of sequences of zoosporic parasites and also a lack of curated sequence databases to correctly identify these parasites (e.g. Frenken et al. 2017).

The influence of environmental factors on the infection susceptibility of four different marine diatom host species to chytrid infection was tested under laboratory conditions, using host and parasite isolates obtained from diverse coastal areas in north-west Iceland in 2015 (Scholz et al. 2017a). Specifically, overall 120 monoclonal host cultures of *Navicula* Bory, *Nitzschia* Hassall, *Rhizosolenia* Brightwell and *Chaetoceros* Ehrenberg were exposed to their chytrid parasites *Chytridium* type I and *Rhizophydium* type I and II in Hellendahl glass staining jars which were subdivided in two compartments by nylon filters (mesh size 5 µm). Infection densities were assessed at different temperatures (5, 15, 20°C), salinities (0, 5, 10, 20, 40), photon fluence rates (PFR; 10, 50, 100, 200 µmol photons m^{-2} s^{-1}) and photoperiods (24 h dark, 8:16 h, 16:8 h light:dark and 24 h light) after 168 h exposure, using the one-factor-at-a-time method. In addition, growth rates and proline concentrations of the non-infected monoclonal host cultures were determined. In this context, proline appears to be the most widely distributed osmolyte accumulated under environmental stress and was utilized in the present study as stress indicator. In most cases of the actual study, decreasing growth rates during the acclimatisation process to abiotic stressors were directly related to increases of proline in the host cells. Significant positive associations of infection densities to cell based proline concentrations were predominantly observed in the high-PFR assays and 24 h daylight treatments. At least in half of the tested host-parasite pairs positive correlations of proline and parasite prevalence were found. Only in *Nitzschia* sp. the parasite density was negatively associated with the biochemical variable proline and showed no significant relationship to the host densities, suggesting that other physiological/biochemical factors related to stress might have an impact on the susceptibility of this peculiar host diatom species (Scholz et al. 2017a). Cross-infection experiments showed that

Rhizophydium type III infected two host species (*Rhizosolenia* and *Chaetoceros*), whereas type I and II as well as *Chytridium* type I were strictly host specific (Scholz et al. 2017b).

To hypothesis II Hypothesizing that environmental stress parameters affect parasite-host recognition, four chytrid-diatom tandem cultures were used to test the chemotaxis of chytrid zoospores and the presence of potential defense molecules in a non-contact-co-culturing approach. As potential triggers in the chemotaxis experiments, standards of eight carbohydrates, six amino acids, five fatty acids and three compounds known as compatible solutes were used in individual and mixed solutions, respectively. In addition, aqueous extracts of the four susceptible host-diatoms (*Navicula* Bory, *Nitzschia* Hassall, *Rhizosolenia* Brightwell, *Chaetoceros* Ehrenberg) grown under standard culture conditions (10 °C, 12:12 h light:dark (L:D), 50 $\mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$) and 24 h light regime with additional UVR exposure, were also tested. Although it was planned in the present project to analyze the biochemical composition of the parasites, the plan was altered due to the restricted biomass of zoospores during the experiments. In all tested cases, the whole-cell extracts of the light-stressed hosts attracted the highest numbers of zoospores (86%), followed by the combined carbohydrate standard solution (76%), while all other compounds acted as weak triggers only, suggesting that multiple attractants drive chemotaxis and may act synergistically. Altogether, this suggests that taxis in zoosporic parasites might not be specific in terms of host selection and is consistent with observations that zoospore attachment to hosts can be reversible in some taxa (Scholz et al. 2017b; Frenken et al. 2017).

To hypothesis III Testing pooled ethanolic, methanolic and n-hexanic extracts of resistant and susceptible diatom taxa for the presence of potential defense compounds (e.g. polyunsaturated fatty acids (PUFAs), carbohydrates, glycosides, phenols, phytosterols and triterpenoids) yielded only minor differences between the resistant and susceptible diatom taxa. Particularly, aldehydes were more abundantly found in biomass and supernatant extracts of resistant diatom taxa, being significant in biomass extracts of *Chaetoceros* sp. and supernatant extracts of *Navicula* sp. and *Nitzschia* sp. The accumulation of carbohydrates and PUFAs was also significant in extracts of unsusceptible *Navicula* and *Nitzschia* biomass and supernatant extracts ($p<0.05$).

While alkaloids and tannins were in general not present in neither biomass nor supernatant extracts, the froth tests for saponins was positive for biomass and supernatant extracts of *Nitzschi*a sp. with no significant concentration difference between the susceptible and the resistant strain ($p>0.05$). In addition, positive colorimetric reactions in the Alkaline Reagent Test indicated the presence of a flavonoid in the biomass extracts of susceptible and resistant *Rhizosolenia* sp. In contrast, the test gave a positive test result only for the resistant *Rhizosolenia* also in supernatant extract (Scholz et al. 2017b).

The infection rates of the four diatom hosts (*Navicula* sp., *Nitzschia* sp., *Rhizosolenia* sp. and *Chaetoceros* sp.) exposed to 2 mL n-hexanic, methanolic and ethanolic extracts re-dissolved in 100 ml aqueous EtOH (40%), obtained from the phytochemical screening showed distinct differences between susceptible and resistant diatom hosts. Inhibitive effects on the infections were predominantly caused by ethanolic biomass and supernatant extracts of the resistant diatom strains, being significant for *Rhizosolenia* and *Chaetoceros* infected by *Rhizophydium* type II a and b, respectively. In addition, methanolic extracts of the resistant diatom strains were also found to inhibit infections by the parasites with significant differences between biomass and supernatant extract ($p<0.05$; Scholz et al. 2017b).

Publications

in peer reviewed Scientific Journals

1. **Scholz, B.**; Guillou, L.; Marano, A.V.; Neuhauser, S.; Sullivan, B. K.; Karsten, U.; Küpper, F.C.; Gleason, F.H. (2016). Zoosporic parasites infecting marine diatoms - A black box that needs to be opened. *Fungal Ecology*, 19, 59–76. https://doi.org/10.1016/j.funeco.2015.09.002
2. **Scholz, B.**; Küpper, F.C.; Vyverman, W.; Ólafsson, H.G.; Karsten U. (2017a). Effects of environmental parameters on chytrid infection prevalence of four marine diatoms – A laboratory case study. *Botanica Marina*, https://doi.org /10.1515/bot-2016-0105.
3. **Scholz, B.**; Küpper, F.C.; Vyverman, W.; Ólafsson, H.G.; Karsten U. (2017b). Chytridiomycosis of marine diatoms - The potential role of chemotactic triggers and defense molecules in parasite-host interactions. *Marine Drugs*, 15, 26; doi:10.3390/md15020026; http://www.mdpi.com/1660-3397/15/2/26

4. Frenken, T.; Alacid, E.; Berger, S.A.; Bourne, E.C.; Gerphagnon, M.; Grossart, H.-P.; Gsell, A. S.; Ibelings, B. W.; Kagami M.; Küpper, F.C.; Letcher P.M.; Loyau, A.; Miki T.; Nejstgaard, J. C.; Rasconi, S.; Rene, A.; Rohrlack, T.; Rojas-Jimenez, K.; Schmeller, D.S.; **Scholz, B**.; Seto, K.; Sime-Ngando, T.; Sukenik, A.; Van de Waal, D.B.; Van den Wyngaert, S.; Van Donk, E.; Wolinska, J.; Wurzbacher, C.; Agha, R. (2017). Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. *Environmental Microbiology*, doi: 10.1111/1462-2920.13827.

Book Chapters

1. Jephcott, T.G.; Gleason, F.H.; van Ogtrop, F.F.; Macarthur, D.J.; **Scholz, B.** (2017). "The ecology of chytrid and aphelid parasites of phytoplankton**"**. In *Fungal Community, its organization and role in the ecosystem, fourth edition*. Edited by John Dighton, James F. White; pp 239–256, CRC Press Taylor & Francis Group, Boca Raton, USA.
2. Gleason, F.H.; **Scholz, B.**; Jephcott, T.G.; van Ogtrop, F.F.; Henderson, L.; Osu Lilje, O.; Kittelmann, S.; Macarthur, D.J. (2017). Key ecological roles for zoosporic true fungi in aquatic habitats. Pre-publicized in: American Society for Microbiology (ASM) Microbiology Spectrum 5(2): doi:10.1128/microbiolspec.FUNK-0038-2016.

In preparation

1. **Scholz, B.**; Küpper, F.C.; Vyverman, W.; Ólafsson, H.G.; Einarsson, H. & Karsten U. (in preparation a). Seasonal succession patterns of zoosporic parasites infecting marine diatoms and their impacts on intertidal community compositions in the Húnaflói.
2. **Scholz, B.**; Küpper, F.C.; Vyverman, W.; Ólafsson, H.G. & Karsten U. (in preparation b). The temperature dependence of zoosporic parasites infecting marine diatoms and their effects on planktonic community compositions in the Húnaflói.
3. **Scholz, B.**; Küpper, F.C.; Vyverman, W. & Karsten U. (in preparation c). Infections by zoosporic parasites (Chytridiomycota and Oomycota) of epiphytic diatoms on selected seaweeds in the Húnaflói.
4. **Scholz, B.**; Küpper, F.C.; Vyverman, W. Ólafsson, H.G.; Einarsson, H. & Karsten U. (in preparation d). The impact of sediment characteristics on the infection

prevalence of benthic marine diatoms to zoosporic parasites in northern Icelandic coastal areas.

<u>Other main achievements</u>:

- Contribution at the **"**1st Phytoplankton Chytridiomycosis Workshop" (23rd and 24th July in Berlin, Germany). Title of the presentation: "Chytrid infections in northern Icelandic coastal habitats **–** project status and perspectives"
- Organization of the 2nd Phytoplankton Chytridiomycosis Workshop in Skagaströnd. Overall 23 participants from 11 countries were present at the workshop held in Skagaströnd from September 15th until 17th. Two grants were successfully captured for this event.
- Co-supervision of master student Daniel Liesner (2016-2017). Main supervisor: Prof. Dr. Ulf Karsten, University of Rostock, Germany. Theme: *„Chytridiomycosis in near coastal phytoplankton communities from the Baltic and Greenland Sea with special emphasis on the influence of abiotic parameters on infection prevalence"*. Final grade: 1.4 (very good). March 20th, 2017. The student was financed for 5 months through BioPol ehf.

4. A short news release about the project, to be published on the RANNIS website

Infections by epi- and endobiotic, zoospore-producing parasites are in most cases lethal for the host. During a long-term monitoring conducted in the Húnaflói (north-west Iceland), foccusing originally on chytrids and oomycetes, the presence of several zoosporic parasites in parallel was observed infecting individual host-diatoms in the microphytobenthos and phytoplankton (Scholz 2015; Scholz et al. 2016). It was found that the infections followed seasonal succession patterns of zoosporic parasites with up to 98% overall infection rates of the total diatom community in the phytoplankton. Hence, such parasites have significant impacts on the abundance of individual hosts and modulating thereby the composition of communities at both producer and consumer trophic levels of food webs (Jephcott et al. 2017; Gleason et al. 2017; Frenken et al. 2017). Further laboratory test series with marine host-diatom and chytrid isolates indicated the potential of the diatoms to defend themselves against the infection by chytrid zoospores (Scholz et al. 2017a) as well as demonstrated a direct link between environmental stressors and host-susceptibility (Scholz et al. 2017b).

The results have been internationally recognized and particularly the research related to host-parasite interactions will be continued on international level.

Scholz, B., 2015. Host-pathogen interactions between brackish and marine microphytobenthic diatom taxa and representatives of the Chytridiomycota, Oomycota and Labyrinthulomycota. Status report for the Icelandic Research Fund from May to June 2014. doi: 10.13140/RG.2.1.4769.6087

Scholz, B.; Guillou, L.; Marano, A.V.; Neuhauser, S.; Sullivan, B. K.; Karsten, U.; Küpper, F.C.; Gleason, F.H. (2016). Zoosporic parasites infecting marine diatoms - A black box that needs to be opened. *Fungal Ecology*, 19, 59–76. https://doi.org/10.1016/j.funeco.2015.09.002.

Scholz, B.; Küpper, F.C.; Vyverman, W.; Ólafsson, H.G.; Karsten U. (2017a). Chytridiomycosis of marine diatoms - The potential role of chemotactic triggers and defense molecules in parasite-host interactions. *Marine Drugs*, 15, 26; doi:10.3390/md15020026; http://www.mdpi.com/1660-3397/15/2/26

Scholz, B.; Küpper, F.C.; Vyverman, W.; Ólafsson, H.G.; Karsten U. (2017b). Effects of environmental parameters on chytrid infection prevalence of four marine diatoms – a laboratory case study. *Botanica Marina*, https://doi.org /10.1515/bot-2016-0105.

Frenken, T.; Alacid, E.; Berger, S.A.; Bourne, E.C.; Gerphagnon, M.; Grossart, H.-P.; Gsell, A. S.; Ibelings, B. W.; Kagami M.; Küpper, F.C.; Letcher P.M.; Loyau, A.; Miki T.; Nejstgaard, J. C.; Rasconi, S.; Rene, A.; Rohrlack, T.; Rojas-Jimenez, K.; Schmeller, D.S.; Scholz, B.; Seto, K.; Sime-Ngando, T.; Sukenik, A.; Van de Waal, D.B.; Van den Wyngaert, S.; Van Donk, E.; Wolinska, J.; Wurzbacher, C.; Agha, R. (2017). Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. *Environmental Microbiology*, doi: 10.1111/1462-2920.13827.

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Gleason, F.H.; Scholz, B.; Jephcott, T.G.; van Ogtrop, F.F.; Henderson, L.; Osu Lilje, O.; Kittelmann, S.; Macarthur, D.J. (2017). Key ecological roles for zoosporic true fungi in aquatic habitats. Pre-publicized in: American Society for Microbiology (ASM) Microbiology Spectrum 5(2): doi:10.1128/microbiolspec.FUNK-0038-2016.

APPENDIX

Total cost and financing of the project (in thousand ISK)

2014 (01.06.2014-31.12.2014)

Item	Awarded funding from IRF	Other financing	Total cost
Wages and wage related expenses	3.150	685	3.835
Operating expenses	330	2.171	2.501
Contracted services	0	0	0
Travel expenses	393	83	476
Overhead	0	0	0
Total	**3.873**	**2.939**	**6.812**

2015 (01.01.2015-31.12.2015)

Item	Awarded funding from IRF	Other financing	Total cost
Wages and wage related expenses	5.760	808	6.568
Operating expenses	519	113	632
Contracted services	0	442	442
Travel expenses	731	7	728
Overhead	0	0	0
Total	**7.000**	**1.370**	**8.370**

2016 (01.01.2016-31.12.2016)

Item	Awarded funding from IRF	Other financing	Total cost
Wages and wage related expenses	5.760	2.183*[1]	7.943
Operating expenses	634	16	650
Contracted services	0	0	0

Travel expenses	606	-108	498
Overhead	0	0	0
Total	**7.000**	**2.091**	**9.091**

*[1] including master thesis student Daniel Liesner (5 months at BioPol ehf)

2017 (01.01.2017-30.05.2017)

Item	Awarded funding from IRF	Other financing	Total cost
Wages and wage related expenses	2.400	1.077	3.477
Operating expenses	520*	238	758
Contracted services	0	0	0
Travel expenses	207	93	300
Overhead	0	0	0
Total	**3.127**	**1.408**	**4.535**