The nature and pathogenicity of apicomplexan parasites associated with mass mortality events in scallop (Bivalvia: Pectinidae) populations in the North-Atlantic Ocean

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Dissertation submitted in partial fulfillment of a
Philosophiae Doctor Degree in Biology

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Abstract

Apicomplexans comprise a group of unicellular, often highly pathogenic, obligate parasites infecting both vertebrates and invertebrates, exploiting either one (monoxenous) or two hosts (heteroxenous) to complete a full reproductive life cycle. Their pathogenicity varies considerably between species and/or their hosts but as most species are obligate, intracellular parasites they cause some level of pathology in all cases.

In relation to abnormal mortality events experienced in scallop populations in the North-Atlantic, four different species of scallops, i.e. Iceland scallop, queen scallop, king scallop and sea scallop, were examined for infectious agents, with the aim of shedding light on these events. Most emphasis was made on the Iceland scallop stock in Icelandic waters that unexpectedly collapsed during the 2000s. Its health status was annually monitored for 14 years. In addition to the scallops, whelks and other mollusc species were examined at later stages in the study, in context with the presumable life cycle of a pathogen observed.

Two different apicomplexan species, both of which appeared to be previously unknown, were identified: 1) *Margolisiella islandica*, a novel species infecting the heart auricles and highly prevalent in Iceland scallop, without causing any significant pathology, but absent in the three other scallop species. It is a monoxenous species, with all life stages present in a single host, i.e. the Iceland scallop. 2) Initially anonymous species, infecting muscular- and connective tissues, found in all four scallop species examined. Except for scallops from the UK, infections were generally heavy, causing severe histopathological changes leading to significant reduction in the general condition of the scallops, especially adductor muscles, which became abnormally reduced and discoloured. Furthermore, it hampers normal gonad development, at least in the Iceland scallop. Molecular studies and *in situ* hybridization, revealed that it is conspecific with *Merocystis kathae*, an apicomplexan described from the common whelk, *Buccinum undatum,* more than 100 years ago. Consequently, its life cycle is heteroxenous, involving the common whelk as a definite host and scallops as intermediate hosts. This is the first dual mollusc life cycle described for an apicomplexan.

M. kathae forms a fully resolved monophyletic clade with *Aggregata* spp. This aggregatid clade is associated with a sister clade containing *Filipodium phascolosomae* and *Platyproteum vivax* (Archigregarinorida (Squirmida)) from sipunculids. *M. islandica* and *Pseudoklossia pectinis* do not form part of this clade, but form a group of apicomplexans that infect marine bivalves and polychaetes (Family Rhytidocystidae). This entire group forms a weakly supported clade of apicomplexans that are found in marine invertebrates.

The results strongly suggest that *M. kathae* played a major role in the collapse of the Iceland scallop stock in Breidafjördur, Iceland. Furthermore, it caused abnormal condition of Faroese queen scallops and is a suspected aetiological agent behind mass mortality events of sea scallops in the eastern USA as well as a number of unresolved mass mortalities and abnormal condition experienced in various other populations of scallops. Scallops seem able to regulate low-level infections of *M. kathae,* as they exist in normal scallop populations. However, during prolonged or high levels of exposure from localised infected whelks, disease outbreaks occur. Hence, reasonable fisheries from both whelk- and scallop stocks could lower infectious load and minimize the occurrence of *M. kathae* epidemics and prevent damaging economic losses.

Útdráttur

Fylking Apicomplexa, samanstendur af hópi sjúkdómsvaldandi, einfruma sníkjudýra sem sýkja bæði hryggdýr og hryggleysingja. Til þess að ljúka lífsferli sínum þarfnast þau ýmist eins hýsils (monoxenous) eða tveggja hýsla (heteroxenous). Meinvirkni þessa hóps sníkjudýra er mismikil, eftir tegundum og/eða hýslum þeirra. Þar sem þetta eru innanfrumusýklar, valda þeir þó einhverjum vefjaskemmdum í öllum tilfellum.

Í tengslum við óeðlilega umfangsmikinn náttúrulegan dauða í ýmsum stofnum og tegundum hörpudiska í Norður-Atlantshafi, voru fjórar tegundir hörpudisks, frá Íslandi, Færeyjum, Bandaríkjunum og Bretlandi, rannsakaðar m.t.t. sjúkdóma, með það að markmiði að varpa ljósi á ástæður þessa mikla náttúrulega dauða. Megináherslan var lögð á stofn íslenska hörpudisksins í Breiðafirði, en algert hrun varð í stofninum á fyrstu árum þessarar aldar. Íslenski stofninn var vaktaður árlega í samfellt 14 ár. Til viðbótar hörpudiskum, voru beitukóngur, auk fleiri tegunda lindýra, rannsökuð á síðari stigum í tengslum við lífsferil sníkjudýrs sem greindist.

Tvær tegundir af fylkingu Apicomplexa greindust í hörpuskeljunum, báðar að því er virtist áður óþekktar: 1) Tegund sem fékk nafnið *Margolisiella islandica* sem sýkir hjarta og fannst eingöngu í íslensku hörpuskelinni. Smittíðnin var há, en vefjaskemmdir samfara sýkingum voru óverulegar. 2) Tegund sem í fyrstu virtist áður óþekkt; sýkir vöðva og stoðvefi (connective tissues) og fannst í öllum fjórum hörpudiskstegundunum. Að frátöldum skeljum frá Bretlandseyjum, voru sýkingar almennt umfangsmiklar og ollu svæsnum vefjaskemmdum. Sýkingar hafa verulega neikvæð áhrif á almennt ástand skelja, einkum aðdráttarvöðva, sem verða óeðlilega rýrir auk þess að verða grá/brúnleitir og lausir í sér miðað við þétta, ljósa, heilbrigða vöðva. Að auki, valda sýkingar verulegum skemmdum á kynkirtlum sem leiðir til þess að þeir þroskast ekki með eðlilegum hætti. Frekari greiningar sýndu fram á að tegundin reyndist vera *Merocystis kathae* sem lýst var úr nýra beitukóngs fyrir meira en 100 árum en veldur sniglinum óverulegu heilsutjóni. *M. kathae* nýtir þ.a.l. tvo hýsla til að ljúka lífsferli sínum, hörpudisk og beitukóng. Þetta er fyrsti lífsferlill sem lýst er fyrir tegund af fylkingu Apicomplexa sem felur í sér tvær mismunandi tegundir lindýra.

Þróunarfræðilega, myndar *M. kathae* einættaða (monophyletic) grein/hóp (clade) með *Aggregata* tegundum. Systur-grein/hópur *M. kathae* og *Aggregata* tegundanna samanstendur af *Filipodium phascolosomae* og *Platyproteum vivax*, tegundum af ættbálki Archigregarinorida sem sýkja liðorma, möttuldýr, akarnorma og sæbelgi. *M. islandica* og skyld tegund sem sýkir tegund hörpudisks, *Pseudoklossia pectinis*, tilheyra ekki þessari grein/hópi, en mynda sérstaka grein/hóp með *Rhytidocystis* tegundum, sem sýkja burstaorma og tveimur ónefndum tegundum úr sjávarsamlokum. Allar áðurnefndar greinar/hópar mynda svo saman grein/hóp tegunda sem sýkja sjávarhryggleysingja.

Niðurstöður þessara rannsókna benda eindregið til þess að *M. kathae* sé meginástæða hruns íslenska hörpudiksstofnsins og slæmu ástandi hörpudiskstegundarinnar við Færeyjar. Auk þessa, eru vísbendingar um að þetta sníkjudýr valdi, og hafi valdið, stórfelldum náttúrulegum dauða í skeljastofnum við austurströnd Bandaríkjanna og mögulega miklum og óútskýrðum afföllum í öðrum stofnum ýmissa tegunda hörpudisks í Norður-Atlantshafi í gegnum tíðina. Þar sem *M. kathae* sýkingar finnast í heilbrigðum hörpudiksstofnum, virðist sem skeljar nái að höndla vægar sýkingar. Hins vegar, þegar mikið og langvarandi smitmagn berst frá smituðum beitukóngum á búsvæðum hörpudisks, geta komið upp faraldrar. Þar af leiðandi, gætu hófsamar veiðar, á bæði hörpudiski og beitukóngi, minnkað smitpressu og dregið úr líkum á uppkomu sjúkdómsfaraldra og tilheyrandi fjárhagstjóni samfara þeim.

I dedicate this thesis to my family, in the broad context of the word. My spouse, son, father (2006†), my mother and my siblings and their spouses

When I went to study biology at the University of Iceland, then almost 30 years of age, this good people, supported my beyond my imagination. At the University, I met my future spouse and my son was born while I was doing my undergraduate studies

Preface

The contents of my thesis is related to the collapse of the Iceland scallop population in Icelandic waters and furthermore, several unexpected extensions of the work during the process. It now covers 16 years of research with the initial onset of the project being in November 2002, when scientists from the Marine Research Institute in Iceland brought scallops for examination to the Fish Disease Laboratory at the Institute for Experimental Pathology at Keldur, University of Iceland. The reason for this was to check whether diseases could play a role in the aforementioned population collapse. Following prelimary results from the initial examinations, a 10 years research plan was formed which included more thorough examination for the infectious agents detected, as well an annual monitoring of the collapsed scallop stock with regard to disease causing agents. Due to the importance of these events, which were a severe economic blow to small towns in western Iceland, a grant was awarded directly by the Ministry of Fisheries in Iceland to conduct further research. Early in the research process, it became evident that one of the infectious agent identified seemed highly pathogenic and likely to have severe negative effect on the scallop population. However, this was not an easy task to resolve. In the project´s initial phases, two apicomplexan species were observed, both of which with immense numbers of different developmental stages, making it difficult to determine which stages belonged to each species. An extension of the project solved that problem; samples of Faroese queen scallops were sent to the laboratory, following a request from the scallop fisheries sector in the Faroe Islands, due to an abnormal condition of the scallop stock. Atlthough the Faroese scallops were heavily infected, they only had one of the apicomplexan parasite found in the Icelandic one. Consequently, a reliable discrimination between the two species was possible. The work was further extended when a colloboration with a researcher from the UK started with a visit to Scotland in autumn 2007, with the aim of collecting and examining queen- and king scallops from Scottish waters. The presence of one of the parasite was confirmed, extending its geographic distribution. It was, however, at very low level, in apparently healthy scallop populations. A new angle of the project was created when suspicions evoked that one of the apicomplexan species possibly had a two-host life cycle, but not one as initially suggested. Phylogenetic analysis of the parasite revealed that all its closest relatives had

two-host life cycles. This lead to the search for the definite host, which was successfully found, i.e. the common whelk. In relation to these novel findings, a king scallop ranch in the NW Scotland was visited in 2015 and samples collected. According to the farmer, whelks were almost absent in the nearest surroundings of the farming area. It is naturally so, but furthermore, the few ones seen during regular divings around the farming site, all whelks found are removed as they are scallop predators. Examination of the scallops supported our two-host theory.

A further extention of the project occurred when scientist from SMAST (School for Marine Science & Technology) at the University of Massachusetts sought after collaboration, in relation to phenomenon termed "gray meat" in sea scallops associated with mass mortality events.

I believe it is fair to say that the contents of this thesis have had a significant international impact, predominantly in North America. The pathogen detected in the Iceland scallop is becoming one of the priority research topics in the aim of resolving the abnormal "gray meat" condition of highly commercially valuable scallop stocks of the Atlantic coast of North America and the analogous "weak meat" condition of weathervane scallops in Alaskan water.

List of Publications

This thesis is based on the following publications, referred to in the text by their Roman numerals:

I. Árni Kristmundsson, Sigurður Helgason, Slavko Helgi Bambir, Matthías Eydal & Mark Andrew Freeman (2011). *Margolisiella islandica* sp. nov. (Apicomplexa: Eimeridae) infecting Iceland scallop *Chlamys islandica* (Müller, 1776) in Icelandic waters. *Journal of Invertebrate Pathology* 108: 139-146. DOI.org/10.1016/j.jip.2011.08.001

II. Árni Kristmundsson, Sigurður Helgason, Slavko Helgi Bambir, Matthías Eydal & Mark Andrew Freeman (2011). Previously unknown apicomplexan species infecting Iceland scallop, *Chlamys islandica* (Müller, 1776), queen scallop, *Aequipecten opercularis* L., and king scallop, *Pecten maximus* L. *Journal of Invertebrate Pathology* 108: 147-155. DOI.org/10.1016/j.jip.2011.08.003

III. Árni Kristmundsson, Ásthildur Erlingsdóttir & Mark Andrew Freeman (2015). Is an apicomplexan parasite responsible for the collapse of the Iceland scallop (*Chlamys islandica*) stock? *PLOS ONE* 10(12): e0144685. DOI.org/10.1371/journal.pone.0144685

IV. Susan D. Inglis, Árni Kristmundsson, Mark Andrew Freeman, Megan Levesque & Kevin Stokesbury (2016). Gray meat in the Atlantic sea scallop, *Placopecten magellanicus*, and the identification of a known pathogenic scallop apicomplexan. *Journal of Invertebrate Pathology* 141: 66-75. DOI.org/10.1016/j.jip.2016.10.008

V. Árni Kristmundsson & Mark Andrew Freeman (2017). Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere. *Scientific Reports* 8:7865. DOI:10.1038/s41598-018-26158-1

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List of Definitions

Amylopectin	a component of starch that has a high molecular weight and branched structure and does not tend to gel in aqueous solutions
Apicoplast	a relict, non-photosynthetic plastid found in most apicomplexan parasites
Brown meat	a term used for sea scallops, *Placopecten magellanicus*, off the east coast of USA with discoloured and reduced adductor muscles. An intermediate stage between white meat and gray meat.
Definite host	the host in which the parasite usually undergoes sexual reproduction
Disporous	apicomplexan species with a sporocyst with two sporozoites
Endemic pathogen	a pathogen which is characteristic of, or prevalent in a particular field, area or environment
Endoplasmic reticulum	the network of fine tubules in the cytoplasm of cells for structural framework and circulation pathway
Epicellular	attached or in close contact with a cell
Epidemic/epizootic	an occurrence of disease that is temporarily of high prevalence
Exotic pathogen	a pathogen introduced from another country and not native to the place where it is found
Extracellular	situated or occurring outside a cell or the cells of the body
Gamogony	part of sexual reproduction; it generally precedes fertilization of macrogametes by microgametes
Golgi apparatus	a cytoplasmic organelle that consists of a stack of several to many smooth, membranous saccules and associated vesicles active in the modification and transport of proteins
Gray meat	term used for sea scallops, *Placopecten magellanicus*, off the east coast of USA with

	abnormally discoloured and reduced adductor muscles
Hermaphrodite	organisms with both male and female genital systems
Heteroxenous	an apicomplexan parasite which requires two hosts to complete a full reproductive life cycle
Intermediate host	a host in which there is development of the asexual or immature stages of a parasite
Intracellular	inside a cell
Macrogamont	a female gamete
Merogony	the sequence of asexual multiplication (syn. schizogony) to produce a great number of merozoites
Meront	an asexual developmental stage to produce merozoites
Merozoite	a crescent stage formed by meront during asexual reproduction; a stage which may initiate another merogony or gamogony
Microgamete	a male gamete
Microgamont	a cell which produces microgametes
Micronemes	structural and secretory element of the apical complex that facilitates interaction with the host cell
Mitochondria	a filamentus or granular organelle in the cytoplasm; it is the principal site of oxidative reactions by which energy is made available for endergonic processes
Monoxenous	an apicomplexan parasite which uses one host to complete a full reproductive cycle
Oocyst	a one to three layered wall, or membrane bound sack surrounding a sporont or sporocyst
Rophtries	structural and secretory element of the apical complex that facilitates interaction with the host cell
Sporadic disease	a disease occurring occasionally, singly, or in irregular or random instances
Sporoblast	a cell which develops into a spore; it is produced generally by the division of a sporont and eventually develops into sporozoites
Sporocyst	a membrane bound sac which contains

	apicomplexan sporozoites
Sporogony	a phase in the development of an apicomplexan in which the zygote initiates asexual reproduction and results in production of infective sporozoites
Sporont	a developmental stage that gives rise to one or many sporoblasts
Sporozoite	a nucleated infective stage formed by devision of the sporont
Syzygy	side by side or end to end association of gamonts after the formation of gametocysts or gametes
Trophozoite	a vegetative (feeding) stage in the life cycle of a parasitic protist and may undergo asexual multiplication (fission)
Weak meat	a term used for weathervane scallops, *Patinopecten caurinus*, off the Alaskan coast with abnormally discoloured and reduced adductor muscles
White meat	a term used for sea scallops, *Placopecten magellanicus*, off the east coast of USA with normal adductor muscles

*The definitions above are from Woo (2006) and Merriam Webster dictionary as well as non-scientific terms used in the scallop industry

List of Abbreviations

µm	micrometer, 1/1000 of a millimeter
AM	adductor mucle
AM^C	catch adductor muscle
AM^P	phasic adductor muscle
cm	centimeter
CS	crystalline style
DG	digestive gland
Di	digestive cecum
DNA	deoxyribonucleic acid
E	eye
F	foot
g	gram
G	gonad
GI	gonad index – condition of a scallop´s gonad
Gi	gills
GW	total weight of a scallop´s gonad
H	heart
I	intestines
Ki	kidney
m	meter
Ma	mantle
MI	muscle index – condition of a scallop´s adductor muscle
min	minute
mm	millimeter
mm^3	volume millimeter
O	operculum
Os	osphradium
OsO_4	osmium tetroxide
Ov	ovary
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pe	penis
SD	standard deviation
sec	second
SSC	saline-sodium citrate

SSU rDNA	small subunit ribosomal deoxynucleic acid
St	stomach
TBS	tris-buffered saline
Te	testis
tn	ton
Tt	tentacles

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I also want to express by graditute to all my other colleagues at Keldur, especially those at the Fish disease Laboratory. In particular, Slavko H. Bambir, for his guidence regarding histopathology, Sigríður Guðmundsdóttir for her positive attitude and encouragements through the years, and Fjóla Rut Svavarsdóttir and Ásthildur Erlingsdóttir, my former students, who inspired me to write this thesis as well as assisting with various aspects of this project. Furthermore, thanks to Margrét Jónsdóttir, Steinunn Árnadóttir and Eygló Gísladóttir, for processing all the histological slides used in the project, Guðmundur Pétursson (2017†) for translating numerous French litterature and Matthías Eydal for his cooperation during the initial steps of the project.
My sincere thanks to Hrafnkell Eiríksson, Jónas Páll Jónasson and coworkers at the Marine Research Institute in Iceland (presently Marine and Freshwater Research Institute) for excellent cooperation and discussions regarding the collapse of the Iceland scallop stock, as well as sampling the majority of the research material used in this study. Furthermore, I would like to thank Gordon Goldsworthy at Loch Fyne Seafarms Ltd. and Grant Campell at Scot-Hatch Ltd., for providing queen- and king scallops from Scottich waters for examination. Similarly, the staff at the O.C Joensen at Faroe Islands Scallop Fishery for sampling of queen scallop off the Faroe Islands.
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The project received financial support from the Ministry of Agriculture and Fisheries in Iceland, University of Malaya, Ross University School of Veterinary Medicine and SMAST at the University of Massachusetts. This support is highly appreciated.

Last, but not least, genuine thanks to my spouse Ragnhildur and my son Hákon, and also my parents and siblings. All these amazing people supported and encouraged me beyond my imagination. I love you all.

1 Introduction

1.1 Scallops (Mollusca: Bivalvia: Pectinidae)

The pectinids (Family Pectinidae Wilkes, 1810) represent one of the largest group of marine bivalves inhabiting diverse niches worldwide, from the intertidal zone down to 3,000 m depth (Brand, 2016). Scallops represent an ancient monophyletic group of bivalve molluscs with a fossil record dating back the early Triassic (Waller, 1991; Malkowsky & Götze, 2014; Serb, 2016). They display a wide range of shell shapes, size and color and constitute a significant part of the oceans´ benthic community (Brand, 2016). Many species are of high commercial value, most of which inhabiting inshore waters of the continental shelves on gravel or coarse to fine sand (Waller, 1991; Brand, 2016).

1.1.1 Early life

The life cycle of scallops can be divided into a larval pelagic- and an adult benthic stage (Cragg, 2016). They have external fertilization, i.e. releasing their gametes into the sea where fertilization occurs. A planktonic, free swimming, ciliated trochophore larva hatches from the eggs, which subsequently develops into a D-shaped veliger larva with folded two valves, the velum, that it uses for swimming, similar to the shells of the adult stage. With further development, the veliger larva grows a foot, which it uses to crawl and seek out a place to settle. At this stage, it is called a pediveliger larva, which is the end stage of the pelagic phase in the scallop´s life cycle (Cragg, 2016). The pediveliger larva settles on a substrate, by use of byssal threads. Subsequently, it undergoes metamorphosis and becomes a post-larva, termed spat, which includes significant physiological and morphological changes resulting in the formation of a juvenile scallop, which moves onto the seafloor and initiates its benthic life (Figure 1.1.). The length of the larval stage varies significantly between scallop species, lasting approximately two to six weeks (Cragg, 2016).

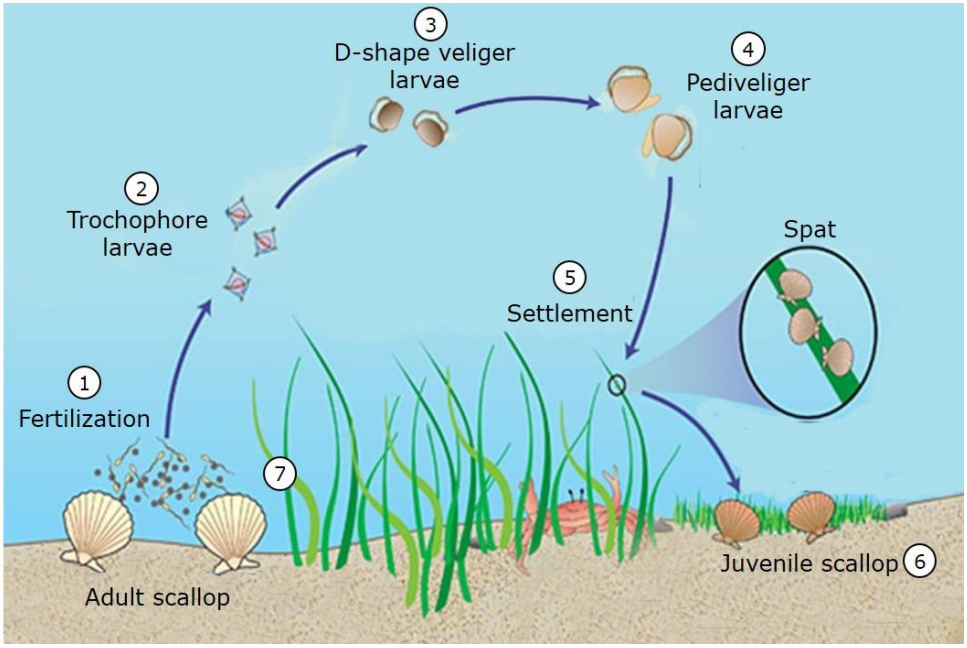

Figure 1.1. Scallop´s life cycle. (1) Gametes are released via the kidneys into the environment (the sea) were an external fertilization occurs. (2) The first larval stage; the ciliated trochophore larva. (3) The D-shaped veliger larva with folded two valves, the velum, which is used for swimming. (4) Pediveliger larvae with a foot, which it uses for seeking out a place to settle. (5) Spat, a settled larvae. (6) Benthic juvenile scallops and (7) adult ones. Redrawn by Árni Kristmundsson from picture at: http://www.whoi.edu/page.do?pid=80696&i=6627.

1.1.2 The adult scallop

As other bivalve species, scallops have two valves, a right and a left one, which are attached together along the hinge line by a triangular shaped elastic ligament. In many scallop species the valves are more or less symmetrical, both bilaterally (equivalved) as well as front/back (equilateral). The hinge side corresponds to an animal´s dorsal or back/top region while the opposite end corresponds to the ventral side. With the hinge orientated towards the top, one side corresponds to an animal's anterior side while the other is the posterior or backside (Drew, 1906; Serb, 2016). The typical scallop shell consists of two similarly circular or prismatic shaped valves with two "ears", an anterior and a posterior one (Duncan et al., 2016). The anterior ear is commonly somewhat larger. Some species have ridged valves, radiating from anterior to posterior (Figure 1.2.).

1.1.3 The anatomy and function of scallops

Mantle

The mantle is a thin and semi-translucent organ that surrounds the animal on both sides of the shell. Its outer side contains a series of tentacles and eyes (Figure 1.3. A), which are the main sense organs of the animal and in the closest contact with its benthic environment. It appears that scallops can sense touch, smell and light (Bourne, 1964). In addition to sensations, the mantle serves an important role in the animal's oxygen uptake, in- and outflow of water, swimming and, last but not least, the formation of the outer shell that is mainly made of calcium carbonate (Gosling, 2003; Beninger & Le Pennec, 2016).

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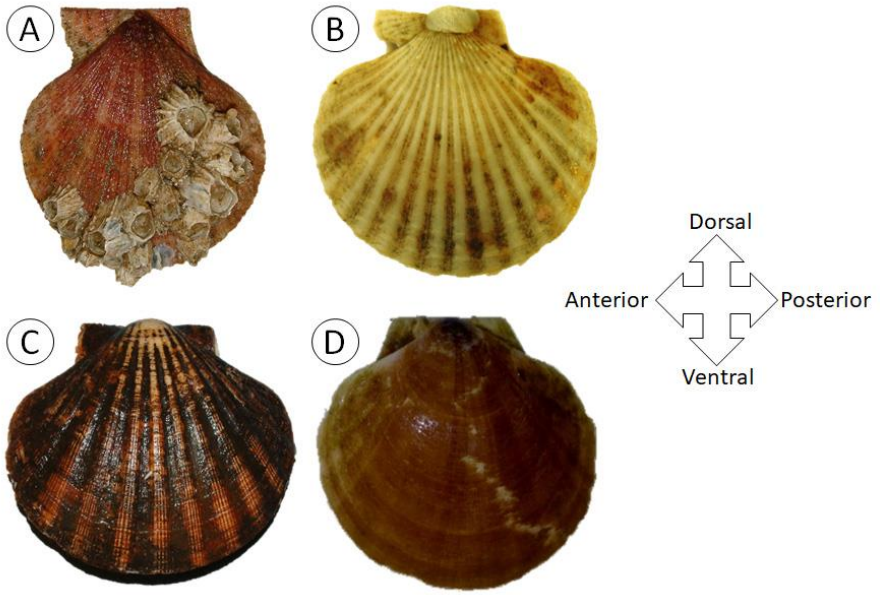

Figure 1.2. The shell shapes of scallops. The four scallop species examined in this thesis and represent different adaptive shell shapes (in parenthesis) discussed by Waller (1991). These shapes refer to forms that have evolved over and over again in the history of the Family Pectinidae: (A) Iceland scallop, Chlamys islandica *(chlamydoid) (B) queen scallop,* Aequipecten opercularis *(aequipectinoid), (C) king scallop,* Pecten maximus *(pectinoid) and (D) sea scallop,* Placopecten magellanicus *(amusioid). Photographs: Árni Kristmundsson.*

Adductor muscles

Under the mantle are the adductor muscles; the most appreciated meat of the scallops, making them a highly valuable seafood (Chantler, 2016). Typically, they are two that, in most scallops, lie closely apposed to one another. The larger one, the striated phasic adductor muscle, facilitates fast, repetitive opening and closing of the valves and enables the scallop to swim by jet propulsion, a distinctive feature of scallops. The smaller smooth tonic muscle, often named catch muscle, has a slower contraction (Figure 1.3. A). However, it can produce substantial force and keep the animal's shell closed for longer periods using minimal amount of energy (Eiríksson, 1986; Beninger & Le Pennec, 2016). The adductor muscles also play a substantial role in storing energy, especially in the form of glycogen, which, at least some scallop species use during maturation of gonads (e.g. Brokordt & Guderley, 2004).

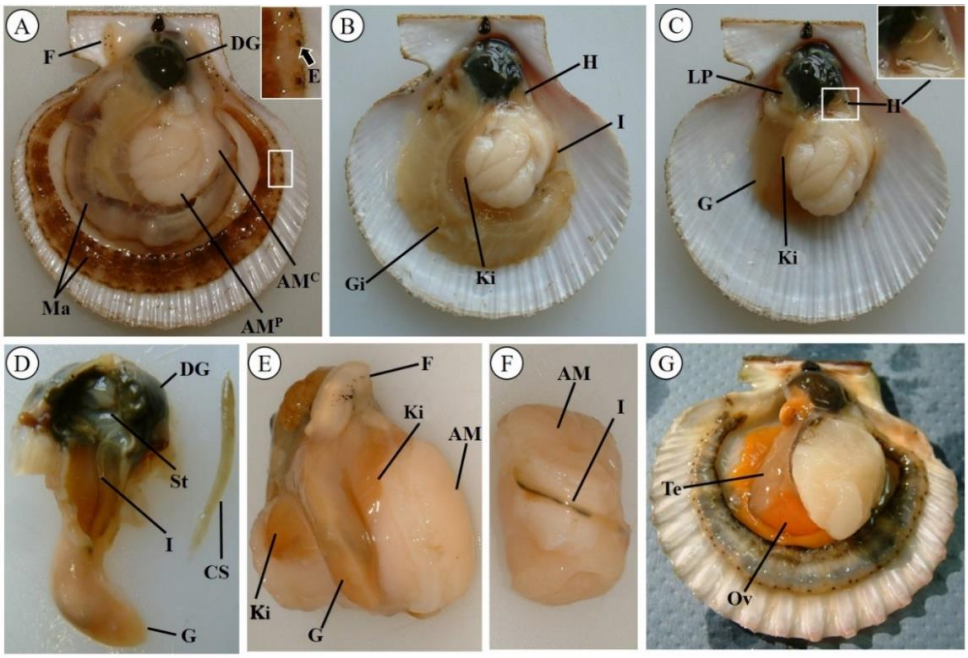

Figure 1.3. Anatomy of scallops. (A-F) Male Iceland scallop. F = Foot; DG = Digestive gland; E = Eye; Ma = mantle; St = stomach; I = intestines; G = gonad; Gi = gills; CS = crystalline style; H = heart; Ki = kidney; AM = adductor muscle; AM ^{P} = phasic adductor muscle; AM ^{C} = catch adductor muscle; (G) Hermaphrodite king scallop, Pecten maximus, *with both testis (Te) and ovary (Ov). Photographs: Árni Kristmundsson.*

Foot and byssal complex

The foot of scallops is small compared to other bivalves, e.g. mussels (Family Mytilidae). It is roughly cylindrical and arises out of the antero-dorsal surface of the gonad (Figure 1.3. A). Its development varies between scallop species, depending on its life mode and the importance of byssal attachment. In most bivalve species, it becomes functional in crawling and attachment of the byssally attached postlarval stages (Gosling, 2003; Beninger & Le Pennec, 2016). An opening of the byssal glands is at the heel of the foot, through which the animal secretes a thread-like, elastic substance called "byssus" by which it can attach itself to a substrate (Gosling, 2003). Some species retain this capability while others do not, in which case the foot is degenerated with no mobility function (Beninger & Le Pennec, 2016).

Respiration, feeding and digestion

The relatively large and paired gills of the scallops have a multifunctional role. In addition to oxygen consumption, they play a major role in the animal's feeding. Scallops are suspensivorous feeders; when water moves over the gills, large part of the food particles become trapped in the ventral groove of the mucous-rich and ciliated gill arches (Beninger et al., 1991). By the aid of the gill´s cilia, the food moves towards the labial palps and thus to the mouth opening (Figure 1.3. B & C). Food particles reach the mouth from the oral groove at the base of the labial palps as a mucoid string, which subsequently is transported to the stomach via the oesophagus (Gosling, 2003; Beninger & Le Pennec, 2016).

Scallops are somewhat selective feeders. A size dependant particle processing is performed by the pallial organs that are comprised of the mantle, gills, labial palps and lips. Unwanted particles, such as grit or too large ones are expelled as mucus-bound mass, the pseudofeces.

The digestive system consists of an oesophagus, stomach, intestines, digestive gland and a crystalline style, which is connected to the stomach and housed in a stylar sac (Gosling, 2003; Beninger & LePennec, 2016) (Figure 1.3. A-F). Due to a syncronized beat of the stomach´s and intestinal cilia the crystalline style rotates against the gastric shield, a chitinous plate inside the stomach, which helps mixing the food, releasing digestive enzymes in the process. Ingested particles are mixed with the liberated digestive enzymes from the crystalline style. This extracellular digestion processes the stomach contents, being influenced by ciliary tracts that cover the stomach. Digestable particles enter the digestive gland ducts while the larger undigestible particles are segregated out and channelled into the intestine along a deep rejection groove on the floor of the stomach. The largest part of the digestion occurs

intracellular in numerous blind-end ducts of the digestive gland (Figure 1.4.) while unwanted waste is passed through the intestines and exits via the anus (Gosling, 2003; Beninger & Le Pennec, 2016).

Circulatory system

The scallop´s heart (Figure 1.3. B & C) has three chambers, which, along with a portion of the intestines, lie in a space called the pericardial cavity, which is enclosed by the thin transparent pericardium. The auricles are two thin-walled input chambers, which receive blood from the gills while the muscular output chamber, the ventricle, contracts to drive the haemolymph into two aortae, an anterior- and posterior one. These aortae divide into many arteries, which break up to a series of thin-walled sinuses, which are tissue spaces lacking an endothelium, or vessel wall filled with circulating blood. A system like this is termed an open circulatory system with the haemolymph in the sinuses bathing the tissues directly. From the sinuses, the haemolymph is carried to the kidneys for purification (Gosling, 2003; Beninger & Le Pennec, 2016).

The haemolymph is the scallop's blood. It participates in a variety of physiological functions, including gas exchange, osmoregulation, nutrient distribution, elimination of wastes and internal defence. It also serves as a hydrostatic skeleton, such as during movements of the labial apparatus, the tentacles and the foot. Most bivalves lack circulating respiratory pigments (Booth & Mangum, 1978; Barnes, 1987) and no reports are of such pigments in any scallop species. Several types of circulating haemocytes are abundant in the haemolymph. They have multifunctional role including: (1) Wound and shell repair, by successive infiltration, clumping and plugging of the wound followed by phagocytosis of necrotic elements. (2) Digestion and transport of nutrients, by absorbing them from the alimentary tract and transporting them to other tissues. (3) Excretion, by absorbing wastes, which they pass directly across the epithelium of the kidney tubules into the kidney lumen and subsequently to the exterior. (4) Internal defences, based on an innate, non-lymphoid system involving both cellular and humoral components. This includes phagocytosis or encapsulation, with subsequent pathogen destruction via enzyme activity and oxygen metabolite release, and humoral components, e.g. opsonins such as lectins or agglutinins, and antimicrobial peptides (Wootton et al., 2003; Beninger & Le Pennec, 2016).

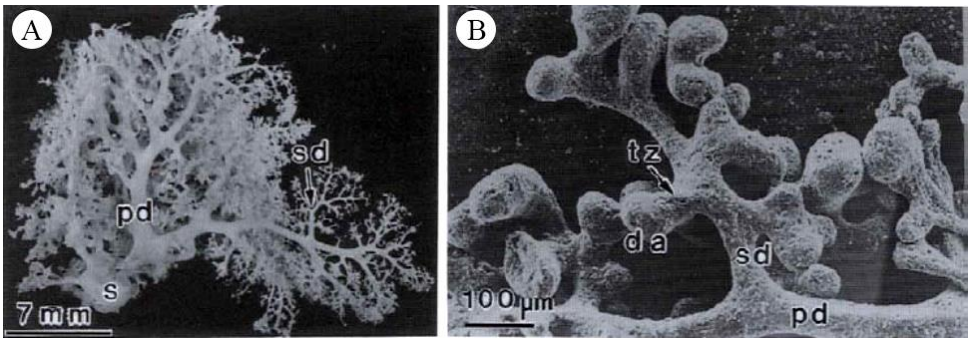

Figure 1.4. The structure of the digestive gland in king scallop, Pecten maximus. (A) Internal mold of digestive gland, showing three-dimentional structure and anatomical relationships of point of attachment to stomach (s), principal duct (pd), and secondary duct (sd). (B) Detail of internal mould, showing ramification of secondary duct (sd) from principal duct (pd), the short tubular zone (tz), terminating in the digestive acinus (da). Pictures from Beninger & Le Pennec (2016).

Nervous system

The nervous system of scallops is relatively simple, consisting of the cerebral- and pedal ganglia and the parietovisceral ganglion. In scallops, the visceral ganglia, which are commenly paired in bivalves, are completely fused, thus forming an elementary brain with distinct optic lobes that innervate the eyes on the edge of the mantle. Numerous other parts of the animals are controlled by the visceral ganglia, i.e. in the gills, heart, pericardium, kidney, digestive tract, gonads, adductor muscle, part or the entire mantle, siphons and pallial sense organs. The cerebral ganglia innervate the palps, anterior adductor muscle, and part of the mantle, as well as the statocysts and osphradia while the pedal ganglia control the foot (Gosling, 2003; Beninger & Le Pennec, 2016).

Urogenital system

The excretory organs in scallops consist of paired kidneys (Figure 1.3. E) and the auricular- and pericardial glands (Gosling, 2003; Beninger & Le Pennec, 2016). The kidneys are brownish, bilateral, flattened organs located on the anterior side of the adductor muscles, dorso-ventrally to the gonad. Each kidney is commonly divided into two sections, a proximal/pericardial one that opens into the pericardium and a distal section, which terminates in the renal or kidney openings, the nephrostomes, into the mantle cavity. Waste accumulates in certain cells of the pericardial glands, which is periodically

discharged into the pericardial cavity, and from there it is eliminated via the kidneys. Other cells of the pericardial glands are involved in filtering the haemolymph, the first stage of urine formation. The filtrate then flows to the glandular part of the kidneys where the processes of secretion and reabsorption of ions occurs. The end-result is urine that has a high concentration of ammonia, and smaller amount of amino acids and creatine (Gosling, 2003; Beninger & Le Pennec, 2016).
The gonads are located on the anterior side of the adductor muscles. The ovary is commonly pink or orange-colored while the testis is whitish. Some scallop species have separate sexes (e.g. Iceland scallop *Chlamys islandica*, sea scallop *Placopecten magellanicus*) while others are hermaphrodites, (e.g. king scallop *Pecten maximus* and queen scallop *Aequipecten opercularis*) (Figure 1.3. C-E & G). During spawning, the scallops release their gametes into the environment through the kidneys´ distal section into the mantle cavity. Given the dual function of the distal parts of the kidneys, i.e. evacuation of kidney fluids and gametes, they are sometimes referred to as reno-genital apartures (Gosling, 2003; Beninger & LePennec, 2016).

1.1.4 Predators

In general, natural mortality rates and factors contributing to natural mortality of scallops are poorly known. However, scallops have various predators, the most significant ones being different species of decapod crustaceans, gastropods (e.g. whelks), octopuses as well as many benthic finfish species (Medcof & Bourne, 1964; Piboubes, 1973; Naidu & Meron, 1986; Minchin, 1991; Halary et al., 1994; Lake & McFarlane, 1994; Grall et al., 1996; Veale et al., 2000; Strohmeier et al., 2006; Boyle & Thompson, 2012). Eider ducks, *Somateria* spp., have also been shown to feed on scallops (Hartnoll, 1967; Brun, 1971).
Predation of scallops is highly related to their body size. Apparently, only few predators can manage large healthy scallops. Therefore, spats and juvenile scallops are more prone to predation than the adult ones (Elner & Jamieson, 1979; Barbeau et al., 1994).

1.1.5 The Iceland scallop, *Chlamys islandica* (Műller, 1776)

The Iceland scallop represents the species with the northernmost distribution. It is however considered a sub-arctic species with its main distribution in the sub-arctic transitional zone. In the NE-Atlantic Ocean, it occurs around Iceland (except for the south coast) and Jan Mayen, along the western coast of Norway, from Bergen in the south and north into the Barents Sea, around Svalbard and Bear Island east to White Sea and Kara Sea. In the NW-

Atlantic, it is found off the west coast of Greenland from Thule to Cape Farewell. On the east coast of Greenland, its distribution is restricted to the King Frederick VI coast in the southeast, with the exception of a population found in the inner part of Franz Josef Fjord (Brand, 2016). Off the east coast of North America, it reaches its northern distribution limit in Cumberland Peninsula, Hudson Bay and Foxe Channel. From there it extends as south as Cape Cod on the American east coast (Simpson, 1910; Ockelmann, 1958; Wiborg, 1963; Greve & Samuelsen, 1970; Lubinsky, 1980; Eiríksson, 1986; Pedersen, 1994). Commonly, the distribution of this species have been described as circumpolar, however, according to Waller (1991) it is absent from the North-Pacific, where closely related species exist previously considered to be subspecies of *C. islandica*, i.e. *C. behringiana*, *C. albida*, *C. rubida* and *C. hastate*. Between Lofoten Islands and Stavanger, the distribution of the Iceland scallop overlaps with two boreal species, i.e. the queen scallop, *Aequipecten opercularis*, and the king scallop, *Pecten maximus*. Furthermore, in the NW-Atlantic, it overlaps with the northernmost distribution area of the sea scallop, *Placopecten magellanicus* (Brand, 2016) (Figure 1.5.).

The Iceland scallop is a slow growing, long-lived species with a recorded maximum age of 23 years (Vahl, 1981). It lives on a bottom with sand, gravel or shell fraction, at depths ranging from 10-100 m. It has separate sexes, with a sex ratio close to 1:1, reaches maturity at approximately 5 years of age (then 4-5 cm) (Eiríksson, 1986; Rubach & Sundet, 1987) and spawns from late June to late July, which seems to be dependent on both sea temperature and food availability (Thórarinsdóttir, 1984; Eiríksson, 1986; Garcia, 2006). The spat settles about six weeks from spawning and prior to metamorphosis; the larvae attach themselves to hydroids and filamentous algae, by the aid of byssal threads (Harvey et al., 1993; Harvey & Bourget, 1995).

Fisheries of Iceland scallop started in 1969 in Icelandic waters (Eiríksson, 1997). For decades, or until the dramatic and sudden collapse in the stock in the beginning of the 2000s (see chapter 1.6. below), these fisheries were of high economic importance, especially for smaller towns in western Iceland. The majority of landed scallops came from Breidafjördur, a large bay off the west coast. During the years 1979-2000, the catch ranged from 8,000-17,000 tn, of which 6,000-12,000 tn were caught in Breidafjördur (Marine Research Institute, 2003). Considerable fisheries have also been conducted off the east coast of Canada since 1965 (Wroblewski et al., 2009). In Norwegian waters, i.e. Svalbard, Bear Island and Jan Mayen, the Iceland scallop was quite intensively fished in the 1980s. However, due to severe declines in the stock, probably somewhat due to overfishing, these fisheries ceased and have still not recovered (Garcia, 2006). Since 1997, no Iceland scallops have been

landed in Norway (Duncan et al., 2016). Commercial fishing for Iceland scallop in West Greenland began in 1983; the main scallops ground being off Nuuk. The catch was never extensive, the maximum reaching around 2,300 tons in 1985. An extensive reduction in the stock was observed during surveys carried in the year 1986 (Pedersen, 1994) and 1995. Since 1999, no scallop fisheries have been conducted in Greenland (Duncan et al., 2016) (Figure 1.6. A).

1.1.6 The queen scallop, *Aequipecten opercularis* (Linnaeus, 1758)

The queen scallop is a boreal species reaching its northern distribution limit in the Lofoten Islands, Norway. From there it extends south along the Norwegian coast and to Skagerrak, Faroe Islands, North Sea, Irish Sea and along the western continental European coast into the Mediterranean (Garcia, 2006) (Figure 1.5.). It is fast growing but short living, commonly reaches 5-6 years of age and a maximum of 8-10 years (Brand et al., 1991; Manx Heritage Foundation, 1992; Lewis & Thorpe, 1994; Brand & Prudden, 1997; Vause et al., 2007). It is found on sandy, gravel and muddy bottoms (Mason, 1983) from shallow subtidal areas to depths of around 180 m, but most commonly at depths between 20-45 m (Tebble, 1966). It is hermaphroditic and reaches maturity around 1 to 2 years of age when its shell height is approximately 40 mm (Brand et al., 1991; Garcia, 2006; Vause et al., 2007). The spawning time varies between populations from different areas. In the North Irish Sea, there are two separate spawning peaks, in summer and autumn, while the spawning seasons in Scandinavia and Mediterranean are June – February and February to June, respectively (Ursin, 1956; Taylor & Venn, 1978; Brand et al., 1980; Paul, 1981; Peña & Canales, 1993; Peña et al., 1996). The length of the pelagic larval stage depends heavily on temperature, food availability and possibly genetic factors (Paulet et al., 1988).

The major fisheries for queen scallops are conducted by the United Kingdom, France, the Faroe Islands and the Isle of Man, with an annual catch of each country ranging from 1,000-15,000 tons (Andrews & Brand, 2012).

1.1.7 The king scallop, *Pecten maximus* (Linnaeus, 1758)

The king scallop is distributed from Myken, Norway, southwards along the Atlantic European coast to Cap Blanc in Mauritania and the Canary Islands and somewhat into the Mediterranean Sea (Figure 1.5.). Furthermore, it is found in the North Sea and the Irish Sea (Garcia, 2006). It lives at depths ranging from very shallow waters down to 180 m, on a sandy, gravel or

muddy bottom. This large and relatively fast growing bivalve can reach more than 200 mm shell height and a life span of 15-20 years (Tang, 1941). However, its growth varies considerably between different regions, depending on factors like temperature and food availability (Tully et al., 2006, Foucher et al., 2010). It is hermaphroditic and reaches maturity at age between 2-4 years and a shell height of 80-100 mm (Beukers-Stewart & Beukers-Stewart, 2009; Foucher et al., 2010).
Similar to the queen scallop, its spawning time varies considerably between populations from different areas. For example, spawning in Scottish- and Norwegian waters occurs in the summertime, while in the Irish Sea and off the west coast of Ireland there are two spawning peaks, in the spring and in July/August (Ursin, 1956; Mason, 1958; Wilson, 1987).
The king scallop is fished commercially throughout much of its range, i.e. from Norway south to Spain. However, the majority of the catch comes from the United Kingdom and France, e.g. 45,000 tons of the 50,000 tons total catch in 2007 (Beukers-Stewart & Beukers-Stewart, 2009; Food and Agriculture Organization of the United Nations, 2018a).

1.1.8 The sea scallop, *Placopecten magellanicus* (Gmelin, 1791)

The distribution of the sea scallop is in the NW-Atlantic Ocean, along the east coast of North America, from the north shore of the Gulf of St. Lawrence south to Cape Hatteras, North Carolina (Black et al., 1993; Hart & Chute, 2004) (Figure 1.5.). They are generally found on firm sand, gravel, shells and rock at depths ranging from 18-119 m (MacKenzie et al., 1978; Langton & Robinson 1990; Thouzeau et al., 1991a; Black et al., 1993; Stewart & Arnold, 1994). The juveniles are often found attached to branching bryozoans, hydroids or algae (Stokesbury & Himmelman, 1995). Common invertebrates associated with the sea scallop include sponges, hydroids, anemones, bryozoans, polychaetes, mussels, moon snails, whelks, amphipods, crabs, lobsters, sea stars, sea cucumbers, and tunicates (Kenchington, 2000).
Like the Iceland scallop, the sea scallop has separate sexes. Although maturity has been observed in females as young as two years old, a noteworthy egg production is commonly not reached until at the age of four, then 85-90 mm in height (Naidu, 1970; MacKenzie, 1979). The spawning time of sea scallops can vary between areas, but a major annual spawning period occurs in August to October (MacKenzie et al., 1978; Barber et al., 1988; DuPaul et al., 1989; Schmitzer et al., 1991; Parsons et al., 1992; Davidson et al., 1993; Almeida et al., 1994; Dibacco et al., 1995). The pelagic larval stage lasts for 35 days or longer after hatching (Culliney, 1974; Posgay, 1982).
The sea scallop is of an extreme commercial value, representing one of the most valuable seafood in the northern Atlantic parts of the United States and

Canada. Furthermore, it supports the most valuable scallop fisheries in the world (Hart & Chute, 2004). The total catch during the years 2000 – 2014 ranged from 200 – 300 thousand tn (Food and Agriculture Organization of the United Nations, 2018b).

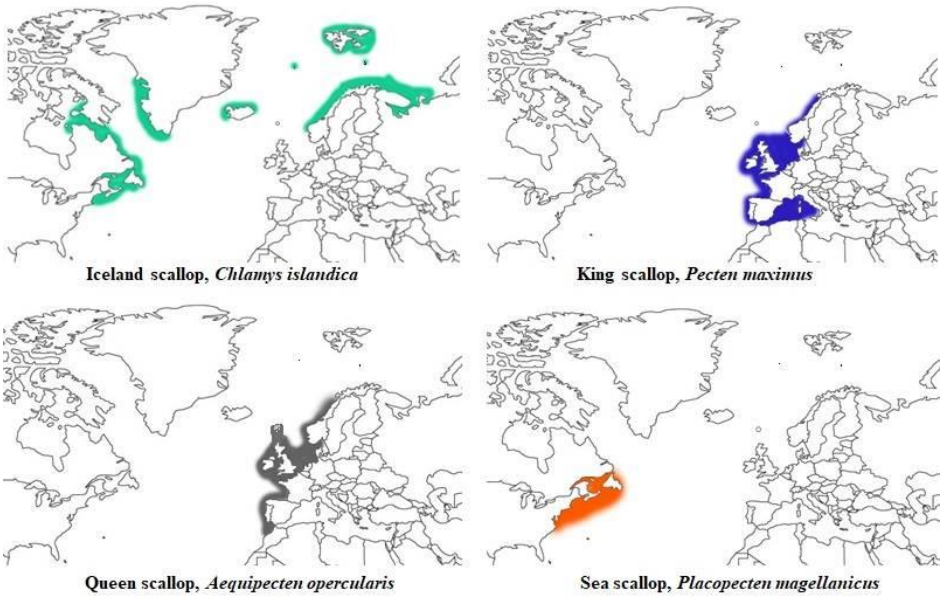

Figure 1.5. The geographic distribution of the four scallop species studied. Figures drawn by Árni Kristmundsson from published data (Simpson, 1910; Ockelmann, 1958; Wiborg, 1963; Greve & Samuelsen, 1970; Lubinsky, 1980; Eiríksson, 1986; Black et al., 1993; Pedersen, 1994; Hart & Chute, 2004; Garcia, 2006; Brand, 2016).

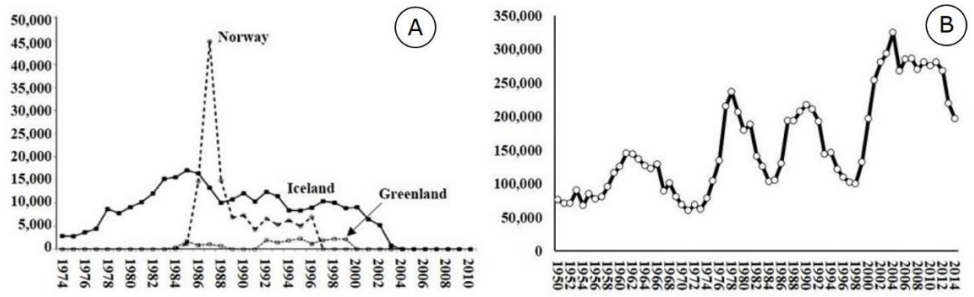

Figure 1. 6. Catch figures for Iceland- and sea scallops. (A) European catch (in tons) of Iceland scallop, C. islandica, from 1974-2010 (Duncan et al., 2016). (B) The global catch (in tons) of sea scallop, P. magellanicus from 1950-2014. Data from Food and Agriculture Organization of the United Nations (2018b).

1.2 Whelks (Buccinidae)

Whelk is the common name used for a group of marine gastropod species within the Family Buccinidae. The whelk family is comprised of hundreds of species belonging to 140 different genera (World register of marine species - WORM). According to WORM, the most specious genus *Buccinum* alone includes 134 valid species, which are distributed all over the world. According to Óskarsson (1982), 22 whelk species belonging to five different genera, are present in Icelandic waters. The largest species, the common or waved whelk, *Buccinum undatum*, is also the most common one.

1.2.1 The common whelk, *Buccinum undatum* (Linneus, 1758)

The common or waved whelk is a marine neogastropod (Fretter & Graham, 1999) most commonly found somewhat below the tidal zone, on sandy, muddy, gravel or rocky bottom, down to around 50 m depth. To some extent, it is found both in the subtidal zone and at greater depths, down to 1,200 m. It is a relatively large gastropod, which can reach up to 150 mm shell length (Óskarsson, 1962; Golikov, 1968). It is widely distributed in the North-Atlantic Ocean; along the European coast from Spain in the south to Svalbard in the north, in the Greenland- and Norwegian Seas, SW Greenland and along the North American coast from New Jersey north to Labrador (Golikov, 1968; Gendron, 1991) (Figure 1.7.).

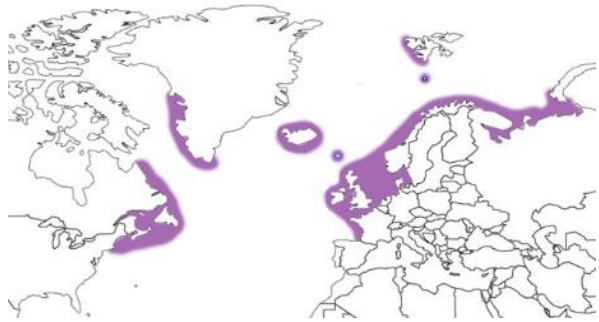

Figure 1.7. The geographic distribution of the common whelk, Buccinum undatum.

The common whelk is both a predator and a scavenger, feeding on various animals, such as polychaetes, bivalves (e.g. scallops), echinoderms, small crustaceans and fish eggs (Nielsen, 1975; Jalbert et al., 1989; Himmelman & Hamel, 1993). The spawning time varies between geographic areas, i.e. being from autumn until mid-winter in European waters and from May – August off the Canadian east coast (Martel et al., 1986a, 1986b; Kideys et al., 1993; Valentinsson, 2002; Henderson & Simpson, 2006). It has an internal fertilization and the female whelk lays egg-masses and attach them to a

substrate, e.g. sea grass, seaweed and rocks (Martel et al., 1986a). Unlike many other molluscs, a planktonic stage is absent. Hence, the typical mollusc´s larval stages, trochophora and veliger, develop inside the eggs and at hatching, the miniature offsprings are morphologically like the adults (Martel et al., 1986b). The proboscis is the feeding apparatus of the common whelk; an eversible organ with the radula at its end, a flexible chitinous apparatus, with a ribbon bearing transverse rows of teeth (Hughes, 1986). A rough anatomy of the whelk is shown in Figure 1.8.

The common whelk and scallops utilize similar habitats and presence of whelks in more than 75% of tows during Iceland scallop fisheries, reflects the cohabitation of these two mollusc species (Chen, 2012).

The common whelk has for long been of commercial value, both as a bait, as in Iceland and the Faroe Islands, and for human comsumption, e.g. in the UK, Belgium and Netherlands (Gunnarsson et al., 1998). There is a long tradition for whelk fisheries in the UK and eastern Canada (Hancock, 1963; Fisheries and Oceans Canada (DFO), 2009; Food and Agriculture Organization of the United Nation, 2018c). In the UK, these fisheries date back to the early 1900s. In 1911, around 4,500 tn were landed in England and Wales (Dakin, 1912). Through the 1900s, a significant increase were in common whelk fisheries. Recently, a significant further increase have been in common whelk fisheries making it among the most important shellfish fisheries in the UK, with an annual landings of 12,900 tn in 2009 to 20,000 – 23,000 tn in 2013-2016 (Haig et al., 2015; Richardson, 2016).

In 1996, experimental fisheries of common whelk, for human consumption, started in Breidafjördur, Iceland. Since then, only small scale and quite sporadic fisheries have been conducted, with no fishing in some years up to 1,300 tn in the year 1997 (Gunnarsson et al., 1998, Marine and Freshwater Research Institute, 2017).

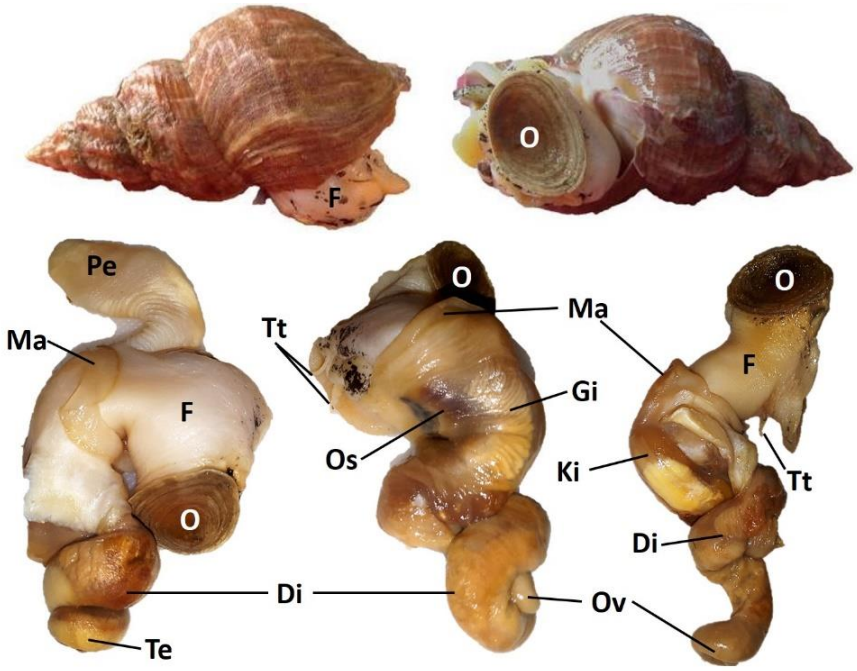

Figure 1.8. The shell and main organs of the common whelk, Buccinum undatum*. F = foot; O = operculum; Pe = penis; Ma = mantle; Te = testis; Di = digestive cecum; Tt = tentacle; Os = osphradium; Gi = gills; Ki = kidney; Ov = ovary. Photographs: Árni Kristmundsson.*

1.3 Diseases and parasites of scallops

Scallop are hosts for all major groups of pathogens as summarized by Getchell et al. (2016). Below, all these groups of pathogens will be briefly discussed. However, as the alveolates (Superphylum Alveolata), especially the Phylum Apicomplexa, is the group most relevant to this thesis, it will be discussed in more details; put in broader spectrum by including other groups of bivalves as well as other mollusc species, necessary to see the larger picture.

1.3.1 Viral infections

Infectious pancreatic necrosis virus (IPNV), a serious pathogen of salmonids, was the first virus observed in scallops, i.e. in king scallops from Norway (Mortensen et al., 1992, 1998; Mortensen, 1993a). No pathology was observed and viral replication in the scallop's tissue was apparently absent. However, scallops might play a significant role as reservoirs of this highly

pathogenic fish virus in the natural environment (Mortensen et al., 1992). "Virus-like particles" were observed associated with significant mortalities in the New Zealand scallop, *Pecten novaezelandiae*. Microscopic examination revealed considerable pathology, especially in the digestive gland (Hine & Wesney, 1997).
In the late 1990s, Ostreid Herpesvirus variant 1 (OsHV-1var), a serious pathogen causing massive problems in aquaculture of bivalves (Hwang et al., 2013), was observed in king scallops exhibiting high mortalities (Arzul et al., 2001). Cellular lesions and viral particles were identified in connective tissues of moribund larvae (Arzul et al., 2001).
In the mid-1990s, Acute Viral Necrosis Virus (AVNV) was identified in cultured *Chlamys farreri* off the northern coast of China. The associated mortilities, up to 90% in juvenile scallops, were shown to be caused be the virus. Histopathological examinations showed severe pathology, starting with necrotic lesions in the gills, mantle, kidneys and the digestive gland (Guo et al., 1999; Song et al., 2001; Wang et al., 2007). Elevated seawater temperature was the suggested influential factor (Tang et al., 2010).

1.3.2 Bacterial infections

Many reports exist of bacterial infections in scallops, most species identified being members of the Gram-negative family Vibrionaceae and the rickettsiales (intracellular *Rickettsia* and *Chlamydia*) (Getchell et al., 2016).
Vibriosis, infections with *Vibrio* species, has been reported from various scallop species, in many cases associated with larval mortality. That includes unidentified *Vibrio* spp. infecting zigzag scallop, *Euvola zizac,* (Freites et al., 1993) and *V. pectinicida* and *V. spendidus* from king scallop (Nicolas et al., 1996; Lambert & Nicolas, 1998; Lambert et al., 1999a,b; Torkildsen et al., 2005; Sandlund et al., 2006).
Rickettsia-like organsims (termed RLOs) seem common in various species of scallops, both captive and wild (Getchell et al., 2016). Some of those have been linked with mass mortality events, e.g. in wild population of sea scallops in the NW-Atlantic (Gulka et al., 1983; Gulka & Chang, 1984; Leibovitz et al., 1984). Furthermore, RLOs causing extreme pathology were observed in other pectinids in European waters, such as queen scallops (LeGall et al., 1992), Peruvian scallop, *Argopecten purpuratus,* (Lohrmann et al., 2000a, 2002), Yesso scallop, *Mizuhopecten yessoensis*, (Elston, 1986; Friedman, 1994) and king scallop (Comps, 1983; LeGall et al., 1988, 1991, 1992; LeGall & Mialhe, 1992; Kellner-Cousin et al., 1993).
A *Mycoplasma* species has been a suspected cause of a disease affecting Yesso scallops, on the Pacific coast of Canada (Bower & Meyer, 1991, 1994; Bower et al., 1992; Bower, 1998). Infection were charecterized by pinkish,

orange pustules, up to 10 mm in diameter in soft tissues such as the adductor muscle. Similar phenomenon were observed in sea scallops in the NW-Atlantic, termed "bacterial abscess disease", from several beds along the Maine and Atlantic Canadian coasts (Sherburne & Bean, 1986).
Other bacteria, such as *Pseudomonas* and *Flavobacterium* species have been associated with larval mortality of scallops (Loderos et al., 1989, 1992).
According to Getchell et al. (2016), bacterial infections are not usually harmful to adult scallops, except at extreme levels.

1.3.3 Fungal and microsporidian infections

Fungi are not among the most common aetiological agents causing diseases in scallop, with only few reported cases despite their ubiquitous distribution. A shell disease, causing a degradation of the outer shell as well as infecting soft parts of larval and adult pelecypods, was suggested to be caused by a fungus (Lauckner, 1983). Furthermore, the fungus *Sirolpidium zoophthorum* has been linked with extensive epizootic mortalities in cultured oyster and bay scallop, *Argopecten irradians*, larvae (Getchell et al., 2016). One report exist on fungal infections affecting the adductor muscle of sea scallop from Main, USA (Getchell et al., 2016).
Fungi of the genera *Aspergillus*, *Aphanocladium, Cladosporium, Penicillium*, *Phialophorophoma* and *Eurotium* were reported from the Yesso scallop, but do not seem to cause any pathology in these species (Borzykh & Zvereva, 2012).
Similar to true fungi, microsporidians are rarely found infecting bivalves, with merely one case reported from scallops (Lohrmann et al., 1999, 2000b), i.e. queen scallops off the English and Welsh coasts of the United Kingdom.

1.3.4 Ascetospora (Marteiliida and Balanosporida)

Marteilia species, such as *M. refringens*, are serious pathogens of bivalves, in particular European flat oysters (*Ostrea edulis*), mussels (*Mytilus* spp.), and the Manila clam (*Venerupis philippinarum*) (e.g. Naoki et al., 2005; Balseiro et al., 2007; Murrey et al., 2012). However, merely one reported case of an unidentified *Marteilia* sp. exists from scallops, i.e. the calico scallop, *Argopecten gibbus*, off the Atlantic coast of Florida in 1989 - 1990. Infections were heavy, causing rapid weakening and subsequent mortalities (Moyer et al., 1993, 1995).
Along with the Marteiliida, the Balanosporida represent some of the most serious parasites affecting bivalve molluscs, including *Haplosporidium* species infecting Pacific oysters, *Crassostrea virginica*, and *Bonamia ostreae* and *B. exitiosus* infecting oyster species *Ostrea edulis* and *O. lutaria*, respectively (OIE, 2017). Presently, two cases involving Balanosporida

species have been reported from scallops (Chu et al., 1996; Wang et al., 2012): Firstly, light but highly prevalent infections of an unidentified Balanosporida species suspected of causing post-spawning mortality in two populations of cultured bay scallops in the Yellow Sea, China (Chu et al., 1996). Secondly, a known bivalve pathogen, *Haplosporidium nelsoni*, identified in Yesso scallops suffering mass mortalities (Wang et al., 2012).

1.3.5 Superphylum Alveolata

General characteristics and phylogeny

Superphylum Alveolata (alveolates) forms a group of primarily single-celled eukaryotes that have adopted extremely diverse modes of nutrition, such as predation, photoautotrophy and parasitism. Generally, they are split into three major phyla: Apicomplexa (apicomplexans), Ciliophora (ciliates) and Dinoflagellata (dinoflagellelates), which are now known to be close relatives based on various ultrastructural and genetic similarities (Molnár, 2006). Three additional lineages, the colpodellids, chromerids and perkinsids, are also considered alveolates, but do not fit within any of the three aforementioned major phyla. The alveolates form a sister group to major clades of photosynthetic eukaryotes, the stramenopiles. In addition to the non-photosynthetic groups (apicomplexans, ciliates, colpodellids, perkinsids) the alveolates also contain photosynthetic lineages, such as the chromerids and the dinoflagellates (Portman & Šlapeta, 2014; Moore et al., 2008) (Figure 1.9.).

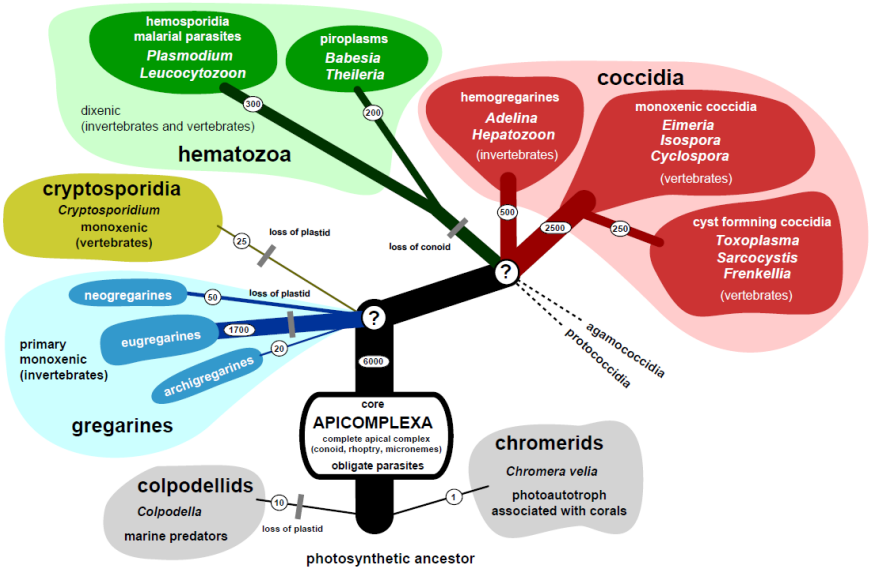

Figure 1.9. The phylogenetic status of the apicomplexans. Their closest ancestral relatives are the photosynthetic chromerids and the predatory colpodellids. Figure taken from Portman & Šlapeta (2014).

Phylum Dinoflagellata

Some mortality of scallops have been associated with the presence of dinoflagellates, including species of the genus *Prorocentrum*. *P. minimum* had toxic affect on postmetamorphic bay scallops, characterized by necrosis in the digestive gland and thrombi in the vascular system, while older juveniles were not affected (Wikfors & Smolowitz, 1993). In addition, Leibovitz et al. (1984) reported mortalities, linked to *Prorocentrum* sp., in post-metamorphic cultured juvenile bay scallops in Long Island Sound. Clinical signs were characterized by signs of distress, such as gaping shells, injuries to the soft tissues and impaction of the pallial cavity (Leibovitz et al., 1984).

Other dinoflagellates, such as *Alexandrium catenella* and *Gymnodinium catenatum*, have been shown to cause histological abnormalities in organs such as the mantle, gills and muscles, respectively in Peruvian scallop, *Argopecten purpuratus*, and Pacific calico scallop, *A. venticosus* (Escobedo-Lozano et al., 2012; Hegaret et al., 2012).

Phylum Ciliophora

A number of ciliates have been reported from scallops, most of which considered commensals rather than true parasites; feeding mostly on bacteria without causing any damage. However, in case of extreme numbers, they can become pathogenic and cause mortality due to physiological stress in the scallops (Getchell et al., 2016), as experienced in sea scallops in Atlantic Canada (Lauckner, 1983; Beninger et al., 1988; McGladdery et al., 1993a).
More than 150 species of ciliates have been identified in marine bivalves, most of which found in the mantle cavity, on the gills or in the digestive gland (Lauckner, 1983). The most common group reported are trichodinids, e.g. *Trichodina pectenis*, *T. polandiae* and *Trichodina jadranica*, infecting Yesso scallop and *Chlamys* species (Lauckner, 1983; Lohrmann et al., 2000a, 2002; Lohrmann, 2009).
While trichodinids are commonly found in the mantle cavity, other ciliates are found at other sites, e.g. *Licnophora auerbachi*, which was found in high prevalence on the eye´s surface of queen scallop, causing epidermal damage (Harry, 1977, 1980).

Phylum Perkinzoa

Perkinsids, e.g. *Perkinsus marinus* and *P. olseni* are serious and quite thouroughly documented pathogens of oysters, clams, abalones and scallops (Dittman, 1993; La Peyre et al., 1993; Liang et al., 2001; Kwang-Sik & Kyung, 2010). Although *Perkinsus* species are relatively uncommon in scallops, a number of species have been reported, the first one, *Perkinsus* sp. from Yesso scallops from Popov Islands off the east coast of Russia following an import from Japan. Lesions were observed but no description of the associated pathology exists (Kurochkin et al., 1986). *Perkinsus quqwadi*, formerly known as "scallop protistan unknown" or SPX, has been observed in cultured Yesso scallops from British Columbia (BC), Canada (Bower et al., 1990, 1992, 1995, 1997, 1998; Bower & Meyer, 1994; Blackbourn et al., 1998; Naoki et al., 2013). *P. quqwasi* is apparently native in BC waters and causes extensive infections in various organs, leading to mortality (Perkins, 1996; Bower et al., 1998). Other native scallop species in this area, e.g. *Chlamys rubida* and *C. hastata* also seem to be susceptible to *P. quqwasi* infections (Bower et al., 1999).

Phylum Apicomplexa

General characteristics
Apicomplexans form a group of unicellular and spore forming pathogens sharing a defining feature, the apical complex that comprises a system of

structural- and secretory elements that facilitates interaction with the host cell (Molnár, 2006). They are obligate parasites, exhibiting both asexual (merogony) and sexual (gamogony) reproduction followed by the development of infective sporozoites (sporogony), represented by numerous different life forms, either in a single hosts (monoxenous) or two hosts (heteroxenous) (Molnár, 2006) (Figure 1.10). In most cases they develop intracellular, but also a varying degree of epi- and extracellular (e.g. some coccidians, gregarines & rytidocystids) development is also known. Extracellular sporulation of oocysts is common, both inside the hosts and in the surrounding environment after being exposed from the hosts (Bartošová-Sojková et al., 2015).

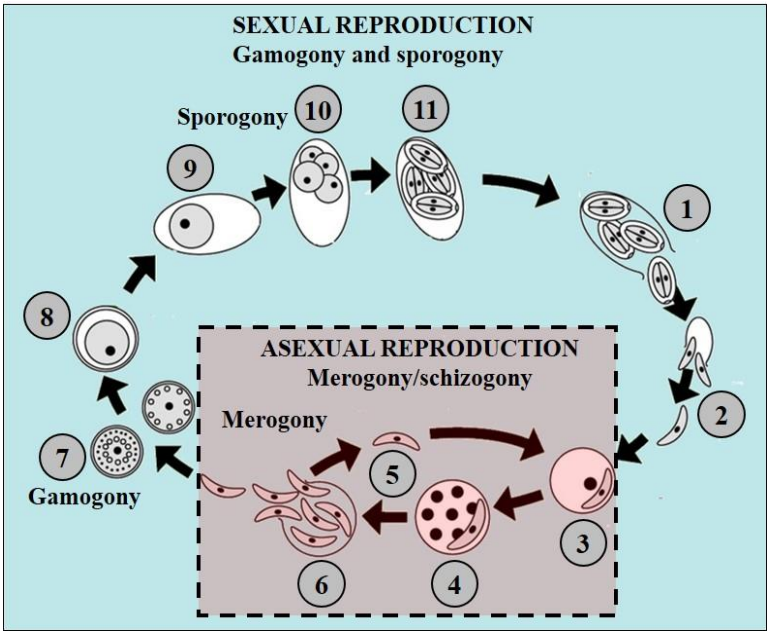

Figure 1.10. A general life cycle of an apicomplexan parasite. (1) Mature infectious sporozoites released from an oocyst. (2) Excystation of sporozoites. (3) Sporozoites infect host cells. (4-6) The merogonic phase; development of meronts and the formation of infectious merozoites, commonly 2-3 generations. (7) The last generation of merozoites infect new host cells and initiate the formation of macrogamonts (eggs) and microgamonts (sperm). (8) Fertilization, the formation of an early oocyst. (9-11). Further development, the final product being a mature oocyst with variable numbers of sporocyst that enclose sporozoites, the infective form. Redrawn by Árni Kristmundsson from: https://www.impextraco.com/es/node/141

Pathogenicity

The pathogenicity of apicomplexan varies considerably between species and/or their hosts. Due to their obligate intracellular location (with some exceptions), they cause some degree of pathology in all cases. However, some are considered to have low pathogenicity while others are highly pathogenic. Some species are serious human pathogens causing diseases such as babesiosis (*Babesia* spp.), malaria (*Plasmodium* spp.), toxoplasmosis (*Toxoplasma gondii*), cryptosporidiosis (*Cryptosporidium parvum*), while others cause coccidiosis in various lower vertebrates and invertebrates (e.g. Seed, 1996; Steinhagen & Hespe, 1998).

Phylogeny

The Phylum Apicomplexa is divided into four main groups, i.e. coccidians, gregarines, spiroplasms and haemosporidians, currently consisting of more than 6,000 nominal species, the great majority described from vertebrate hosts both terrestrial (e.g. humans and livestock) and aquatic (fishes). As apicomplexan are found in all groups of animals, the number of species yet to be discovered is vast and has been estimated to be well over a million species by some scientists (Hausman et al., 2003; Molnár, 2006; Adl et al., 2007). Despite apicomplexans´ obligate parasitism, chromerids (*Chromera velia, Vitrella brassicaformis*) and colpodellids are considered their closest relatives, both of which are non-parasitic; the former group photosynthetic and associated with corals and the latter free living predators (Figure 1.9.) (Kuvardina et al., 2002; Moore et al., 2008; Oberník et al., 2011; Oberník et al., 2012; Portman & Šlapeta, 2014).

To date the apicomplexans are thought to have evolved from algal ancestors and the presence of a non-photosynthetic plastid-like organelle in apicomplexans, called the apicoplast, supports that theory (Arisue & Hasimoto, 2015). The discovery of the chromerid, *Chromeria velia* (Moore et al., 2008), which has been termed *"the mother of all parasites"* (Okamoto & MacFadden, 2008) was a milestone with regard to understanding the evolution of parasitism which is a vital component in protist research, concerning both parasites and free-living unicellular organisms. *C. velia* contains a photosynthetic plastid (chloroplast) and has typical features of the alveolates and phylogenetically related to the apicomplexan, with their non-photosynthetic plastid, the apicoplast (Kuvardina et al., 2002; Okamoto & MacFadden, 2008; Oberník et al., 2011; Oberník et al., 2012; Arisue & Hasimoto, 2015). The chromerids can be regarded as an example of a bridge between two conventional categories, i.e. photosynthetic and parasitic protists (alveolates). Since the discovery of *C. velia*, a number of papers have been published on apicomplexan related lineages (termed ARLs) observed

from environmental samples as well from, or associated with, corals. These researches suggests that ARLs are common in association with coral reefs and probably symbionts of corals (Janouškovec et al., 2012; Janouškovec et al., 2013; Kirk et al., 2013).

Apicomplexans of marine invertebrates

In spite of the fact that researches on apicomplexans infecting marine invertebrates, date back to the 19th century, they are poorly documented and understood, compared to species infecting vertebrate hosts. Of about 6,000 known species (Hausman et al., 2003; Adl et al., 2007), only a small part are reported from invertebrates in the marine environment and the great majority of those are gregarines (Subclass Gregarinasina) and not true coccidians (Subclass Coccidia). Furthermore, the phylogenetic position of the currently described species from marine invertebrates is largely unknown, as no molecular data exists for the vast majority of the species. Considering the generally accepted evolution of life, one should expect to find the most primitive parasitic apicomplexan forms in marine invertebrates.

Scallop apicomplexans

Apicomplexans in scallops are very poorly documented, with only three nominal species known, belonging to two separate families, i.e. the coccidian family Aggregatidae (aggregatids, Order Eucoccidiorida) and the gregarine family Porosporidae (eugregarines, Order Eugregarinorida).

***Pseudoklossids* (*Pseudoklossia* and *Margolisiella* spp.)**. According to the conventional definition, species assigned to the Family Aggregatidae are intracellular in marine invertebrates, with oocysts typically having many sporocysts, mostly heteroxenous, merogony in one host and gamogony in another (Levine, 1973). Several aggregatid species have been reported from scallops, only one of which nominal, i.e. *Pseudoklossia pectinis* which is also the first apicomplexan reported from scallops, i.e. from epithelial cells of the kidney of king scallops from Roscoff in France (Léger & Debusqc, 1917). While all gamogonic stages were present, no asexual, merogonic stages were observed. Hence, it was suggested it had a heteroxenous life cycle with an unknown intermediate host. No pathology was reported associated with the infections. In the original publication, the authors suggested that *P. pectinis* had syzygous division (Léger & Debusqc, 1917), a process were two mature trophozoites eventually pair up and develop into gamonts and characteristic feature of apicomplexans within the family Adeloridae. Consequently, the validity of its current taxonomy within the family Aggregatidae is considered debatable (Desser & Bower, 1997). Few other papers on similar species exist

in the literature, all of which infecting the kidney and some other organs as well. Leibowitz et al. (1984) reported heavy infections with anonymous coccidia in both wild and captive bay scallops. The infections were mostly found in kidney but also, to a lesser extent in the gonads, digestive gland and adductor muscle. As more than one type of oocysts were observed, several apicomplexan species were involved. Infections were more severe in the captive scallops than the wild ones, causing severe pathology resulting in mortalities exceeding 80%, in the former ones (Leibowitz et al., 1984). Similar reports exist for the same scallop species in eastern US and Canada, all of which primarily infecting the kidney but also other organs, similar to Leibowitz et al., (1984) findings. In each of these reports, only one coccidian species was present, *P. pectinis*-like (Karlsson, 1991), *Pseudoklossia* sp. (Cawthorn et al., 1992) and an anonymous one (Whyte et al., 1994). DiSalvo (1994) reported another coccidian in the kidneys of broodstock Peruvian scallops, *Argopecten purpuratus*. This was related to production of poor-quality gametes, but no further descriptions of the infection have been reported. Other studies of this scallop species have not revealed the presence of this parasite (Lohrmann et al., 1991, 2000a; Lohrmann & Smith, 1993), and it is speculated that the infection detected by DiSalvo (1994) may have been opportunistic.

A major taxonomic change was made on *Pseudoklossia* species in 1997, when the genus *Margolisiella* was created for *Pseudoklossia* species with suggested monoxenous life cycles, while the remaining, possibly heteroxenous species, were left within the genus *Pseudoklossia* (Desser & Bower, 1997). However, the validity of this change is debatable and some authors believe that all *Pseudoklossia* species have monoxenous life cycle although the merogonic stages have not been found in all cases (Duszynski et al., 1999). Furthermore, presently these former congeneric species belong to two different families, i.e. Aggregatidae (*Pseudoklsossia* spp.) and Eimeridae (*Margolisiella* spp.) (Desser & Bower, 1997; Desser et al., 1998).

***Gregarines*.** The gregarines represent the most specious group of apicomplexan parasites infecting invertebrates, both terrestrial and aquatic; with more than 1,500 known species assigned to hundreds of genera (Clopton, 2002). They are split into three separate orders, i.e. Neogregarinorida, Archigregarinorida and Eugregarinorida, which are commonly named neogregarines, archigregarines and eugregarines. The first one only infects terrestrial hosts while the archigregarines are solely found in marine habitats and eugregarines in marine, freshwater and terrestrial ones. Gregarines are characterized by their large trophozoites/gamonts infecting the extracellular space of the intestines and conducting syzygy (Clopton, 2002).

The second group (in addition to the pseudoklossids) known to infect scallops are eugregarines of the family Porosporidae. Species of *Nematopsis* Schneider, 1892 (Apicomplexa) are hetereoxenous gregarine parasites commonly infecting commercially important marine molluscs as intermediate hosts and decapods as their definitive hosts (Schneider, 1892; Prytherch, 1938, 1940; Théodoridès, 1962; Cheng, 1967; Sprague 1970; Lauckner, 1983; Perkins, 1991; Bradbury, 1994; Jiménez et al., 2002). *Nematopsis* species have been reported to have monozoic oocysts (spores or sporocysts according to some authors) with a thick wall enclosing a single sporozoite (Sprague & Orr, 1955; Théodoridès, 1962; Sprague, 1970; Desportes et al., 1977; Azevedo & Cachola, 1992; Azevedo & Matos, 1999; Padovan et al., 2003). Although gregarines are primarily parasites of annelids and arthropods, this group of gregarines conduct intermediate development in marine bivalves (Getchell et al., 2016). All the species reported from scallops belong to the genus *Nematopsis*, a genus characterised by sporozoites encapsulated by a resistant spore, which is actually the oocyst of the parasite, according to Lauckner (1983). The first species identified in scallops was *Porospora pectinis* from *Chlamys varia* in European waters (Léger & Dubosqc, 1925). It was later transferred to the genus *Nematopsis* (Sprague, 1970). The intermediate host for this species is unknown. Species similar to *N. pectinis* have also been found in Japanese scallop *Mizuhopecten yessoensisi* and *Pecten caurinus* (Getchell et al., 2016). Other species of this genus include *N. ostrearum* and *N. duorari* from bay scallop, *A. irradians* (Kruse, 1966; Sprague, 1970), and the final host for the latter species being a shrimp, *Penaeus duodarum*. *Nematopsis* species are generally not considered pathogenic in bivalves (Sprague and Orr; 1955). They can however be highly prevalent, e.g. in Tehuelche scallop, *Aequipecten tehuelchus*, in Argentina, where infections were close to 100% (Cremonte et al., 2005).

Apicomplexans infecting other molluscs

***Aggregata* spp. (Family Aggregatidae)**. Cephalopods are infected by a number of apicomplexan species, mostly of the genus *Aggregata* (e.g. Gestal et al., 2010; Mayo-Hernándes, 2013), the first one *A. eberthi* (syn. *Klossia eberthi*) described in the late 19th century (Léger, 1897; Léger & Duboscq, 1906). Currently, close to 20 nominal species have been reported, two of which with available molecular data (*A. octopiana*, *A. eberthi*). Species of this genus have a two-host life cycle where sexual stages occur in the digestive tracts of cephalopods, and asexual stages in the digestive tracts of crustacean intermediate hosts (Gestal et al., 2002, 2005, 2010).

***Pseudoklossia*/*Margolisiella* (Families Aggregatidae/Eimeridae).** To date, roughly 15 nominal and several un-named *Pseudoklossia*/*Margolisiella* species are known from the literature. All these species infect molluscs, mostly bivalves, including scallops, as noted above, but also gastropods and chitons. They are most commonly found in the kidney although species infecting other organs exist, such as *P. tellinovum* (ovary) (Buchanan, 1979). Many of the currently known species were described in the late 19th century and early 20th. Since then, several major taxonomic revisions have been made. Firstly, species originally assigned to genera such as *Merocystis* and *Hyalloklossia* were transferred to the genus *Pseudoklossia* (Léger, 1897; Léger & Duboscq, 1906; Léger & Duboscq, 1915; Léger & Duboscq, 1917; Debaisieux, 1922; Buchanan, 1979). Furthermore, as previously mentioned, a second major change was made in 1997 when the genus *Margolisiella* was created for *Pseudoklossia* species with apparent monoxenous life cycles (Desser & Bower, 1997).

***Merocystis* spp**. Currently this genus consists of a single species, *M. kathae*, infecting the kidney of the common whelk (Dakin, 1911; Foulon, 1919; Patten, 1935). One further species described, *M. tellinovum* (Buchanan, 1979) was moved to the genus *Pseudoklossia* few years after its original description. Due to its high relevance to this thesis, *M. kathae* will be discussed further text in chapter 1.4.2. below.

Piridium sociabile is a presumed apicomplexan species infecting the common whelk (Patten, 1936). Its taxonomic position is unresolved. This parasite is discussed further in chapter 1.4.2. below.

The adeleorines (Suborder Adelelorina). These are parasites of both vertebrates and invertebrates and characterized by a phenomenon called syzygy, a pairing of gamonts prior to fertilization, a process also utilized by gregarines. Currently, this group does not represent a major clade of apicomplexans infecting marine invertebrates and the only species reported, i.e. *Klossia tellinae*, described from the kidney of the bivalve *Tellina tenuis* from Scottish waters (Buchanan, 1979). No molecular data exist for this species.

Some nominal and un-named species with unresolved taxonomy / phylogeny

Apicomplexan of flat oysters. This apicomplexan played a significant role in mass mortalities events causing a 91% reduction in a population of commercial-sized oysters (*Ostrea chilensis)* in New Zealand waters from late 1985 to 1993 (Diggles et al., 2016). This apicomplexan species is further discussed in the chapter 1.8.2.

Unidentified apicomplexans from the giant clam (Tridacna crocea) and oysters (Ostrea edulis). Both these apicomplexan species are anonymous. The one infecting the giant clam has been partially described, but the only life stages observed were trophozoites (Nakayama et al., 1998). The one infecting *O. edulis* is unpublished but an 18S rDNA sequence is available in Genbank (ref.nr. U83331.1).

Apicomplexans infecting other marine invertebrates

The gregarines are common parasites of invertebrates, mostly annelids although a number of species, e.g. *Nematopsis* spp. and *Porospora* spp., are known from molluscs, including scallops as noted above (Clopton, 2002; Desportes & Schrével, 2013). Although much work is yet to be done, the knowledge on their phylogenetic status within the phylum Apicomplexa is probably more holistic than other groups of apicomplexans infecting marine invertebrates. Recently, a major phylogenetic revision was made to gregarines, were some members of the archigregarines (genera *Platyproteum* and *Filipodium*) were given a higher taxonomic hierarchy (Class Squirmidea; Order Squirmida) where these former gregarines are not even considered true apicomplexans; rather a sister group to all other apicomplexans (Cavalier-Smith, 2014).

The rhytidocystids (Agamococcidiorida: Rhytidocystidae). According to the original definition, species assigned to this order are parasites of annelids, with large, flattened and oval trophozoites. Extracellular development, gamogony (no fertilization) and merogony absent, trophozoites become oocysts with many sporocysts, each with two banana-shaped sporozoites (Levine, 1979). The presumed lack of gamonts and fertilization as well as merogony makes this group unique among other apicomplexans. The Order Agamogoccidiorida consists of two separate families: (i) Rhytidocystidae, with one genus *Rhytidocystis* and five separate species according to Rueckert & Leander (2009), all of which parasites of marine polychaetes. Molecular data exist for two *Rhytidocystis* species, *R. cyamus* and *R. polygordiae* (Leander & Ramey, 2006; Rueckert & Leander, 2009) (ii) Gemmocystidae, including one genus, *Gemmocystis*, including merely a single species, *G. cylindrus*, infecting scleractinian corals in the Caribbean (Upton & Peters, 1986) and presently the only nominal coral apicomplexan. The description of *G. cylindrus*, which created the basis for the Family Gemmocystidae within the Order Agamococcidiorida, was exclusively based on morphological characteristics (Upton & Peters, 1986). However, to date the valitidy of this family is debatable, as DNA sequences, presumed to be from *G. cylindrus*, do

not group with the rhytidocystids (Toller et al., 2002; Šlapeta & Linares, 2013).

1.3.6 Helminthes

As in more or less all animals, numerous helminth species, i.e. trematodes, cestodes, turbellarians and nematodes, have been reported from scallops. These groups of parasites are however generally not considered major pathogens of scallops.

The exception from this are bucephallid digenean trematodes, which are known to cause significant damage to the reproductive systems, causing castration in some cases (Getchell et al., 2016).

Cestodes, particularly larval forms, are commonly found in scallops, e.g. in the intestinal tract, gonodal tissues and viscera. However, these infections are generally not considered pathogenic (Getchell et al., 2016).

Although turbellarian flatworms are common parasites of bivalves (Lauckner, 1983), they have rarely been reported in scallops. Leibovitz et al., (1984) reported an unidentified turbellarian from the digestive gland of bay scallop, *Argopecten irradians*, apparently causing no pathological changes.

In some cases, nematode infections can affect scallops´ commercial value. As an example, at least two scallop species are known to serve as intermediate hosts for the ascarid nematode, *Sulcascaris sulcata*, which adult stage inhabits the stomach of sea turtles (Sprent, 1977). Two scallops species, *Amusium balloti* and *Chlamys* sp., are known intermediate hosts for *S. sulcate*, both of which inhabiting Australian waters (Cannon, 1978; Lester et al., 1980).

1.3.7 Polychaetes

Polychaetes worms are generally free-living animal and not considered true parasites. However, a number of spionid species of genera *Polydora, Boccardiella* and *Boccardia* are well known for their boring activities in mollusc shells, including scallops, which they use as a habitat (Evans, 1969; Mori et at., 1985). Although they do not generally invade soft tissues, they can cause damage to the scallop´s shell to the extent of affecting their health by weakening the adductor muscle attachment that can impair their shell closure, swimming, feeding, and escape behaviour (Getchell et al., 2016). Furthermore, in cases where the scallops are marketed as whole, i.e. in their shell, this can significantly reduce their commercial value (Getchell et al., 2016).

1.3.8 Crustaceans

Several scallop species, e.g. *Argopecten irradians*, *A. gibbus*, *Placopecten magellanicus*, *Chlamys farreri* and *Aequipecten tehuelchus*, are known to be hosts for parasitic pinnotherid crabs (pea crabs), such as *Tumidotheres* (*Pinnotheres*) *maculatus* and *Amusium pleuronectis*, which parasitize their mantle cavity (Getchell et al., 2016). Although there are no indications that these species feed directly on host tissues, their presence in the mantle cavity can cause irritation and even severe structural alteration and pathology, leading to emaciation, reduced filtering capacity, damage to the gills, palps, and mantle, and compression of the gonad, which may affect gonadal development (Lauckner, 1983).

1.3.9 Gastropods

Odostomids (Family Pyramidellidae) are small gastropods, which parasitize marine bivalves, including scallops, and feed on their haemolymph (Lauckner, 1983; McGladdery et al., 1993a). Leibovitz et al. (1984) reported mortality among captive bay scallops due to *Odostomia* spp. The gastropods were found in large numbers on the valves, mantle, and pallial cavity of captive scallops but also within the soft tissues. Another case, including *Odostomium seminuda* was reported from two scallop species from North Carolina (Wells & Wells, 1961).

1.3.10 Algea

A number of algal species are considered symbionts of marine bivalves. Several species of the green algae *Zoochlorella* spp. live in the mantle, eyes and tentacles of bay- and sea scallops, off Cape Cod in the western Atlantic. They can have pathological effect, such as granulomatous lesion and infiltration of haemocytes (Leibovitz et al., 1984).
The microalga, *Coccomyxa parasitica*, is a facaultive parasite infecting sea scallop, in particular its mantle fold. Hovever, it is not considered highly pathogenic (Stevenson & South, 1975).

1.3.11 Foraminiferans

The foraminiferan *Cibicides refulgens* is commonly found living on the valves of the Antarctic scallop, *Adamussium colbecki*. It aquires nutrition by eroding through the scallop shell (Getchell et al., 2016). Although, *C. refulgens* was shown to cause significant erosion in shell of its host, their detrimental effect on the scallops is not fully known (Alexander & DeLaca, 1987). The presence of the foraminiferan, *C. lobatulus*, has also been observed in the queen scallop (Haward & Haynes, 1976).

1.3.12 Porifera

In addition to polydorid polychaetes, clionid sponges are also known to colonize bivalve shells, both dead and living (Lauckner, 1983), and cause damage or destruction of the shell, e.g. in the sea scallop in Newfoundland waters (Getchell et al., 2016).

1.3.13 Cnidaria

The colonial hydroid, *Hydractinia echinata*, commonly inhabits the outer part of the shell of the sea scallop and in some cases also its internal surface. Its presence can impair the scallop´s activities and cause deformities of the shell (Getchell et al., 2016). Another hydroid species, *Eutima japonica*, has been linked with mortalities in juvenile Yesso scallops (Baba et al., 2007).

1.3.14 Neoplastic disease

Neoplastic disease, also termed haemic neoplasia, haemocytic neoplasia, disseminated neoplasia, haematopoietic neoplasia, disseminated sarcoma and gonadal neoplasia, is a serious, leukaemia-like disease known from multiple bivalve species (Barber, 2004). As implied in the terms used for this condition, it affects the bivalves' blood cells, the haemocytes. It was first described from blue mussels, *Mytilus edulis*, from Yaquina Bay, Oregon USA, in the late 1960s (Farley, 1969). It is characterized by the presence of large, anaplastic cells in the connective tissues, blood vessels and sinuses of the visceral mass, muscle and mantle tissues (Barber, 2004). Neoplastic cells are hypertrophied (2-4 times larger than normal haemocytes), with hyperchromatic and often a pleomorphic nucleus containing one or more prominent nucleoli. Consequently, the nucleus vs cytoplasm ratio is higher than in normal haemocytes. Furthermore, the rate of cellular division of neoplastic cells is much greater than in normal cells (Barber, 2004). The disease is progressive; the replacement of normal cells by neoplastic cells causes loss of normal tissue and organ architecture that can result in significant mortalities of the affected hosts (Barber, 2004). Recently, Metzger et al. (2016) showed that neoplastic cells (or cancer, as they name it), can be transmitted both between individuals of the same species but also between different species. These findings are fundamental, as it now appears that neoplasia can be "infectious". According to the available literature, scallops are not prone to neoplastic diseases. However, gonadal neoplasia (termed germinoma) has been reported from bay scallop (Landsberg, 1996). The reasons for neoplasia are not fully understood but several presumable influencing factors, such as biotoxins and viral infections, have been suggested (Oprandy et al., 1981; Landsberg, 1996).

1.4 Whelk diseases

The common whelk is poorly studied with regard to diseases and parasites. The most frequently reported ones are larval trematodes infecting the digestive gland and gonads.

1.4.1 Trematodes

A number of helminth species have been reported from the common whelk, especially larval trematodes. The first reports on trematodes infecting the whelk date back to the late 1800s and early 1900s. A number of species were observed in these early studies, including *Cercaria Zoogonoides viviparous*, *Cercaria buccini*, *C. neptuneae* and *C. Neophasis lageniformis* (Køie, 1969). However, none of these studies considered the influence of these parasites on the whelk (Køie, 1969). Køie (1969) studied endoparasites of the whelk in Scandinavian waters and observed four larval trematodes (*Cercaria Neophasis lageniformis, C. Zoogonoides viviparus, C. buccini* and a xiphidiocercaria of the genus *Renicola*), all infecting the digestive gland and the gonads, one turbellarian, *Graffilla buccinicola,* in the stomach and digestive gland and one unidentified nematode in the digestive gland. Køie (1969) stated that all the larval trematodes could cause castration of the host. Other papers have reported a castration of the whelk due to larval trematodes, such as Tetreault et al., (2000) from common whelks from the Mingan Islands, eastern Canada, by larval stages of a species of *Neophasis.* As several previous authors, Declerck (1990) also found *C. buccini* and *Z. viviparus* in the Belgian North Sea, as well as two adult digenean, *Protoceces buccini* and an unidentified species. *C. buccini* and *Z. viviparus* were observed once again by Siddall et al. (1993) as well as two additional species of larval trematodes, i.e. *Stephanostomum baccatum* and the xiphidiocercariae and sporocysts of a *Renicola* sp. Siddall and colleagues (1993) concluded that toxic trace metals affected the survival of the trematodes´ miracidia, resulting in reduced parasite load. Sommerville (1978) reported severe infections in whelks with *Stephanochasmus baccatus,* causing significant pathology in the digestive glands. The only reports of trematodes from whelks in Iceland are from Magnúsdóttir (2010), who found *Neophasis* sp. in the gonad and/or digestive gland of whelks in Breidafjördur.

1.4.2 Apicomplexa

Merocystis kathae (Dakin, 1911)

Merocystis kathae is an apicomplexan infecting renal tissues of the common whelk, *Buccinum undatum* in northern Europe. It is the type species for the

genus, originally discovered and described by Dakin (1911) from Port-Erin, Isle of Man. Subsequently, Foulon (1914) and Patten (1935) published papers including a more detailed description of the different life stages of the parasite. Dakin (1911) placed *M. kathae* within the Family Polysporocystidae but later Foulon (1919), who described all life stages of the parasite in details, transferred it to the Family Aggregatidae and suggested it should be moved the genus *Aggregata*, as Dakin (1911) seemed to have confused many of the developmental stages. Patten (1935), who studied the seasonal occurrence of different developmental stages of the parasite (Figure 1.11.),

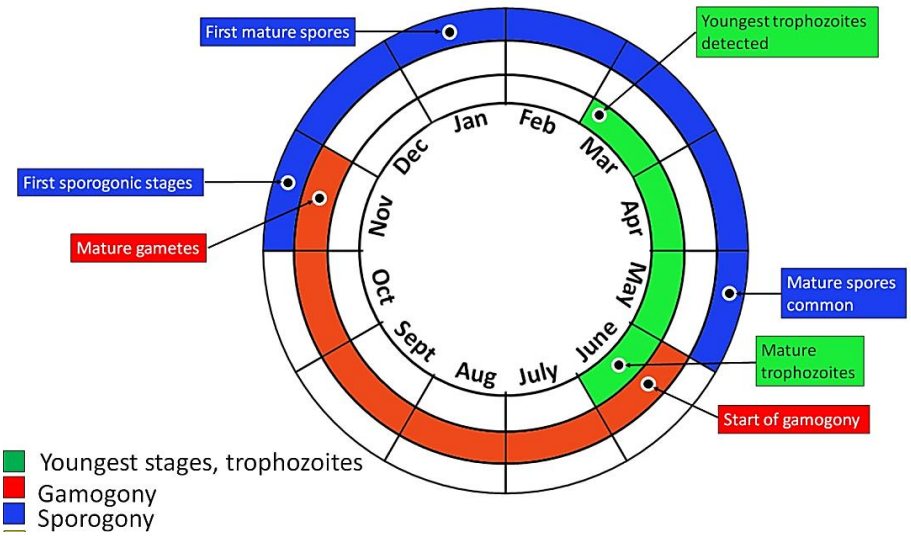

Figure 1.11. The seasonal development of Merocystis kathae *in the common whelk,* Buccinum undatum. *Drawn by Árni Kristmundsson from data published in Patten (1935).*

supported the suggestion that *M. kathae* should be placed within the Family Aggregatidae, but believed the development of male gametes was different enough from *Aggregata* species to retain the genus *Merocystis*. In these original descriptions (Dakin, 1911, Foulon, 1919, Patten, 1935), only gamogonic and sporogonic stages were observed. The merogonic stages were thought to be in an unknown intermediate host. Patten (1935) pointed out the similarity of the life histories of *M. kathae* and *Aggregata* species and the possibility, that the missing merogonic stages of this apicomplexan could occur in crabs or other crustaceans, which was already known to be the case for *Aggregata eberthi* (Setna & Bhatia, 1934). To date, this parasite has been reported from the Irish Sea, Belgian part of the North Sea (Declerck, 1990),

in Sound (Øresund) and Gullmarfjord in Danish- and Swedish waters (Køie, 1969). In 2007, the presence of *M. kathae* was confirmed in few whelks from Icelandic waters, with examination of fresh mounts and histological sections (Árni Kristmundsson, unpublished data). In 2010, the distribution and prevalence of this species was evaluated in whelks from ten different locations in Breidafjördur Iceland, examining macroscopic signs typical for the infections under a dissecting microscope (Magnúsdóttir, 2010). *M. kathae* was found at all ten locations, the prevalence ranging from 5-70% (Magnúsdóttir, 2010). Most probably, the prevalence was underestimated as no microscopic examination was performed and hence low-level infections most likely not detected.

Piridium sociabile (Patten, 1936)

Piridium sociabile is an exceptionally unusual parasite, and believed to be an apicomplexan. It is the only known species of this genus and infects connective tissues of the foot of the common whelk. According to Patten (1936), its development in the whelk takes up to one year. All developmental forms are morphologically similar, except for their size. The initial forms detected are around 2 µm but gradually grow up to 30 µm (Patten, 1936). Except for few papers reporting its presence in the common whelks (Køie, 1969; Magnúsdóttir, 2010), no studies have been performed on this species since its original description. Therefore, much is yet to explore of this unusual parasite, including its phylogenetic status.

1.5 Host – parasite - environment interaction.

1.5.1 Epidemiological terms

Endemic pathogen (or disease) is *"characteristic of, or prevalent in a particular field, area, or environment"* (Merriam-Webster dictionary). It can be clinically expressed or not, but is constantly present in a population in a given region.

Exotic pathogen is *"introduced from another country and not native to the place where found"* (Merriam-Webster dictionary). However, in a broad sense, most diseases causing agents could qualify as exotic because one can find an unaffected region or country for any given disease. Indeed, the exotic connotation is relative to the country of interest, because each disease is

indigenous to countries where it occurs and exotic where it does not (Kaoud, 2008).

Sporadic disease is defined as one **"***occurring occasionally, singly, or in irregular or random instances"* (Merriam-Webster dictionary), i.e. it occurs with low frequency and with no obvious temporal pattern. However, this does not mean that a sporadic disease occurs totally at random, i.e., without any pattern, nor does it mean that the disease is of no consequence (Kaoud, 2008).

Epidemic/epizootic is defined as **„***an occurrence of disease that is temporarily of high prevalence. An epidemic occurring over a wide geographical area (e.g., worldwide) is called a pandemic. The rise and decline in epidemic prevalence of an infectious disease is a probability phenomenon dependent upon transfer of an effective dose of the infectious agent from an infected individual to a susceptible one. After an epidemic has subsided, the affected host population contains a sufficiently small proportion of susceptible individuals that reintroduction of the infection will not result in a new epidemic. Since the parasite population cannot reproduce itself in such a host population, the host population, as a whole is immune to the epidemic disease, a phenomenon termed herd immunity"* (Encyclopaedia Brittannica).
An epidemic generally results from either a significant increase in the exposure to a new agent or an increase in susceptibility of a population to an endemic agent (Calow, 1998). Figure 1.12. clarifies the difference between some of the terms discussed above.

Emerging disease is defined as a one „*recently increasing in incidence, geographic range or virulence, recently moved into a new host, newly discovered or caused by a newly evolved pathogen*" (Brown 2000; Daszak et al., 2000a).

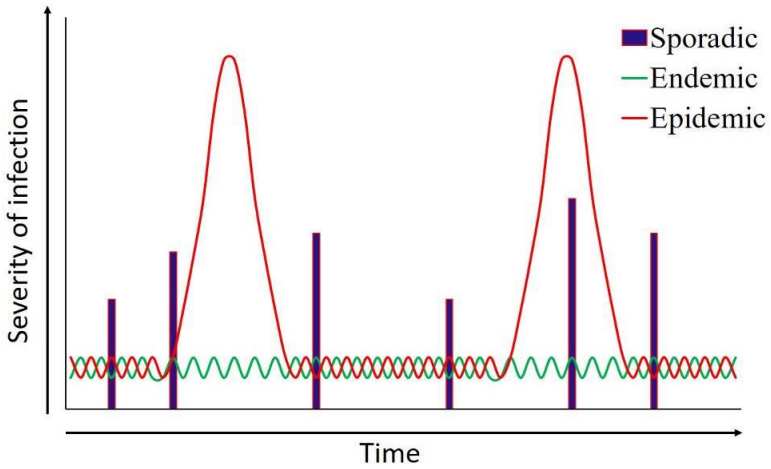

Figure 1.12. A schematic representation of endemic infectious agent, a sporadic disease and an epidemic. Drawn by Árni Kristmundsson from definitions in text above.

1.5.2 The disease triangle and mollusc epidemiology

It is widely acknownledged that for a disease to arise, a synergy among three main factors is required. Commonly, this synergy is called the disease- or epidemiological triangle, which is composed by a host, an infectious agent and their environment (Zannella et al., 2017). The theory of the disease or epidemiological triangle was introduced in 1960 in order to *"to study the interrelationship of various factors in an epidemic"* and to understand how epidemics might be predicted, limited or controlled (McNew, 1960). Although originally attributed to parasites of plants, it has been shown to be equally attributable to other hosts and pathogens. McNew (1960) broadly defined the parameters of the disease triangle as *"the inherent susceptibility of the host, the inoculum potential of the parasite, and the impact of the environment on parasitism and pathogenesis"*. According to McNew´s theory, an interaction of six factors determine the potential for a host to develop a disease after being exposed to a pathogen under favourable environmental and temporal conditions. These factors are: (1) the severity of the physical environment (for example, temperature, salinity, humidity or rainfall); (2) the duration of the infection period (time); (3) prevalence of the pathogen; (4) virulence of the pathogen; (5) the age or maturity of the host and; (6) its inherent susceptibility to a disease.

McNew's disease triangle gained immediate acceptance in the community of plant pathology, perhaps because it provided means to visualize and articulate observations that three interlocking participants, i.e. the host,

pathogen and environment, determine disease outcome during the life history of the host. Later it has been widely used in epidemiological studies of different pathogens and host, often with some modifications (e.g. Scholthof, 2007).
Let´s consider these three factors, i.e. host, pathogen and environment in context to mollusc's epidemiology.

Host-parasite-environment interaction

A host (such as a bivalve) can be resistant or very susceptible to a certain pathogen, and everything in between. A pathogen can be apathogenic, highly pathogenic to a certain host, and everything in between. A susceptibility of a certain host can be influenced by many factors, e.g. the hosts themselves, in particular their genetic traits, age/developmental stage, density and the condition and immune function. The influence of host age can be neutral, in favour of the host or disadvantage. For example, juvenile bivalves are generally more susceptible to viral- and bacterial infections (Lane & Birkbeck, 2000; Renault & Novoa, 2004; Travers et al., 2015) while the prevalence of bonamiasis among flat oysters, *Ostrea edulis*, generally increases with age and size (Cáceres-Martínez et al., 1995; Culloty & Mulcahy, 1996). Logistically, older hosts have had more time to accumulate disease-causing parasites and viruses than younger hosts have (Lafferty & Kuris, 2009). Furthermore, they generally inhale more food than the juveniles do, which increases the possibility of consuming pathogens (Mouritsen et al., 2003). The energy consuming process of maturation and spawning is also disadventurous as it causes reduction in host fitness and immunosuppression, making them more susceptible to diseases (Taskinen & Saarinen, 1999). On the other hand, older molluscs have had more time available than juvenile molluscs to develop defence mechanisms, for example thicker shells that are more effective, for example to resist boring parasites (Stefaniak et al., 2005).
The influence of high density of molluscs is generally thought to have negative influences, especially in case of directly transmissible disease agents, by increasing contact between infected and uninfected individuals (Anderson & May, 1991). Furthermore, it can cause stress in the population, which can make them more susceptible to infections (e.g., Ford et al., 2002). In contrast, a lack of gene flow between affected and disease free areas might hamper the development of disease resistance (Hofmann et al., 2009).
Life cycles and modes of transmission of pathogens are of great importance in the onset of a disease (Coen & Bishop, 2015). Other issues relevant to host-parasite interaction is whether a pathogen is exotic to its host or they have co-evolved. An introduction of a new virulent parasite can have catastrophic effects on the native population. Strong evidence exist showing

that such an introduction, commonly through human activities, is one of the most important factor driving disease emergence in wildlife populations (Daszak et al., 2000a,b). Vice versa, when host species are introduced to a new environment, e.g. Pacific oysters to European waters, the introduced host bivalve can be heavily affected by parasites of native molluscs (Peeler et al., 2011).

The environment is a key element in epidemiology. It can be favourable to the pathogen or the host, and everything in between. In fact, these interactions are very complex and almost endless numbers of different combinations. The environment is composed of abiotic factors (related to non-living things) e.g. temperature, salinity, acidity (pH), light, minerals etc, and biotic factors (related to living things), i.e. other organisms in the environment. In cases where both the host and the pathogen are native or endemic in a certain environment, an equilibrium in the host-pathogen-environment interaction exists, to a certain point. Environmental changes, can alter this equilibrium, e.g. in favour of the pathogen by making it more pathogenic or making the host more susceptible often due to immunosuppression. For example, an interaction of abiotic factors are known to influence the production and enzyme activities associated with cellular defences of a mollusc host (e.g., Akberali & Trueman, 1985; Jenny et al., 2002; Edge et al., 2012; Soudant et al., 2013). Abiotic factors in the marine environment are numerous and, according to the literature, they seem to trigger most bivalve epidemics (e.g., Hofmann et al., 2001; Soniat et al., 2009), the two most important ones being changes in temperature and salinity (Coen & Bishop, 2015). They can influence disease patterns in many ways, e.g. the distribution of the host, the microhabitat of the pathogen in their host, the parasite´s survival in the environment (i.e. the sea in this case) and the host-parasite interaction. The can be naturally occurring or be a result of coastal development (e.g. pollution) or climate change (e.g. increased temperature, decreased salinity, ocean acidification) and may impact the host directly or indirectly (e.g., Lafferty & Kuris, 2005; Morley, 2010, Coen & Bishop 2015). In fact, an infectious agent may be pathogenic or apathogenic to a certain host species, however, its life stage (larval, juvenile or adult form) and immune system, on which the environmental factor plays a key role (Zannella et al., 2017). For example, extreme temperatures, non-optimal salinity levels, human handling, extreme density in rearing systems and co-infection with other parasites or disease agents, are likely to reduce the host´s immune defences, and increase the pathogenicity of the infectious agent at the same time (Zannella et al., 2017). Considering this, environmental changes due to global warming, such as increasing sea temperatures, reduced salinity and increased acidity of the oceans, are likely to cause misbalance in

the interaction of the key factors. In many cases there are multiple interacting abiotic factors influencing the patterns and dynamics of a molluscan disease. For example, according to Soniat et al. (2009), low temperatures can enable a serious bivalve pathogen to tolerate low salinities. It is therefore obvious that in case of mass mortality events in wild populations, determining the factors responsible for the epidemics can be extremely difficult.

The disease triangle illustrates the interaction of the three above-mentioned factors. Each factor is represented by a circle. The strength of each factor is indicated by the size of the circle, i.e. larger pathogen circle means it is more pathogenic, larger host circle means that the host is more susceptible to the pathogen and larger environmental circle means it is more hostile to the host. A disease occurs in the intersection of all the three factors. Figures 1.13. A-F, demonstrate different combinations of interactions between these three essential factors, host, pathogen and environment.

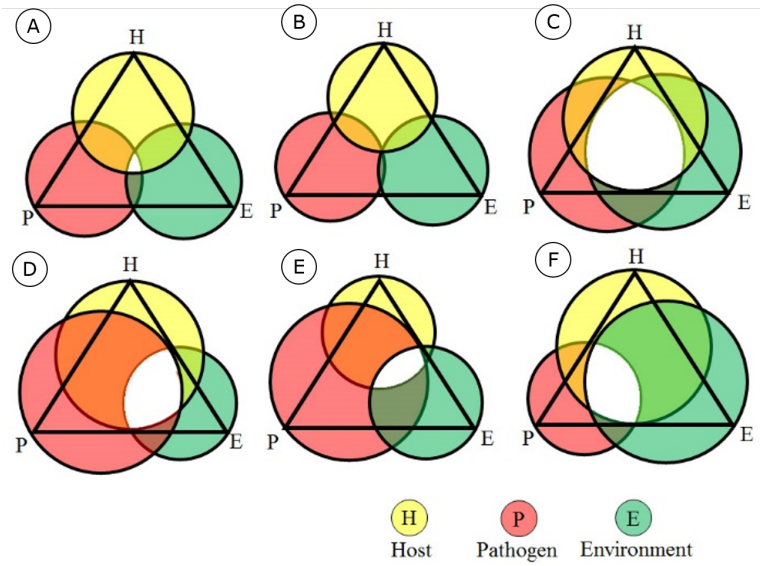

Figure 1.13. The epidemiological triangle. The size of the intercept of all the three factors (the circles) represent the severity of diseases in a population. (A) An equilibrium between the three factors; a normal scenario as infectious agents contribute to the natural mortality of all animal/plant populations. (B) A scenario of a nonexisting, perfect world with no diseases. (C) Worst case scenario, i.e. hostile environment, highly pathogenic agent and a highly susceptible host. (D) A pathogenic agent and a highly susceptible host, e.g. an exotic pathogen and a naïve host. (E) Similar scenario as in (D) but the host is more resistant. (F) A common scenario caused by a hostile environment, i.e. causing immunosuppression of the host making the host more susceptible to infectious agents. Drawn by Árni Kristmundsson.

1.5.3 The influence of diseases in marine environment

In general, epidemiological studies of aquatic, especially marine, ecosystems are relatively new, compared to terrestrial ones (Coen & Bishop, 2015). Consequently, the knowledge of the effects of diseases on the population dynamics of marine organisms is quite limited. With regard to molluscs, much of the present knowledge of impacts of diseases derive from relatively few intensive marine parasite-host systems, i.e. gastropod – trematode, cockle – trematode, and oyster – protistan interactions (Mouritsen & Poulin, 2002; Coen & Bishop, 2015). It seems logistic to presume that the scarcity of epidemiological studies of wild populations is at least partly due to the complexity of the marine ecosystem. Many of the epidemiological studies have been performed on captive animals, probably due to two main reasons: (1) it is easier to get an overview of the whole population studied. (2) for economic reasons, i.e. a thorough understanding of diseases, preventive measures, management and treatment, is vital for a successful aquaculture; in fact a great part of the knowledge on host-parasite dynamics, especially those involving microparasites, have been conducted in response to mass-mortality events in mollusc aquaculture (Elston & Ford, 2011). However, it is certain that diseases play a role in population dynamics of all living organisms.

Although limited, a number of examples on disease epidemics, affecting both marine fish and invertebrate population, exist in the literature (e.g. Hine, 2002; Óskarsson et al., 2010). However, case studies on the importance of diseases affecting populations of marine molluscs are increasing. A large part of the reasons for the growth of such research are thought to be related to: (1) expanding aquaculture; (2) current and future climate change; (3) increasing movement of non-native species; and (4) coastal development modifying molluscan disease dynamics, ultimately leading to complex relationships between diseases and cultivated and natural molluscan populations (Coen & Bishop, 2015).

The effect of diseases on the marine environment is at both individual level and population level but also at community-and ecosystem-levels (Coen & Bishop, 2015). Many pathogens have been linked to altering the condition of individual molluscs as well as their phenotypes (Combes, 1991; Poulin, 2007; Coen & Bishop, 2015). That includes direct death or indirect by weakening them and thereby making them more prone to predation or other pathogens (Poulin et al., 1998; Thomas & Poulin, 1998). Furthermore, a pathogen can alter the host´s phenotype, e.g. shell shape (e.g., Lagrue et al., 2007; Miura & Chiba, 2007) or even an adaptation that enhances the proliferation of the parasite and its transmission through manipulated behaviours (Coen & Bishop, 2015). A number of research have shown that parasites and diseases can have great influence on population sizes. In addition of causing direct

mortality to the hosts, they can influence populations in other manners, such as hampering reproduction of the host, leading to reduced recruitment in the stock (Hine, 2002, Cranfield, et al., 2005; Jónasson, 2006; Coen & Bishop, 2015, Lafferty et al., 2015, Magalhães et al., 2015). Parasites and diseases can also have severe effect at community- and ecosystem levels. A modification of an individual, e.g. its morphology or movement, and an effect at population level, e.g. density, can potentially modify interactions between different species inhabiting the ecosystem and significantly alter the community structure (Coen & Bishop, 2015). Figures 1.14. & 1.15. show some examples of the outcomes of such an effect.
In the next chapters, some selected mass mortality events of bivalve populations, and therefore related to the topic of this thesis, are discussed.

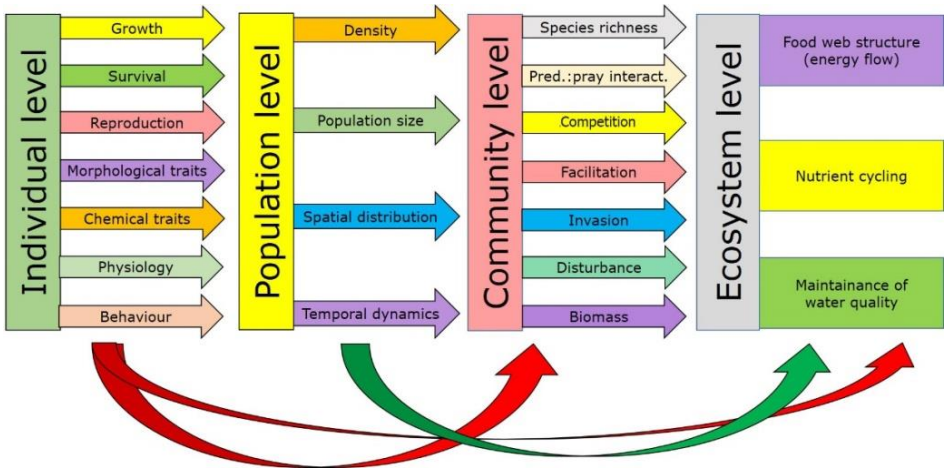

Figure 1.14. Summary of the variables that diseases may affect, at individual-, population-, community- and ecosystem levels. At higher levels of ecological organization, effects of disease(s) may arise directly or, alternatively, indirectly, due to lower levels of organization affected. Figure redrawn by Árni Kristmundsson from Coen & Bishop (2015).

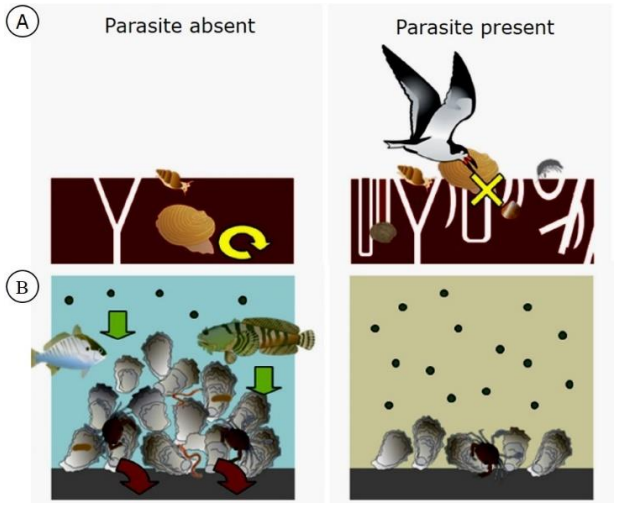

Figure 1.15. Examples of how parasites can affect communities and ecosystems. (A) Trematode infecting the foot of cockles preventing them from burrowing, making them more susceptible to predation of the definite host. (B) Oysters support dense and diverse communities of fish and invertebrates and provide important regulating services by cycling nutrients and maintaining water quality. Hence, parasites that reduce the abundance of oysters may have large effects on ecosystem services. Figures and data from Coen & Bishop (2015).

1.6 The collapse of the Iceland scallop stock

Almost from the onset of scallop fisheries in Iceland, in 1969, the Marine Research Institute (MRI – currently Marine and Freshwater Research Institute) in Iceland have annually conducted a biomass survey in Breidafjördur, the most important scallop ground in Iceland (Marine and Freshwater Research Institute, 2017). With some fluctuations, the stock index was relatively steady until the beginning of the 2000s. First indications of abnormalities in the scallop stock in Breidafjördur appeared in the year 2000, when high prevalence of diminished and abnormally coloured adductor muscles were noticed during processing. The following year, evidence of severe increase in natural mortalities where noticed when high numbers of cluckers (empty shells still attached by the hinge) were observed, which were almost exclusively restricted to mature scallops (shells > 5 cm). As these abnormally high natural mortalities were in many cases observed at scallop grounds where little or no fisheries had been undertaken, it was apparently

unrelated to fishing pressure (Marine Research Institute, 2003). Over a three years period, 2000-2003, a 70% decrease was experienced in the stock index, compared to its average size in the 1990s. The following years, it continued to decline, reaching its minimum in 2007 and 2008, being merely 13% of its average size in the 1990s (Marine Research Institute, 2009) (Figure 1.16.). A

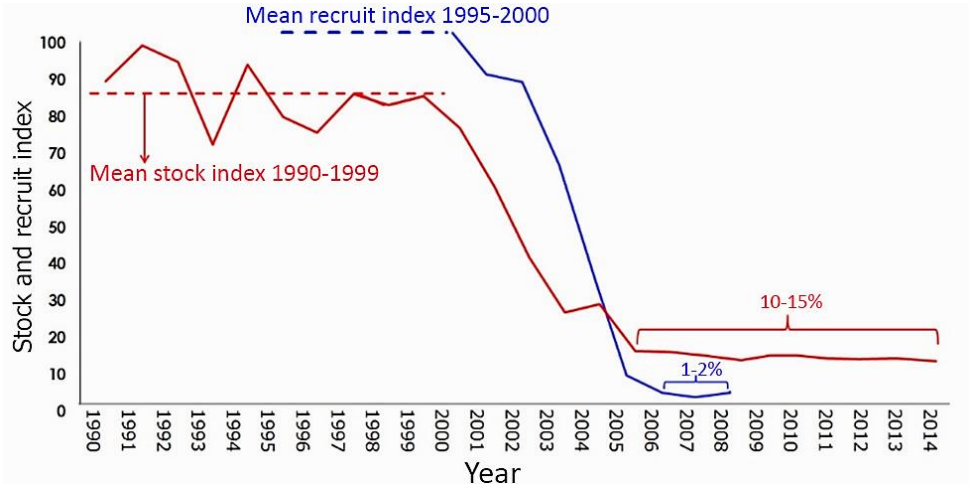

Figure 1.16. The stock and recruit indices of Iceland scallop in Breidafjördur, the most important scallop ground in Iceland. Drawn by Árni Kristmundsson from data from the Marine Research Institute (2015) and Eiríksson et al. (2010).

similar pattern was evident in other scallop grounds around Iceland, e.g. in the Westfjords in the northwest, Húnaflói in the north and Hvalfjördur in the southwest, where the scallop stocks collapsed despite minimal or no fishing in these areas (Marine Research Institute, 2002). Due to these findings, a total fishing ban was instructed in Icelandic waters in 2003.

However, what caused a total collapse in a stock that had been relatively stable for 30 years? None of the most common factors considered the most probable ones, i.e. increasing temperature, overfishing and food availability (Garcia, 2006; Jónasson et al., 2006), passed inspection. In 2003 and 2004, the sea temperature in Breidafjördur reached the highest mean in 100 years and the maximum temperature observed in August 2003 (12.2°C) was assumed to exceed the upper temperature tolerance of the species, previously thought to be 10°C (Garcia, 2006). However, subsequent experiments made on scallops from Icelandic waters showed that they can tolerate up to 13.0°C for at least three weeks with no negative effects but considerable mortality was observed at 14.0°C (Jónasson et al., 2004). Furthermore, the abnormal mortality in the stock had already been observed during the spring survey of

the MRI in Iceland in 2001, a period when the sea temperature was considerably lower (Jónasson et al., 2006). This makes temperature increases as the main factor for causing the mortality an unlikely explanation. Similarly, sea temperature alone cannot explain the mass mortality events in the nearby site of Hvalfjördur in 1983 or the fjords and bays in eastern Iceland in the 1990s, as sea temperature there was considerably lower than observed in Breidafjördur during the associated collapses. Based on these findings, Garcia (2006) stated that overfishing was left as the most likely factor responsible for the collapse in the scallop stock. However, that is contradictory to the observed mortalities which seemed unrelated to fishing intensity (Marine Research Institute, 2003) and the most extensive mortalities during the collapse were actually observed in scallop stocks where none or very minimal fisheries were undertaken (Jónasson et al., 2006), e.g. in the Húnaflói and the northeastern part of the Westfjords (Marine Research Institute, 2000, 2002). The same argument can be applied to the mass mortality observed in Hvalfjördur in 1983 (Eiríksson, 1986). When considering lack of food availability as a factor of importance, the abnormal mortality and macroscopic disease signs were not observed in immature scallops but restricted to the larger ones (Marine Research Institute, 2003). For unavailability of food to be considered as a major factor of importance, one would expect the whole populations to be more or less equally affected, regardless of scallop size or maturity. The most likely cause for the collapse was, by far, infectious diseases, as demonstrated in this thesis.

1.7 The "gray/weak meat" phenomenon

"Gray- and weak meat" are terms used for an abnormal condition of sea scallop (*Placopecten magellanicus*) and weathervane scallop (*Patinopecten caurinus*), respectively while the term "white meat" is used for normal adductor muscles and "brown meat" the stage in between. This condition is similar to the one observed in the collapsed Iceland scallop stock, characterized by an abnormally small, and discoloured: brown/gray, loosely bound and stringy adductor muscle (Stokesbury et al., 2007; Levesque et al., 2016). The "gray meat" phenomenon dates back to 1936 (Stevenson, 1936) and quite many different reasons have been given for this condition. Since it was originally described, it has episodically been observed in commercial sized sea scallops on the eastern coast of the United States (Stokesbury et al., 2007; Levesque et al., 2016). Stevenson (1936), described darkened, gray, stringy meat from sea scallops from the Bay of Fundy, which he thought was linked to senescence. Thirteen years later, Medcof (1949) described a ''darkened meat" condition in sea scallops off Digby, Nova Scotia. He

concluded that it was due to chronic infestation of older scallops by boring sponges (*Cliona* sp.). Furthermore, Medcof (1949) reported that as the meat became darker, the meat yield decreased, and suggested that it might be due to energy transfer from growth to shell structure repairing. In 1979-1980, a mass mortality event associated with "gray meat" was experienced in sea scallops in Narragansett Bay, Rhode Island. Prokariotic infections were observed and believed to be the cause (Gulka et al., 1983). Furthermore, Stokesbury et al. (2007) reported "gray meat" in adult scallops during a mass mortality in Nantucket Lightship Closed Area (NLCA) between 2004 and 2005. The cause for this event was believed to be a synergistic effect of senescence and parasitism by shell borers and prokaryotic infections. After a three-year fishing closure, a large numbers of discolored "gray and brown meat" scallops were observed in the Closed Area 1 (CA1) rotational scallop management area of Georges Bank in 2011. These scallops were discarded by fishermen due to low meat yield, and the low market value of the discoloured and stringy meat. Similarly, due to high numbers of "gray meat" scallops, fishermen were only able to collect 32% of their total allowable catch in the CA1 access fishing area in 2013 (SAW 59, 2014). As the adductor muscle is the most commercially valuable part of the scallops, comprising around 33% of the somatic tissue and 10-18% of the total weight in this scallop species (Mottet, 1979), the "gray meat" phenomenon has had a huge impact on the Atlantic sea scallop fisheries in eastern USA.

The term "weak meat" is analogous to "gray meat". The only real difference is that it is used for an abnormal muscle condition in weathervane scallop, off the Alaskan coast (Brenner et al., 2012). Similar to "gray meat" in sea scallop, it has had severe negative impact on commercial fisheries of the weathervane scallop, which have been conducted in Alaska since 1967 (Brenner et al., 2012). From the start, poor scallop adductor muscle quality, characterized by off-white to greyish colour, tissue that tears easily during shucking with notable stringy texture, and a spongy consistency, have been observed (North Pacific Fishery Management Council, 2008; Brenner et al., 2012).

Similar condition was observed in the Faroese queen scallop population in the mid-2000s and beyond, where signifant proportion of the population showed signs of abnormally reduced and discoloured adductor muscles. That lead to an examination of such scallops with regard to infectious diseases; a request from the scallop fisheries sector in the Faroe Islands (a part of this thesis).

Macroscopic changes similar to those observed in the Icelandic scallop populations have been reported from other populations of Iceland scallop as well as in other scallop species from different geographic areas, including the

Barents Sea. In 2006, a disease only affecting mature larger Iceland scallop, was reported from Svyatoy Nos, Russia. The macroscopic changes were dull grey coloured adductor muscles and changes in colour of the gonads. Histopathological investigation performed showed severe necrotic changes in the mantle, adductor muscle and gonads (ICES, 2006). At that time, the associated mortalities had not been determined and to the best of knowledge, the cause for this condition is still unknown.

1.8 Other mass mortality events of bivalve populations

1.8.1 Scallop mortalities

In addition to the Icelandic- and "gray meat" epizootics, a number of mass mortality events exists in wild scallop populations, most of which unresolved. Commonly, these mortalities have been attributed to increasing sea temperature and/or overfishing. However, these conclusions are in many cases highly speculative and the potential role of infectious diseases not even considered, as no examinations were performed (Wiborg, 1963; Eiríksson, 1986; Garcia, 2006). In addition to the mass mortality of Iceland scallop in Hvalfjördur in SW Iceland in 1983, which was briefly discussed in section 1.2.4 (Eiríksson, 1986), other scallop beds around Iceland have suffered similar mass mortality episodes. In 1985, previously unknown scallop beds were found in several fjords and bays in eastern Iceland (Eiríksson, 1986) and subsequently fisheries were conducted in that area in 1985 and 1986 (Jónasson et al., 2006). However, a research survey in 1998 revealed that these previously commercially valuable scallop beds only consisted of dead shells (Gudfinnsson & Gunnarsson, 2001). In both the above-mentioned mortality events, the reasons remain unknown. In neither cases, the possible role of infectious agents were considered, as no such research was performed. Similar mass mortalities events exist from other northern areas. Wiborg (1963) reported an apparent extinction of numerous scallop populations in Norwegian waters; scallop beds that only consisted of empty shells (cluckers). He suggested that changes in environmental conditions, such as a sudden rise in sea temperature, were the cause. In the early 1980s, extensive beds of Iceland scallops had been discovered in the Barents Sea, in the Jan Mayen and Svalbard areas and the potential for scallop fisheries was considered to be very promising (Sundet, 1985; Garcia, 2006). However, Sundet (1985) noted that high percentages of dead scallop shells in the catches would make fishing methods less efficient. Surveys in this area in 1986 (Rubach & Sundet, 1987) indicated further favourable prospects for

scallop fisheries. However, as soon as 1987, some of the major scallop beds in the Barents Sea had already collapsed (Garcia, 2006), with the suggested cause of overfishing. A similar situation occurred in the scallop fisheries in Greenland, where massive declines or total losses of scallop beds were experienced, despite limited fisheries of about 10% of the stock size (Garcia, 2006). In addition, considerable decrease in gonad and muscle yield have also been reported from scallop populations around Greenland (Garcia, 2006). Mass mortality of scallop populations have also been reported from the NW-Atlantic Ocean, in both Iceland scallop and sea scallop stocks in Canadian waters. Despite some research effort, the causes for these abnormal mortalities could not be determined (Giguere et al., 1995).

1.8.2 The oyster epizootics in New Zealand

Mass mortality events have regularly been experienced in oysters, *Ostrea chilensis*, in Foveaux Strait, New Zealand. These events seem to follow a cyclical pattern, emerging every 20 – 30 years. A known pathogen, *Bonamia exitiosa*, was associated with these mortalities (Hine, 1996, 2002). Later it became evident that an apicomplexan species played a significant role in these events (Hine, 2002). This apicomplexan is also known to infect two mussel species in New Zealand, *Mytilus galloprovincialois* and *Perna canaliculus*, and is to date considered one of the most serious pathogens of bivalves in New Zealand waters (Diggles et al., 2016).

The mass mortality event among *O. chilensis* in Foveaux Strait, New Zealand during the years 1985-1993, is probably the most severe one related to apicomplexan infections in bivalves. Over this period, a co-infection of this apicomplexan and *Bonamia exitiosa* reduced the population of commercial-sized oysters by 91% (Hine, 2002). Hine (2002) concluded that the apicomplexan infection had a significant contribution, both directly but also by increasing the susceptibility of wild flat oysters to infection with *B. exitiosa*. It primarily infects connective tissues, causes severe pathology and may hamper gonad development (Hine, 2002; Webb, 2008; Diggles et al., 2016). It is morphologically different to any other apicomplexan identified in molluscs (Hine, 2002). Although it is still an anonymous species, a recent paper describes the phylogenetic position of this apicomplexan. It does not group with any known mollusc pathogens, its sequences forming the nearest sister lineage to the clade consisting of *Toxoplasma gondii*, *Hepatozoon catesbianae* and *Adelina bambarooniae*, though with weak homology support (Suong et al., 2017). As only one of the developmental forms of the parasite is detected in the oyster, it probably has an alternative host, which is presently unknown (Hine, 2002; Suong et al., 2017).

High density of the oysters is the most commonly named driver of these epidemics. However, some scientists thought that other factors played a major part, e.g. direct effect of fisheries (fishing mortality) or indirect ones due to a stress causing distruption of bivalve habitats and alteration of the benthic communities interfering with normal interspecies interaction of the species that inhabit it (Anonymous, 2006).

2 The present study – background and objectives

2.1 Background

The driver of this project was the collapse of the Iceland scallop population in Icelandic water in the beginning of the 2000s (discussed in chapter 1.2.4.). Over a period of three years, i.e. 2000-2003, the population declined by 73% and reached its minimum size of 13% few years later, compared to its mean size in the 1990s (Marine Research Institute, 2004, 2010). This collapse was sudden, its magnitude unprecedented and no obvious reasons in hand. In the autumn of 2002, the first scallops were brought to the Fish disease Laboratory at the Institute for Experimental Pathology at Keldur, University of Iceland, for diseases examination. Following initial examination and the observation of presumable disease causing agents, a 10 years project, including both further examinations of the observed infecious agents as well as disease monitoring of the scallop´s stock, was formed. This monitoring has, however, exceeded the original 10 years planned, and has annually been performed up to present.

In 2004, I was contacted by the Faroese Fisheries sector as similar clinical signs, typical for the scallops from the collapsed Iceland scallop, had been observed in queen scallops in Faroese waters causing significant reduction in the commercial value of this species.

To examine further the presence of this apicomplexan, two scallop species, queen- and king scallops, were sampled from Scottish waters, initially in the autumn of 2007 and again in 2016.

The project was further extented in 2013, when a collaboration with scientist from the University of Massachusetts began. That was due to periodic mass mortality events associated with high prevalence of clinical signs, termed “gray meat” (see chapter 1.7. above) in sea scallops of the eastern coast of North America, which were apparently analogous to those observed in the collapsed Iceland scallop stock.

2.2 Objectives

The main objective of this study was to identify infectious agents associated with the collapse in the Iceland scallops stock in Breidafjördur in Iceland.

- To identify and thouroughly describe infectious agents observed in Iceland scallops, determine their pathogenicity and analyse long-term data to evaluate their potential role in the collapse of the scallop stock.
- To examine their host specificity and geographic distribution in different species of molluscs.
- To analyse the impact of the observed infectious agents on other scallop populations, some of which experiencing mass mortalities associated with abnormally reduced and discoloured adductor muscles, similar to the Iceland scallop population in Icelandic waters.
- To resolve the transmission and life cycle of the parasites observed.
- To determine the phylogenetic position of the observed apicomplexans among known but related species from vertebrates and invertebrates.

3 Material and methods

3.1 Research material

In the whole study, 1782 mollusc individuals were examined. Of those, 1632 were scallops (i.e. 1502 Iceland scallops; 80 queen scallops; 50 king scallops and 50 sea scallops), 40 were whelks and furthermore, 90 individual belonging to two species of gastropods and three species of non pectinid bivalves, were examined.
A detailed summary of the numbers, sampling sites and -times of all species collected are shown in Tables 3.1.-3.4. and Figures 3.1.-3.3.below.

Table 3.1 Sampling times and –sites of all scallops examined from Breidafjördur in 2002 – 2016. Abbreviations: AU = autumn; SP = spring; SU = summer; NOV = November. See Figure 3.2. for more detailed location of sampling sites.

	Papers I & II	**Papers III & V**																			
Year	2002	2003	2004	2005		2006		2007		2008		2009	2010	2011		2012	2013	2014	2015	2016
Time	NOV	AU	AU	SP	AU	SP	AU	SP	AU	SU	AU	AU	AU	SU	AU	AU	AU	SP	AU	NOV
Site																				
11			74	50	57	61	60	50	50	60	57	46	31	24	30	30		30		
12.1			83	50	54	31	56	25												
12.2							26	40	55		59	14	30		30	30				
2											45									
32.2	60	10		22	5												38		15	
31													20							
33.2										60										
42	60																			15

Total number of scallops examined: 1502 individuals

*Table 3.2. Sampling times and –sites of queen- and king scallops (**Papers I & II**) and sea scallops (Paper IV). See Figures 3.1. and 3.3. for more detailed location of sampling sites. Abbreviations: CA1 = Closed Area 1; CA2 = Closed Area 2.*

	Papers I & II			**Paper IV**		**Paper V**	
Species	Queen scallops		King scallop	Sea scallop		King scallop	
Sampling site	Scottich water	Faroese waters	Scottish waters	US - Georges Bank CA1	CA2	Scottish waters	
Samping time	September 2007	February 2005	September 2007	Sept/Oct 2013 and April 2014		December 2015	June 2016
Number examined	10	60	10	25	25	10	10

Total number of scallops examined: 130 individuals

*Table 3.3. Sampling time and number of common whelks collected in Breidafjördur, Iceland (**Paper V**).*

Sampling time	June 2006	July 2014	November 2015	August 2016
Number examined	10	10	10	10

Total number of whelks examined: 40 individuals

*Table 3.4. Sampling time and number of other bivalves and gastropods examined from Breidafjördur, Iceland (**Paper V**).*

	Gastropods		Bivalves		
	Rejected neptunes *Neptunea despecta*	Dog whelk *Nucella lapillus*	Blue mussel *Mytilus edulis*	Horse mussel *Modiolus modiolus*	Ocean quahog *Arctica islandica*
Samping time	July 2014	June 2014	May 2006 June 2014	May 2006	July 2014
Number examined	10	10	30	10	30

Total number of other molluscs examined: 90 individuals

*Figure 3.1. Sites where scallops were sampled for **Papers I & II** (white circles); Breidafjördur in Iceland (Iceland scallop), the east coast of the Faroe Islands (queen scallops) and the west coast of Scotland (queen- and king scallops). Furthermore, the site off the northwest coast of Scotland sampled from a king scallop ranch for **Paper V** (red circle).*

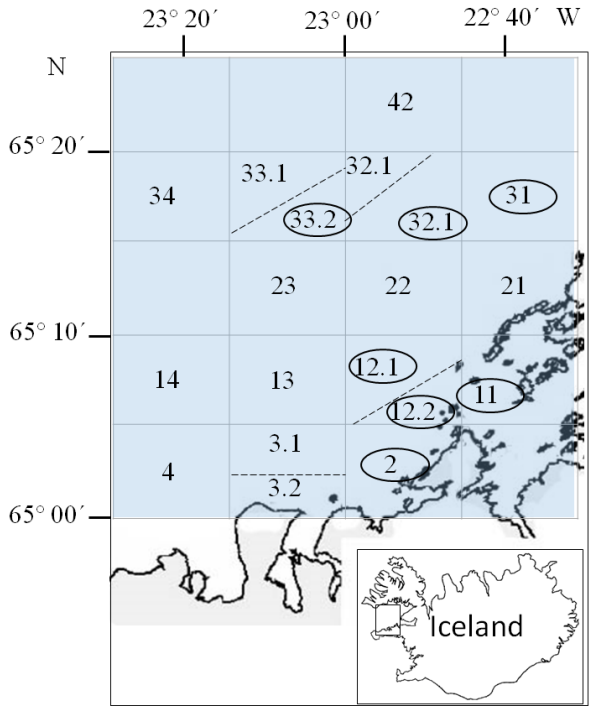

*Figure 3.2. Iceland scallops´ sampling sites in Breidafjördur in the years 2003-2016 (**Papers III & V**). The numbers represent the main scallop grounds in the bay and the encircled ones where samples were collected.*

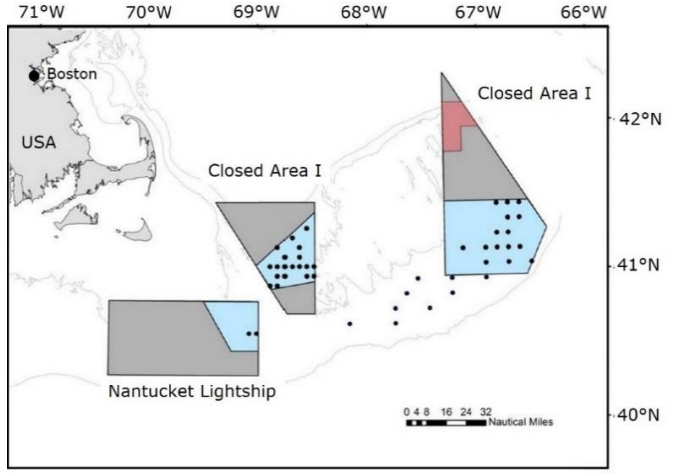

*Figure 3.3. Sampling sites of sea scallops in Georges Bank off the US east coast (**Paper IV**). The samples were collected within the blue zone of Closed Areas I and II.*

3.2 Methods

3.2.1 Dissection – gross observations

All scallops and whelks were brought live to the laboratory and held in seawater until examined. When dissected, the internal organs from all individuals were removed from the shells, the shell height measured (cm) for both mollusc groups and the wet weight (g) determined for gonads and adductor muscles of the scallops.

Macroscopic changes of the scallops´ adductor muscles were graded on the scale from 0–3 as follows:

Grade 0 = normal muscle, white and compact.

Grade 1 = muscle light coloured but less compact and with increasing fluid content.

Grade 2 = greyish/light brown coloured and loosely bound with high fluid content.

Grade 3 = dark grey or brown, very loosely bound with high fluid content and visible holes or hollow areas in the muscle when cut in half.

Subsequently, samples for various examinations were taken as described below.

3.2.2 Fresh preparations

Fresh mounts (**Papers I, II, IV & V**); samples from each organ pressed between a glass slide and a coverslip. Parasite forms detected were measured (µm) and photographs taken using Leica DMLB microscopes equipped with a digital camera (Leica DC300F and Nikon DS-Filc).

3.2.3 Histology

During dissection, all major organs, from both scallops and whelks, were fixed in Davidson's fixative for 48 h and subsequently dehydrated in 70% ethanol, embedded in paraffin wax, sectioned (4 µm), and stained with Giemsa and/or Hemotoxyllin and Eosin (HE), according to routine histological protocols.

Histopathological examination included evaluating the distribution and morphological characteristics of all developmental forms of the apicomplexans observed (**Papers I, II, IV & V**) and the histopathological effect of the infections (**Papers I, III, IV & V**) on scallops and whelks.

3.2.4 Imprints

Tissue imprints from the adductor muscles of the scallops were prepared (**Paper III**) by cutting them in half and pressing their inner side to a microscopic slide. Subsequently, the slides were air dried, fixed in methanol for 3 min, stained with May–Grünwald–Giemsa and mounted in resin-based medium. The intensities of infections were determined by calculating the mean number of apicomplexan zoites present in 10 microscopic fields from the tissue imprints at 250x magnification. Six of the fields were randomly selected, however due to uneven distribution of the parasite in the adductor muscle; four of the fields were selected where apicomplexan zoites had accumulated. The levels of infections were graded on a scale of 1–5 as follows:

Grade 1 = ≤ 20 zoites per microscopic field
Grade 2 = 21–50 zoites per microscopic field
Grade 3 = 51–100 zoites per microscopic field
Grade 4 = 101–200 zoites per microscopic field
Grade 5 = > 200 zoites per microscopic field

3.2.5 Molecular analysis

DNA extraction and amplification (PCR)

Freshly dissected, infected tissues were fixed in 95% ethanol for DNA analyses. Small (approximately 20 mg) parts from adductor muscles (**Papers III, IV & V**), heart auricles (**Paper I**) and kidney (**Paper V**) from scallops and whelks (**Paper V**), were used for DNA isolation. The total DNA was extracted using GeneMATRIX kit (EURx Poland) following the tissue protocol (**Papers I, III, IV & V**).
The apicomplexan small subunit ribosomal DNA (SSU rDNA) was amplified using several primer pairs, both general ones for initial amplification, and more specific ones designed from sequence alignments performed in CLUSTAL_x (Thompson et al., 1997) and/or published ones, in context with the initial reads to allow complete sequencing of the SSU rDNA (Table 3.5.).

*Table 3.5. Primers/primer pairs used for PCR amplification in the study (**Papers I, III, IV &V**).*

Primer names and pairing	Primer sequence	Reference
SFC-340f	5′ AGTTTCTGACCTATCAGC 3′	Present study
SFC-1260r	5′ TCAGCCTTGCGACCATACTC 3′	Present study
SC1-1185f	5′ TCACGATTTGACACTTTCAGC 3′	Present study
18 gM rev	5′ CTTCCGCTGGTTCACCTACG 3′	Freeman et al. (2008)
SC1-590r	5′ ACTCGTGTGAAGCTTTACTTCC 3′	Present study
18e fwd	5′ CTGGTTGATCCTGCCAGT 3′	Hillis & Dixon (1991)
18e fwd	5′ CTGGTTGATCCTGCCAGT 3′	Hillis & Dixon (1991)
SC2-1370r	5' TCCTTCATATGTCTGGCACTAG 3'	Present study
SFC-1120f	5' GAACGAAAGTTRGGGGMTCG3'	Present study
18 gM rev	5′ CTTCCGCTGGTTCACCTACG 3′	Freeman et al. (2008)
18e-Merf*	5′CTGCCAGTAGTTATACGT3′	Present study
Mer-790r*	5′ACACSCTTGAAGCACCCTAC3′	Present study

*Diagnostic PCR for *Merocystis kathae*

PCR conditions, sequencing and phylogenetic analyses

The conditions for all PCR reactions of **Paper I**, were as follows: initial denaturing 5 min at 95°C followed by 35 cycles of: 94°C for 30 sec, 55°C for 45 sec, 72°C for 1 min, with a terminal extension of 72°C for 7 min. PCR bands of the expected sizes were recovered from the PCR products using a GeneMATRIX PCR products cleanup kit (EURx Poland). For **Papers III, IV & V**, the conditions were as described above but used an annealing temperature of 64°C with an extension time of 30 sec.

Sequencing reactions were performed using BigDyeTM Terminator Cycle Sequencing chemistry utilising the same oligonucleotide primers that were used for the original PCRs. DNA sequencing was performed in both forward and reverse directions for all PCR products and nucleotide BLAST searches performed for each sequence to confirm an apicomplexan origin. Contiguous sequences were obtained manually using CLUSTAL_X and BioEdit (Hall, 1999).

For phylogenetic analyses, taxa were chosen from BLAST searches that had similarities to the novel sequences and additional apicomplexan sequences chosen to represent recognised clades for the groups. CLUSTAL_X was used for the initial sequence alignments with the settings for gap opening/extension penalties being adjusted manually to achieve optimum alignments. Regions of ambiguous sequence alignments were manually edited using the BioEdit sequence alignment editor.

Phylogenetic analyses were performed using the maximum likelihood methodology in PhyML with the automatic smart model selection (selection

criterion: Akaike Information Criterion (AIC)) (Guindon et al., 2010), typically running the general time-reversible substitution model (GTR +G6 +I) with 1000 bootstrap repeats. For the Bayesian inference analysis, models of nucleotide substitution were first evaluated for the alignment using MrModeltest v. 2.2 (Nylander et al., 2004). The most parameter-rich evolutionary model based on the AIC was the general time-reversible, GTR+I+G model of evolution. Therefore, the settings used for the analysis were nst = 6, with the gamma- distributed rate variation across sites and a proportion of invariable sites (rates = invgamma). The priors on state frequency were left at the default setting (Prset statefreqpr = dirichlet (1, 1, 1, 1)). Posterior probability distributions were generated using the Markov Chain Monte Carlo (MCMC) method with four chains being run simultaneously for 2,000,000 generations. Burn in was set at 2500 and trees were sampled every 100 generations making a total of 7,500 trees used to compile the majority rule consensus trees.

3.2.6 *In situ* hybridization (ISH)

ISH was applied to confirm the conspecificity of the unidentified scallop apicomplexan and the kidney apicomplexan, *Merocystis kathae*, from Iceland scallops and common whelks, *Buccinum undatum*, respectively. All major organs from 10 selected individuals of each mollusc host species were subjected to this methodology (**Paper V**). The methodology roughly followed Morris et al. (1999) and Holzer et al. (2003), with modifications (lower concentration of proteinase K). Histological sections, 7 µm thick, were dewaxed in series of alcohol and subsequently hydrated and permeabilized with 10 µg ml^{-1} proteinase K in Tris-buffered saline (TBS) pH 8 for 12 minutes at 37°C followed by a 2 x 5 min washing in PBS. Samples were then post-fixed in 0.4% paraformaldehyde in PBS for 15 min and subsequently washed for 2 x 5 min in distilled water. In order to prevent non-specific binding, sections were exposed to 10% hydrogen peroxide (H_2O_2) in methanol for 10 min and then washed in distilled water for 2 x 5 min. Following that, the sections were dried in 45°C for 10-12 min to be able to omit the time-consuming pre-hybridization step. Samples were enclosed with Frame-Seal™ (Bio-Rad, Sundbyberg, Sweden) chambers and equilibrated in hybridization buffer consisting of 100 µg ml^{-1} calf-thymus DNA, 1.5 ng ml^{-1} of each of two 5‘ biotin labelled oligonucleotide probes and 4 x saline-sodium citrate buffer (SSC) in TBS containing 0.5% Ficoll, 0.5% polyvinylpyrrolidone, 0.5% bovine serum albumin. The following two probes were used: 790r 5' ACACSCTTGAAGCACCCTAC 3' and SC2-1370r 5' TCCTTCATATGTCTGGCACTAG 3' (sequences from **Paper III**). The sections were sealed, denatured at 95°C for 4 min followed by a 60 min

hybridization at 45°C. Hybridization was followed by non-stringent and stringent washes with 2x SSC and SSC with 0.1% Tween 20 at 42°C, respectively. Signal detection was achieved using incubation with horseradish peroxidase-labelled streptavidin (Dako, Agilent Technologies, Glostrup, Denmark) for 20 min at room temperature followed by 3 x 5 min washing in PBS (pH 7.4) and visualized with a DAB Peroxidase Substrate (Vector Laboratories, Burlingame, USA). Haematoxylin was applied as a counterstain, after which sections were rapidly dehydrated in series of ethanol, transferred to xylol and mounted in resin based medium.

3.2.7 Transmission Electron Microscopy (TEM) and semithin sections

TEM was used to: (1) Examine the ultrastructural characteristics of the apicomplexan observed (**Paper II**). (2) Check for the presence of viral particles (**Paper III**).

For these examinations, small pieces (1 mm^3) of fresh adductor muscles, heavily infected with the apicomplexan and showing obvious clinical sign of these infection (**Papers II & III**), were fixed in 2.5% buffered glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 24 h at 4°C. Subsequently, the samples were washed three times in cacodylate buffer, post-fixed for 1 h in 1% OsO_4, rinsed again with buffer, dehydrated in graded alcohol series, sectioned, stained for 10 min in 5% uranyl acetate and for 6 min in 5% lead citrate.

Semithin sections (0.5 μm thick) were cut and stained with toluidine blue and mounted in resin-based medium. They were used both for conventional histological examination but also to select samples for TEM examination. From those selected samples, ultrathin sections were routinely prepared and examined using a FEI, Tecnai G2 Spirit Biotwin, Transmission Electron Microscope (at 120 kV) at the Institute of Aquaculture, University of Stirling, Scotland.

3.2.8 Handling of data (Paper III)

Fulton´s condition index was determined for adductor muscles (MI) and gonads (GI). Furthermore, to make the figures comparable to available data on gonad weight of 7–8 cm scallops in 1988–1990 (Thórarinsdottir, 1993), when the population was considered to be in a normal condition, the gonad weights of all fully mature scallops sampled in autumn and spring (≥ 5 cm; N = 1170) were extrapolated to shell size of 7.5 cm. These indices were calculated using the following formulas:

$$MI = 100\frac{MW}{SH^3} \qquad GI = 1000\frac{GW}{SH^3} \qquad GW = \frac{7.5^3 GI}{1000}$$

SH is the shell height (cm), MW is the wet weight (g) of the adductor muscles and GW is the wet weight (g) of the gonads.
Parasitological examination was performed on all 1493 scallops sampled. However, to be able to achieve the goals, three different approaches were applied: 1) Relationship of scallop size/maturity and infections. All scallops sampled in 2003-2006 (N = 637) were split into three size groups and compared, i.e. (i) immature scallops – shell height less than 4.0 cm (N = 166); (ii) premature and mature scallops – shell height between 4.0 and 4.9 cm (N = 100); (iii) all mature scallops – shell height 5 cm or more (N = 371); 2) Difference in infections between seasons. All mature scallops (≥ 5 cm, N = 227) from two selected sites (12.1 and 11 – see Figure 3.2. and Table 3.1.), sampled in spring (N = 114) and autumns (N = 113) in the years 2005-2006, were analysed; 3) Progress of infections and macroscopic changes and their relationship with the muscle and gonad indices (MI and GI) of all mature scallops (≥ 5 cm) sampled during 2003-2014 (N = 1218).

3.2.9 Data analysis and statistics (Paper III)

Terminology

Ecological terms are according to previous definitions (Bush et al., 1997). All statistical tests and plots were performed using RStudio (version 0.98.1062).

Statistical analysis

On plots and tests

The data were plotted using boxplots with notches, which provide an approximate 95% test of the null hypothesis that the true medians are equal: if the notches do not overlap, the medians could be described as statistically significant. However, as this is an insufficient way for hyphotesis testing, multiple groups were compared using ANOVA or the non-parametric Kruskal-Wallis test, after determining the normality of the data with the Shapiro-Wilk normality test. In cases of non-normality, a non-parametric test was applied, e.g. when comparing pairs, the non-parametric Wilcoxon test was used.
Grade of infection and microscopic signs were compared between three shell size groups using the non-parametric Kruskal-Wallis test.
Seasonal differences of macroscopic changes and the grades of infection were examined separately with the non-parametric Wilcoxon test.

Grades of infection was compared between grades of macroscopic changes with the non-parametric Kruskal-Wallis test

Muscle index was compared between grades of infection, using ANOVA and subsequently,

Gonad index was compared between grades of infection for each season, spring and autumn, using the non-parametric Kruskal-Wallis test.

When examining each grade of infection between seasons the non-parametric Wilcoxon test was applied.

Gonad index was compared between four groups of macroscopic changes using the non-parametric Kruskal-Wallis test.

4 Results summary

4.1 *Margolisiella islandica* n. sp. (Papers I & IV)

4.1.1 Occurrence and tissue distribution

Margolisiella islandica sp. nov. was found in the Iceland scallops examined from both two sampling sites, from which scallops were examined for the parasite. However, none of the queen scallops and king scallops from the Faroe Islands and Scotland or sea scallops from US waters, were infected. The prevalence of infections was high in the Iceland scallops (95-100%) from both locations and showed no pattern in relation to host size. As intracellular infections were only detected in the auricle, it seems to be the preferred organ of the parasite to develop. All life stages in the apicomplexan reproductive phases, i.e. merogony, gametogony and sporogony, were detected. Syzygy, the pairing of gametes prior to fertilization, was not observed.

4.1.2 Development in the host

Margolisiella islandica is a heteroxenous apicomplexan with all life forms, representing merogony (asexual forms), gamogony (sexual forms) and sporogony, occurring in the same host, i.e. the Iceland scallop. Trophozoites are found intracellular in the auricular endothelium (Figure 4.1. A) with developmental stages bulging from the hypertrophic host cells.
Merozoites of two sizes (6.0-7.0 x 2.8-3.2 µm and 12.0-13.0 x 3.0-4.0 µm), which most probably represent two different generations, were frequently seen in great numbers in fresh specimens (N = 2 x 60). The merozoites showed upright clockwise twirling motility, i.e. when the parasite is attached to the substrate by its posterior end, it produces a clockwise spinning. These merozoites derived from spherical meronts, 10 µm in diameter, which were commonly observed inside endothelial cells of the auricle in histological sections, giving rise to eight merozoites in a cruciform or rosette configuration (Figure 4.1. A & B).

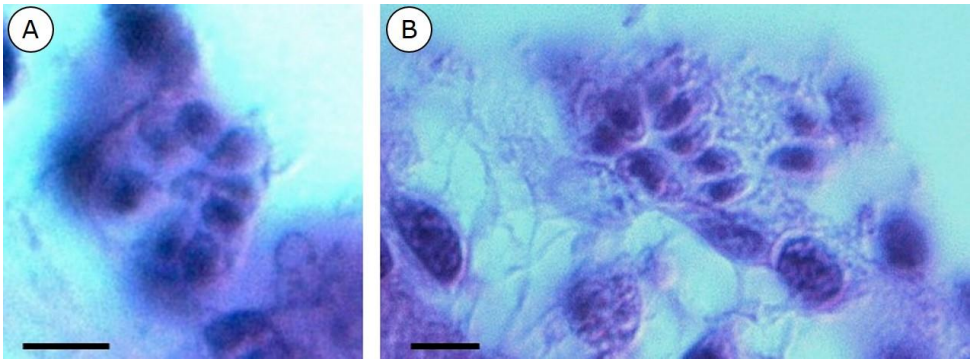

Figure 4.1. Merogonic stages of Margolisiella islandica*. Histological sections from a heart's auricle from a scallop showing* M. islandica *meront with eight developing merozoites arranged in a rosette like fashion (A) and a mature meront with merozoites (B). Scale bars = 5 µm. From* ***Paper I****.*

Gamonts were detected in high numbers; often free in the haemolymph, macrogamonts being much more numerious than microgamonts. Histological examination frequently showed growing gamonts inside endothelial cells of the auricle. Gamont-infected auricular endothelial cells were hypertrophied and appeared to rupture, releasing the parasites, but gamonts were also observed bulging from the endothelial cells. Macrogamonts had a coarsely granular cytoplasm and a large nucleus with a prominent nucleolus. Young macrogamonts were ellipsoidal but at maturity, they became pear- or heart shaped (Figure 4.2.). Live, fully mature macrogamonts measured 40-50 x 30-40 µm (N = 60). Microgamonts, spheroidal or ellipsoidal, with peripherally arranged nuclei, were seen in histological sections. Fully mature microgamonts measuring 30-40 µm (N = 20) had numerous microgametes which occasionally were seen bulging from the microgamonts.

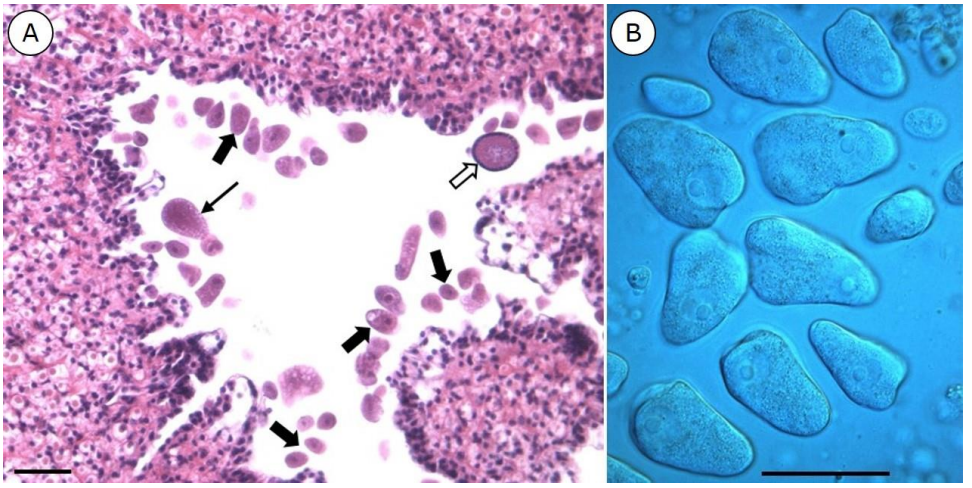

Figure 4.2. Gamogonic stages of Margolisiella islandica*. (A) Histological section through the heart's auricle showing numerous developmental forms of* M. islandica*; macrogamonts (broad black arrows), developing oocyst with peripherally arranged nuclei (white arrow), mature oocyst (thin black arrow). (B) Live specimens showing developing and mature macrogamonts with large nucleus and a prominent nucleolus. Scale bars = 50 µm. From* ***Paper I****.*

Oocysts were only seen free in the haemolymph. Live mature oocysts, ellipse or pear shaped, measured 48-60 µm in length and 40-44 µm at the widest part (N = 30). Each oocyst contained numerous (> 500) densely packed pairs of sporozoites (Figure 4.3. A & B), facing each other at the convex side (Figure 4.3. C), forming spherical sporocysts (live specimens) 5.5-6.5 µm in diameter enclosed with a thin and fragile membrane (N = 30). Ruptured oocysts were common, with sporocysts flowing out to the haemolymph (Figure 4.3. B & C). In such cases the sporocyst membrane commonly broke, the sporozoites detached and straightened out revealing their crescent-shaped appearance and its distinct centred nucleus (Figure 4.3. D). Live straightened sporozoites measured 8.5-10.0 µm in length and 3.0 µm at their widest part (N = 30).

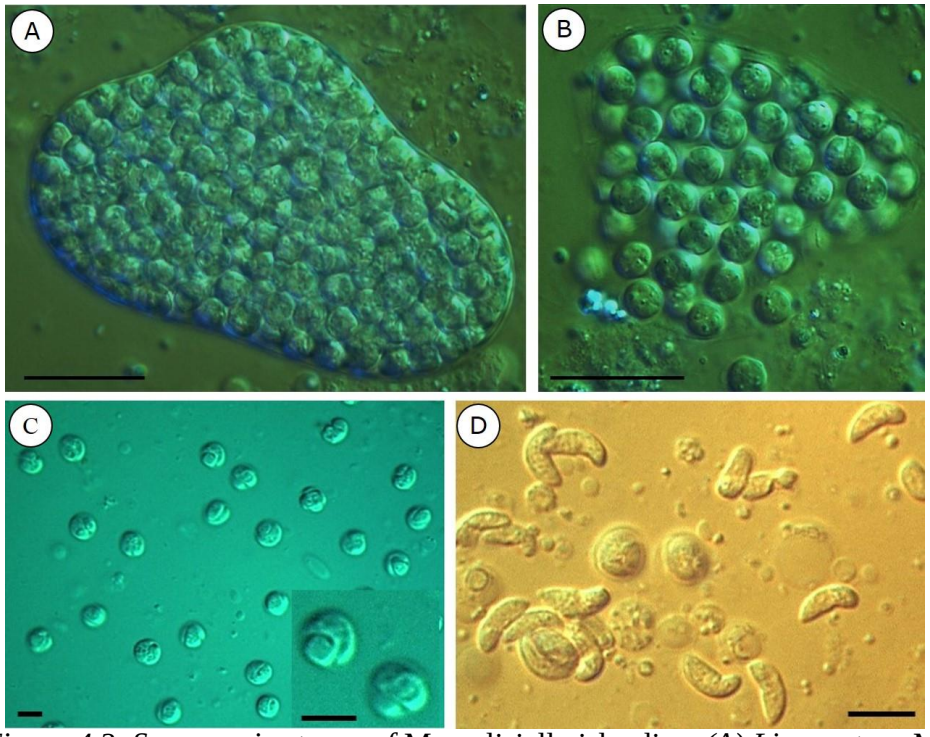

Figure 4.3. Sporogonic stages of Margolisiella islandica*. (A) Live mature* M. islandica *oocyst with numerous sporocysts. (B) Ruptured oocyst, which sporocysts are spreading into the haemolymph. Scale bar = 20 µm. (C) Live, free sporocysts in the auricular lumen. Insert: Sporocysts, higher magnification showing how sporozoites face each other at the convex side. (D) Live sporozoites liberated from broken sporocysts, straightened out revealing their crescent-shaped appearance and distinct centred nucleus. Scale bars: (A) & (B) = 20 µm; (C) = 5 µm; (D) = 10 µm. From* ***Paper I****.*

4.1.3 Pathogenicity

Exept for some focal hypertrophy and a subsequent rupture of infected cells due to the size of the parasite, *M. islandica* does not seem to have negative effect on the scallops´ health (unpublished data).

4.1.4 Molecular analysis and phylogeny

Complete small subunit (SSU) rDNA of 1773 base pairs was successfully amplified and sequenced for *M. islandica* sp. nov. and was deposited in GenBank under the accession number JN227668.

See further analysis in the chapter 4.4 below - Phylogeny of the observed species.

4.1.5 Taxonomic summary of *Margolisiella islandica* sp. nov.

Suborder: Eimeriorina Léger, 1911
Family: Eimeridae Michin, 1903
Type host: Iceland scallop, *Chlamys islandica* (Műller, 1776)
Type locality: Breidafjördur bay, W-Iceland.
Habitat/site of infection: Endothelium of the heart's auricle.
Etymology: The species name refers to both type locality and type host.

This summary is according to the presently acknowledged taxonomy for *Margolisiella* species.

4.2 *Merocystis kathae* (Dakin, 1911) - the whelk/scallop apicomplexan (Papers II, III, IV & V)

4.2.1 *Merocystis kathae* in the definite host; the common whelk (Paper V)

Infections; prevalence, intensity and associated macroscopic signs

Merocystis kathae was observed in all whelks examined from Breidafjördur in Iceland. Infections were commonly intensive. Gross clinical signs of infections were common and seen as small white spherical cysts in the whelks´ kidneys (Fig. 4.4. A and B).

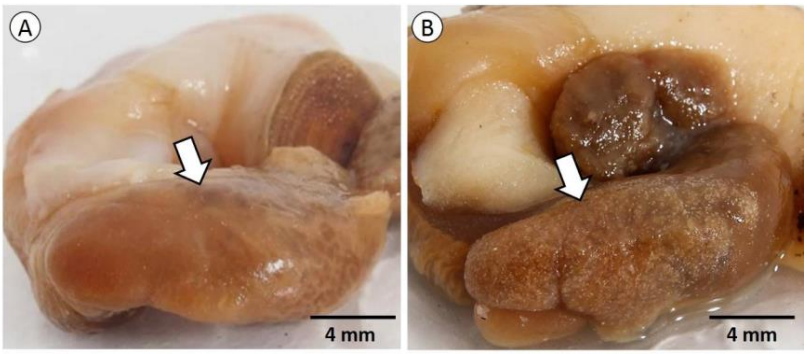

Figure 4.4. Macroscopic signs of Merocystis kathae *in the common whelk. (A) Normal, uninfected kidney of a common whelk. (B) Whelk kidney heavily infected with* M. kathae *characterized by numerous small white spherical cysts visible to the naked eye. From* ***Paper V****.*

Gamongony and sporogony; the sexual reproduction in the whelk

Exept for small forms detected by ISH in the intestinal epithelium, the distribution of the parasite in the whelks were restricted to the kidney in which all developmental stages, representing gamogony and sporogony, were observed. The smallest forms are trophozoites around 10 µm, intracellular in renal cells, which with further development grow into macrogamonts and microgamonts (Figure 4.5. A & B). Post fertilization, oocysts start to form, characterized by nuclear cleavage. At a certain point in development the nuclei are peripherally arranged (Figure 4.5. C & D). Further development involves formation of numerous sporoblasts (Figure 4.5. E & F) which eventually grow into sporocysts, each containing two sporozoites (Figure 4.5. G & H), the infective stage for the intermediate host, the scallop.

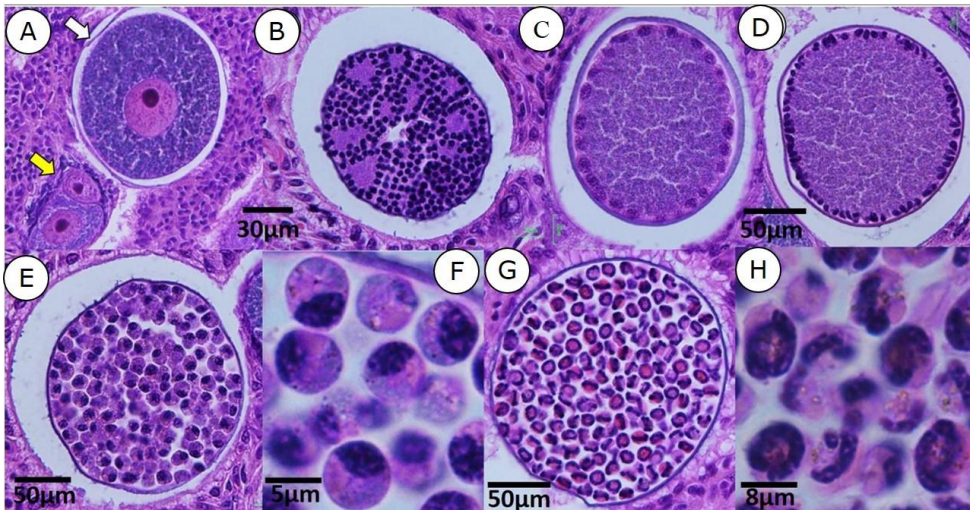

Figure 4.5. Gamogonic- and sporogonic stages of Merocystis kathae. *(A) Mature macrogamont (white arrow) and two growing intracellular trophozoites inside renal cells (yellow arrow). (B) Mature microgamont with numerous microgametes. (C - H) Sporogonic forms. (C & D) Immature oocysts with peripherally located nuclei. (E) Premature oocyst filled with numerous sporoblasts. (F) Higher magnification of sporoblasts. (G) A mature oocyst filled with sporocysts, each containing two sporozoites. (H) Higher magnification of disporous sporocysts. All sections are stained with H&E. From* ***Paper V****.*

Histopathology

Only minor histopathological changes are detected in *M. kathae* infected whelks, even in those with extensive infections. Regardless of infection status, the whelks appear to be in good condition. As an intracellular parasite, it causes some focal pathological changes in affected cells, i.e. the renal epithelial cells, which increase in size as the parasite grows and hence projects into the renal cavity or the underlying connective tissue. The host cell retains its position in the renal epithelium with no signs of penetration of the parasite into other host cells.

4.2.2 *Merocystis kathae* in the intermediate hosts; the scallops (Papers II, III, IV & V)

Infections and associated macroscopic signs (Paper III)

The presence of *Merocystis kathae* was confirmed in four different scallop species from several different geographic locations in the North-Atlantic Ocean, i.e. Iceland scallop from Icelandic waters, queen scallop from Faroese and Scottish waters, king scallop from Scottish waters and sea scallops from eastern US waters.

Iceland scallop (Paper III). All Iceland scallops, 4.0 cm and larger, were infected throughout the study while the prevalence in the juvenile ones (i.e. < 4.0 cm) was 70%. The intensity of infection in mature scallops (≥ 5.0 cm) was highest the first five years (2003-2007), followed by relativelely light infections the remaining years of the study, except for an increase in spring 2014.

Macroscopic signs associated with infections are predominantly characterized by grey/brownish coloured and greatly diminished adductor muscles. Reduced gonads were also commonly observed in heavily infected scallops (Figures 4.6. A & B).

Similar to the intensity of infections, the prevalences (36-90%) and grades of macroscopic signs in the adductor muscles of mature scallops were high over the years 2003-2007. A subsequent decrease in both the prevalence and severity of clinical signs was observed in the year 2008 (prevalence 8-16%). The remaining years of the study, clinical signs were light and in low prevalence (0-4%), except for a sudden increase (40%) of low-grade clinical signs in 2014.

Figure 4.6. Macroscopic signs in Iceland scallops associated with Merocystis kathae infections. (A) The first on the left is from 7.8 cm scallop which has severe macroscopic changes (grade 3; MI = 1.4), it is brownish coloured and greatly diminished and extensively infected while the first on the right is from 7.2 cm healthy looking one with firm and light coloured adductor muscle (MI = 2.5) which had a mild apicomplexan infection. The one in the middle is from a 7.5 cm scallop with grade 2 macroscopic changes (MI = 1.9). (B) An almost ripe female gonad from a healthy individual (upper) and a severely infected one with greatly reduced gonad. Both these gonads are from scallops of similar size and collected at the same time of year, i.e. spring when they should be close the maturity. From ***Paper III****.*

Significant differences in infection´s intensity and severity of macroscopic signs were observed, with regard to both shell size/maturity and season. The smallest shells (< 4.0cm) had the lightest infections and macroscopic signs never detected, whilst the largest ones were most severely infected and the prevalence and grades of macroscopic signs most severe. Mature shells (≥ 5.0 cm) collected in spring were significantly more intensively infected and with higher prevalence and grades of macroscopic signs, than those collected in the autumn.

Queen scallop (Paper II). All 60 scallops collected in Faroese waters were infected. Infections were commonly intense and macroscopic clinical signs common and in many cases severe. However, four of 10 queen scallops, collected from the west coast of Scotland in 2007, were lightly infected and with no clinical signs.

King scallops (Papers II & V). Nine of 10 king scallops, sampled on the west coast of Scotland in 2007, were infected. Infections were light, as in the queen scallops sampled at the same time and site, and no clinical signs

present (**Paper II**). However, 19 of 20 scallops from a virtually "whelk free" scallop-ranching site, on the northwest coast of Scotland in 2015-2016, were free of infections (**Paper V**) and merely few life stages of *M. kathae* detected in the remaining one.

Sea scallops (Paper IV). *Merocystis kathae* was detected in all scallops examined from Georges Bank, USA showing clinical signs in the adductor muscles, termed "gray/brown meat", and occasionally in those exhibiting normal (white) ones (Figure 4.7). In healthy scallops with white meat, the infections were either absent or very light. Conversely, moderate to severe infections were generally detected in all scallops with clinical signs (brown and gray meat), measured by the number of apicomplexan forms present per tissue (according to **Paper III**).

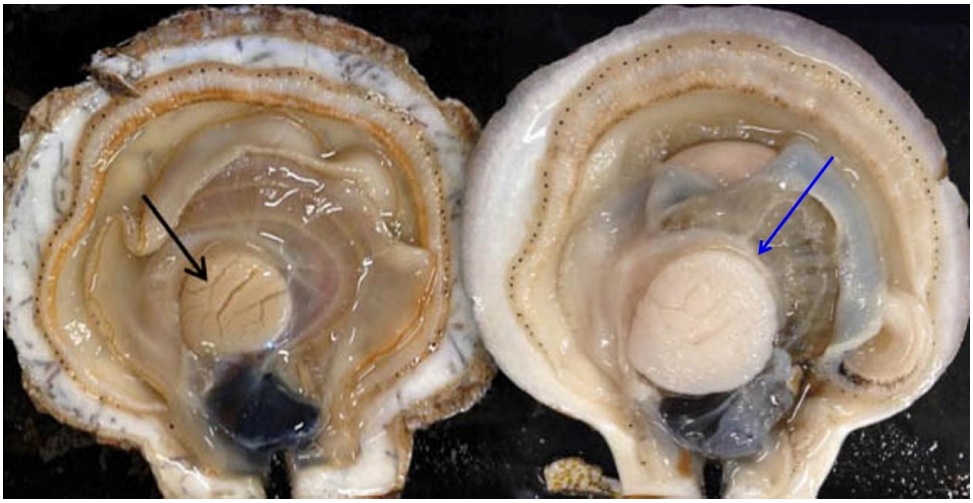

Figure 4.7. Macroscopic signs in sea scallops associated with Merocystis kathae *infections. Infected scallops are characterized by gray/brownish coloured and greatly diminished adductor muscles (black arrow) compared to a normal looking adductor muscle (blue arrow). From* ***Paper IV****.*

Distribution in the scallop hosts (Papers II, III, IV & V)

Merocystis kathae is found in more or less all organs of all the four scallop species. However, apart from the initial infective forms originating from whelks, which are detected in the intestinal epithelium, the presence of *M. kathae* in the scallops is restricted to muscular and connective tissues. Infective sporozoites are widely distributed in all muscular tissues, both striated (including the heart muscle) and smooth, and connective tissues, both intracellular in haemocytes and in the extracellular matrix. Aggregations of

both fully mature sporozoites, but also to some extent sporoblasts (immature sporozoites), were frequently found in loose- and muscular connective tissues surrounding the whole gastrointestinal tract, from the buccal cavity,

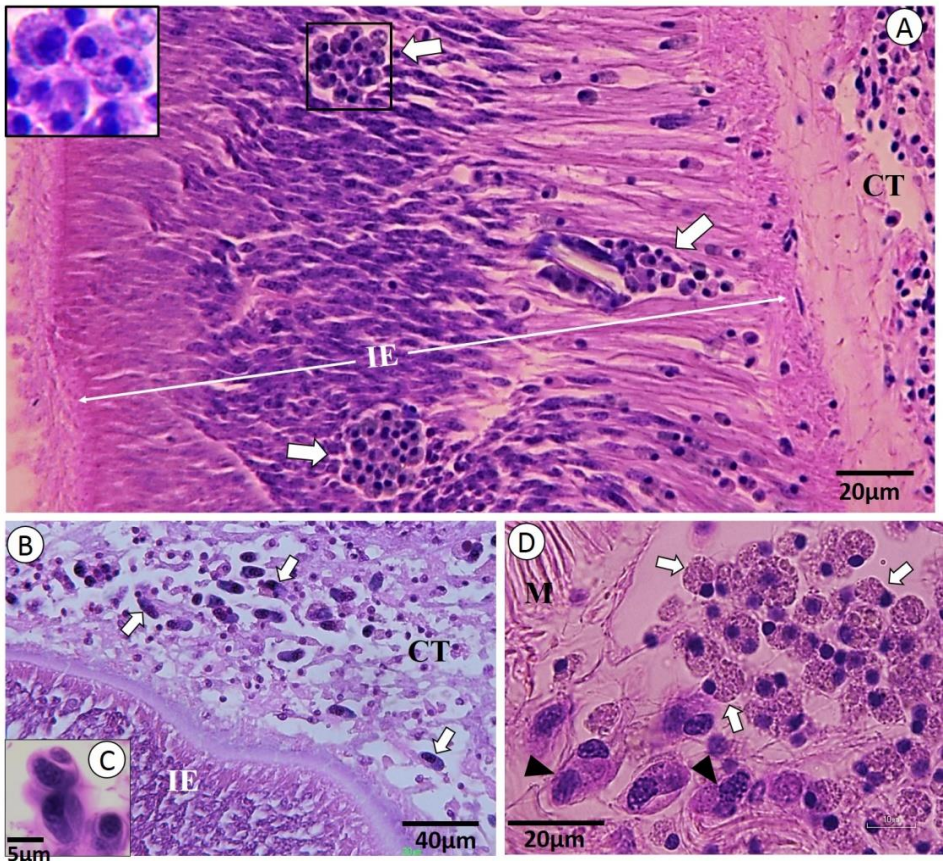

Figure 4.8. Initiation of Merocystis kathae *infections in Iceland scallop. (A) Histological section through the intestinal epithelium (IE) of Iceland scallop showing clusters of sporoblasts (arrows) of* M. kathae *entering the connective tissue (CT) adjacent to the epithelial lining. Inserted picture: Higher magnification of the sporoblasts. (B) Numerous sporozoites (arrows) which have entered the connective tissue between the intestinal epithelium (IE) and the digestive gland of the Iceland scallop. (C) High magnification of two sporozoites inside haemocytes. (D) Cluster of sporoblasts (white arrows) and sporozoites (black arrowheads) in the adductor muscle of an Iceland scallop. From* ***Paper V****.*

oesophagus, stomach and throughout the intestine (Figures 4.8. A-D) as well as in both inter-acinal and peri-gonodal connective tissues of the gonads.

Similarly, the connective tissues surrounding the epithelial lining of the primary and secondary ductus, which connect the stomach to the part of the digestive gland harbouring the digestive cells themselves, were heavily infected as was the interstitial connective tissue surrounding the digestive cells. Furthermore, sporozoites were found in inter-tubular connective and fibromuscular tissues of the kidney, muscular tissues in the base of the gill lamellae and in the quite variable mixture of tissue types making up the mantle, foot and labial palps (i.e. muscle fibres, loose connective tissue, muscular connective tissue and fibromuscular tissues).
Cysts, i.e. the asexual merongonic stages, were exclusively found in muscular tissues, predominantly in the adductor muscles.

Life stages in the scallops (Papers II, III, IV & V)

The infective life stage – the sporozoites
As described above, the gamogonic phase, which end-product are infective sporozoites, predominantly occurs in the whelk. However, it appears that some level of the maturation of the sporozoites occurs also in the scallops. These are the infective stages, which are very abundant in the scallop hosts (**Papers II, III, IV & V**).
Live sporozoites measure 17.5 ± 2.0 x 6.5 ± 1.5 µm (N = 100), the size range most frequently encountered being 18-19 x 6.5-7.5 µm. They are slightly curved and with a distinct and large nucleus (Figure 4.9.) (**Paper II**).

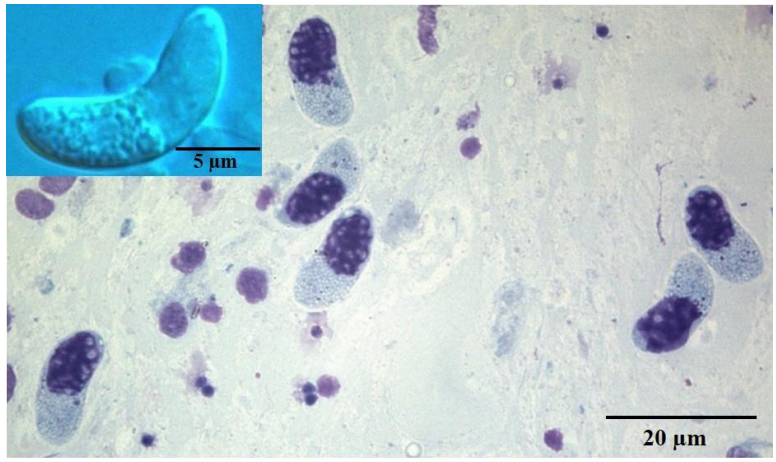

Figure 4.9. Merocystis kathae *sporozoites in a scallop host. May-Grűnwald-Giemsa stained imprint showing six infective sporozoites in a scallop host. Insert: Higher magnification of a live single sporozoite. From* ***Paper II****.*

Ultrastructural examination of the sporozoites, using transmission electron microscopy, showed that they contain all major structures characterizing apicomplexan zoites (Figure 4.10. A & B). A large and round nucleus is located in the posterior half of the parasite occupying almost the whole width of the cell and virtually half of its length. The cell boundary, the pellicle, consists of an outer unit membrane and an inner layer, composed of two unit membranes closely attached to one another. The outer unit membrane and the inner membranous layer are separated by an intermediate osmiophobic space. Approximately 80–85 sub-pellicular microtubules extend from the anterior margin of the nucleus and to the outermost front of the cell. The conoid was detected but not the polar rings. Micronemes typically span from the apical complex to near the anterior surface of the nucleus, but occasionally posterior to the nucleus. Rhoptries are clearly seen and the Golgi cisternae were observed near the anterior surface of the nucleus and the endoplasmic reticulum, between the nucleus and the apical complex. Thick-walled structures are in the anterior part of the cell, which could possibly be apicoplasts. Mitochondria are present at various placements in the cell and occupy a large space in the cytoplasm. Amylopectin granules are present in large numbers but almost exclusively in the anterior part (**Paper II**).

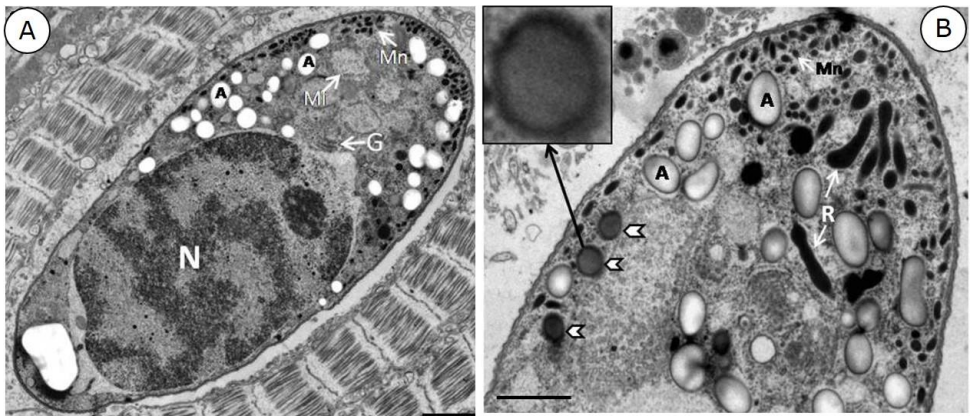

Figure 4.10. Ultrastructure of Merocystis kathae *sporozoite. (A) Longitudinal section through a sporozoite in the adductor muscle showing many of the major components of a zoite, e.g. a nuclues (N), Golgi apparatus (G), mitochondria (Mi) and micronemes (Mn). (B) Section through the anterior part of a sporozoite showing rhoptries (R), micronemes (Mn), amylopectin granules (A) and a thick walled structure (arrowhead), possibly apicoplasts. Scale bars: (A) = 2 µm, (B) = 1 µm. From* ***Paper II****.*

Merogony; the asexual reproduction in the scallops (Papers II & V)

The initiation of merogony is detected by the presence of young trophozoites developing inside an adductor muscle cells (Figure 4.11.)

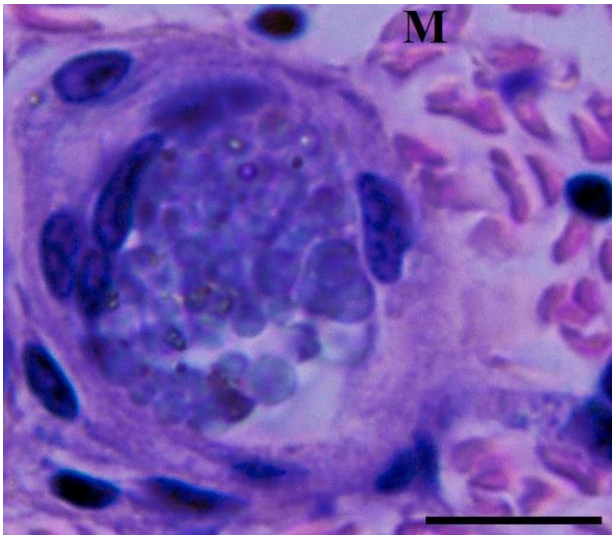

Figure 4.11. Initiation of the merogonic phase of Merocystis kathae *in scallops. A young trophozoite developing inside an adductor muscle cell, which subsequently develops into an early meront. M = muscle fibres. Scale bar = 10 µm. From* ***Paper V****.*

The trophozoites grow in size and turn into immature meronts (Figure 4.12. A), followed by a series of nuclear cleavages (Figures 4.12. B & C) and a formation of a premature multinucleated meronts (Figure 4.12. D), which subsequently develop into mature meronts with numerous merozoites (Figure 4.12. E-H). Two generation of merozoites are produced originating from two types of meronts. Type I, size range: 300 ± 25 µm x 75 ± 20 µm (Figure 4.12. E), with shorter merozoites and with both ends somewhat pointed (Figure 4.12. F) and type II, size range 200 ± 80 µm x 75 ± 20 µm (Figure 4.12. G), with convex, more slender and sausage-shaped merozoites (Figure 4.12. H). The latter generation of merozoites are the infective stage for whelks (**Papers II & V**).

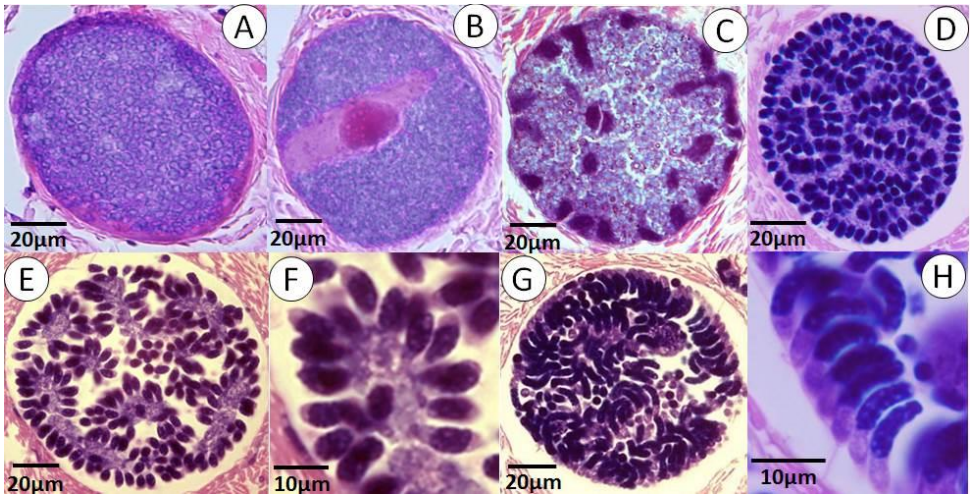

Figure 4.12. Merogonic stages of Merocystis kathae *in the adductor muscle of Iceland scallop. (A) Premature meront. (B) A meront with a spindle-like apparatus indicating the initiation of nuclear cleavage. (C and D) Further development of a meront characterized by further nuclear divisions. (E-H) Two different types of meronts representing two generations of merozoites. (E) Mature type I meront with numerous merozoites arranged in a rosette like fashion. (F) Higher magnification of merozoites from image (E). (G) Mature type II meront with numerous merozoites, more convex and slender in appearance and differently arranged compared to type I meronts. (H) Higher magnification of merozoites from image (G). All sections are stained with H&E. From* ***Paper V****.*

Effect on the condition of mature scallops (Papers III & IV)

Iceland scallop (Paper III). Infections severely affected the condition of both the adductor muscle and the gonads (using Fulton´s condition index; MI = Muscle index and GI = Gonad index).

A highly significant positive relationship was observed between grades of infections and macroscopic signs in the adductor muscles (Figure 4.13. A). Furthermore, a significant negative relationship was observed between grades of infections and the MI (Figure 4.13 B.). Similar to the MI, the GI decreased with higher grade of infection, both for scallops sampled in spring and autumn (Figure 4.14.).

The gonad weights (extrapolated to 7.5 cm) of all scallops, were compared with analogous data from 1988-1990 (Thórarinsdóttir, 1993) when the scallop stock was considered to be normal. The comparison showed that the gonad wet-weight in 2003-2006 was much lower than the normal gonad

weight, both in spring and autumn. In spring 2007, the weight was approaching normal and from autumn 2007 and until the end of the study it was within a normal range.

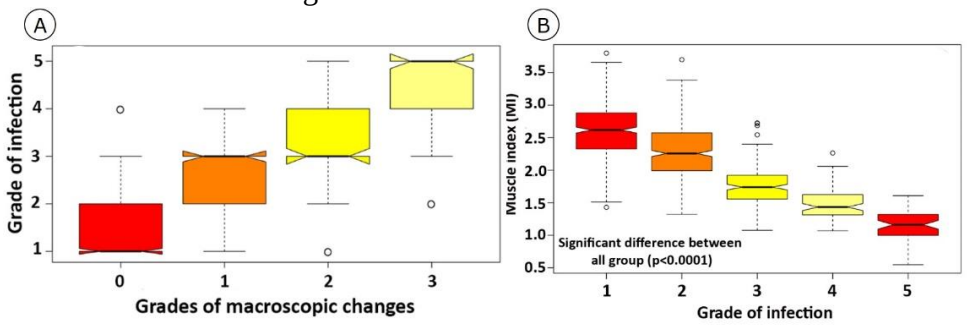

Figure 4.13. The effect of infections on muscle condition of Iceland scallops (N = 1218). (A) The relationship of the grades of infections with Merocystis kathae *and macroscopic signs in the adductor muscle of Iceland scallops. (B) The relationship of the MI and the grades of infections from all mature scallops examined. The MI deceases significantly with increasing grades of infection (p < 0.0001 between all grades). From* ***Paper III****.*

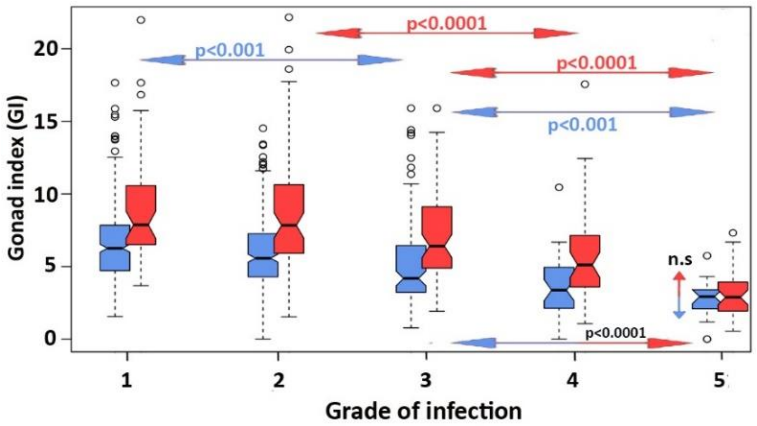

Figure 4.14. The effect of infections on the condition of gonads of Iceland scallops. The relationship of the GI and the grades of infections from all mature scallops sampled in the spring (red boxes; N = 297) and autumn (blue boxes; N = 794). A reduction in the GI is apparent, especially in the spring (red double arrow line). Note that in spring the GI should be much higher as these shells should be close to full maturity. However, scallops with grade 5 infections are not significantly different between autumn and spring. Furthermore, the GI for grade 5 spring scallops is significantly lower than the one for grade 1–3 autumn scallops (blue/red double arrow line). From ***Paper III****.*

Queen scallops (Paper II). Most of the scallops from the Faroe Islands appeared in suboptimal condition. As previously described in previous section, infections were commonly intense and macroscopic clinical signs common and often severe. Because these examinations were just based on a single sampling, no basis was for further analysis. The queen scallops, collected from the west coast of Scotland in 2007, were all in good condition and had very lightly infected and no clinical signs present.

King scallops (Papers II & V). No indications were of abnormal condition of king scallops sampled in Scottish waters.

Sea scallops (Paper IV). As noted above, scallops exhibiting clinical signs, termed "gray and brown meat" were commonly severely infected with *Merocystis kathae*, while the assymtomic ones were either uninfected or with light infections.
The occurrence of "gray meat" was weakly correlated with shell height only explaining 8.49% of the variance in a generalized additive model (GAMS). However, "gray meat" weights were significantly lower than "white meat" and had a dramatic reduction in protein and carbohydrate content as well as an increase in moisture (Table 4.1.), associated with extensive myodegeneration.
No correlation was observed between the reproductive stage and the "gray meat" condition. However, analysis of "gray meat" samples, collected from the NLCA during August, showed that they had significantly ($p < 0.05$) lower gonad somatic index than those from normal white adductor muscles.

Table 4.1. Proximate composition (mean % wet weight ± SD) of adductor muscle in Atlantic sea scallops from Georges Bank. There was a significant reduction in % protein and carbohydrate and inverse increase in moisture content in "brown and gray" meat compared to white meat scallops N = 88; (ANOVA, $p < 0.05$). M:P = Moisture vs protein ratio. From ***Paper IV****.*

Analysis	White (N = 33)	Brown (N = 26)	Gray (N = 29)
Moisture	77.86 ± 2.56	80.82 ± 2.33	90.33 ± 3.05
Protein	17.68 ± 1.68	14.81 ± 2.57	6.97 ± 1.01
Carbohydrate	2.56 ± 0.87	0.62 ± 0.92	0.08 ± 0.76
Ash	2.90 ± 0.24	3.67 ± 0.14	2.77 ± 0.14
Lipid	0.08 ± 0.02	0.08 ± 0.01	0.03 ± 0.01
M:P	4.40 ± 0.89	6.46 ± 1.36	12.96 ± 4.20
M:P = Moisture vs protein ratio.			

Histopathological changes associated with *Merocystis kathae* infections (Papers III & IV)

Iceland scallop (Paper III). Histopathological changes were restricted to the presence of apicomplexan zoites and no pathology was associated with cysts, i.e. the meronts. Some degree of histopathology was observed in all organs. In healthy looking scallops the histopathological changes were minor, mostly affecting the adductor muscle in which focal necrosis was present in close vicinity to the apicomplexan zoites. However, scallops with clinical signs in the adductor muscle, had severe histopathological changes. Apicomplexan zoites were found in clusters either intracellular in muscle fibres, extracellular and associated with necrotic muscle cell debris or in the endomysium, a connective tissue surrounding the muscle cells. Striated and smooth muscles were equally affected. A destruction was evident in large parts of the adductor muscle in the form of extensive liquefactive necrosis, a loss of striations, muscular fragmentation and hyalinization (Figure 4.15. A & B). Pathological changes were common in the heart´s ventricle, associated

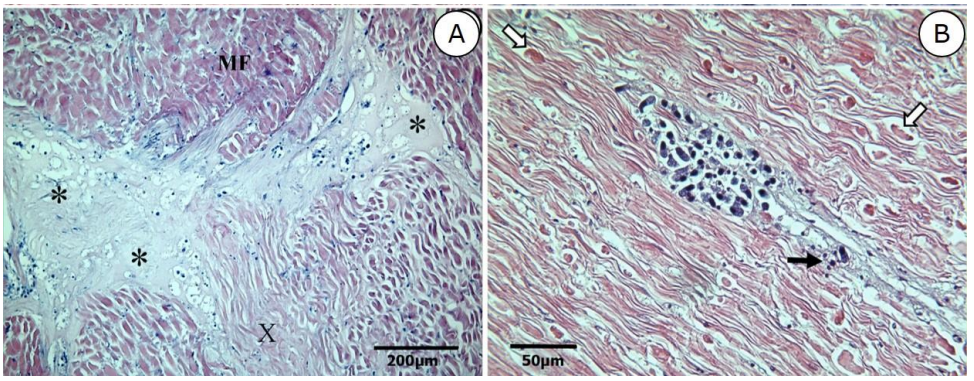

Figure 4.15. Histopathological changes observed in the muscles of Iceland scallop due to Merocystis kathae *infection. (A) Severely affected muscle with loss of striation in degenerating muscle fibres (X) and liquefactive necrosis of large areas (*). (B) A cluster of apicomplexan zoites causing focal necrosis (black arrow), fragmentation, and hyalinization (white arrows) of the surrounding muscle. MF = Normal muscle fibres. From* ***Paper III****.*

with an accumulation of apicomplexan zoites, especially in the myocardium and to some extent, in the epithelial and connective tissue layers of the epicardium. Degeneration of cardiac muscle fibres was common, characterized by loss of striation followed by nuclear degeneration and substantial necrosis.

Severe histopathological changes were associated with aggregations of parasite zoites in loose- and muscular connective tissues surrounding the gastrointestinal tract, from the buccal cavity, oesophagus, stomach and throughout the intestines. Similarly, the connective tissues surrounding the epithelial lining of the primary and secondary ductus, which connect the stomach to the part of the digestive gland harbouring the digestive cells themselves, were heavily affected. The apicomplexan infections were both intracellular in haemocytes and in the extracellular matrix and commonly causing total destruction of these tissues leading to a separation of the basement membrane from the gastrointestinal epithelium. Focal or disseminated necrosis was commonly observed in the interstitial connective tissue surrounding the digestive cells (Figure 4.16. A). In the most severe cases, the epithelial layer surrounding the intestine and the surrounding digestive cells was almost completely destroyed.

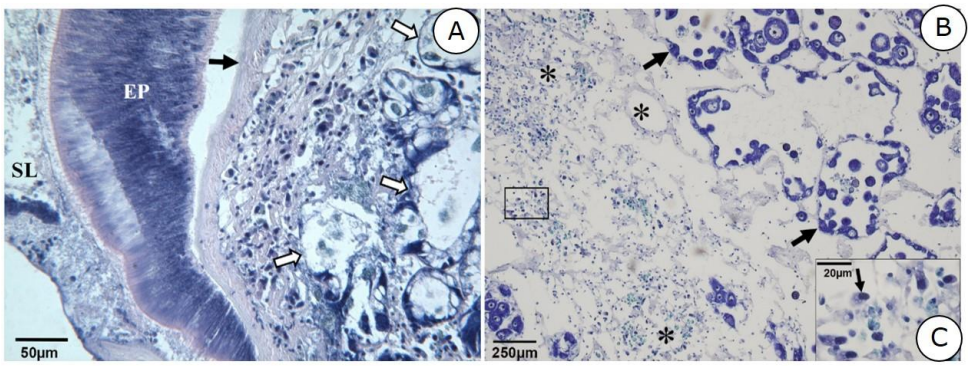

Figure 4.16. Histopathological changes in the digestive gland and gonads of Iceland scallop due to Merocystis kathae *infections. (A) Sections through the stomach and the digestive gland of a heavily infected scallop showing extensive accumulation of apicomplexan zoites in connective tissues surrounding the stomach epithelium and in the digestive gland interstitium causing separation of the basement membrane (black arrow) from the stomach epithelium (EP) and necrosis of the connective tissues and digestive cells (white arrows). (B) An ovary where the inter-acinal tissues are massively infected with apicomplexan zoites which have caused a total destruction of the acini in large areas (*). The remaining acini have merely premature eggs (arrows) and their development asynchronous with time of year. (C) Higher magnification of the area within the black square of (B) showing apicomplexan zoites (arrow). EP = Epithelial layer; SL = Stomach lumen. From* ***Paper III****.*

Extensive aggregations of parasites were routinely observed in both inter-acinal and peri-gonodal connective tissues of the gonads causing disruption in the inter-acinal tissues and a destruction of the wall enclosing the acini, which subsequently lead to a degeneration of primordial germ cells. In addition to the observed destruction of large parts of the gonads (Figure 4.16. B & C), the development of the remaining parts of the gonads was asynchronous with season/time, i.e. gonads of scallops harvested in May, when they should be almost fully mature, were only at the initial stages of maturity, normally observed in autumn.
Although parasites were normally found in inter-tubular connective and fibromuscular tissues of the kidney, they were commonly lightly affected. Occasionally, some pathology was evident, associated with an infiltration of parasites and haemocytes in the kidney interstitium. These pathological changes were characterized by vacuolization and necrosis of the tubular epithelial cells.
The gills were generally lightly affected by the apicomplexan parasite. Although infections were commonly detected, they were characterized by isolated parasites, or groups of a few apicomplexan zoites, which were more or less restricted to muscular tissues at the base of the gill lamellae. The associated pathology was normally mild, characterized by a focal necrosis in the vicinity to the apicomplexan zoites.
Apicomplexan zoites were routinely found in great numbers in the mantle, foot and labial palps, often causing extensive tissue disruption.
Host responses to infections were generally minor. Light infiltration of haemocytes was commonly observed associated with the presence of the apicomplexan zoites. In some cases, especially in the heart´s myocardium, the foot, the mantle and in the labial palps, accumulations of haemocytes were extensive. Occasionally, fibroblast like cells were seen surrounding a clusters of apicomplexan zoites or developing cysts and on several occasions, in the foot, some signs of tissue repairing were observed, where muscular- and fibromuscular tissues were substituted by fibroblast-like cells.

Queen scallop from the Faroe Islands (unpublished data). Histopathological changes were similar to the ones described above for Iceland scallop.

Queen and king scallop from Scottish waters (***Papers II & V).*** As all these scallops were either uninfected or very lightly infected, histopathological changes were minor and similar to lightly infected Iceland scallops described above, i.e. some focal necrosis in the adductor muscle in close vicinity to the apicomplexan zoites (unpublished data).

Sea scallop (Paper IV). As in other scallop species, histopathological changes were restricted to the presence of the apicomplexan parasite. In normal "white meat" scallops, histopathology was minor and similar as described in low-level infections of other scallop species above.

The histopathology in more heavily infected "gray meat" scallops were similar to those observed in heavily infected Iceland scallops exhibiting clinical signs. Some degree of pathology was observed in most organs and the most extensive ones being in the adductor muscle, which were characterized by degeneration of muscle fibers (myodegeneration) at moderate to extensive level, with extensive thinning of muscle fibers, loss of striations, muscular fragmentation and hyalinization. In the most severe cases a total necrosis of extensive areas of the adductor muscle were observed (Figure 4.17. A & B). Different degrees of pathological condition were also observed in other organs, e.g. necrosis in muscular connective tissues in the inter-acinal area of the gonads and the connective tissues adjacent to the epithelial layer of the gastrointestinal tract.

Furthermore, in some cases, an aggregation of abnormal neoplastic-like haemocytes were observed in connective tissues in the digestive gland and inter-acinal areas, which seemed anaplastic and abnormally large. However, this condition was apparently unrelated to both the extent of apicomplexan infections as well as the abnormal discoloration of the adductor muscles as it was equally detected in white, "brown- and gray meat" scallops.

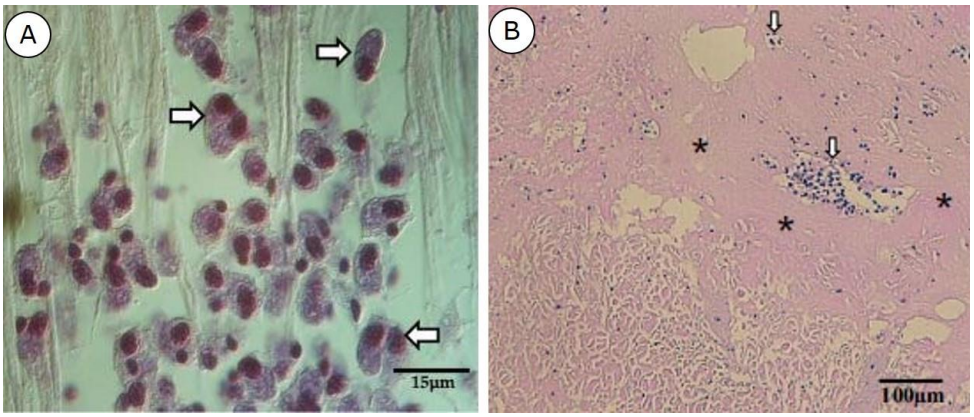

Figure 4.17. Examples of histopathological changes in sea scallops due to Merocystis kathae*. (A) High magnification of a cluster of apicomplexan zoites (arrows) associated with degeneration of muscle fibres. (B) Extensive liquefactive necrosis (asterisk) in an adductor muscle associated with apicomplexan zoites (arrows). From* ***Paper IV****.*

The novel life cycle of *Merocystis kathae* (Paper V)

The life cycle of *Merocystis kathae* is proposed in Figure 4.18. In brief, whelks acquire infections via the gastrointestinal tract. The apicomplexan merozoites migrate to the kidney where the sexual phase occurs, leading to formation of sporozoite, the infective stage for the scallops. Scallops aquire infectious sporozoites via the gastrointestinal tract by their unselective filter feeding. The parasite actively invades the intestinal epithelium, reaching its targeted muscular tissues with the haemolymph, either free or within haemocytes, where the asexual merogonic phase occurs, leading to the formation of merozoites, which are the infective stages for the whelks.

Molecular analysis of *Merocystis kathae*

A contiguous sequence of 1811 bp of SSU rDNA was successfully obtained for the scallop apicomplexan and *Merocystis kathae* (GenBank accession no. MH348777). These showed that the SSU rDNA of *M. kathae* and SAP was 100% identical.
See further analysis in the chapter 4.4. below.

4.3 *Pseudoklossia pectinis* from king scallops (Paper V)

Pseudoklossia pectinis was observed from king scallops from NW Scotland. The parasite was microscopically identified from the kidney and subsequent molecular analyses to assist with the phylogenetic placement of aggregatids from bivalves.
The complete SSU rDNA of 1750 base pairs were amplified and sequenced for *P. pectinis* and was deposited in GenBank under the accession number MH348778.
See further analysis in the chapter 4.4. below.

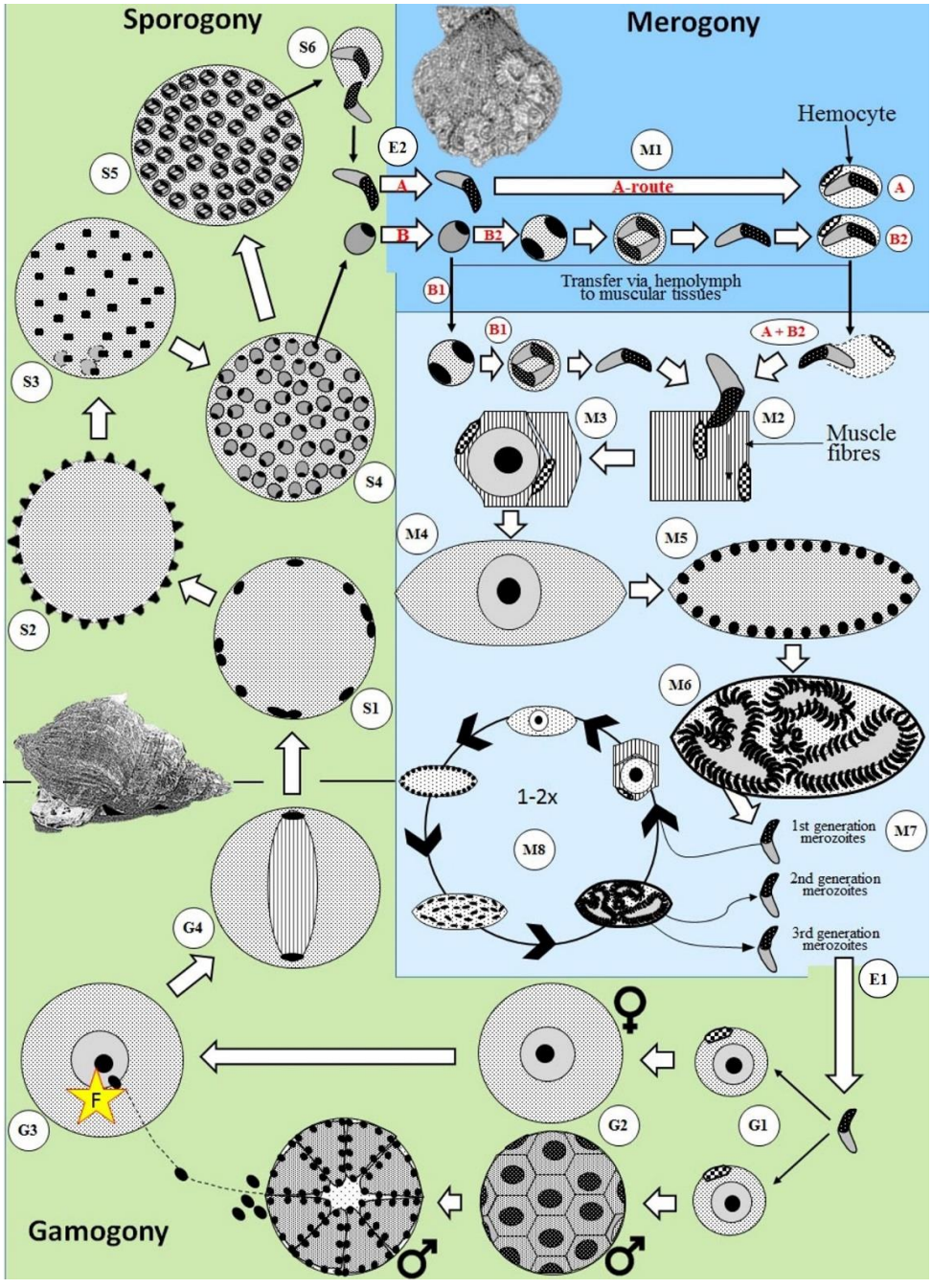
Sporogony
Merogony
Hemocyte
S6
S5
E2
M1
A
A-route
A
B
B2
B2
B1
Transfer via hemolymph
to muscular tissues
B1
A + B2
S3
M3
M2
Muscle
fibres
S4
M4
M5
S2
M6
S1
1-2x
M8
1st generation
merozoites
M7
2nd generation
merozoites
3rd generation
merozoites
E1
G4
G1
G3
F
G2
Gamogony

Figure 4.18. The life cycle of Merocystis kathae. *Merozoites invade the whelk through the intestinal tract (E1) and migrate to the kidney where they infect renal cells and gamogony (G) starts (G1). Some develop into macrogamont (♀) while others become microgamonts (♂) (G2) eventually leading to fertilization (F) (G3) and a formation of a zygote which nucleus starts dividing (G4) initiating the sporogony process (S). Subsequently recurrent nuclei cleavage occur at the periphery of the zygote (S1) resulting in a cyst with regularly arranged nuclei at the periphery (S2). With further development, the nuclei migrate into the cyst and start forming uninucleate sporoblasts, each containing a cytoplasm (S3 and S4). Then the sporoblasts divide to form an oocyst with numerous sporocysts, each containing two sporozoites (S5). Sporogonic stages are released from the common whelk, either in the form of mature sporozoites (route A) or sporoblasts (route B), and enter the Iceland scallop via the gastrointestinal tract to invade the host via the intestinal epithelium (E2) and into adjacent connective tissues. In case of route A, the sporozoites are transmitted via haemolymph, commonly inside haemocytes, to muscular tissues. In case of route B, the sporoblasts are either transmitted directly to muscular tissues where they sporulate (B1) or they sporulate in the connective tissues (Sct) surrounding the gastrointestinal tract prior to transportation to muscular tissues (B2). The merogonic phase (M) starts when the sporozoites invade muscle cells (M1). The muscle cells become hypertrophied as the pre-meront increases in size (M2), eventually leading to a burst of the muscle cell (M3). Further development of the meront is characterized by recurrent nuclei cleavage resulting in a multi-nucleated cyst (M4) which forms into a mature meront containing numerous merozoites (M5). Free merozoites, which are released from the meronts (M6), then infect new muscle cells starting a new merogonic cycle in the scallop´s muscle. After the formation of 2-3 generations of merozoites (M7), the last generation merozoites infect the whelk (E1) where the gamogonic phase starts again (G1). From* ***Paper V****.*

4.4 The phylogeny of the observed species (Papers I & V)

Complete contiguous sequences from three different apicomplexan species were successfully obtained:
(1) A sequences of 1811 bp of the complete SSU rDNA, for the scallop apicomplexan and *Merocystis kathae* which were identical, confirming conspecificity of these apicomplexans in these two mollusc species (**Paper V**). (2) A sequence of 1773 bp, covering the complete SSU rDNA from

Margolisiella islandica (**Paper I**) and (3) a sequence of 1750 bp of the complete SSU rDNA for *Pseudoklossia pectinis* (**Paper V**).

Phylogenetic analyses consistently and robustly place *Merocystis kathae* in a clade with other members of the Family Aggregatidae (Figure 4.18.). This aggregatid clade is weakly, but consistently, associated with a sister clade containing *Filipodium phascolosomae* and *Platyproteum vivax* (Archigregarinorida (Squirmida)) from sipunculids.
Margolisiella islandica and *Pseudoklossia pectinis* do not form a part of this clade but groups with an unidentified apicomplexans infecting the giant clam *Tridacna crocea*, European oyster (available genbank sequence: U83331) and species of the Family Rhytidocystidae, which infect polychaetes (**Papers I & V**).
This entire group forms a weakly supported clade of apicomplexans that are found in marine invertebrates (Figure 4.19.).

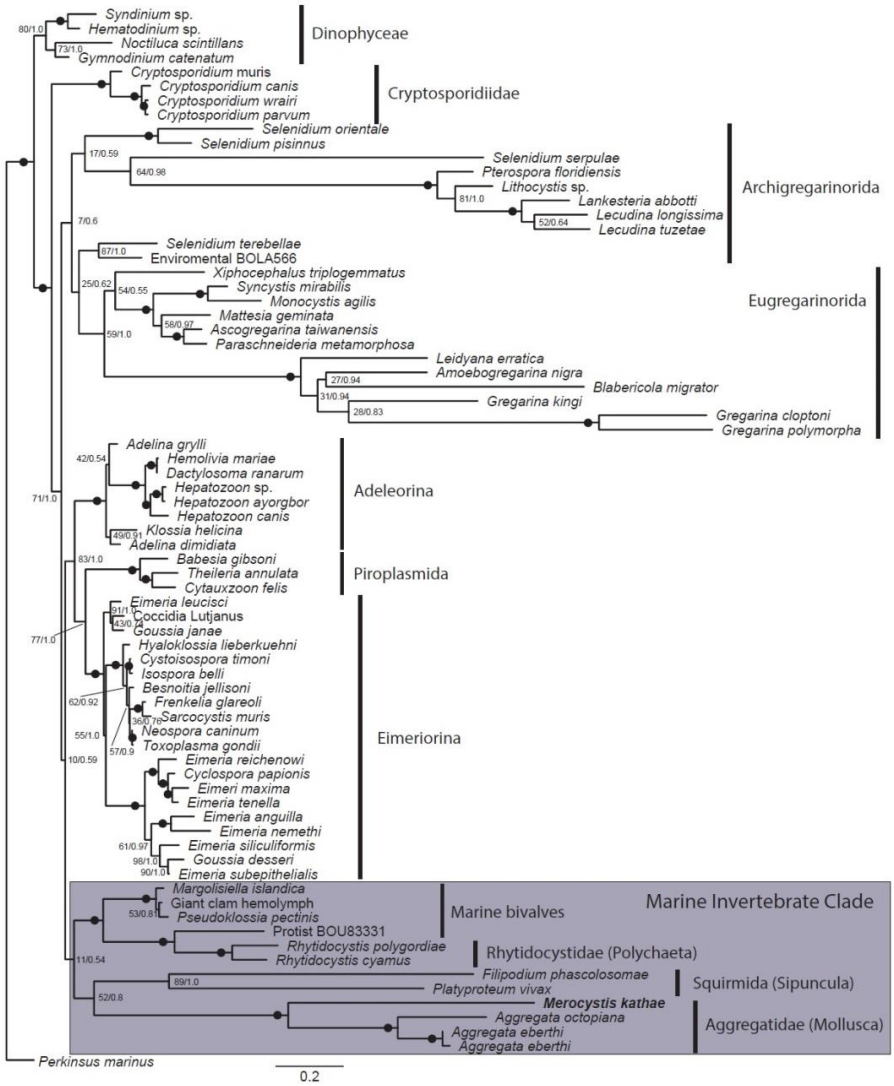

Figure 4.19. Phylogenetic status of the apicomplexan species observed. Maximum Likelihood (ML) topology of alveolate taxa focused on apicomplexans and rooted to Perkinsus marinus*. The phylogenetic tree was inferred using the GTR + G + I model of substitution on an alignment of 74 small subunit (SSU) rDNA sequences and 1,916 sites. Numbers at the branches denote ML bootstrap percentages and Bayesian posterior probabilities; black dots on branches denote bootstrap and Bayesian posterior probabilities support of 95% and higher.* Merocystis kathae *is fully supported in the marine invertebrate clade; a clade with taxa from the genus* Aggregata*. From* ***Paper V****.*

5 Discussion and conclusions

In the beginning of the 2000s, an extensive increase in natural mortality was experienced in the population of Iceland scallop (*Chlamys islandica*) in Breidafjördur Iceland, resulting in a total collapse in the stock. The condition of scallops, within the abnormal population, was characterised by high prevalence of abnormally reduced and discoloured adductor muscles (Marine Research Institute, 2003, 2009). A similar pattern was evident in other scallop grounds around Iceland, e.g. in the Westfjords in the northwest, Húnaflói in the north and Hvalfjördur in the southwest, where the scallop stocks collapsed despite minor or no fishing (Marine Research Institute, 2002). Therefore, overfishing seemed like an unlikely cause. This collapse was sudden, its magnitude unprecedented and no obvious reasons in hand. Subsequently, a total fishing ban was instructed in Icelandic waters in 2003 (Marine Research Institute, 2004).

In the autumn of 2002, scallops were brought to the Fish disease Laboratory at the Institute for Experimental Pathology at Keldur to examine the possible role of diseases in this mass mortality event.

Two apicomplexan species, both of which seemed previously unknown, were identitfied; one infecting the heart and the other infecting muscular- and connective tissues (**Papers I & II**). Early in the research process, it became evident that the species infecting muscular- and connective tissues (presently known to be *Merocystis kathae* (**Paper V**)) seemed highly pathogenic in Iceland scallops and likely to have severe negative effect on the population, while the other one (presently known as *Margolisiella islandica* (**Paper I**)) appeared to be harmless to its host (unpublished data).

Later on, the pathogenic apicomplexan species was identified in four other scallop populations in the North-Atlantic, some of which experiencing mass mortality and abnormally reduced and discolored adductor muscles, similar to Iceland scallops (**Papers II, III & IV**).

5.1 *Margolisiella islandica* n. sp.

Margolisiella islandica is a novel species and the first apicomplexan parasite described from Iceland scallop (**Paper I**). As it was only detected in Iceland scallop but not in related bivalve species, i.e. the queen-, king- (**Paper I**) and sea scallop (**Paper IV**), suggests that it might be host specific.

The presence of both asexual- (merogony) and sexual stages (gametogony and sporogony) of *M. islandica*, confirms a monoxenous life cycle (**Paper I**). The genus *Margolisiella* was established by Desser and Bower (1997) for species of the genus *Pseudoklossia* with known monoxenous life cycles, but left the remaining, possibly heteroxenous species, within the genus *Pseudoklossia*. Therefore, as merogonic forms were not detected in the first and only known *Pseudoklossia* species from scallops, *P. pectinis* (Léger & Duboscq, 1917), it was left within that genus. However, four other *Pseudoklossia* species, i.e. *P. patellae*, *P. chitonis*, *P. tellinovum* and *P. haliotis* were transferred to the new genus *Margolisiella* (Desser & Bower, 1997). Since then, several further species have been described following this criterium, e.g. *P. semiluna* (Desser et al., 1998). On that basis, *M. islandica* from Iceland scallop was placed in the genus *Margolisiella* rather than *Pseudoklossia* (**Paper I**). Presently, these two genera, which are merely separated by their mode of life cycle, belong to two different families as well. When the genus *Margolisiella* was defined, it was placed in the Family Eimeriidae (Desser & Bower, 1997), while *Pseudoklossia* species were retained in the Family Aggregatidae. Some scientists believe it is likely that all *Pseudoklossia* species are monoxenous and therefore the genus *Margolisiella* should be considered a junior synonym of *Pseudoklossia* (Duszynski et al., 1999). Furthermore, the phylogenetic analysis, presented in **Paper V**, somewhat supports this, as well as making the current classification of the genus *Pseudoklossia* within the family Aggregatidae questionable. *Pseudoklossia pectinis* and *Margolisiella islandica* form a group with the rhytidocystids (Family Rhytidocystidae), apicomplexans that infect marine polychaetes, and species infecting the giant clam, *Tridacna crocea* (Nakayama et al., 1998) and the European oyster (Genbank sequence available), but do not form part of the clade including the true aggregatids. Therefore, the current phylogenetic analyses, presented in **Paper V** suggest that the genera *Pseudoklossia* and *Margolisiella* should be moved away from the true aggregatids.

5.2 *Merocystis kathae* (Dakin, 1911)

During the initial identification of this scallop apicomplexan, a great number of different developmental forms were detected in infected scallops, suggesting that it had a monoxenous life cycle. However, the possibility of an obligate alternate host could not be fully excluded, e.g. because the resemblance of developmental stages during merogony and gamogony, especially at the earlier stages of development (**Paper II**). When the phylogenetic placement, as an aggregatid apicomplexan, became clear

(**Paper V**), suspicions arose that an obligate definite host was required for the parasite to finish a full reproductive life cycle. The fact that all nominal aggregatid species, which life cycles were known, required two hosts, suggested that the same was the case for the scallop apicomplexan. The search for this definite host was successful and shown to be the common whelk and the parasite´s SSU rDNA was identical to *Merocystis kathae*, an aggregatid apicomplexan described more than 100 years ago from the common whelk, *Buccinum undatum* (**Paper V**). At the same time, it became clear that in the initial identification (**Paper II**), some merogonic stages were misidentified for gamonts and oocysts.

M. kathae is the first apicomplexan parasite known to require two mollusc hosts to complete its life cycle, the common whelk and pectinid bivalves (scallops) (**Paper V**). It was originally detected in the renal organ of common whelks from Port-Erin, Isle of Man (Dakin, 1911). It is the type species for the genus. Its unusual, and currently unique life cycle, might reflect the limited examination of invertebrate apicomplexans rather than it being an unusual occurrence.

The transmission of *M. kathae* occurs via the gastrointestinal route in both the definitive and intermediate hosts (**Paper V**). As unselective filter feeders, scallops consume a range of particle sizes from their surroundings by movement of ciliated cells in the gills; particles become entangled in mucus and are subsequently transferred along rejection tracts to the mouth palps, where they enter the digestive tract (Jørgensen, 1996).

The present knowledge of the geographical distribution of *M. kathae* in the definitive host is poorly understood, mostly due to limited research on parasites of the common whelk and related species. To date, this parasite has been reported in common whelk in the Irish Sea (Dakin, 1911; Foulon, 1919; Patten, 1935), the Belgian part of the North Sea (Declerck, 1990), in Øresund and Gullmarfjord in Danish- and Swedish waters (Køie, 1969), and now Iceland (**Paper V**).

This novel discovery of the conspecificity of *M. kathae* and the SAP (scallop apicomplexan) has extended the known distribution of this parasite. Its presence in the intermediate scallop host, was first reported in 2011, from three different scallop species, *Chlamys islandica*, *Aequipecten opercularis* (queen scallop) and *Pecten maximus* (king scallop), in Icelandic-, Faroese- and UK waters, respectively (**Paper II**). Later it was also confirmed in sea scallop, *Placopecten magellanicus*, on the western side of the Atlantic, i.e. off the east coast of the USA and Canada (**Paper IV**). All the scallop species found infected with *M. kathae* were collected within the known distribution of the common whelk. In fact, the distribution of these bivalve species is almost identical to that of the common whelk (**Paper V**). The confirmation of

the presence of *M. kathae* and its high prevalence, off the coast of Iceland, Scotland, Faroe Islands (**Paper II**) and eastern US waters (**Paper IV**), suggests it is widely distributed, and most likely present on the continental coast of Europe, although it has not yet been confirmed.

With regard to host specificity of *M. kathae*, the knowledge is limited, especially in the definite host. Obviously, pectinid scallops serve as intermediate host for the parasite (**Papers II, III & IV**), but the fact that it was not found in other non-pectinid bivalve species examined from sites endemic for the parasite, suggests that it is not a generalist parasite infecting a number of unrelated bivalve species (**Paper V**).

Presently, *M. kathae* has only been observed in a single definite host species, i.e. the common whelk (**Paper V**). Whelks, are however, poorly studied with regard to parasites. It seems quite possible that some of the 134 valid species of the genus *Buccinum*, which are distributed all over the world (Palomares & Pauly, 2018), could serve as hosts. This is indicated by the presumable presence of *M. kathae* in the weathervane scallop, *Patinopecten caurinus*, in the Alaska Bay NE-Pacific Ocean (**Paper IV**). The common whelk is absent from this area, while several other whelk species of the genus *Buccinum* inhabit it (Palomares & Pauly, 2018).

The two *Aggregata* species with known life cycles seem more specific with respect to their cephalopod definitive hosts than their intermediate hosts, which include various crustacean species of different families (Gestal et al., 2002; Gestal et al., 2005). Therefore, it might be reasonable to expect the same in the case of *M. kathae*, perhaps being limited to certain whelk species but numerous pectinid hosts.

In addition to the present study (**Paper V**), all other available studies indicate that *M. kathae* does not negatively affect the the health of the common whelk definitive host (Dakin, 1911; Foulon, 1919; Patten, 1935) as they are in good condition and the histopathological effect of the parasite is minor, even in extreme infections. However, *M. kathae* is a serious pathogen of scallops (**Papers II, III & IV**). It causes extensive histopathological changes in all major organs of Iceland scallops, which severely affects its condition. In particular, it causes severe myodegeneration as well as hampering normal gonad development (**Paper III**). As thoroughly analysed in **Paper III**, all data points towards it playing a major role in the sudden 90% decline in populations of Iceland scallop in Breidafjördur Iceland as well as severely affecting the queen scallop population around the Faroe Islands (**Papers II & III**). Histopathological changes were similar in sea scallops, associated with infections and the occurrence of “gray meat” scallops. *M. kathae* is therefore a suspected cause for the abnormal condition in the sea scallops in eastern US

waters, i.e. the regular occurrence of “gray meat” and associated mass mortality events (**Paper IV**).

It should be reasonable to speculate whether it might have played a role in numerous other unresolved mass mortality events and abnormal condition in various other scallop populations. Other scallop populations in Icelandic water have suffered abnormal mortality at several occasions (Eiríksson, 1986; Gudfinnsson & Gunnarsson, 2001; Jónasson et al., 2006), but also populations in Norway (Wiborg, 1963), Jan Mayen and Svalbard (Sundet, 1985; Rubach & Sundet, 1986), Greenland (Pedersen, 1987), Russian Barents Sea (ICES, 2006) and northern shore of Quebec eastern Canada (Gigurere et al., 1995). Furthermore, the abnormal condition of adductor muscles, similar to the one caused by *M. kathae*, observed in the weathervane scallop, *Patinopecten caurinus* in the Alaska Bay North East Pacific Ocean (Brenner, 2012; Armstrong, 2016). This condition is associated with an apicomplexan infection, which according to **Paper IV** is likely to be *M. kathae*.

According to Patten (1935), *M. kathae* follows a seasonal pattern in whelks, with the earliest developmental stages appearing between March and June while the first mature sporozoites form in January and become increasingly common up to May. Consequently, the scallops are most extensively exposed to infective sporozoites in late winter and spring. During the epizootic in the Iceland scallop population in Breidafjördur Iceland in the 2000s, scallops caught in the spring were significantly more infected with *M. kathae* and associated macroscopic signs more severe, than those caught in autumn (**Paper III**). Although the energy demanding maturation process, being close to spawning at that time of year, might make the scallops more vulnerable to infections, the extensive influx of infective sporozoites into their surroundings during this time must also play a major role. Whelks are known to be predatory, with scallops forming a regular part of their diet, but they are also scavengers, feeding on moribund and dead animals (Himmelman & Hamel, 1993; Chen, 2012). Thus, during such mass mortality events (**Paper III**), the availability of dead or moribund scallops would be plentiful, resulting in whelks intensifying their infection. Subsequently, substantial amounts of infective sporozoites are released into the surroundings, which infect the remaining naive filter feeding scallops. The very high prevalence of *M. kathae* in both whelks and Iceland scallops, many of those heavily infected, reflects this situation (**Paper III**).

Although *M. kathae* has been shown to severely affect scallops (**Papers II, III & IV**), it appears that it only occurs when infections reach high intensities, as low-level infections exist in scallop populations under normal conditions (**Papers II & III**). After an almost complete collapse, the scallop population in Iceland has been slowly recovering. Macroscopic disease signs have rarely been detected the last few years and condition of muscles and gonads appear normal. However, low-level infections remain in high

prevalence in the stock (**Paper III**). Similarly, highly prevalent but low level infections of *M. kathae* were observed in both king and queen scallops from UK waters in 2007, but no abnormal clinical signs were reported (**Paper II**). This might suggest that the scallops' immune system can suppress light infections, to some extent.

Historical data on stock indices and mass mortality events in scallop populations are generally poorly documented and in most the aforementioned cases, the causes cannot be verified. However, considering the wide distribution of *M. kathae* and the fact that almost all these events occurred within the known distribution of the common whelk (**Supplementary Information Paper V**), it seems plausible that it was a major factor influencing these events. Although scarce, some reports exist from scientists and fishermen showing that mass mortality events in some scallop populations are cyclical. That is the case for sea scallops on the east side of North America, where mass mortality events associated with a condition termed "gray meat" have periodically occurred since 1936 (Stevenson, 1936; Medcof 1949; Gulka et al., 1983; Stokesbury et al., 2007) (**Paper IV**). Furthermore, some indications of such periodic events exist in Icelandic waters (**Paper III**). As *M. kathae* has been reported in relation with such events in both these scallop populations, it seems possible that epizootics occur regularly as a consequence of a host-parasite relationship, a phenomenon widely recognized in nature and in many ways comparable to prey – predator systems. In both cases, severe fluctuations occur in the population size of both groups (Krebs, 2009).

The main commercial fishing grounds for both scallops and whelks in Iceland is Breidafjördur. Until the collapse of the scallop population, commercial fisheries of that species had been reliably conducted since 1969. Whelk fisheries have a much shorter history, starting with some experimental collections in 1996. Following that, they have been quite intermittent to the present, with no harvesting during some years (Marine Research Institute, 2016). Conversely, whelk fisheries are among the most important shellfish fisheries in the United Kingdom, dating back to the early 1900s (Elliott et al., 2013). As low-level infections do not appear to have a negative impact on the scallops, it should be possible to lower the infectious load with reasonable fisheries from both the whelk and scallop stocks. This would minimize the chance of epizootics caused be *M. kathae*, and create an optimal host - parasite equilibrium. The present study showed that infections were almost absent in king scallops from a "whelk free" area (**Paper V**). Furthermore, only light infections were reported in both king- and queen scallops from other UK locations, collected in 2007 (**Paper II**). The extensive fisheries for whelks in the UK might help to explain this phenomenon.

6 General conclusions

In general, wild scallops are poorly studied with respect to parasites and diseases and of all commercially exploited species, the Iceland scallop, *Chlamys islandica*, is probably the least studied (Ball & McGladdery, 2001; McGladdery et al., 2006; Getchell et al., 2016). Hence, the results from this study adds significantly to the present knowledge on parasites infecting pectinid bivalves.

This project is unique in several ways. It presents long-term epidemiological data on a pathogenic apicomplexan parasite during a severe mass mortality events experienced in a mollusc population, which appears to be unprecedented in the literature (**Paper III**). Furthermore, it confirms the presence of this pathogen, and suggests its role in similar mass mortality events experienced in other scallop populations as well as shedding light on the presumable causes of unresolved mass mortality events in numerous other scallop populations through the decades (**Papers II, III & IV**). It also reveals a unique, two-mollusc host life cycle of an apicomplexan parasite; the first one described and offers a way to minimize epizootics by reasonable fisheries of both these hosts (**Paper V**). Lastly, a novel apicomplexan species is described (**Paper I**) and molecular data retrieved from three different apicomplexan species, which brings increased resolution to the poorly documented phylogeny of basal apicomplexan infecting marine invertebrates (**Paper V**).

7 Future research

When considering future research, many ideas come into mind, three of which I decide to discuss further.

(1) Epidemiology and management. There is no doubt in my mind that the results from this thesis could prove to be a basis for changed management policies. The unexpected collapse, experienced in the scallop stock in Iceland, had a huge impact on the economy of smaller towns on the Snæfellsnes Peninsula (Jónasson et al., 2006). Results from my thesis indicate that *Merocystis kathae* played a major role in this event and both host species for this parasite are known (**Papers III & V**). In the light of this, it is my belief that the management of the scallop stock would benefit from annual monitoring of both scallops and whelks from all main scallop grounds in Icelandic waters. Such monitoring could give valuable indications for an imminent epidemic, timely enough to take preventive acts to lower the infectious load in the scallops´environment, e.g. be removal of whelks and a reasonable fishing from scallop beds, especially those with high biomass.

(2) *Merocystis kathae* and other scallop populations. There is little doubt that *Merocystis kathae* has a potential to have severe negative impact on the condition of the Iceland scallop (**Paper III**). *Merocystis kathae* might also explain, at least to some extent, the reasons for mass mortality events in sea scallops off the eastern coast of the United States and weathervane scallop off the Alaskan coast (**Paper IV**). Both these events have had huge economic impact. However, to be able to make reliable statements in that respect, further research are needed. These would include extensive long-term examinations of the parasite in affected scallop populations and the definite hosts for the parasite, the whelks. Although the apicomplexan species, identified in diseased weathervane scallops, is most likely *M. kathae*, the common whelk *Buccinum undatum*, is absent in Alaskan waters. Hence, an alternative definite host, or hosts, probably exist. The most likely candidates are other species of the genus *Buccinum*, of which several inhabit Alaskan waters. The common whelk is widely distributed off the eastern coast of the US, but also other *Buccinum* species (Palomares & Pauly, 2018). Possibly, many different whelk species serve as definite host there. It would also be interesting to examine whether *M. kathae* exists in scallops from areas where

mass mortality events have occurred through the decades, e.g. Russian- and Norwegian Barents Sea and Greenland. Lastly, the question whether different strains of *M. kathae* infect scallops from different geographic areas should be addressed.

(3) Increased resolution of apicomplexans of marine molluscs. The present knowledge on diversity, distribution, host specificity and phylogenetic position of apicomplexan parasites infecting marine molluscs is very limited. Furthermore, of the relatively few species described, molecular data exist for just a fraction of them. Apicomplexan are probably common in a range of marine molluscs but very under-sampled (especially mollusc species of low or no commercial value) with undefined molecular phylogenetic placement and may represent some of the most basal apicomplexans known. It would be interesting to increase the resolution of mollusc apicomplexans and create a more robust phylogeny for basal (hard to place) apicomplexan parasites infecting marine molluscs as well as other invertebrates.

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Paper I

Margolisiella islandica sp. nov. (Apicomplexa: Eimeridae) infecting Iceland scallop *Chlamys islandica* (Müller, 1776) in Icelandic waters

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Margolisiella islandica sp. nov. (Apicomplexa: Eimeridae) infecting Iceland scallop *Chlamys islandica* (Müller, 1776) in Icelandic waters

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ABSTRACT

Wild Iceland scallops *Chlamys islandica* from an Icelandic bay were examined for parasites. Queen scallops *Aequipecten opercularis* from the Faroe Islands and king scallops *Pecten maximus* and queen scallops from Scottish waters were also examined. Observations revealed heavy infections of eimeriorine parasites in 95–100% of *C. islandica* but not the other scallop species. All life stages in the apicomplexan reproduction phases, i.e. merogony, gametogony and sporogony, were present. Trophozoites and meronts were common within endothelial cells of the heart's auricle and two generations of free merozoites were frequently seen in great numbers in the haemolymph. Gamonts at various developmental stages were also abundant, most frequently free in the haemolymph. Macrogamonts were much more numerous than microgamonts. Oocysts were exclusively in the haemolymph; live mature oocysts contained numerous (>500) densely packed pairs of sporozoites forming sporocysts.

Analysis of the 18S ribosomal DNA revealed that the parasite from *C. islandica* is most similar (97.7% identity) to an unidentified apicomplexan isolated from the haemolymph of the giant clam, *Tridacna crocea*, from Japan. Phylogenetic analyses showed that the novel sequence consistently grouped with the *Tridacna* sequence which formed a robust sister clade to the rhytidocystid group.

We propose the name *Margolisiella islandica* sp. nov., referring to both type host and type locality.

1. Introduction

The Iceland scallop, *Chlamys islandica* (Müller, 1776) (Mollusca: Pectinidae), is a sub-arctic species with its main distribution in the sub-arctic transitional zone. It occurs in the North Atlantic Ocean south to Massachusetts, around Iceland, Greenland, the Barents Sea, the northern part of Norway south to Bergen. It has also been recorded in the Bering Strait and extends into the Pacific Ocean (Brand, 2006).

Although apicomplexans infecting bivalve molluscs are poorly studied, such studies go back to 1897, when Léger (1897) described the first species, *Hyalloklossia pelsineeri* from *Donax* sp. and *Tellina* sp. Before that, bivalves were thought to be free of apicomplexans (Léger, 1897). Subsequently, Léger and Duboscq (1915) established the genus *Pseudoklossia* to accommodate an apicomplexan species, *Pseudoklossia glomerata*, from *Tapes floridus* and *T. virgineus*, and they also transferred *H. pelsineeri* to this genus. Later *Pseudoklossia pectinis* was described from *Pecten maximus* by Léger and Duboscq (1917), and *Pseudoklossia patellae* and *Pseudoklossia chitonis* from the gastropod *Patella vulgaris* by Debaisieux (1922). More recently, *Merocystis tellinovum* was described from the ovary of *Tellina tenuis* by Buchanan (1979), which later was transferred to the genus *Pseudoklossia* by Levine (1988). A new genus, *Margolisiella*, was established for an apicomplexan of littleneck clams, *Protothaca staminae*, which was named *Margolisiella kabatai* by Desser and Bower (1997). Both sexual and asexual stages occurred in the same host, confirming a monoxenous life cycle. They proposed this new genus for *Pseudoklossia* species with known monoxenous life cycles, but left the remaining, possibly heteroxenous species, within the genus *Pseudoklossia*. Subsequently they proposed a transfer of *P. patellae*, *P. chitonis*, *Pseudoklossia tellinovum* as well as *Pseudoklossia haliotis* (described from gastropods of the genus *Haliotis*) to this new genus, *Margolisiella*. Since then, one species, *Pseudoklossia semiluna*, has been described from the bivalve *Mytilus* spp. (Desser et al., 1998). A *P. pectinis*-like coccidian was reported from the scallop *Argopecten irridians* by Karlsson (1991), and *Pseudoklossia* sp. from *A. irridians* by Cawthorn et al. (1992) and from the cockle *Cerastoderma edule* by Carballal et al. (2001). Unidentified apicomplexans have also been reported from other bivalves (Leibovitz et al., 1984; Morado et al., 1984; Whyte et al., 1994; Nakayama et al., 1998; Hine, 2002). To date, no parasite species have been described from the Iceland scallop and very limited DNA data exists for apicomplexan parasites of bivalves.

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In the year 2002, live Iceland scallops were sent to the Fish disease Laboratory at the Institute for Experimental Pathology at Keldur, for examination. Observation revealed infection with two previously unknown apicomplexan species.

The aim of the studies that followed was to describe these parasites, one of which is the subject of the present paper; the second one is described in a separate paper (Kristmundsson et al., 2011).

2. Materials and methods

In November 2002, live Iceland scallops, *C. islandica*, were sampled from the bay of Breidafjördur, western Iceland ($n = 2 \times 60$). Queen scallops *Aequipecten opercularis* L. ($n = 60$) from the east coast of the Faroe Islands were sampled in February 2005. In September 2007 queen scallops ($n = 10$) and king scallops *P. maximus* L. ($n = 10$) were sampled from the west coast of Scotland (Fig. 1).

The scallops were either examined immediately after sampling or kept for a maximum of one week in 170 L tanks supplied with seawater at 9 °C.

The scallops were dissected, shell height measured and samples from all major organs examined with a compound microscope. Parasites present were identified and their distribution in organs evaluated, using: (1) fresh mounts; samples from each organ pressed between a glass slide and a coverslip and (2) histology; all major organs fixed in Davidson's fixative for 48 h and subsequently dehydrated in 70% ethanol, embedded in paraffin wax, sectioned (4 μm), and stained with Giemsa according to routine histological protocols. Parasite forms detected were measured (μm) and photographs taken using Leica DMLB microscope equipped with a digital camera (Leica DC300F).

Freshly dissected infected tissue was fixed in 95% ethanol for the DNA analysis. Total DNA was extracted using a GeneMATRIX kit (EURx Poland) following the tissue protocol. Small subunit ribosomal DNA (SSU rDNA) was amplified from the parasite using the primer pair SFC-340f 5′ agtttctgacctatcagc 3′ SFC-1260r 5′ tcagccttgcgaccatactc 3′ designed from alignments of apicomplexan taxa made in CLUSTAL_X (Thompson et al., 1997). From the initial sequence reads two additional more specific primers were designed for use with universal primers to complete the sequencing of the SSU rDNA. SC1-1185f 5′ tcacgatttgacactttcagc 3′ was used with the reverse primer 18gM (Freeman et al., 2008) and SC1-590r 5′ actcgtgtgaagctttacttcc 3′ was used with the forward primer 18e (Hillis and Dixon, 1991). The conditions for all PCR reactions were as follows: Initial denaturing 5 min at 95 °C followed by 35 cycles of: 94 °C 30 s, 55 °C 45 s, 72 °C 1 min, with a terminal extension of 72 °C 7 min. PCR bands of the expected sizes were recovered from the PCR products using a GeneMATRIX pcr products extraction kit (EURx Poland). PCR reactions were performed in triplicate (three infected scallops). Sequencing reactions were performed using BigDyeTM Terminator Cycle Sequencing chemistry utilising the same oligonucleotide primers that were used for the original PCRs. DNA sequencing was performed in both forward and reverse directions for all PCR products and nucleotide BLAST searches performed for each sequence to confirm an apicomplexan origin. The contiguous sequence was obtained manually using

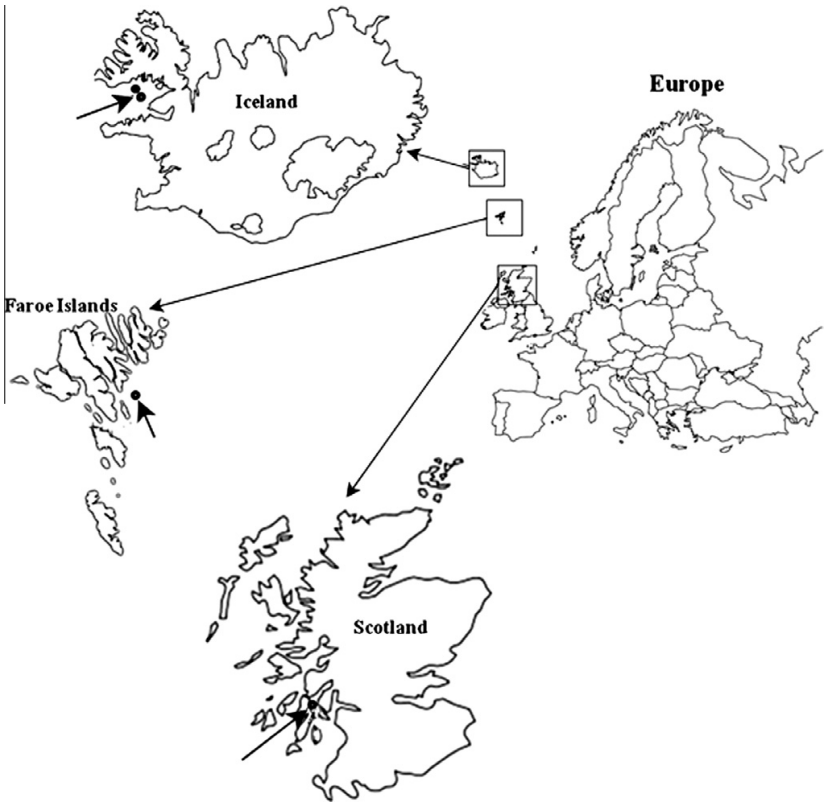

Fig. 1. Sampling sites of scallops from Iceland, Scotland and the Faroe Islands (●).

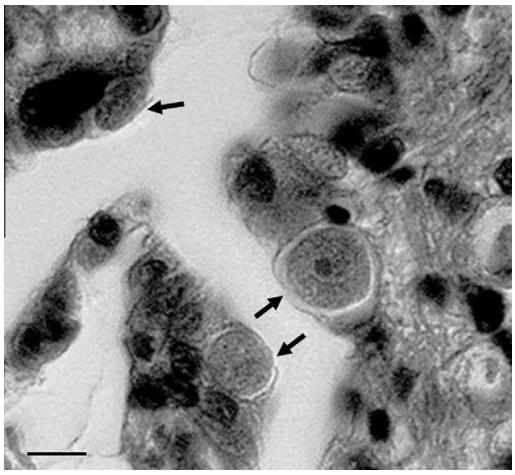

Fig. 2. Histological section showing *Margolisiella islandica* trophozoites inside auricular endothelial cells (arrows). Scale bar = 10 μm.

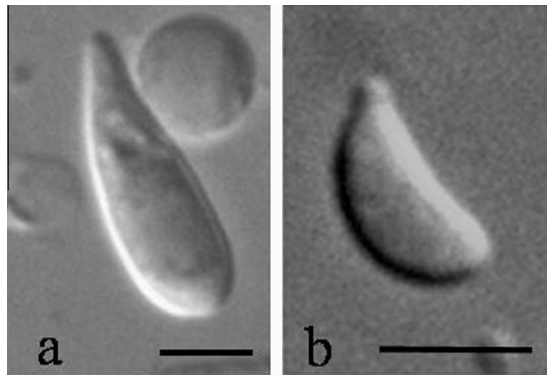

Fig. 4. Live specimens of two generations of *Margolisiella islandica* merozoites found free in the haemolymph. Scale bar = 4 μm.

CLUSTAL_X and BioEdit (Hall, 1999). For phylogenetic analyses, taxa were chosen from BLAST searches that had similarities to the novel sequence and additional apicomplexan sequences chosen to represent the majority of recognised clades for the group. CLUSTAL_X was used for the initial sequence alignments with the settings for gap opening/extension penalties being adjusted manually to achieve optimum alignments. Regions of ambiguous sequence alignments were manually edited using the BioEdit sequence alignment editor.

Phylogenetic analyses were performed using the maximum likelihood methodology in PhyML (Guindon et al., 2010) with the general time-reversible substitution model selected and 1000 bootstrap repeats, and Bayesian inference (BI) analysis using MrBayes v. 3.0 (Ronquist and Huelsenbeck, 2003). For the BI analysis models of nucleotide substitution were first evaluated for the alignment using MrModeltest v. 2.2 (Nylander et al., 2004). The most parameter-rich evolutionary model based on the AIC was the general time-reversible, GTR+I+G model of evolution. Therefore, the settings used for the analysis were nst = 6, with the gamma-distributed rate variation across sites and a proportion of invariable sites (rates = invgamma). The priors on state frequency were left at the default setting (Prset statefreqpr = dirichlet (1, 1, 1, 1)). Posterior probability distributions were generated using the Markov Chain Monte Carlo (MCMC) method with four chains being run simultaneously for 1000,000 generations. Burn in was set at 2500 and trees were sampled every 100 generations making a total of 7500 trees used to compile the majority rule consensus trees.

Taxa used in phylogenetic analyses: *Adelina grylli* DQ096836; *Ascogregarina taiwanensis* DQ462454; *Babesia gibsoni* AF231350; *Besnoitia jellisoni* AF291426; *Colpodella pontica* AY078092; *Cryptosporidium muris* AB089284; *C. parvum* AF108865; *Cyclospora papionis* AF111187; *Cystoisospora timoni* EU200792; *Cytauxzoon felis* AY679105; *Eimeria maxima* U67117; *E. tenella* U67121; *Frenkelia glareoli* AF009245; *Goussia janae* AY043206; *Hepatozoon ayorgbor* EF157822; *H. canis* AY461378; *Hyaloklossia lieberkuehni* AF298623; *Isospora belli* U94787; *Mattesia geminata* AY334568; *Monocystis agilis* AF457127; *Neospora caninum* L24380; *Noctiluca scintillans* AF022200; *Rhytidocystis cyamus* GQ149767; *R. polygordiae* DQ273988; *Sarcocystis muris* M64244; *Theileria annulata* DQ287944; *Toxoplasma gondii* U03070; *Tridacna apicomplexan* AB000912; *Oyster apicomplexan* U83331.

3. Results

3.1. Occurrence and tissue distribution

Eimeriorin parasites were found in nearly all of the Iceland scallops examined from both sampling sites. However, none of the queen scallops and king scallops from the Faroe Islands and Scotland were infected. The prevalence of infections was high in Iceland scallops (95–100%) from both locations and showed no pattern in relation to host size. As intracellular infections were only detected in the auricle, it seems to be the preferred organ of the parasite to develop. Occasionally, macrogamonts were detected in other organs,

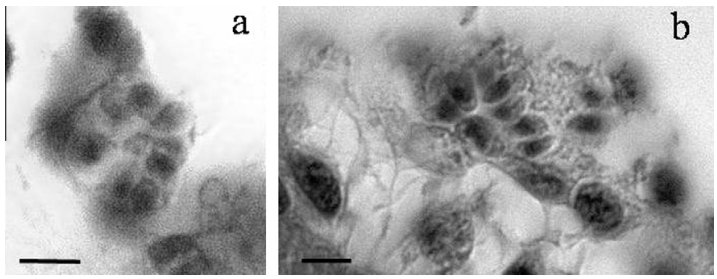

Fig. 3. Histological sections from a scallop heart's auricle. *Margolisiella islandica* meront with eight developing merozoites arranged in a rosette like fashion (a). Mature meront with merozoites (b). Scale bar = 5 μm.

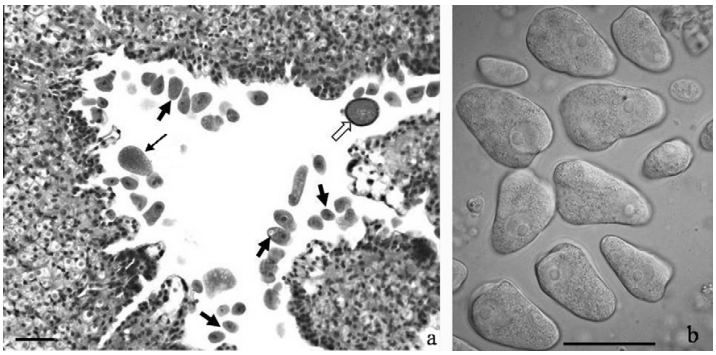

Fig. 5. Histological section through the heart's auricle showing numerous developmental forms of *Margolisiella islandica*; macrogamonts (broad black arrows), developing oocyst with peripherally arranged nuclei (white arrow), mature oocyst (thin black arrow) (a). Live specimens showing developing and mature macrogamonts with large nucleus and a prominent nucleolus (b). Scale bar = 50 μm.

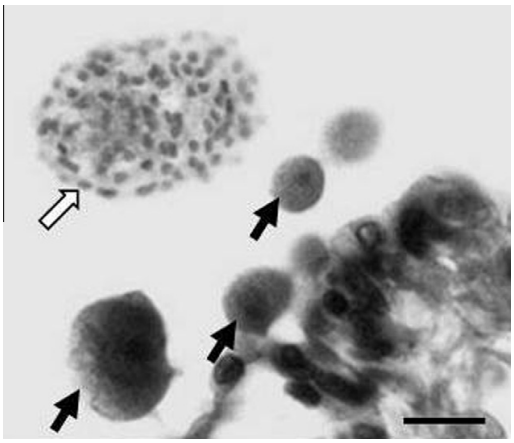

Fig. 6. Histological section showing mature *Margolisiella islandica* microgamont (white arrow) packed with numerous microgametes and developing macrogamonts (black arrows). Scale bar = 10 μm.

especially the adductor muscle; its presence there is most likely due to transport in haemolymph. All life stages in the apicomplexan reproductive phases, i.e. merogony, gametogony and sporogony, were detected. Syzygy, the pairing of gametes prior to fertilisation, was not observed. We propose the name *Margolisiella islandica* sp. nov., referring to both type locality and type host.

3.2. Parasite development

Trophozoites were intracellular in the auricular endothelium (Fig. 2), with developmental stages bulging from the hypertrophied host cell.

3.2.1. Merogony

Meronts, spherical and 10 μm in diameter by histology during division, giving rise to eight merozoites in a cruciform or rosette configuration (Fig. 3) were commonly seen inside endothelial cells of the auricle. Merozoites of two sizes (6.0–7.0 × 2.8–3.2 and 12.0–13.0 × 3.0–4.0) which most probably are two generations of merozoites, were frequently seen in great numbers in fresh specimens (Fig. 4) ($n = 2 \times 60$). The merozoites showed upright clockwise twirling motility, i.e. when the parasite is attached to the substrate by its posterior end, it produces a clockwise spinning.

3.2.2. Gametogony

Gamonts were very numerous, often free in the haemolymph, with more macrogamonts than microgamonts. Histological examination frequently showed growing gamonts inside endothelial cells of the auricle. Gamont-infected auricular endothelial cells were hypertrophied and appeared to rupture, releasing the parasites, but gamonts were also observed bulging from the endothelial cells.

Macrogamonts had a coarsely granular cytoplasm and a large nucleus with a prominent nucleolus. Young macrogamonts were

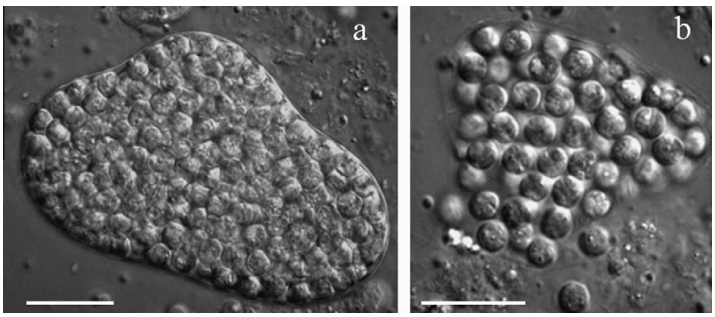

Fig. 7. Live mature *Margolisiella islandica* oocyst with numerous sporocysts (a). Ruptured oocyst, sporocysts spreading into the haemolymph (b). Scale bar = 20 μm.

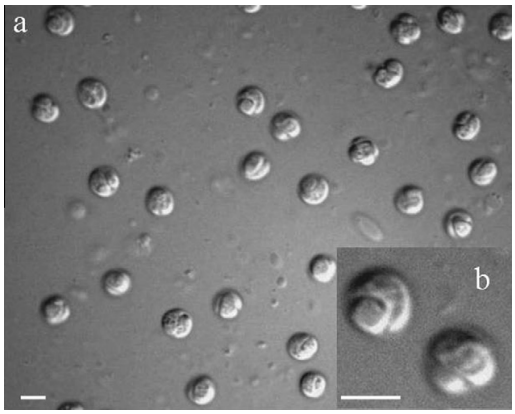

Fig. 8. Live specimen of free sporocysts in the auricular lumen (a). Sporocysts, higher magnification showing how sporozoites face each other at the convex side (b). Scale bar = 5 μm.

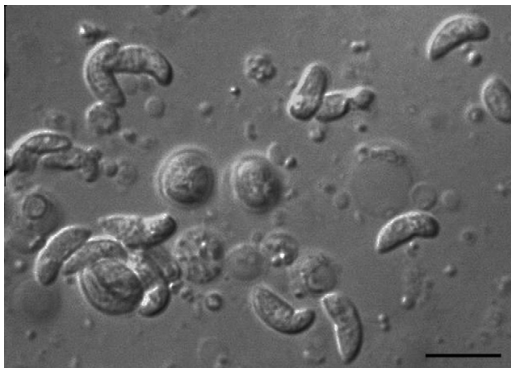

Fig. 9. Live specimen of free sporozoites from broken sporocysts, straightened out revealing their crescent-shaped appearance and distinct centred nucleus. Scale bar = 10 μm.

ellipsoidal but at maturity they became pear- or heart shaped (Fig. 5). Live, fully mature macrogamonts measured 40–50 × 30–40 μm (n = 60).

Microgamonts, spheroidal or ellipsoidal, with peripherally arranged nuclei, were seen in histological sections (Fig. 6). Fully mature microgamonts measuring 30–40 μm (n = 20) had numerous microgametes which occasionally were seen bulging from the microgamonts.

3.2.3. Sporogony

Oocysts were only seen free in the haemolymph. Live mature oocysts, ellipse or pear shaped, measured 48–60 μm in length and 40–44 μm at the widest part (n = 30). Each oocyst contained numerous (>500) densely packed pairs of sporozoites facing each other at the convex side (Fig. 7a) forming spherical sporocysts (live specimens) 5.5–6.5 μm in diameter enclosed with a thin and fragile membrane (n = 30). Ruptured oocysts were common with sporocysts flowing out to the haemolymph (Figs. 7b and 8a). In such cases the sporocyst membrane commonly broke, the sporozoites detached and straightened out revealing their crescent-shaped appearance and its distinct centred nucleus (Fig. 9). Live straightened sporozoites measured 8.5–10 μm in length and 3.0 μm at their widest part (n = 30).

3.3. Molecular data

Complete SSU rDNA of 1773 base pairs was successfully amplified and sequenced for *M. islandica* sp. nov. and has been deposited in GenBank under the accession number JN227668. A nucleotide BLAST search showed that an unidentified apicomplexan parasite that infects the haemocytes of the giant clam *Tridacna crocea* from Japan had the highest sequence identity with 97.7% identity over 1768 bases of comparable sequence data. Phylogenetic analyses, irrespective of method used, consistently and robustly group *M. islandica* sp. nov. with the *Tridacna* apicomplexan, forming a well-supported sister clade to the rhytidocystids group (Fig. 10). However, this rhytidocystid/bivalve grouping is only weakly supported as a sister clade to the neogregarine/*Cryptosporidium* group (50/0.85), but is well supported (91/1.0) at the major division of the tree as a group that lies away from the main eimerid coccidian group (Fig. 10).

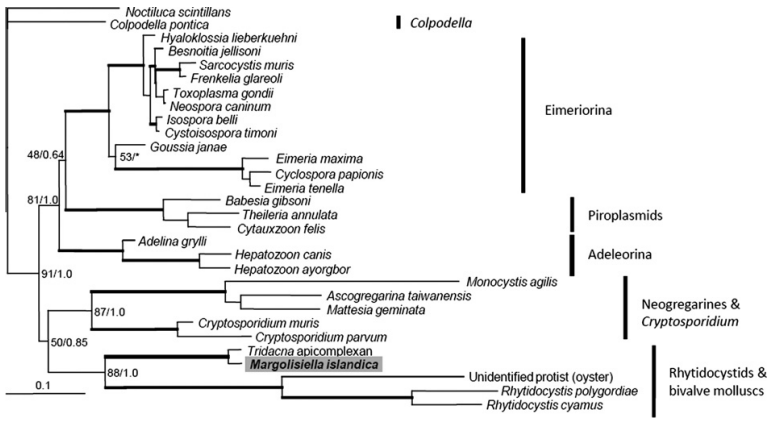

Fig. 10. Maximum likelihood tree as inferred using the GTR model of nucleotide substitutions in PhyML. Based on an alignment of 30 SSU rDNA sequences and 1909 characters. Numbers at the nodes represent maximum likelihood bootstrap percentages and Bayesian posterior probabilities. Bold branches lead to a node with full support of (100/1.0). The tree is rooted to the dinoflagellate *Noctiluca scintillans* and the major clades are labelled to the right of the tree. An asterisk (*) indicates a different topology obtained in Bayesian analysis (*G. janae* formed an unresolved polytomy in the Bayesian analysis).

Table 1
Comparison of apicomplexan species described from molluscs. All dimensions in μm.

Name of apicomplexan	Host Class/species	Infected organs	Meronts	Oocystsize	Sporocyst size	No of sporocysts/ oocyst	No of sporozoites/ sporocyst
Bivalve clam							
Pseudoklossia pelseneeri[1]	*Donax* sp. *Tellina* sp.	Kidney	–	75–80	4–6	n.d.	n.d.
P. glomerata[2]	*Tapes floridas* *Tapes virginius*	Kidney	–	≈35	4–5	n.d.	n.d.
P. (*Merocystis*) *tellinovum*[3,4,*]	*Tellina tenuis*	Ovary	+	≈27	≈4	n.d.	n.d.
Unnamed[5]	*Protothaca staminae*	Kidney	+	≈35	≈6	20–24	n.d.
Margolisiella kabatai[6]	*Protothaca staminae*	Kidney	+	≈41	9 × 10	≈32	n.d.
Unnamed[7]	*Tridacna crocea*	Hemolymph	n.d.	n.d.	n.d.	n.d.	n.d.
Bivalve cockle							
Pseudoklossia sp.[8]	*Cerastoderma edule*	Kidney	–	≈26	≈5	n.d.	2
Bivalve Scallop							
P. pectinis[9]	*Pecten maximus*	Kidney	–	32–35	3–5	n.d.	n.d.
Many species[10]	*Argopecten irradians*	Kidney and other organs	n.d.	n.d.	n.d.	n.d.	n.d.
Pseudoklossia sp.[11]	*Argopecten irradians*	Kidney and other organs	n.d.	n.d.	n.d.	n.d.	n.d.
P. pectinis-like[12]	*Argopecten irradians*	Kidney	n.d.	n.d.	n.d.	n.d.	n.d.
Unnamed[13]	*Argopecten irradians*	Kidney and other organs	n.d.	n.d.	n.d.	n.d.	n.d.
Margolisiella islandica[14]	*Chlamys islandica*	Heart	+	48–60 × 40–44	5.5–6.5	>500	2
Bivalve mussel							
P. semiluna[15]	*Mylilus* spp.	Kidney	–	22–25	6 × 3	≈24	2
Gastropoda							
P. haliotis[*,16]	*Haliotis* spp.	Kidney	+	≈30–32	8 × 9	≈36	2
Polyplacophora							
P. patellae[*,17]	*Acanthochites fasciularis*	Intestine hepatopancreas	+	n.d.	n.d.	n.d.	n.d.
P. chitonis[*,17]			+	n.d.	n.d.	n.d.	n.d.

n.d. = No data.
[1] Léger (1897).
[2] Léger and Duboscq (1915).
[3] Buchanan (1979).
[4] Levine (1988).
[5] Morado et al. (1984).
[6] Desser and Bower (1997).
[7] Nakayama et al. (1998).
[8] Carballal et al. (2001).
[9] Léger and Duboscq (1917).
[10] Leibovitz et al. (1984).
[11] Cawthorn et al. (1992).
[12] Karlsson (1991).
[13] Whyte et al. (1994).
[14] Present study.
[15] Desser et al. (1998).
[16] Friedman et al. (1995).
[17] Debaisieux (1922).
[*] Species transferred to the genus *Margolisiella* by Desser and Bower (1997).

3.4. Taxonomic summary – *M. islandica sp. nov.*

Suborder: Eimeriorina Léger, 1911
Family: Eimeridae Michin, 1903
Type host: Iceland scallop, *C. islandica* (Müller, 1776)
Type locality: Breidafjördur bay, W-Iceland.
Type material: Histological slides and stained tissue imprints will be deposited at The Institute for Experimental Pathology, University of Iceland, IS-112 Reykjavik, Iceland.
Habitat/site of infection: Endothelium of the heart's auricle.
Etymology: The species name refers to both type locality and type host.

4. Discussion

M. islandica, along with a novel but an unidentified apicomplexan species described from the same material (Kristmundsson et al., 2011), is the first apicomplexan species described from Iceland scallop. The presence of both asexual (merogony) and sexual stages (gametogony and sporogony) of *M. islandica*, confirms a monoxenous life cycle. Desser and Bower (1997) established a new genus, *Margolisiella* for *Pseudoklossia* species with known monoxenous life cycles, but left the remaining, possibly heteroxenous species, within the genus *Pseudoklossia*. On that basis this new apicomplexan species from Iceland scallop belongs to the genus *Margolisiella* rather than *Pseudoklossia*.

Unlike *M. islandica*, which was only found in Iceland scallop, the aforementioned unidentified apicomplexan infected all the scallop species examined, i.e. Iceland scallop, queen scallop and king scallop (Kristmundsson et al., 2011). Developmental forms of this apicomplexan detected, apparently included both sexual and asexual stages of the parasite which strongly suggests a monoxenous life cycle. It was found in all muscular tissues of its hosts, both intracellular but also in the extracellular space. Furthermore, zoites

were commonly found inside haemocytes. This parasite is morphologically different from *M. islandica* and apparently all other apicomplexan species previously described from bivalves. In addition, it seems to be the only one infecting muscle cells (Kristmundsson et al., 2011).

Apart from the present paper and the one of Kristmundsson et al., 2011, the only paper published on parasites of Iceland scallop is that of Giguere et al. (1995), who studied the cause of mass mortalities of sea scallops *Plagopecten magellanicus* and Iceland scallops occurring in Canadian waters. They found the Iceland scallops and sea scallops infected with unidentified turbellarians and ciliates as well as rickettsia-like organisms.

In general, wild scallops are poorly studied with respect to parasites and diseases and of all commercially exploited scallop species, *C. islandica* is probably the least studied (Ball and McGladdery, 2001; McGladdery et al., 2006). *M. islandica* together with *P. pectinis* from king scallop *P. maximus* (Léger and Duboscq, 1917), are apparently the only two apicomplexans infecting scallops which have been identified to a species level. Other apicomplexans reported from scallops are either unnamed (Leibovitz et al., 1984; Whyte et al., 1994) or assigned to certain genera (Karlsson, 1991; Cawthorn et al., 1992). *M. islandica* differs in many ways from other eimeriorin species previously described from molluscs. It is the only species which infects the heart while the majority of other species described primarily infect the kidney. Furthermore, the oocysts differ in size and also the number of sporozoites inside each oocyst. The number of sporozoites in *M. islandica* is much greater than in other species, i.e. >500 compared to 20–36 in other known species (Table 1). Morphologically, *P. pectinis*, from *P. maximus* (Léger and Duboscq, 1917) resembles *M. islandica*, especially the development of macrogamonts which become pear or heart shaped at later developmental stages. However, the oocysts and sporocysts of *P. pectinis* are considerably smaller (oocysts: 32–35 μm compared to 40–44 × 48–60 μm; sporocysts: 3–5 μm compared to 5.5–6.5 μm) and contain fewer sporocysts. Furthermore, the sporozoites are not enclosed within a membrane, unlike *M. islandica*, and *P. pectinis* infects the kidney (Léger and Duboscq, 1917).

The robust grouping of *M. islandica* as a sister taxon to the rhytidocystid clade in the phylogenetic analyses indicates that it is more closely related to the poorly understood coccidian-like lineage of agamococcidians (order: Agamococcidiorida Levine 1979) than to the true eimeriorine parasites (order: Eucoccidiorida Léger & Duboscq, 1910). *Rhytidocystis* spp. are monoxenous parasites of polychaetes that penetrate the host intestine and develop in the connective tissues, gonads and the coelom (Rueckert and Leander, 2009). Two other apicomplexan sequences that form the rhytidocystid/bivalve grouping (Fig. 10) are from unidentified apicomplexan parasites infecting the giant clam, *T. crocea*, and the European oyster, *Ostrea edulis*, both bivalve molluscs. The apicomplexan infecting the giant clam has been partially described, but the only life stages observed were trophozoites (Nakayama et al., 1998). No oocysts or sporozoites were observed which are the forms we would use for comparison, consequently it is hard to morphologically compare this species to *M. islandica*. Nevertheless, they have a high percentage similarity (97.7%) with respect to the SSU rDNA sequence data.

Molecular data from apicomplexans infecting bivalves is currently very limited and none are available for scallop-infecting species. More data from other apicomplexan parasites infecting invertebrates is required to fully validate this phylogenetic placement for *Margolisiella*. In general, apicomplexan parasites of invertebrates are poorly represented in phylogenetic reconstructions compared to the well-studied vertebrate-infecting groups (Kopečná et al., 2006).

The infection prevalence and intensity of *M. islandica* was high in Iceland scallops from both sampling sites in Breidafjördur bay. However, no infections were detected in the queen scallops and king scallops from Faroese and Scottish waters. A number of things could explain that: (1) the parasite is host specific; (2) the sample size is too small to detect low levels of infection, or (3) these other species have not been exposed to infection. *Pseudoklossia* and *Margolisiella* species are generally not considered highly pathogenic (Leibovitz et al., 1984; Morado et al., 1984; Karlsson, 1991; Whyte et al., 1994; Desser et al., 1998; Carballal et al., 2001). However, heavy infections, which seem more restricted to artificial conditions, have the potential of causing tissue damage (e.g. Leibovitz et al., 1984; Cawthorn et al., 1992; Carballal et al., 2001).

Long term studies on the potential pathological effect of *M. islandica* on the health status of the Iceland scallop population are in process and will be discussed in a separate paper.

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Paper II

Previously unknown apicomplexan species infecting Iceland scallop, *Chlamys islandica* (Müller, 1776), queen scallop, *Aequipecten opercularis* L., and king scallop, *Pecten maximus* L.

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Previously unknown apicomplexan species infecting Iceland scallop, *Chlamys islandica* (Müller, 1776), queen scallop, *Aequipecten opercularis* L., and king scallop, *Pecten maximus* L.

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ABSTRACT

Examination of three scallop species from three separate locations: Iceland scallop from Icelandic waters, king scallop from Scottish waters and queen scallop from Faroese and Scottish waters, revealed infections of a previously unknown apicomplexan parasite in all three scallop species. Developmental forms observed in the shells appeared to include both sexual and asexual stages of the parasite, i.e. merogony, gametogony and sporogony, which suggests a monoxenous life cycle. Meronts, gamonts, zygotes and mature oocysts were solely found in the muscular tissue. Zoites, which could be sporozoites and/or merozoites, were observed in great numbers, most frequently in muscles, both intracellular and free in the extracellular space. Zoites were also common inside haemocytes. Examination of the ultrastructure showed that the zoites contained all the major structures characterizing apicomplexans. This apicomplexan parasite is morphologically different from other apicomplexan species previously described from bivalves. Presently, its systematic position within the phylum Apicomplexa cannot be ascertained.

1. Introduction

Wild populations of Iceland scallop *Chlamys islandica*, queen scallop *Aequipecten opercularis* (syn. *Chlamys opercularis*), and king scallop *Pecten maximus*, are all commercially exploited shellfish species. Iceland scallop has the most northern distribution, occurring mainly in the sub-arctic transitional zone. In the Atlantic Ocean it is found around Iceland, Greenland, in the Barents Sea, off the northern coast of Norway south to Bergen and along the east coast of Canada south towards Massachusetts. It has also been recorded in the N-Pacific Ocean (Brand, 2006). King- and queen scallops have a similar geographic distribution, which extends more south than that of Iceland scallop. The queen scallop occurs in the NE-Atlantic from northern Norway and the Faroe Islands south to the Iberian Peninsula, the Azores and the Canary Islands. Its distribution also extends into the Mediterranean and Adriatic seas. The distribution of king scallop spans from northern Norway, along the west coast of Europe and south to North Africa (Nicolajsen, 1997; Brand, 2006).

Although reports on apicomplexans infecting bivalves date back to 1897, when Léger (1897) described the first species, *Hyalloklossia pelsineeri* from *Donax* sp. and *Tellina* sp., they are poorly studied; this is especially so for scallops. Only few studies on parasites in the scallop species examined in the present paper exist in the literature, i.e. the king scallop, the queen scallop and the Iceland scallop. One apicomplexan species, *Pseudoklossia pectinis*, has been reported from king scallop off the French coast (Léger and Duboscq, 1917), and one from Iceland scallop in Icelandic waters, i.e. *Margolisiella islandica* (Kristmundsson et al., 2011). The only parasitic examination performed on queen scallop seems to be that of Lohrmann et al. (2000), who reported a microsporidian infection in this species. Mortenson (1993) published a health survey on selected bivalve stocks in Norwegian waters. The king scallop was included in the survey, but no protozoan parasites were detected. To date, *Argopecten irradians* is probably the most examined scallop species with regard to apicomplexan parasites, and several papers have been published (Karlsson, 1991; Cawthorn et al., 1992; Leibovitz et al., 1984; Whyte et al., 1994). However, none of the apicomplexans encountered in these surveys have been identified to a species level.

Most apicomplexan species known to infect bivalves have been described from clams. These include *Pseudoklossia* (syn. *Hyalloklossia*) *pelsineeri* from *Donax* sp. and *Tellina* sp. (Léger, 1897), *Pseudoklossia glomerata*, from *Tapes floridus* and *Tapes virgineus* (Léger and Duboscq, 1915), *Pseudoklossia* (syn. *Merocystis*) *tellinovum* from *Tellina tenuis* (Buchanan, 1979; Levine, 1988) and *Margolisiella kabatai* from the littleneck clam *Protothaca staminae* (Desser and Bower, 1997). In addition, Morado et al. (1984) and Nakayama et al.

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(1998) reported unidentified apicomplexans from the littleneck clam *P. staminae* and the giant clam *Tridacna crocea*, respectively.

Some apicomplexan species infecting bivalves have direct life cycles, i.e. both asexual and sexual stages are present in the same host (e.g. Desser and Bower, 1997), while in others, only sexual stages have been found, which could suggest the need for an additional host (Desser et al., 1998). Another possibility is that the asexual forms are present in the same host, but have not been detected. However, in some cases, such as *Nematopsis* spp., an indirect life cycle has been confirmed where sexual stages are found in various bivalve species and schizogonic multiplication occurs in crustaceans (Lauckner, 1983; Soto et al., 1996; Darriba et al., 2010). Finally, there are documentations of apicomplexan parasites in bivalves where certain parasitic forms have been detected, which could not be identified as either asexual- or sexual forms (Nakayama et al., 1998; Hine, 2002). According to papers published to date on apicomplexans of bivalves, it seems there are no examples where only the asexual reproduction, i.e. schizogony, occurs in bivalves.

In the year 2002 live scallops were sent to the Fish Disease Laboratory at the Institute for Experimental Pathology at Keldur for examination due to abnormal mortality in the stock. Observations revealed infections with two previously unknown apicomplexan species.

In this paper one of the apicomplexan species is described, but the second one is the subject of a separate paper (Kristmundsson et al., 2011). Due to abnormal conditions of queen scallops off the Faroe Islands at that time, a Faroese company in the Scallop fishery industry requested an observation of the scallops with regard to diseases. In addition, queen- and king scallops from Scottish waters were sampled at a later stage and examined to further check the geographic distribution of the parasites.

2. Materials and methods

2.1. Scallop collection

Live Iceland scallops from two locations in the Bay of Breidafjördur were sampled in November 2002 ($n = 2 \times 30$; size 3.0–9.0 cm). Queen scallops ($n = 60$; size 6.0–7.0 cm) from the east coast of the Faroe Islands were sampled in February 2005, and in September 2007 queen scallops ($n = 10$; size 4.5–7.0 cm) and king scallops ($n = 10$; size 10.0–13.0 cm) from the west coast of Scotland (Fig. 1). The scallops were sampled from natural beds by dredging, except the queen scallops from Scottish waters, which were collected by scuba diving.

The Iceland scallops were sampled from a population suffering extensive natural mortality leading to a collapse in the stock index (Anonymous, 2003). An examination of the queen scallops from Faroese waters was requested by a company in the Faroese Scallop fishery industry due to poor condition of the adductor muscle. No data on natural mortality of the scallop population off the Faroe Island was available. Macroscopic signs of disease were apparent in adductor muscles of both Iceland scallops and queen scallops from the Faroe Islands. The muscles were small compared to shell size, had a brownish appearance, were less compact than normal and with a high fluid content. The scallops from Scottish waters were sampled from an apparently healthy population experiencing no abnormal mortality or signs of disease.

2.2. Microscopic observations

Shells were dissected and all major organs (adductor muscle, kidney, mantle, gonads, palps, gills, heart, digestive diverticula, stomach, intestine) examined microscopically for parasites using the following methods: (1) Fresh mounts: samples from each organ pressed between a glass slide and a coverslip, (2) Stained imprints: tissue imprints of organs (i.e. adductor muscle, heart and kidney) were air dried, fixed in methanol for 3 min and stained with May–Grünwald–Giemsa, (3) Davidson's fixed histological sections of all organs prepared by conventional methods and stained with Giemsa. Parasites present were described and their tissue distribution analysed. All dimensions were measured (μm) and photographs taken using a Leica DMLB microscope equipped with a digital camera (Leica DC300F).

For examination of parasite ultrastructure, small pieces ($\approx 1\ mm^3$) of infected muscles were fixed in 2.5% buffered glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 24 h at 4 °C, washed three times in cacodylate buffer, post-fixed for 1 h in 1% OsO_4, rinsed again with buffer, dehydrated in a graded alcohol series, sectioned, stained for 10 min in 5% uranyl acetate and for 6 min in 5% lead citrate. Ultrathin sections were examined using a FEI, Tecnai G2 Spirit Biotwin, Transmission Electron Microscope (at 120 kV) at the Institute of Aquaculture, University of Stirling, Scotland.

3. Results

All Iceland scallops and the queen scallops sampled in Faroese waters were infected with an apicomplexan parasite. Infections were commonly intense. Identical developmental forms were also found in both queen scallops (detection frequency 40%) and king scallops (90%) from Scottish waters, but infections were very light in all cases.

3.1. Fresh mount observations

Various types of large cysts at different developmental stages are present in the adductor muscle. Their size and outer and inner morphology is quite variable. Thin walled elongated cysts, $320 \pm 50\ \mu m \times 75 \pm 25\ \mu m$ ($n = 10$) (Fig. 2a), with granular cytoplasm are common. Sometimes, these have membranous protrusions at each end. In other cases, similar cysts contain a clear area, which resembles a formation of spindle apparatus, which becomes evident when zygotes undergo the first nuclear division (Fig. 2b). Another type of cysts, commonly found, are usually more slender and with pointed ends. These cysts, $285 \pm 25\ \mu m \times 45 \pm 10\ \mu m$ ($n = 10$), are filled with numerous round spheres or nuclei, 3.5–4.0 μm in diameter (Fig. 2c). Yet another type, $297 \pm 40\ \mu m \times 98 \pm 25\ \mu m$ ($n = 10$), has a very thick wall (5–7 μm) (Fig. 2d and e) with regular villar protrusions (Fig. 2f). Due to the thick wall, its inner structure is not clearly seen in wet mounts.

Zoites, i.e. sporozoites and/or merozoites, are abundant in all muscular tissue of moderately and heavily infected individuals. The adductor muscle is generally most heavily infected. Live zoites measure $17.5 \pm 2.0 \times 6.5 \pm 1.5\ \mu m$ ($n = 100$), the size range most frequently encountered being $18–19 \times 6.5–7.5\ \mu m$. They are slightly curved with a distinct and large nucleus (Fig. 3a and b).

3.2. Cyst morphology

Histological examination showed numerous cysts intracellular in the adductor muscle of infected individuals, presenting different developmental stages of the parasite. These include cysts with uniformly granular cytoplasm, cyst containing variable numbers of nuclei and zoite-forming cysts, apparently representing merogony, gametogony and sporogony. Syzygy, the pairing of two gametes prior to fertilization, was absent. Suggested developmental forms for merogony, gametogony and sporogony are presented in Figs. 4 and 5.

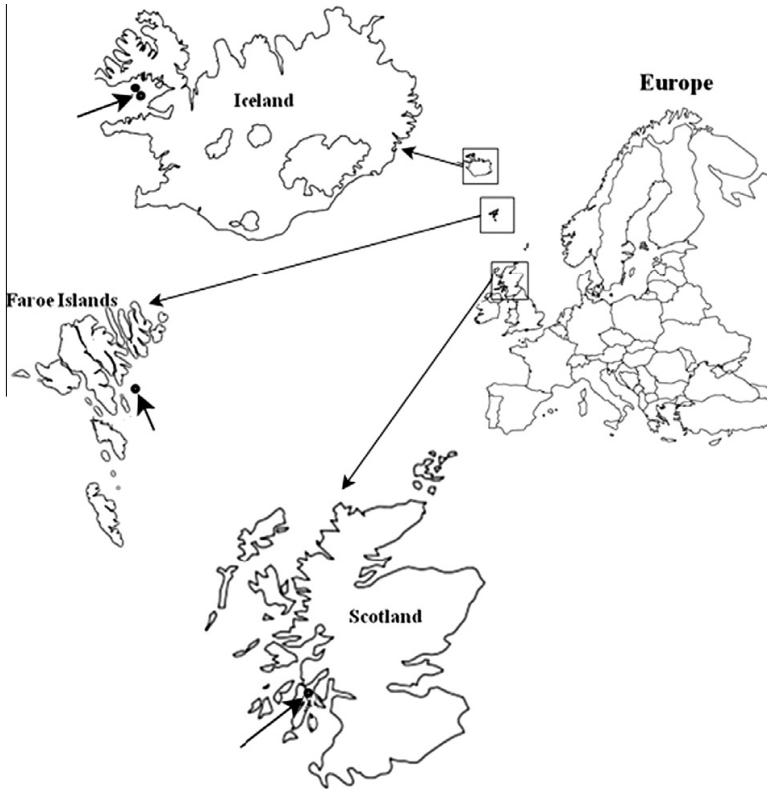

Fig. 1. Sampling sites of scallops from Iceland, Scotland and the Faroe Islands.

3.2.1. Merogony

Apparently, the first sign of merogony involves a formation of cysts with few slightly elongated and peripherally arranged nuclei (Fig. 4a). With further development, these nuclei divide and become uniformly arranged inside the cyst (Fig. 4b). Subsequently, a series of nuclei cleavage occurs forming cysts, 75 ± 5 μm (diameter), containing slightly elongated nuclei without obvious cytoplasm, forming several elliptical forms within the cyst (Fig. 4c). Mature meronts, 300 ± 25 × 100 ± 20 μm (n = 20), contain merozoites with obvious cytoplasm arranged in a rosette like fashion around residual bodies (Fig. 4d and e).

3.2.2. Gametogony

Macrogamont, 330 ± 50 × 75 ± 25 μm (n = 20), are the most common type of cysts observed. They are elongated, with a granular cytoplasm, commonly with pointed ends and enclosed with a thin membrane (Fig. 5a). Presumed microgamonts, 180 × 100 ± 10 μm (n = 5), contain numerous curved and slender microgametes, 13–14 × 2.5 μm (n = 20), irregularly distributed inside the cysts. The microgametes differ from zoites by their apparent lack of cytoplasm (Fig. 5b).

3.2.3. Sporogony

Fertilization is detected by the formation of cysts with a spindle like apparatus representing a zygote undergoing its first nuclear division (Fig. 5c). The onset of oocyst formation seems to involve numerous nuclei divisions resulting in cysts containing a variable number of round uniformly spread nuclei. These cysts, which are found in great numbers, contain up to several hundred nuclei (Fig. 5d and e). With further development, these nuclei elongate and a cytoplasm becomes evident (Fig. 5f). Mature oocysts, 200 ± 80 × 75 ± 25 μm (n = 20), enclose numerous regularly arranged sporozoites, 12–13 μm in length and 3–4 μm in width (n = 20), with a clear and distinct cytoplasm (Fig. 5g and h).

In some cases, developing cysts could be identified as belonging to merogony (Fig. 4), gametogony or sporogony (Fig. 5), while in other cases, their situation in the life cycle is more speculative.

3.5. Characterization of zoites

In histological sections, zoites were found in all muscular tissues. In the adductor muscle they were found in large clusters (Fig. 6a and b) in necrotic areas where they infected individual muscle cells (Fig. 6c). They were also common in the intercellular space of various organs, but also inside haemocytes, most often one in each haemocyte, but in some cases up to four (Fig. 6d–f). Zoites were not seen in the alimentary canal or its epithelium. Two zoite size groups were observed. The larger ones (14–15 × 7–8 μm) were most common, but the smaller ones (10–12 × 3–4 μm) were only occasionally seen. The smaller zoites appeared to have a more definite curled form with a nucleus covering most of it and cytoplasm only at each end (Fig. 6g).

Ultrastructurally, the larger zoites contained all major structures characterizing apicomplexan zoites (Fig. 7a–i). A large and round nucleus is located in the posterior half of the parasite occupying almost the whole width of the cell and almost half of its

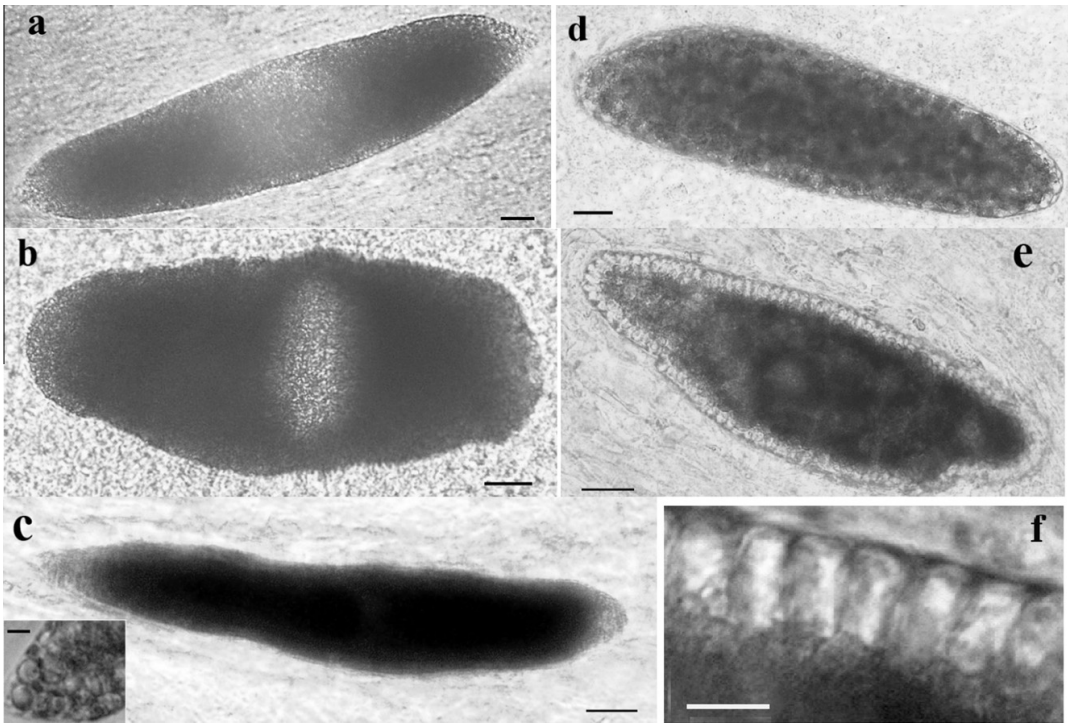

Fig. 2. Live specimens of cysts from adductor muscle. Slender cyst with granular cytoplasm (a). Cyst with granular cytoplasm and a visible fertilization spindle (b). Slender cyst with pointed ends containing numerous round spheres or nuclei (inserted picture) (c). Cyst where a thickening of the cyst wall becomes evident (d). Thick walled cyst with villar protrusions on the wall (e and f). Scale bar (a–c) = 25 μm; (inserted picture and f) = 5 μm.

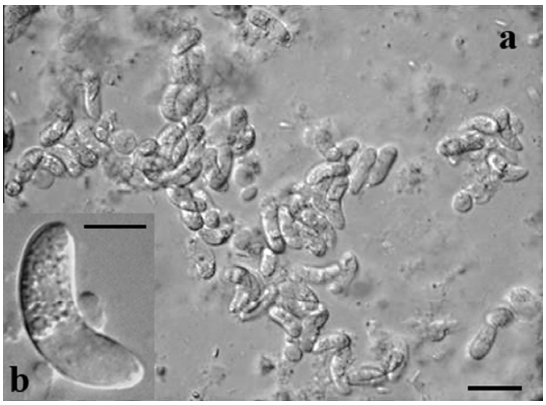

Fig. 3. Cluster of free zoites (a) and a single zoite at high magnification (b) Live specimens. Scale bar: (a) = 20 μm; (b) = 5 μm.

length (Fig. 7a). The cell boundary, the pellicle, consists of an outer unit membrane and an inner layer, which is composed of two unit membranes closely attached to another. The outer unit membrane and the inner membranous layer are separated by an intermediate osmiophobic space (Fig. 7f). Approximately 80–85 sub-pellicular microtubules extended from the anterior margin of the nucleus and to the outermost front of the cell (Fig. 7g). The conoid was detected but not the polar rings (Fig. 7e). Micronemes spanned from the apical complex to near the anterior surface of the nucleus, but were occasionally posterior to the nucleus. Rhoptries were clearly seen but were not serially sectioned and counted (Fig. 7b,c and g). The Golgi cisternae were observed near the anterior surface of the nucleus (Fig. 7h) and the endoplasmic reticulum between the nucleus and the apical complex (Fig. 7e). Thick-walled structures were detected in the anterior part of the cell, which could possibly be apicoplasts (Fig. 7b). Mitochondria were seen in many sections at various placements in the cell and seemed to occupy a large space in the cytoplasm (Fig. 7a and i). Amylopectin granules were found in large numbers and almost exclusively in the anterior part (Fig. 7a and h).

4. Discussion

Only few papers have been published on apicomplexans infecting scallops and only two species have been identified to a species level, i.e. *P. pectinis* from *Pecten maximus* (Léger and Duboscq, 1917) and *M. islandica* from Iceland scallop (Kristmundsson et al., 2011). Other reports of apicomplexans in scallops are from *A. irradians*, such as *P. pectinis*-like apicomplexan (Karlsson, 1991), *Pseudoklossia* sp. (Cawthorn et al., 1992), and few other unnamed examples (Leibovitz et al., 1984; Whyte et al., 1994).

Apart from *M. islandica*, described by Kristmundsson et al. (2011) from the same material as in the present paper, the only report on parasites of Iceland scallop is that of Giguere et al. (1995), who found Iceland scallops and sea scallops *Placopecten magellanicus* on the lower shore of Quebec infected with unidentified turbellarians and ciliates as well as rickettsia-like organisms. Regarding the queen scallop, the only reported parasite is a

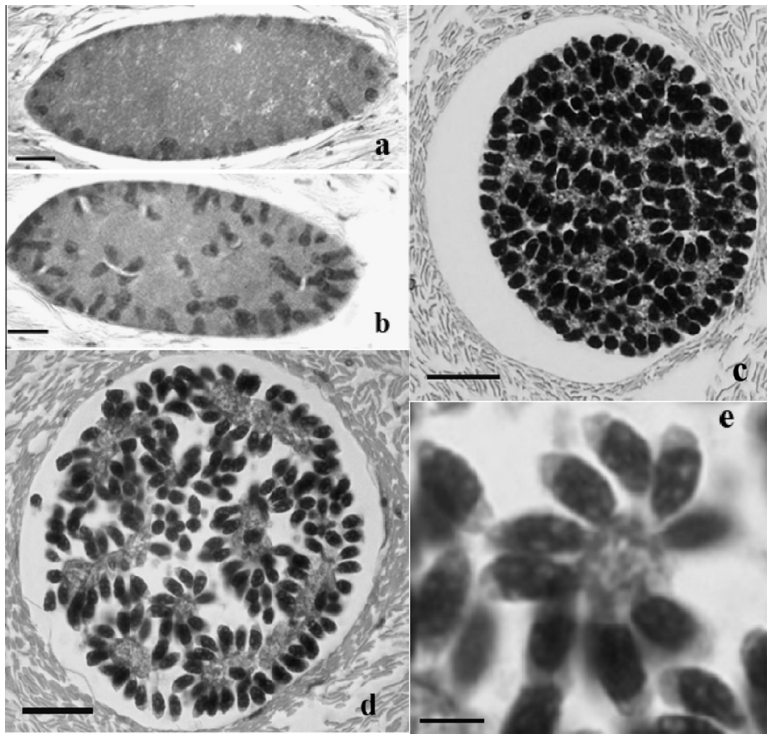

Fig. 4. Meront development. Giemsa stained histological sections showing: Formation of multiple, slightly elongated, nuclei at the periphery signalling the onset of meront formation (a). Further development; nuclei multiplication (b and c). Mature meront with merozoites arranged in a rosette fashion around a residual body (d). Rosette arranged merozoites (e). (a and b) = longitudinal sections, (c–e) transverse sections. Scale bar: a–d = 20 μm; e = 5 μm.

microsporidian infecting the connective tissue of the digestive gland (Lohrmann et al., 2000). The only parasite described from *Pecten maximus* is the aforementioned species *P. pectinis* (Léger and Duboscq, 1917).

The apicomplexan parasite described in this paper was found in three scallop species of three different, but related genera, which indicates a relatively low host specificity of this parasite. The host specificity of apicomplexans infecting bivalves is poorly known, but those infecting fish differ considerably in their host specificity (Molnár, 1995). The study confirms the presence of this parasite off the coast of Iceland, Scotland and the Faroe Islands. Infections were detected in both queen- and king scallop off the Scottish coast despite only 10 shells being examined of each species. Although there are no reports of this parasite from the continental coast of Europe and the east coast of Canada, our results might indicate that this parasite is common among scallops at least in the eastern part of the North Atlantic.

The authors feel confident that all life stages are present in infected shells. The merogonic stages, presented in Fig. 4a–e, resemble comparable life stages reported by e.g. Desser and Bower (1997, Fig. 2) and Lindsay et al. (2000, Figs. 1i and 2a and b). Similarly, the presence of macrogamonts and a fertilization spindle (Fig. 5a and c) confirms the gametogony. Microgamonts were not observed with certainty. An early development of microgamont resembles those of young meront (Fig. 4a) (see Morado et al., 1984, Fig. 13). The cyst in Fig. 5b, which we speculate might be microgamonts, contains many curved elongated bodies, which, because of no apparent cytoplasm, differ from zoite-forming cysts (Fig. 4d,e and 5a,h). Apart from these, there are various other developmental forms whose position within the developmental cycle cannot be definitely ascertained (Fig. 5b,d,e).

The presence of both sexual and asexual stages in individuals of all three scallop species examined in the present study suggests a monoxenous life cycle, whereby a direct horizontal transmission occurs between individuals with the possibility of autoinfection. All stages, i.e. zoites and cysts, of the parasite are present inside adductor muscle cells. Zoites were also present inside muscle cells of other organs, inside haemocytes and widely distributed in the extracellular space of the host. No zoites were detected in the intestinal lumen or intestinal mucosa. The route of entry of the parasite, its complete development within the host and the release of the infective stages is not known at present. Whether this is through active filter feeding of the host, subsequent parasite penetration through the gastrointestinal mucosa, spread with haemocytes or via hemolymph, and release of the infective stage with faeces and/or decomposing dead host, remains speculative. Infection trials with serial sampling would be a feasible approach to analyze the successive entry and development of the parasite.

Presently, the systematic position of this new species within the phylum Apicomplexa cannot be determined. It is morphologically different from all other apicomplexan species previously described from bivalves and apparently the only one infecting muscle cells. The gamonts and oocysts of this parasite are elongated and much larger than observed in other known species infecting bivalves. The huge amount of free sporozoites inside each oocyst (hundreds) and the lack of sporocysts also differ significantly from previously described species. Although the sporozoites are crescent shaped, a common morphology of apicomplexan zoites, they do not

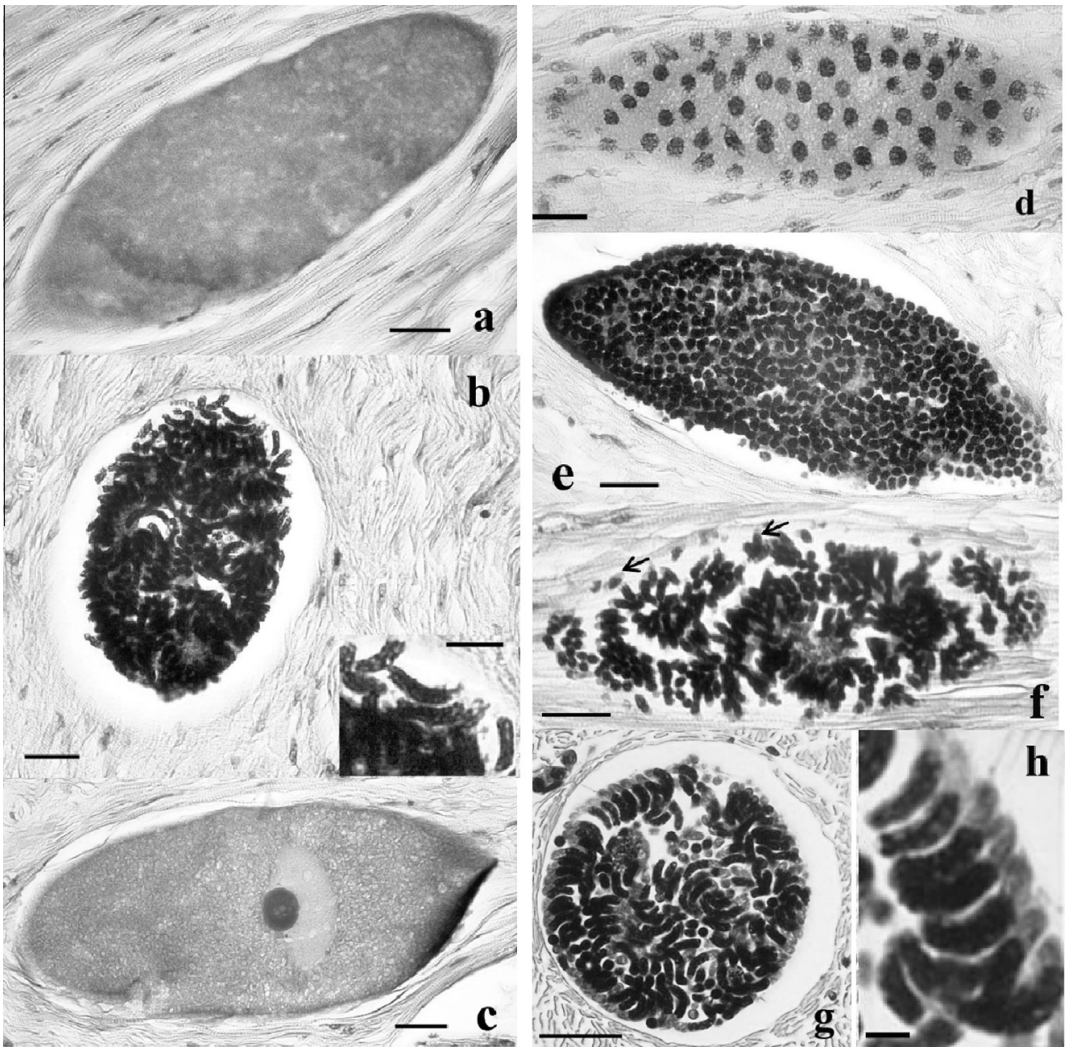

Fig. 5. Giemsa stained histological section showing: Macrogamonts with granular cytoplasm closely packed in muscular tissue (a). Numerous presumable microgametes emerging from a mature microgamont (b). Fertilization spindle becoming visible whereby the chromatids migrate to the periphery – indication of first nuclear division of the zygote (c). Developing oocyst tightly packed with numerous small spherical nuclei derived from series of cleavage (d and e). Oocyst with multiple nuclei which are beginning to elongate and a cytoplasm becoming evident (arrows) (f). Mature oocyst containing numerous sporozoites (g). Mature sporozoites inside an oocyst (h). (a and f) = longitudinal sections; (g and h) = transverse section. Scale bars: (a–g) = 25 μm; h = 5 μm.

resemble sporozoites of other known species, i.e. *Pseudoklossia* sp. and *Margolisiella* sp., which are common apicomplexan species found in bivalves (see Kristmundsson et al., 2011, Table 1). They are larger and furthermore, the nucleus is relatively bigger, occupying a significant part of the cytoplasm. Of other apicomplexans reported from bivalves, the zoites found in dredge oysters *Ostrea chilensis* around New Zealand (Hine, 2002) show the most resemblance with the zoites of those found in the present study, such as the great number (>80) of sub-pellicular microtubules in both these species. However, Hine (2002) merely detected zoites and did not observe any sign of other developmental forms. Regarding the cysts observed in our material, which were exclusively found in muscular tissue, they apparently show considerable similarity with *Sarcocystis* spp., an apicomplexan species commonly found in various muscles of herbivores and omnivores (Rommel et al., 2000). There is, however, also a big difference as the developmental forms of *Sarcocystis* species found in the muscles of herbivores and omnivores are only asexual stages, but those found in our material are both sexual and asexual. Furthermore, the oocysts in the present study contain no sporocysts but numerous free sporozoites. *Sarcocystis* spp., however, have oocysts with four sporozoites in each sporocyst (Rommel et al., 2000).

Possible negative health effects on the Iceland scallop population caused by this undefined apicomplexan parasite, described in this paper along with *M. islandica* (Kristmundsson et al., 2011), also detected in the Icelandic scallop, cannot be ruled out. Long term studies, which are presently in process, will shed light on this issue. These studies will be discussed in a separate paper.

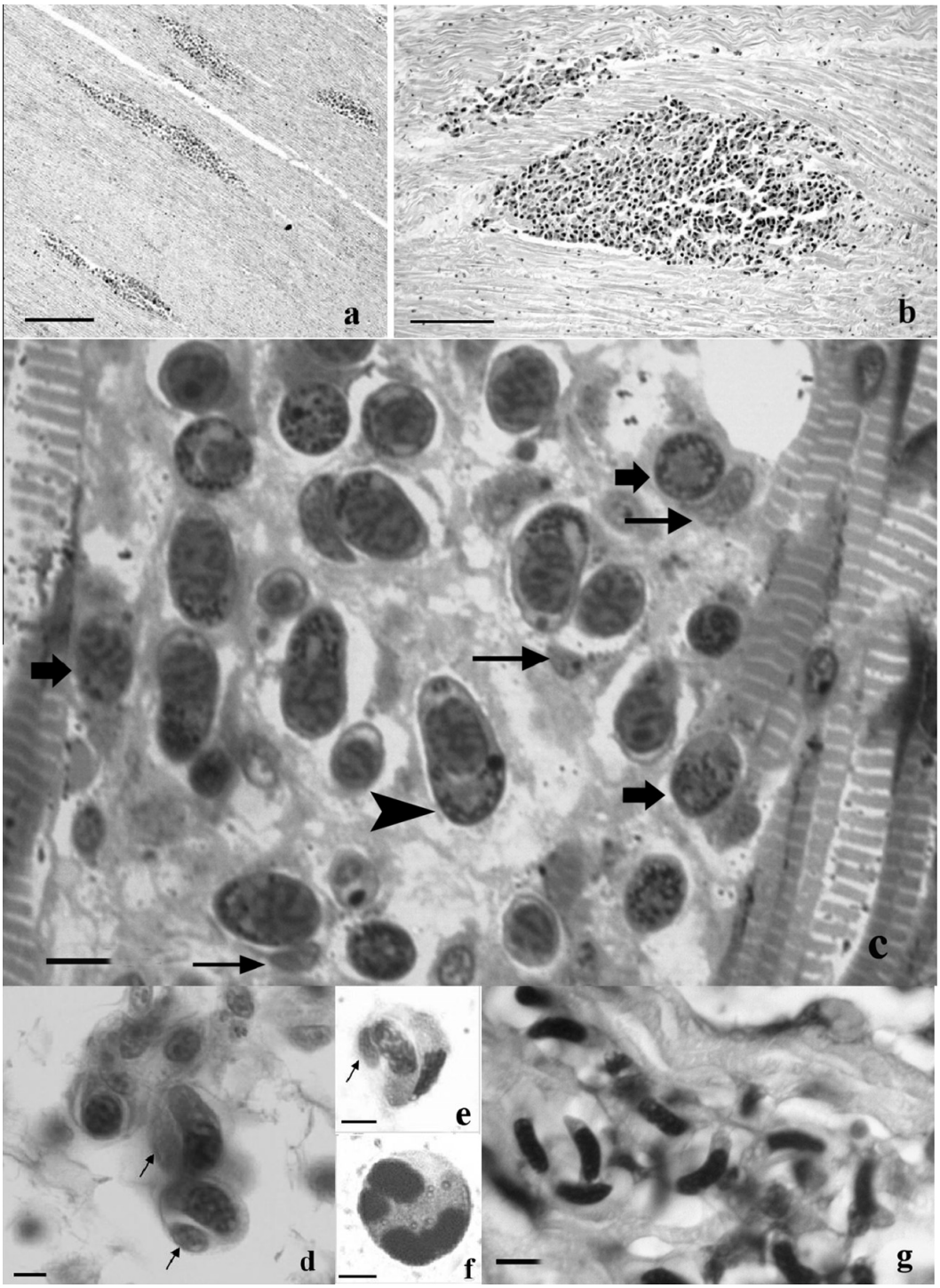

Fig. 6. Histological section of zoite clusters in the adductor muscle (a and b). Numerous zoites in a necrotic area of adductor muscle. Host nuclei (thin arrow). Zoites within degenerating muscle cells (broad arrow). Zoites in necrotic muscle cell debris (arrowhead) (c). A single zoite inside haemocytes (d). Multiple zoites inside haemocytes (May–Grünwald–Giemsa stained imprints). Note host cell nucleus (arrow) (e and f). Cluster of smaller size zoites (g). Scale bars: (a and b) = 100 μm; (c and g) = 5 μm.

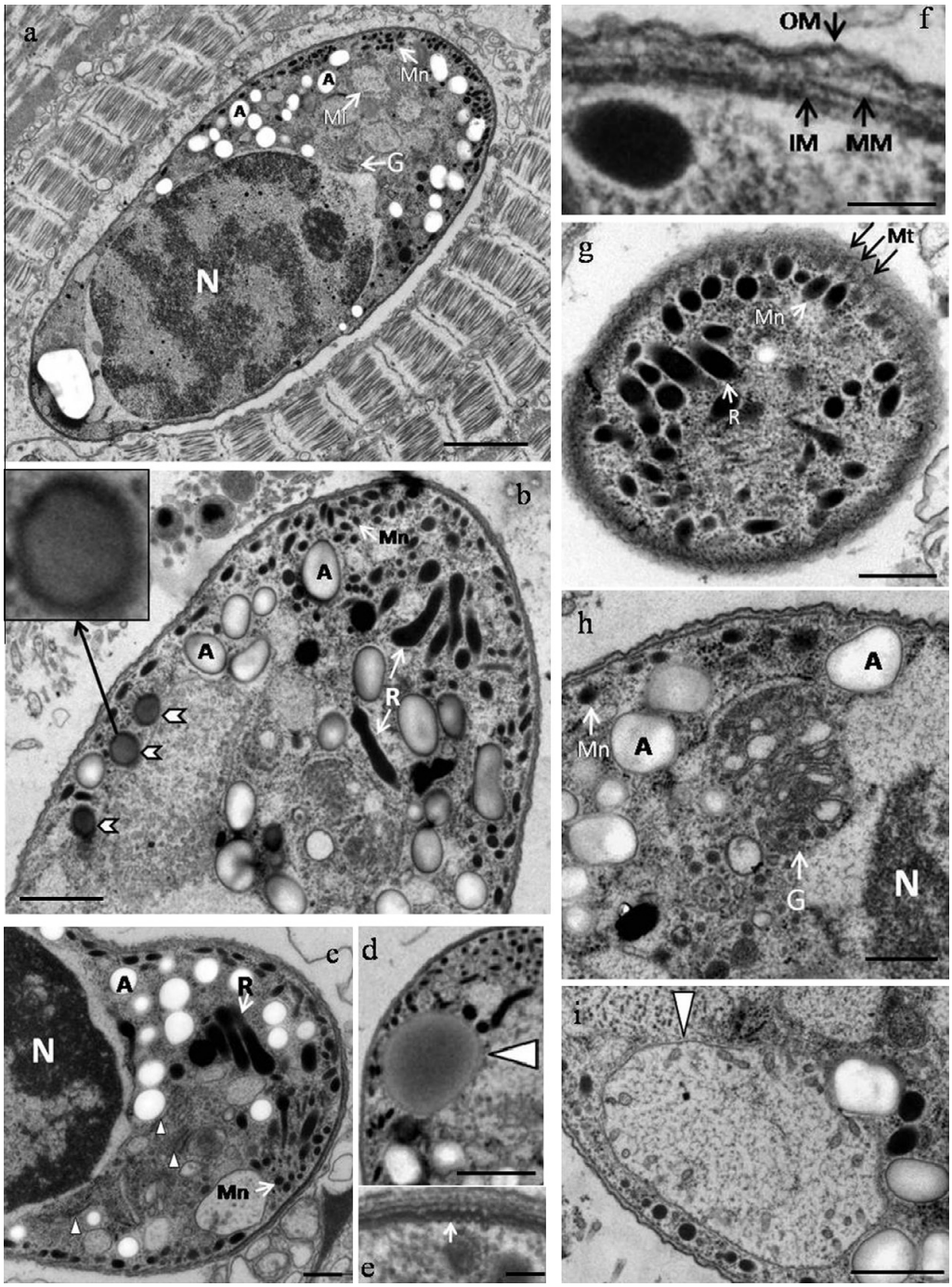

Fig. 7. Longitudinal section through a sporozoite in muscle showing many of the major components of a zoite (a). Section through the anterior part of a sporozoite showing rhoptries, micronemes, amylopectin granules and a thick walled structure (arrowhead), possibly apicoplasts (b). Anterior part of a sporozoite showing various cell structures, i.e. endoplasmic reticulum (arrowhead) (c). A large lipid vacuole (arrowhead) (d). Section through edge of the conoid (e). The pellicle, with an outer membrane (OM) and an inner membrane (IM) and middle membrane (MM). Between IM and OM is an osmiophobic space (f). Transverse section through the anterior part of the sporozoite showing approximately 80–85 sub-pellicular microtubules (Mt) as well as micronemes and rhoptries (R) (g). The Golgi apparatus (G) along with nucleus (N), micronemes (Mn) and amylopectin granules (A) (h). Mitochondria (arrow) (i). Scale bars: a = 2 μm, b = 1 μm, c = 0.4 μm, d = 1 μm, e = 0.2 μm, f = 0.05 μm, g = 0.3 μm, h = 0.4 μm, I = 0.5.

Acknowledgments

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Paper III

Is an apicomplexan parasite responsible for the collapse of the Iceland scallop (*Chlamys islandica*) stock?

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All authors read and approved the final manuscript.

PLOS ONE

RESEARCH ARTICLE

Is an Apicomplexan Parasite Responsible for the Collapse of the Iceland Scallop (*Chlamys islandica*) Stock?

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Abstract

Due to the total and unexpected collapse of the Iceland scallop, *Chlamys islandica*, stocks around Iceland during the 2000s, a commercial fishing ban has been imposed on this valuable resource since 2003. Following the initial identification of an apicomplexan parasite in the scallops, a long-term surveillance program was established to evaluate the effect of the parasite on the population. The infections were highly prevalent in all shell sizes throughout the study. However, the parasite only impacts mature scallops where they cause severe macroscopic changes, characterized by an extensively diminished and abnormally coloured adductor muscle. A highly significant relationship was observed between infection intensity and gonad and adductor muscle indices. The first four years of the study, were characterized by high infection intensity and very poor condition of the adductor muscle and gonads, whilst during subsequent years, infections gradually decreased and the condition of the scallops improved. Histopathological changes were restricted to the presence of apicomplexan zoites which were widely distributed, causing varying degrees of pathology in all organs. In heavy infections, muscular and connective tissues were totally necrotized, destroying significant parts of numerous organs, especially the adductor muscle, digestive gland and gonads. The progression of the disease was in good synchrony with the mortality rates and the subsequent decline observed in the scallop stock and recruitment indices. Our findings strongly suggest that the apicomplexan parasite played a major role in the collapse of the Iceland scallop stock in Breidafjordur. In addition to causing mortality, the infections significantly impact gonad development which contributes further to the collapse of the stock in the form of lower larval recruitment. Furthermore, compelling evidence exists that this apicomplexan pathogen is causing serious disease outbreaks in other scallop populations. Similar abnormal adductor muscles and the parasite itself have been identified or observed in association with other mass mortality events in several different scallop species and commercial stocks in the northern hemisphere.

Introduction

Iceland scallop, *Chlamys islandica* (Muller, 1776) (Mollusca: Pectinidae) is a cold water bivalve species inhabiting the boreal-subarctic transition zone. In the NE Atlantic it is found around Iceland, in the Barents Sea and from the northern part of Norway, south to Bergen and the Stavanger Fjord. In the NW Atlantic, it is found in West Greenland, from Thule in the north to Cape Farewell in the south and along the eastern coast of Canada, from Cumberland Peninsula, Hudson Bay and south to Massachusetts [1].

Commercial fisheries of Iceland scallop have mainly been in Icelandic waters, but also on a smaller scale off West Greenland, in the Barents Sea and off the Atlantic coast of North America [1]. The fisheries in Iceland date back to 1969 with the main fishing grounds in Breidafjordur West Iceland, constituting 60–100% of the total catch, with the scallop industry forming a vital part of the economy in that region [2]. Other fishing grounds of less importance are the Westfjords in NW Iceland, Húnaflói in northern Iceland and Hvalfjordur in the southwest. From 1980 to 2000, the annual catch ranged from 8,500–17,000 tn, reaching a peak in the years 1983–1986 followed by a stable 8,000–9,000 tn catch from 1990–2000 [3].

The Iceland scallop is a relatively slow growing and long-lived bivalve species, its maximum observed age being 23 years [4]. It is dioecious (separate sexes) [5] similar to the Atlantic sea scallop, *Placopecten magellanicus* [6], while many species in the scallop family are hermaphroditic, such as the king scallop, *Pecten maximus* [7] and queen scallop, *Aequipecten opercularis*, [8]. The most commercially valuable part of the scallop is its large adductor muscle although gonads or even whole scallops are also utilized to some extent. In Iceland, the scallop fisheries are carried out by dredging and mostly just the adductor muscle is utilized and marketed as a high value sea food product [9].

In autumn 2000, the first indications of abnormalities in the scallop stock in Breidafjordur appeared, when diminished and abnormally coloured adductor muscles were noticed during processing. Subsequently, evidence of natural mortalities (not fishing-associated) in the stock emerged in 2001 when high numbers of cluckers (empty shells still attached by the hinge) were observed, which were almost exclusively restricted to mature scallops (shells $>$ 5 cm). These high natural mortalities were confirmed not to be associated with fishing pressure, as they were, in many cases, observed at scallop grounds where little or no fisheries had been undertaken [3]. Surveys of scallop stocks in Breidafjördur, the main scallop grounds in Iceland, over the years 2000–2006, performed by the Marine Research Institute (MRI) in Iceland, showed an 84% decrease compared to its average size in the years 1993–2000 [10]. In the years 2007 and 2008 the stock index reached a historical minimum, being merely 13% of its average size in the 1990s [11]. At other scallop grounds around Iceland a similar pattern was also emerging: in the Westfjords in the northwest, Húnaflói in the north and Hvalfjordur in the southwest, where the scallop stocks collapsed despite little or no fishing in these areas [12]. Due to these findings, no scallop fishing has been allowed in Icelandic waters since 2003.

In November 2002, when the downtrend in stock abundance became evident, live scallops were sent to the Fish Disease Laboratory at the Institute for Experimental Pathology at Keldur for examination. This initial examination of affected scallops revealed the presence of two apicomplexan parasites, one of which was found infecting muscular tissues and the other, *Margolisiella islandica*, the heart auricles [13,14]. Following the initial identification, a monitoring program was established where samples from affected scallop beds were examined for these infections at regular intervals. These examinations have shown that infections of *M. islandica* in the heart auricles are equally prevalent and intense in all shell sizes and that they do not negatively affect the scallops (unpublished data). Here we present the results of this survey, which now spans 12 years, with regards to the apicomplexan species infecting the adductor muscle

[13] its infection prevalence and intensity and impact on the condition of the stock of the Iceland scallop in Iceland.

Materials and Methods

Sampling

The field studies did not involve endangered or protected species. All the sampling sites are a property of the Icelandic state and no specific permissions were required for these locations/activities. The Marine Research Institute (MRI– www.hafro.is), responsible for all sampling, was established in 1965 and is working under the direct authority of the Ministry of Industries and Innovations in Iceland and conducts various marine research and provides the Ministry with scientific advice based on its research on marine resources and the environment, according to specific legislations (1965 nr. 64—see: http://www.althingi.is/lagas/140a/1965064.html).

During routine expeditions by the MRI in Iceland in the years 2003–2014, a total of 1493 scallops (size range 1.5–9.1 cm) were sampled from seven different sites in Breidafjordur in West Iceland (Fig 1). The scallops were sampled by dredging from natural beds of populations suffering extensive natural mortality. In the years 2003–2006, a mixture of all shell sizes were sampled (N = 637) but from 2007 to 2014 (total N = 854), almost exclusively sexually mature shells (5 cm or larger) (N = 847) were taken. The sampling times and sites and the number of scallops collected are shown in S1 Table.

Scallop examination

All scallops were brought live to the laboratory and held in seawater until examined, which was within 48 h of collection. When dissected, the internal organs were removed from the shells, the shell height measured (cm) and the wet weight (g) determined for gonads and adductor muscles. Macroscopic changes of the adductor muscles were graded on the scale from 0–3 where: Grade 0 = normal muscle; Grade 1 = muscle light coloured but less compact and with increasing fluid content; Grade 2 = greyish/light brown coloured and loosely bound with high fluid content; Grade 3 = dark grey or brown, very loosely bound with high fluid content and visible holes or hollow areas in the muscle when cut in half.

For each scallop, a tissue imprint from the adductor muscle was made by cutting it in half and pressing their inner side to a microscopic slide. Subsequently the slides were air dried, fixed in methanol for 3 min, stained with May–Grünwald–Giemsa and mounted in resin based medium. The intensities of infections were determined by calculating the mean number of apicomplexan zoites present in 10 microscopic fields from the tissue imprints at 250x magnification. Six of the fields were randomly selected, however due to uneven distribution of the parasite in the adductor muscle; four of the fields were selected where apicomplexan zoites had accumulated. The levels of infections were graded on a scale of 1–5 where: Grade 1 = $\leq$ 20 zoites per microscopic field; Grade 2 = 21–50 zoites per microscopic field; Grade 3 = 51–100 zoites per microscopic field; Grade 4: 101–200 zoites per microscopic field; Grade 5: $>$ 200 per microscopic field.

Handling of data

Fulton´s condition index was determined for adductor muscles (MI) and gonads (GI) using the following formulas:

$$MI = 100\frac{MW}{SH^3} \quad \text{(i)}$$

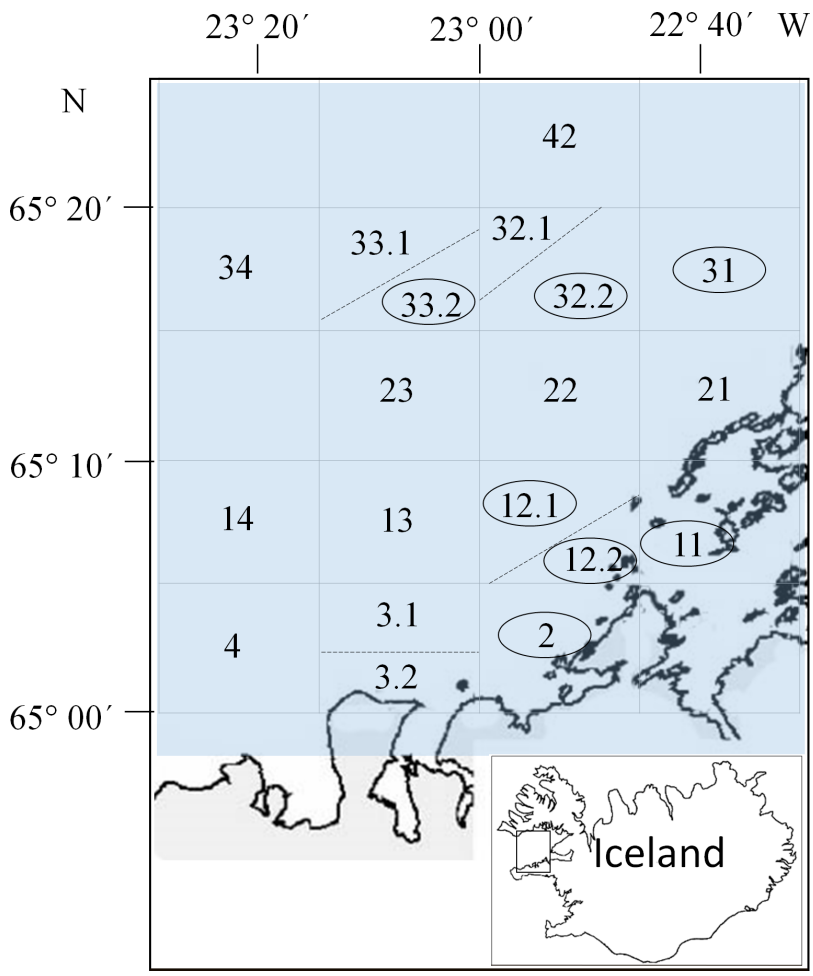

Fig 1. The research site; Breidafjordur, Iceland. The numbered squares represent all the main fishing grounds in the area. The encircled numbers are the sites from which scallops were sampled. Details of samples collected at each site are shown in S1 Table.

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and

$$GI = 1000\frac{GW}{SH^3} \quad \text{(ii)}$$

SH is the shell height (cm), MW is the wet weight (g) of the adductor muscle and GW is the wet weight (g) of the gonads. To make the figures comparable to available data on gonad weight of 7–8 cm scallops in 1988–1990 [15], when the population was considered to be in a normal condition, the weights of the gonads of all fully mature scallops sampled in autumn and spring ($\geq$ 5 cm; n = 1170) sampled were extrapolated to shell size of 7.5 cm with the

formula:

$$GW = \frac{7.5^3 GI}{1000}$$

Parasitological examination was performed on all 1493 scallops sampled. However, to be able to achieve our goals, three different approaches were applied: 1) Relationship of scallop size/maturity to infection. All scallops sampled in 2003–2006 (N = 637) were split into three size groups and compared, i.e. (i) immature scallops–height less than 4.0 cm (N = 166); (ii) pre-mature and mature scallops–height between 4.0 and 4.9 cm (N = 100); (iii) all mature scallops–shell height 5 cm or more (N = 371); 2) Difference in infections between seasons. All mature scallops ($\geq$ 5 cm, N = 227) from two selected sites (12.1 and 11 –see S1 Table), sampled in spring (n = 114) and autumns (n = 113) in the years 2005–2006, were analysed; 3) Progress of infections and macroscopic changes and their relationship with the muscle and gonad indices (MI and GI) of all mature scallops ($\geq$ 5 cm) sampled during 2003–2014 (N = 1218).

Histopathology

To study the histopathological effect of infection, all major organs from 200 scallops, from normal-looking to ones with variable degree of macroscopic changes in the adductor muscle, were fixed in Davidson's fixative [16] for 48 h and subsequently dehydrated in 70% ethanol and processed according to routine histological protocols. Giemsa stained sections (4 μm thick) were examined for histopathological changes related to infections. Furthermore, small pieces ($\approx$ 1 mm^3) of muscles from five heavily infected scallops were fixed in 2.5% buffered glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 24 h at 4°C, washed three times in cacodylate buffer, post-fixed for 1 h in 1% OsO4, rinsed again with buffer, dehydrated in a graded alcohol series. Subsequently, semithin sections (0.5 μm thick) were cut and stained with toluidine blue and mounted in resin based medium.

Transmission electron microscope (TEM)

Muscles samples from 20 scallops with severe macro- and histopathological changes were processed as described for the semithin sections in the histopathology section above. Ultrathin sections were cut, stained and examined for the presence of viral particles associated with histopathological changes in the muscle, using a FEI, Tecnai G2 Spirit Biotwin TEM.

Molecular work

Freshly dissected adductor muscles, from both normal-looking scallops and individuals showing typical gross signs of infections, were fixed in 95% ethanol for molecular analysis; five samples from each sampling site whenever sampled (total number of samples = 180). Total DNA was extracted using a GeneMATRIX kit (EURx Poland) following the tissue protocol. Apicomplexan small subunit ribosomal DNA (SSU rDNA) was amplified from the parasite using the primers and PCR conditions as previously described by Kristmundsson et al. [14]. In addition, the primer pairs 18e / SC2-1370r 5' tccttcatatgtctggcactag 3' and SFC-1120f 5'gaacgaaagttrggggmtcg3' / 18gM [17] were used following the same PCR protocol. From the initial sequence reads two more specific primers were designed from related apicomplexan sequence alignments to be used as a diagnostic PCR for this apicomplexan; 18e-Mer 5' ctgccagtagttatacgt 3' and Mer-790r 5'acacscttgaagcaccctac amplify a 772 bp section on the 18S including the variable v1-v4 regions. PCR conditions were as previously described but used an annealing temperature of 64°C with an extension time of 30 s.

PCR bands of the expected sizes were recovered from the PCR products using a GeneMATRIX PCR extraction kit (EURx Poland). All PCR reactions were performed in triplicate (five infected scallops). Sequencing reactions were performed using BigDyeTM Terminator Cycle Sequencing chemistry utilising the same oligonucleotide primers that were used for the original PCRs. DNA sequencing was performed in both forward and reverse directions for all PCR products and nucleotide BLAST searches performed for each sequence to confirm an apicomplexan origin. The contiguous sequence was obtained manually using CLUSTAL_X and BioEdit [18].

Terminology and statistics

Ecological terms are according to previous definitions [19]. All statistical tests and plots were performed using RStudio (version 0.98.1062). See S1 Statistics) for an overview of all statistical analyses applied in the study.

Results

Shell size/maturity

The prevalence of infection, determined by examination of stained imprints, was high in all size groups; 100% in size groups 4.0–4.9 cm and > 5 cm and 84% of shells < 4.0 cm. Macroscopic changes in the adductor muscle varied considerably between the size groups as none were observed in < 4.0 cm group, 27% in the 4.0–4.9 cm group and 70% in shells larger than 5.0 cm. Furthermore, the median grade of macroscopic changes was significantly higher ($p < 0.0001$) in the > 5.0 cm group than in the 4.0–4.9 cm group. Similar to macroscopic changes, the grades of infection (examination of stained imprints) was highest in the largest scallops and lowest in the smallest ones with highly significant differences ($p < 0.0001$) between all groups (Fig 2A).

Seasonal difference

A seasonal difference was observed in both prevalence and mean grades of macroscopic signs and mean intensity of infection. For both sampling sites and years tested for this purpose (sites 12.1 and 11), the prevalence and macroscopic changes in the adductor muscle were considerably higher in the springtime than in the autumn. Furthermore, both the grades of infections and macroscopic changes in the adductor muscles were significantly higher in spring ($p < 0.0001$) (Fig 2B).

Mature scallops; progress of infections and macroscopic changes

All mature scallops were found to be infected throughout the study. From 2003–2007, the occurrence of macroscopic changes in the adductor muscle was high, ranging from 36–90% with a subsequent decrease to 8–16% in 2008. During the following five years, the prevalence of macroscopic changes was low, not exceeding 4% but increased to 40% in a limited numbers of scallops from one site in spring 2014. The median grade of macroscopic changes varied somewhat between sampling times being highest the first four years and more severe in the springtime, with the exception of autumn 2003. Conversely, although 40% of the scallops showed macroscopic changes in spring 2014, they were commonly light and only of grades 1 and 2 (Fig 2C). The grades of infection showed a similar pattern to the prevalence and severity of macroscopic changes, i.e. being high the first 4–5 years of the survey, followed by a gradual decrease over the next six years and a sudden increase in spring 2014 (Fig 2D). A highly

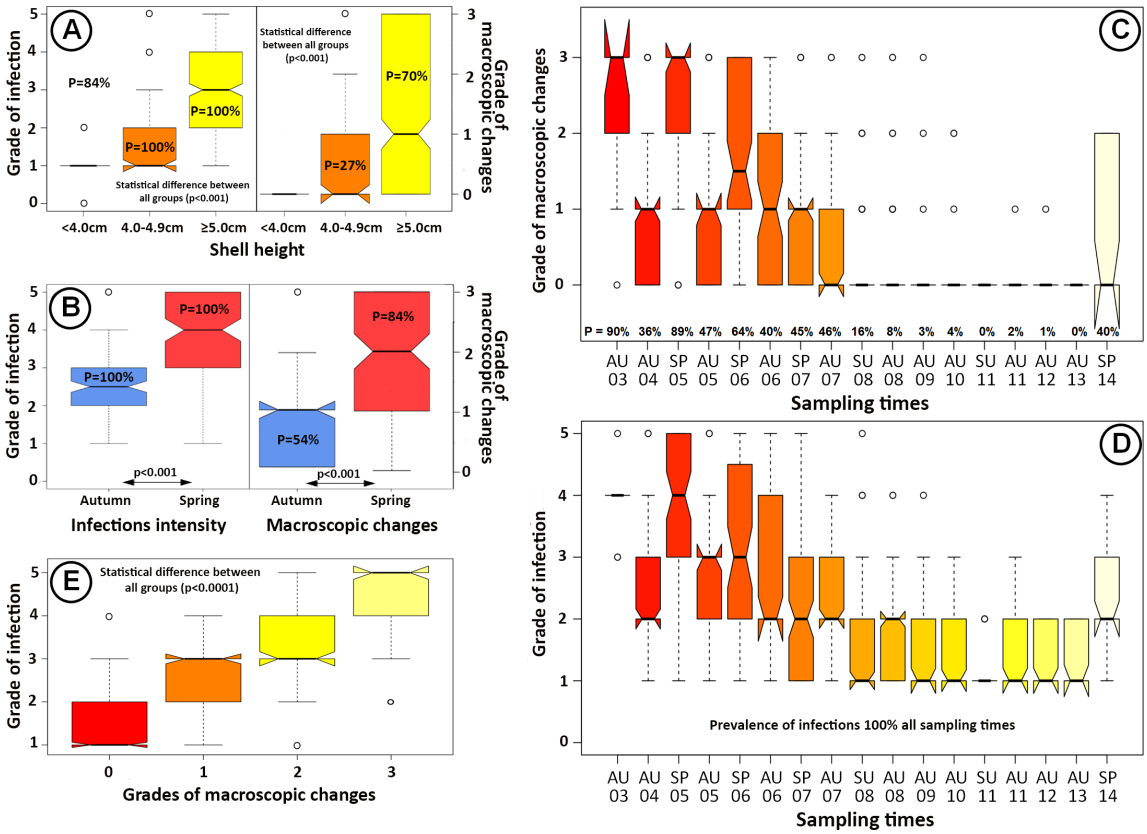

Fig 2. Seasonal and size difference and infection´s progress in mature scallops. (A) Comparison of infections between three size groups of scallops sampled in 2003–2006 (n = 637). The prevalence of infections (P) is high in all size groups. No macroscopic changes are visible in immature scallops (< 4.0 cm; N = 166), while 27% of those reaching maturity (4.0–4.9 cm) (N = 100) have macroscopic signs and 70% of fully mature scallops (≥ 5.0 cm; n = 371). Both the grades of infections and macroscopic signs increase with maturity/size of the scallops and the differences between the median values highly significant between all size groups, both for macroscopic changes and infections intensity. **(B)** Comparison of the seasonal variation in prevalence and median grade of infections and macroscopic changes of 227 scallops (spring = 114; autumn = 113) sampled from two different sites (12.1 and 11) in 2005 and 2006. The prevalence of macroscopic changes is considerably higher in the spring (84%) than in the autumn (54%). The median grades of infections and macroscopic changes are significantly higher in the spring in all cases ($p < 0.0001$). **(C and D)** The prevalence (P) and severity of macroscopic changes (C) and infections (D) for all mature scallops (≥ 5cm) sampled in 2003–2014 (N = 1218). Both the prevalence (P) and the grades of macroscopic changes and infections are most prominent from 2003–2006 with a subsequent gradual decrease from 2007–2013. In 2014, an increase is observed; however, in most cases the grades of the macroscopic changes are mild. AU = autumn; SP = spring; SU = summer. *The notches on the boxplots provide an approximate 95% test of the null hypothesis that the true medians are equal: if the notches do not overlap, the medians could be described as statistically significant.

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significant positive relationship ($p < 0.0001$ between all groups) was evident between the grades of infections and macroscopic changes (Fig 2E).

Effect on the condition of mature scallops

The most apparent macroscopic changes observed were greyish/brownish and greatly diminished adductor muscles compared to the normally sized, light coloured normal-looking ones (Fig 3A). Infections severely affected the condition of both the adductor muscle and the gonads (using Fulton´s condition index; MI = Muscle index and GI = Gonad index). A significant

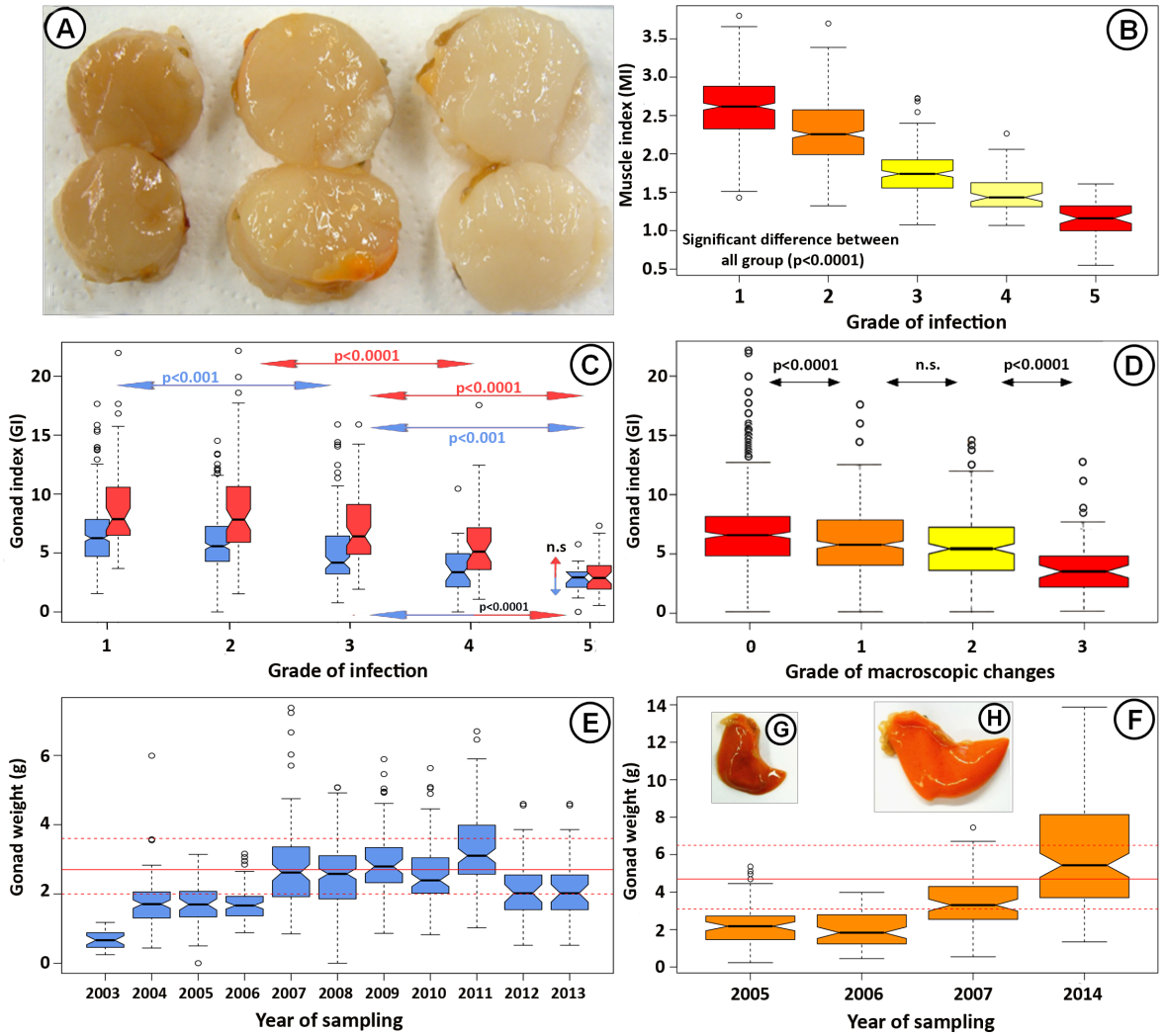

Fig 3. Effect of apicomplexan infections on the scallop condition. (A) Adductor muscles from three mature scallops. The first on the left is from 7.8 cm scallop which has severe macroscopic changes (grade 3; MI = 1.4), it is brownish coloured and greatly diminished and extensively infected while the first on the right is from 7.2 cm healthy looking one with firm and light coloured adductor muscle (MI = 2.5) which had a mild apicomplexan infection. The one in the middle is from a 7.5 cm scallop with grade 1 macroscopic changes (MI = 1.9). (B) The relationship of the MI and the grades of infections from all mature scallops examined (N = 1218). The MI deceases significantly with increasing grades of infection ($p < 0.0001$ between all grades). (C) The relationship of the GI and the grades of infections from all mature scallops sampled in the spring (red boxes; N = 297) and autumn (blue boxes; N = 794). A reduction in the GI is apparent, especially in the spring (red double arrow line). Note that in spring the GI should be much higher as these shells should be close to full maturity. However, scallops with grade 5 infections is not significantly different between autumn and spring. Furthermore, the GI for grade 5 spring scallops is significantly lower than the one for grade 1–3 autumn scallops (blue/red double arrow line). (D) The relationship of the GI and the grades of macroscopic changes observed in adductor muscles. The GI significantly decreases with severity of the macroscopic changes. (E and F) Comparison of the gonad weigh of 7.5 cm scallops caught in autumn (E) and spring (F) in 1988–1990 [15] and 2003–2014 (extrapolated to 7.5 cm). The whole and broken horizontal red lines represent the normal mean range of gonad weight observed by Thorarinsdottir [15] from a healthy population of scallops in 1988–1990. (E) Scallops sampled in autumn (N = 794). The gonad weight of scallops from 2005 and 2006 is greatly reduced but from 2007 their weigh is similar to those observed in 1988–1990. (F) Scallops sampled in spring (N = 297). The gonad weight of scallops from 2005 and 2006 is greatly reduced. In 2007, their weigh is approaching normal and from that time their weight is similar to those in 1988–1990. (G) Macroscopic changes observed in the gonad caught in the spring and (H) healthy

looking gonad caught at the same time. *The notches on the boxplots provide an approximate 95% test of the null hypothesis that the true medians are equal: if the notches do not overlap, the medians could be described as statistically significant.

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negative relationship was observed between all different grades of infections and the MI ($p < 0.0001$ in all cases) (Fig 3B). Macroscopic changes were also commonly observed in gonads with low GI. These were diminished and dark compared to the orange coloured normal ones (Fig 3G and 3H). Similar to the MI, the GI decreased with higher grade of infection, both for scallops sampled in spring and autumn, although a significant difference was not observed between all grades of infections (Fig 3C)**.** A significant difference was observed between GI for scallops caught in spring and autumn ($p < 0.001$), except for the most severely infected scallops (grade 5). Furthermore, the GI of spring caught scallops with grade 5 infection (median GI ≈ 3), was statistically lower ($p < 0.001$) than the GI of autumn caught scallops with grade 1, 2 and 3 (median GI ≈ 6, 5 and 4, respectively) (Fig 3C). In addition, the GI decreased with higher grades of macroscopic signs, being statistically different ($p < 0.0001$) for all grades, except grades 1 and 2 (Fig 3D).

The gonad weights (extrapolated to 7.5 cm) of all scallops, were compared with analogous data from 1988–1990 [15], when the scallop stock was considered to be normal. The comparison showed that the gonad wet weight in 2003–2006 was much lower than the normal gonad weight, both in spring and autumn. In spring 2007, the weight was approaching normal and from autumn 2007 and till the end of the study it was within the normal range of gonad weight (Fig 3E and 3F).

Histopathology

Histopathological changes were restricted to the presence of apicomplexan zoites and no pathology was associated with cysts, i.e. gamonts, meronts and oocysts. The apicomplexan zoites were almost exclusively restricted to muscular and connective tissues and the cysts exclusively in the adductor muscles. The apicomplexan zoites were widely spread, causing varying degrees of damage to all scallops examined, depending on the organ infected and the intensity of infections.

Adductor muscle and heart. The parasite was commonly found in large numbers in both parts of the adductor muscle, i.e. the striated phasic adductor and the smaller smooth tonic adductor muscle (catch muscle). In healthy looking scallops the histopathological changes were minor, mostly affecting the adductor muscle in which focal necrosis was present in close vicinity to the apicomplexan zoites. However, in scallops with macroscopic changes in the adductor muscle, severe histopathological changes were observed (Fig 4). Apicomplexan zoites were found in clusters either intracellularly in muscle fibres, extracellular and associated with necrotic muscle cell debris or in the endomysium, a connective tissue surrounding the muscle cells (Fig 4B). Striated and smooth muscles were equally affected. In mild infections of normal looking shells, some light focal necrosis was observed while in the heavily infected scallops, destruction of large parts of the muscle was evident in the form of extensive liquefactive necrosis, a loss of striations, muscular fragmentation and hyalinization (Fig 4C–4E).

Pathological changes were common in the heart ventricle, associated with an accumulation of apicomplexan zoites, especially in the myocardium and also, to some extent, in the epithelial and connective tissue layers of the epicardium. Degeneration of cardiac muscle fibres was common, characterized by loss of striation followed by nuclei degeneration and substantial necrosis. In some cases, a massive infiltration of haemocytes was observed which in some cases formed clusters of hematopoietic centres (Fig 4F and 4G).

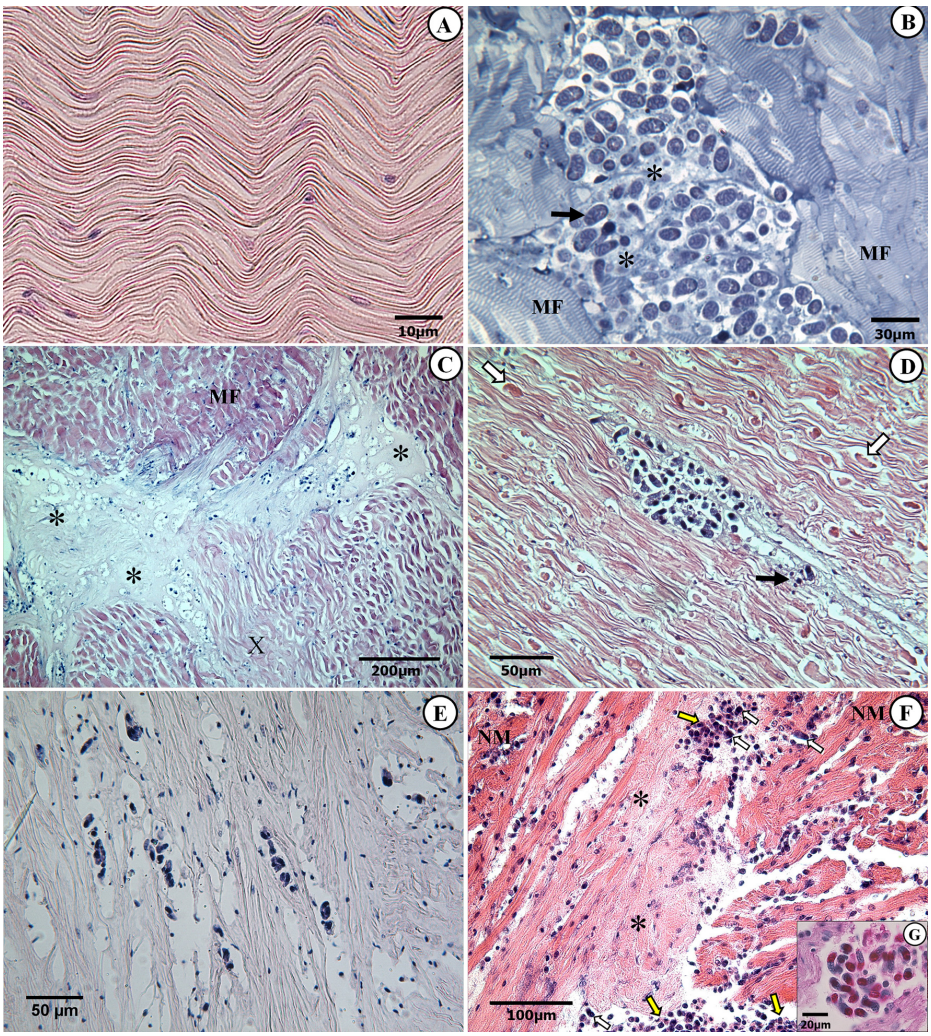

Fig 4. Histopathology of the adductor muscle and heart. Stained histological sections of adductor muscles from heavily infected scallops (B-E) and heart (F and G). (A) A normal striated muscle fibres in a scallop´s adductor muscle. (B) A semithin section showing numerous apicomplexan zoites (black arrow) in the extracellular space associated with necrotic muscle cell debris (*). (C) Severely affected muscle with loss of striation in degenerating muscle fibres (X) and a liquefactive necrosis of large areas (*). (D) A cluster of apicomplexan zoites causing focal necrosis (black arrow) and fragmentation and hyalinization (white arrows) of the surrounding muscle. (E) Necrotized muscle fibres with cytoplasmic vacuolisations, pyknotic nuclei and no well-defined cross-striation. (F) Histological section through a ventricle from an infected scallop showing degeneration of the muscle fibres of the myocardium and infiltration of haemocytes (yellow arrows) mixed with apicomplexan zoites (white arrows); loss of striations in the muscle fibres (*) and muscular necrosis. (G) Higher magnification of a cluster of apicomplexan zoites associated with necrotic cardiac muscle cell debris. Abbreviations: MF = Muscle fibres; NM = Normal muscle.

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Gastrointestinal tract. Aggregations of parasite zoites were frequently found in loose and muscular connective tissues surrounding the whole gastrointestinal tract, from the buccal cavity, oesophagus, stomach and throughout the intestine. Similarly, the connective tissues

surrounding the epithelial lining of the primary and secondary ductus, which connect the stomach to the part of the digestive gland harbouring the digestive cells themselves, were heavily infected as was the interstitial connective tissue surrounding the digestive cells (Fig 5A–5D). The apicomplexan infections were both intracellular in haemocytes and in the extracellular matrix and commonly causing total destruction of these tissues leading to a separation of the basement membrane from the gastrointestinal epithelium. Focal or disseminated necrosis was commonly observed in the interstitial connective tissue surrounding the digestive cells (Fig 5B). In the most severe cases the epithelial layer surrounding the intestine and the surrounding digestive cells was totally destroyed (Fig 5C and 5D). Furthermore, on some occasions, unidentified parasitic forms, possibly growing trophozoites, were observed as intracellular in the gastrointestinal epithelium.

Gonads. Extensive aggregations of parasites were routinely observed in both inter-acinal and peri-gonodal connective tissues of the gonads causing disruption in the inter-acinal tissues and a destruction of the wall enclosing the acini, which subsequently lead to the degeneration of primordial germ cells (Fig 5E–5I). In addition to the observed destruction of large parts of the gonads (Fig 5F–5I), the development of the remaining parts of the gonads were asynchronous with season/time, i.e. gonads in scallops harvested in May, when they should be almost fully mature, were only at the initial stages of maturity, normally observed in autumn (Fig 5E and 5F). Similar asynchronous development was observed in scallops sampled in late summer and autumn during the period when infections were most severe (2003–2006). At that time of year, when the scallops are normally in their initial phases of gonad maturation, scallops with a mixture of mature, semi-mature and decaying egg- or sperm acini were observed, indicative of an unsuccessful spawning event.

Other organs. Although parasites were normally found in inter-tubular connective and fibromuscular tissues of the kidney, the kidney tubules were usually fairly unaffected. Occasionally some pathology was evident, associated with an infiltration of parasites and haemocytes in the kidney interstitium. These pathological changes were characterized by vacuolization and necrosis of the tubular epithelial cells.

The gills were, in general, lightly affected by the apicomplexan parasite. Although infections were commonly detected, they were characterized by isolated parasites, or groups of a few apicomplexan zoites, which were more or less restricted to muscular tissues in the base of the gill lamellae. The associated pathology was normally mild, characterized by a focal necrosis in the vicinity of the apicomplexan zoites.

A quite variable mixture of tissue types make up the mantle, foot and labial palps, the most common ones being muscle fibres, loose connective tissue, muscular connective tissue and fibromuscular tissues, i.e. a musculature featuring an irregular meshwork of fibrous tissue. Normally the extracellular matrix of the connective tissues in these organs contains high numbers of various types of haemocytes. The outer parts of these organs are enclosed by a lining of epithelial cells. Apicomplexan zoites were routinely found in great numbers in these organs, often causing extensive disruption to all the formerly named tissue types with extensive necrosis associated with the presence of the parasites.

Host response. Host responses to infections were generally light. Light infiltration of haemocytes was commonly observed associated with the presence of the apicomplexan zoites. In some cases, especially in the heart myocardium, the foot, the mantle and in the labial palps, the accumulation of haemocytes was extensive. Occasionally, fibroblast like cells were seen surrounding a clusters of apicomplexan zoites (Fig 6A) or developing cysts and on several occasions, in the foot, some signs of tissue repairing were observed, where muscular and fibromuscular tissues were substituted by fibroblast like cells. (Fig 6B)

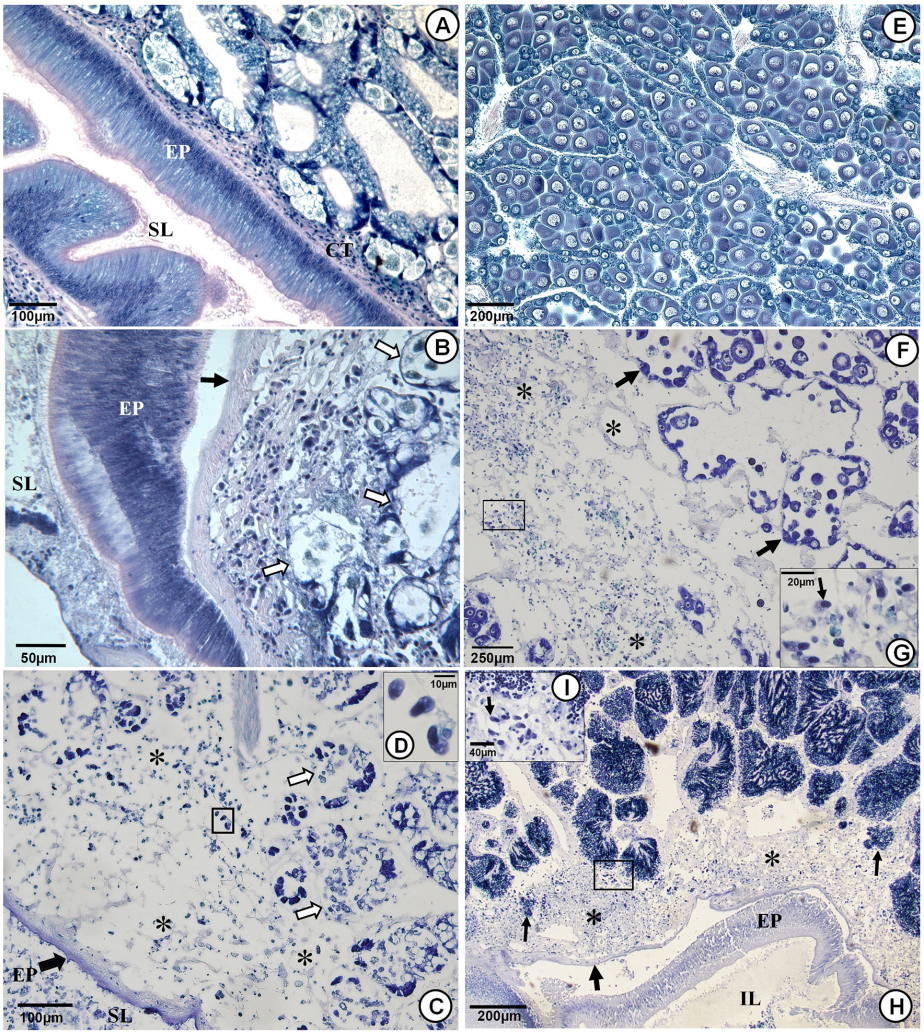

Fig 5. Histopathology of the gastrointestinal tract, digestive gland and gonads. (A) Normal histology of the stomach epithelium and the adjacent digestive gland tubules. Note the firm connective tissue layer (CT) separating the stomach wall and the digestive gland tubules. (B) Histological sections through the stomach and the digestive gland of a heavily infected scallop showing extensive accumulation of apicomplexan zoites in connective tissues surrounding the stomach epithelium and in the digestive gland interstitium causing separation of the basement membrane (black arrow) from the stomach epithelium (EP) and necrosis of the connective tissues and digestive cells (white arrows). (C) A total destruction the digestive gland associated with mixture of cellular debris and numerous apicomplexan zoites (*). The stomach epithelium (EP), connective tissues and digestive cells (white arrows) are heavily necrotized. (D) Higher magnification of the area within the black square of (D) showing apicomplexan zoites. (E and F) Female gonads from scallops caught in early May 2005. A normal one (E), with large acini filled with semi-mature eggs, and a diseased one (F) where the inter-acinal tissues are massively infected with apicomplexan zoites which have caused a total destruction of the acini in large areas (*). The remaining acini have merely premature eggs (arrows) and their development asynchronous with time of year. (G) Higher magnification of the area within the black square of (F) showing apicomplexan zoites (arrow). (H) Section through a heavily infected gonad and intestine of a male scallop. The connective tissue surrounding the intestinal epithelium and inter-acinal tissues are heavily infected with apicomplexan zoites causing the basement membrane to separate from the epithelial lining (broad arrow) and degeneration of the epithelial cells (EP). The apicomplexan causes destruction of the connective tissue which makes up the wall surrounding the acini which subsequently decay (thin arrows). (I) Higher magnification of the area within the black square of (H) showing apicomplexan zoites. Abbreviations.
EP = Epithelial layer; IL = Intestinal lumen; SL = Stomach lumen.

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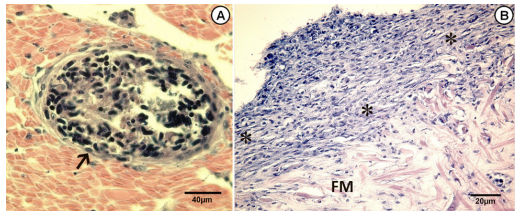

Fig 6. Host responses to infections. Host responses to the apicomplexan infections. (A) An isolation of a cluster of apicomplexan zoites with fibroblast like cells (arrow). (B) Repairing of scallop foot where fibromuscular tissue is substituted by fibroblast like cells (*). NM = Normal muscle; FM = Fibromuscular tissue.

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TEM

TEM examination of adductor muscles samples, which showed severe macroscopic and histopathological changes, revealed the presence of numerous apicomplexan zoites in association with degenerative muscle fibres and/or muscle cell debris. No viral particles were detected.

Molecular analysis

A contiguous sequence of 1811 bp of SSU rDNA was successfully obtained for the apicomplexan parasite from infected adductor muscles. BLAST searches showed that the sequence was most similar to those from the apicomplexan genus *Aggregata*, found infecting cephalopod molluscs, with an 81% identity. The diagnostic PCR worked well and was able to successfully detect parasite DNA in adductor muscle samples with no macroscopic changes of infection. The same apicomplexan was detected in all 180 samples tested. No other apicomplexan species were identified in the samples.

Discussion

The progression of the scallop stock and apicomplexan infections

The almost complete collapse in the population of the Iceland scallop, *Chlamys islandica*, experienced in Breidafjordur in West Iceland became apparent in the years 1999–2000 [3,20,21] and in 2005–2006 the stock index was only at 16% of its average size from previous years 1993–2000 (MRI, 2007). Concurrently, the recruitment index decreased by approximately 98% [22]. The period from 2000–2006 was characterized by extremely high rates of natural mortality in larger scallops, independent of fishing activities. Associated with these mortalities was a high prevalence of scallops with diminished adductor muscles with an abnormal grey or brown colouration [13,21]. Since that time, no significant changes have been observed in the stock index [10,23].

The observed progression of the apicomplexan infection is in good synchrony with the mortality rates and the subsequent decline observed in the scallop stock and recruitment indices. With respect to the severity of infections and their effect on the condition of the scallops, the first four years of the study (2003–2006), were characterised by high infection intensity and a high prevalence of scallops with abnormally grey or brownish adductor muscles which was loosely bound with a high fluid content and low MI and GI. Conversely, in the latter eight years, a gradual decrease in infection intensity and prevalence and levels of macroscopic changes were observed along with a considerable improvement in both MI and GI. Although the stock index has remained low despite decreasing infections, some signs of recovery are

emerging [23,24]. Along with decreasing levels of apicomplexan infections and natural mortality, the proportion of adult scallops (i.e. the affected individuals) have increased significantly, and furthermore, shells exceeding 8.0 cm, which were almost completely depleted, are now found in considerable numbers in the stock. With regards to changes in recruitment, the abundance of juvenile scallops remained poor in the MRI dredge surveys until 2012 when year-classes from 2010 were observed. Furthermore, during the MRI scallop expeditions in 2013 and 2014, juvenile scallops were found in numerous scallop beds, both in the conventional areas and new ones, in considerable amounts; cohorts that will hopefully contribute to the fishing stock in few years time [23,24].

The effect of infections on the scallop host

Similar to the abnormal natural mortality observed, the negative impact of the apicomplexan infections was restricted to larger mature scallops [3]. Immature scallops were significantly less infected and abnormally looking adductor muscles never observed in scallops of less than 4 cm, whilst a high proportion of the adductor muscles of fully mature scallops (> 5 cm), caught at the same time and site, had macroscopic changes. The Iceland scallop reaches maturity at sizes 4.0–5.0 cm and all scallops below 4 cm are considered immature and all above 5.0 cm fully mature while the sizes in-between include a mix of pre-mature and mature animals [9]. Therefore, the prevalence and grade of macroscopic changes, which correlate with parasite intensity, observed in 4.0–4.9 cm scallops, seems logical. In addition to scallop size, the infection intensity was significantly higher and macroscopic changes more prevalent and intense in scallops caught in the springtime (April–May) than in the autumn (Sept.–Oct.). It therefore seems that size and level of maturity plays a major role with respect to infections and the impact on the host.

The apicomplexan infection severely affects the condition of both the adductor muscle and the gonads. The infection intensity showed a significant positive relationship with the grade of macroscopic changes and negative relationship with MI and GI. Furthermore, the histopathological examination not only showed severe histopathological changes in the adductor muscle and the gonads, but also in most other organs. A comparison of the gonad weight of mature scallops (size 7.5 cm) observed in the current study to those in 1989–1990 [15], when the scallop population was considered to be in a normal condition reflects the decrease in the condition of the scallop stock. The gonad weight during the years 2003–2006 was extremely poor i.e. when the infections were most severe. At this time the gonad weights, in both spring and autumn, were far from normal and in many cases not even half the normal weight of gonads recorded from 1988–1990. In 2007 and 2008 the weights were recovering and approaching normal, and from 2009–2014 their condition had returned to normal. This is fully consistent with the progress of infection intensity, the prevalence of macroscopic changes as well as the natural mortality and stock index during these years. As in other animals, gonad maturation in scallops requires a significant metabolic input. In the case of the Iceland scallop, the gonad development depends largely on energy reserves, such as carbohydrates and proteins from somatic tissues, especially from the adductor muscle, but also other organs such as the digestive gland [25]. The results of the present study show that infections cause varying degrees of pathology in more or less all organs of the Iceland scallops. However, muscle tissue is the main target tissues of the parasite, where it is commonly found in high abundance. Heavy infections cause severe myodegeneration with a total destruction of large parts of the muscle, characterized by severe pathology in both striated and smooth muscle fibres. In addition to causing direct pathology in the gonads, the bad condition of the adductor muscle would further contribute to the poor gonads condition and hamper its normal development, as a result of a lack

of stored energy required for normal gonad maturation. Indications of a delayed or failed gonad development and spawning were routinely observed in this study, where scallops harvested in May, when they should be almost fully mature, were merely in the initial developmental stages, and in August–October, some scallops had a mixture of mature, semi-mature and decaying egg or sperm sacs. A good example how severely the gonads were affected by the infections, the GI of spring caught scallops with grade 5 infection (median GI ≈ 3), was statistically lower than the GI of autumn caught scallops with grade 1, 2 and 3 (median GI ≈ 6, 5 and 4, respectively). Under normal conditions this should naturally be the opposite, i.e. around 11 (on average) in the spring compared to 6 in the autumn. The fact that the reduction in the recruitment index exceeded the decline in the stock index support that some of the remaining mature scallops did not contribute to the recruitment due to spawning failure. Examples exist from dredge oysters, *Ostrea chilensis*, where severe apicomplexan infections affected normal gametogenesis by causing a total destruction of the connective tissues surrounding the gonad acini [26], similar to the scallops in the present study.

Unresolved mass mortality events and abnormal condition of scallop populations

Many examples exist of mass mortality events in wild populations of Iceland scallop, both abroad and in Icelandic waters. Most commonly, these mortalities have been attributed to increasing sea temperature and/or overfishing. However, these conclusions are in most cases highly speculative and the potential role of pathogens and diseases not even considered as no examinations were performed [1,5,9].

In the year 1983, a mass mortality of Iceland scallop occurred in Hvalfjordur in SW Iceland [9]. The reasons for this were unclear but the effect of unusually high sea temperature the previous year was speculated as the cause. In 1985, previously unknown scallop beds were found in several fjords and bays in eastern Iceland [9] and subsequently fisheries were conducted in that area in 1985 and 1986 [21]. However, a research survey in 1998 revealed that these previously commercially valuable scallop beds only consisted of dead shells [27]. In both the above mentioned mortality events, the reasons remain unknown. In either cases, the possible role of infectious agents were not considered as no such research was performed. Similar examples of mass mortalities exist from other northern areas. In 1963, Wiborg [5] reported an apparent extinction of numerous scallop populations in Norwegian waters; scallop beds which only consisted only of empty shells (cluckers). He suggested that changes in environmental conditions, such as a sudden rise in sea temperature, were the cause. In the early 1980s, extensive beds of Iceland scallops had been discovered in the Barents Sea, in the Jan Mayen and Spitzbergen areas and the potential for scallop fisheries was considered to be very promising [1,28]. However, Sundet [28] noted that high percentages of dead scallop shells in the catches would make fishing methods less efficient. Surveys in this area in 1986 [29] indicated further favourable prospects for scallop fisheries. However, as soon as 1987, some of the major scallop beds in the Barents Sea had already collapsed [1], with the cause given as overfishing. A similar situation occurred in the scallop fisheries in Greenland, where massive declines or total losses of scallop beds were experienced, despite limited fisheries of about 10% of the stock size [1]. In addition, considerable decreases in gonad and muscle yield have also been reported from scallop populations around Greenland [1]. Mass mortality of scallop populations have also been reported from the NW Atlantic Ocean, in both Iceland scallop and sea scallop (*Placopecten magellanicus*) stocks in Canadian waters. The causes for these abnormal mortalities could not be determined [30].

In addition to mass mortality events, macroscopic changes similar to those observed in the Icelandic scallop populations have been reported from other populations of Iceland scallop as

well as in other scallop species from different geographic areas, including the Barents Sea, NW Atlantic and NE Pacific Ocean [31–33]. In 2006, a disease only affecting mature larger Iceland scallop, was reported from Svyatoy Nos, Russia. The macroscopic changes were dull grey coloured adductor muscles and changes in colour of the gonad. Histopathological investigation were performed which showed severe necrotic changes in the mantle, adductor muscle and gonads [31]. At that time, the associated mortalities had not been determined and to our best of knowledge, the cause for this condition is still unknown. Similar macroscopic changes in adductor muscles were also reported by Kristmundsson et al. [13] from a wild population of queen scallops (*Aequipecten opercularis*) from the Faroe Islands which were associated with heavy infections of the same apicomplexan affecting the Iceland scallop. In the NW Atlantic, darkened and reduced adductor muscles, termed "grey meat", also restricted to larger Atlantic sea scallops, *Placopecten magellanicus*, have periodically been reported since 1949 [32,34–37]. Recently, large numbers of "grey meat" scallops have been observed in the rotational management areas of Georges Bank after extended fishing closures [38,39]. In 2013, access to certain fishing areas on Georges Bank ended early due to the high number of "grey meat" scallops landed, which have a low meat yield and the discoloured appearance reduces market value [39]. The cause for these mortalities and macroscopic changes is unidentified although effects of senescence and parasitism by shell borers and prokaryotic infections have been suggested as a cause [31,37]. The prokaryotic infections reported by Gulka et al. [37] have been cited as a possible cause by many authors. Gulka et al. [37] reported a sudden and total extinction of a population of sea scallops in Narrangansett Bay, Rhode Island in 1979–1981. Associated macroscopic changes were greyish, flaccid adductor muscles and histology showed extensive myodegeneration. Gulka et al. [37] identified prokaryotic infections in 88% of 34 animals, in gills, plicate membranes and other epithelial surfaces of the body. Associated histopathology was characterized by fragmentation of muscle fibres, hyaline change with loss of cross striations and necrosis with foci of amoebocytic accumulations. When examining Fig 4 in this paper [37], which shows the histopathology in the adductor muscle, many of the structures referred to as amoebocytes within the necrotic area look remarkably like apicomplexan zoites, similar to those observed in the Iceland scallops in the present study. Furthermore, no prokaryotic infections were observed in the adductor muscle where the most extensive pathology was observed. Gulka et al. [37] stated that the heavy gill infection and extensive myodegeneration suggested that gill dysfunction caused metabolic stress causing pathological changes in muscle tissue. However, recently the apicomplexan parasite described infecting the Iceland scallop was identified in sea scallop from both US and Canadian waters, by both morphological and molecular methods [38,39]. In the NE Pacific Ocean and the Bearing Sea, the weathervane scallop, *Patinopecten caurinus*, has been harvested commercially since 1967. Poor quality adductor muscles, termed "weak meat", characterized by an off-white to greyish colour, with a notable stringy texture, and a spongy consistency causing difficulty marketing them, has resulted in an underutilization of the resource in the eastern Gulf of Alaska [33]. The authors discuss the similarity of this phenomenon to that in the sea scallop, *P. magellanicus*, on the eastern coast of North America and the potential causes named in that scallop species, i.e. clionid infestation, prokaryotic infection, and age-related senescence [32,33,37,40,41] but suggest further exploration is required on potential impact of environmental factors or parasites and diseases.

The apicomplexan parasite–distribution, host specificity and transmission

To date, this apicomplexan pathogen has been identified, using the diagnostic PCR, in four different scallop species from different geographic areas. In addition to the Iceland scallop, king

scallop, *Pecten maximus* from UK waters, queen scallop, *A. opercularis*, from UK and Faroese waters and sea scallop, *P. magellanicus*, off the eastern coast of USA, have been found infected [13,39,40]. Therefore, its geographic distribution is wide and it is non-specific with regards to its pectinid hosts.

Kristmundsson et al. [13] described the morphology of this apicomplexan in three scallop species; the Iceland scallop, the queen scallop and the king scallop, and stated that apparently all life forms necessary for the parasite to complete its life cycle, i.e. merogony, gamogony and sporogony, were present in the scallops. If that is the case the life cycle is monoxenous, i.e. a direct transmission between scallops without an obligate intermediate host being required. However, that can only be fully confirmed by trials where naïve scallops would be exposed to infective sporozoites and samples routinely taken to follow the development of the parasite. Although data on life cycles of apicomplexan infecting marine molluscs are quite scarce, both direct and indirect modes of transmission are known for apicomplexan species, e.g. the heteroxenous *Aggregata* species from cephalopods [42] and monoxenous *Margolisiella* species reported from bivalves and other molluscs [14]. Given that the apicomplexan in the present study is monoxenous, the scallops would most probably get infected via the oral route while unselectively filtering feeding and ingesting infective sporozoites from the environment. This also seems logical considering the observed histology, where massive accumulation of the parasites were observed along the entire gastrointestinal tract. Furthermore, some unidentified parasite stages, which could be developing trophozoites, were seen inside the epithelial cells surrounding the gastrointestinal tract. According to Kristmundsson et al. [13] most of the development seems to take place in the adductor muscle as developing cysts were almost exclusively found there. Two routes of exposure of infective spores seem logical: 1) excretion through the kidney with a subsequent exposure into the environment with feces; 2) from dead decaying scallops which would be especially effective during mass mortality events as well as when the biomass of shells is high. Furthermore, a direct life cycle offers the possibility of an autoinfection which can contribute severely to the proliferation of the pathogen in an infected individual.

Although a direct transmission between scallops seems the most plausible route, the possibility of a two host life cycle cannot be excluded. In that case, the biomass and distribution of proper intermediate hosts would be crucial for an effective dispersal of the pathogen. Furthermore, knowledge on such an intermediate host would be very important to study the epidemiology of the pathogen.

The results show a high prevalence of the infections in all size groups. However, a great difference in the severity of infections is apparent between scallop sizes. Therefore, the scallops seem to get infected at a young age but for some reasons the infections do not seem to intensify in the younger individuals. An age related difference in parasite infections is a well-known phenomenon in case of helminth infections. Larval helminths are thought to remain alive in fish for long periods, often the whole life span of the fish [43]. Due to that, such infections accumulate in the fish over long periods and consequently cause an age related mortality of the fish [43]. However, this is unlikely to be the reason for the difference in the apicomplexan infections in scallops as microparasites, such as apicomplexans, have a much shorter generation time which can be counted in days or weeks but not years [44,45]. Consequently, the explanation for this age difference in the scallops must have some other causes. Age related susceptibility to various infectious agents, such as viruses, are well known in fishes, e.g. the IPNV in which acute infections occur in 1- to 4-mo-old fish and may cause mortalities up to 90% while the susceptibility to the virus decreases with increasing age. Similarly, nodavirus infection predominantly affects the larval or juvenile stages of fish, such as Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*), in which mortality may be very high [46].

Although purely speculative, this difference could reflect different physiology with age, related to maturity and different immune responses.

Is the apicomplexan responsible for the collapse of the Icelandic scallop stock?

In addition to the apicomplexan infections, the most common factors considered likely to cause the collapse of the Iceland scallop stock in Breidafjordur in the 2000s were temperature increases, overfishing and food availability [1,21,47]. As in Breidafjordur, unusually high bottom sea temperature was the first factor named as a cause for the mass mortality event in the scallop stock in Hvalfjordur in Iceland in 1983 [3,9]. In 2003 and 2004, the sea temperature in Breidafjordur reached the highest mean in 100 years and the maximum temperature observed in August 2003 (12.2°C) was assumed to exceed the upper temperature tolerance of the species, previously thought to be 10°C [1]. However, subsequent experiments made on scallops from Icelandic waters showed that they can tolerate up to 13°C for at least 3 weeks with no negative effects but considerable mortality was observed at 14°C [20]. Furthermore, the abnormal mortality in the stock had already been observed during the spring survey of the MRI in Iceland in 2001, a period when the sea temperature was considerably lower [21]. This makes temperature increases as the main factor for causing the mortality an unlikely explanation. Similarly, sea temperature alone cannot explain the mass mortality events in the nearby site of Hvalfjordur in 1983 or the fjords and bays in eastern Iceland in the 1990s, as sea temperature there was considerably lower than observed in Breidafjordur during the associated collapses. Based on these findings, Garcia [1] stated that overfishing was left as one of the major factors responsible for the collapse in the scallop stock. However, that is contradictory to the observed mortalities which were unrelated to fishing intensity [3] and the most extensive mortalities during the collapse were actually observed in scallop stocks where none or very minimal fisheries were undertaken [21]. The same argument can be applied to the mass mortality observed in Hvalfjordur in 1983 [9]. When considering lack of food availability as a factor of importance, the abnormal mortality and macroscopic changes were not observed in immature scallops but restricted to the larger ones [3]. For unavailability of food to be considered as a major factor of importance, one would expect the whole populations to be more or less equally affected, regardless of scallop size or maturity.

In general, wild bivalves are poorly studied with respect to apicomplexan parasites and not many examples exist of this group of parasites causing mass mortalities in bivalve populations. The most common apicomplexan species reported from bivalves belong to the genera *Pseudoklossia* and *Margolisiella*. Species of these genera are generally not considered highly pathogenic [14,48–53]. Indeed, this is also the case with *M. islandica* which has been described from Iceland scallop in Breidafjordur [14] (unpublished data). The most severe mortalities related to apicomplexan infections in bivalves have occurred among the oyster, *Ostrea chilensis*, on the south coast of New Zealand from late 1985 to 1993 that reduced the population of commercial-sized oysters by 91% [25]. Single or dual infections of two parasite species were identified; a previously unknown apicomplexan species and a known pathogen of oysters. Hine [26] stated that the apicomplexan infection had a significant contribution to this mass mortality event. This apicomplexan, which remains unidentified, also infects two mussel species in New Zealand, *Mytilus galloprovincialois* and *Perna canaliculus*, and is to date considered one of the most serious pathogens of bivalves in New Zealand waters. It primarily infects connective tissues and hampers gonad development [26,54,55]. Therefore, it has been demonstrated that apicomplexan species can cause high mortality rates in wild bivalve populations.

So, is the apicomplexan parasite responsible for the collapse of the Iceland scallop stock in Iceland? Although the prevalence of the apicomplexan infections was high in all sizes of scallops over the study period regardless of the condition of the scallops, the intensity of infections and the prevalence and grades of macroscopic changes of the adductor muscle were in good alignment with the progress of the stock index according to MRI estimation during the 1990s and until 2014 [23]. Furthermore, the extensive histopathological changes associated with the infections indicate that they severely affect the survival of the scallops. The infections mostly affect sexually mature scallops and the pathology observed in the gonads as well as their apparent abnormal development, indicates that the infections seriously impact successful spawning of scallops. This leads to low recruitment, which in turn further contributes to a decrease in the stock. The fact that the recruitment index decreased considerably more than the stock index in these years seems to support this [22].

Since 2009, mostly low level infections have been found in the population, macroscopic changes rarely detected and muscle and gonad condition were normal. Similar findings were reported from UK waters [13] where highly prevalent but low level infections of this apicomplexan were observed in both king and queen scallops but no abnormal condition associated. This might suggest that under normal conditions, low level infections exist in populations of scallops but under hitherto unknown circumstances epidemics can occur. Epidemics, like the one observed in the Iceland scallop, might periodically occur which would make them a major factor in the population dynamics of scallops. What causes such an epidemic is difficult to identify. However, studying the well-known epidemiological triangle, i.e. the host, the infectious agent and the environment is helpful. The infectious agent can be relatively apathogenic, have high pathogenicity, low pathogenicity or even be an opportunistic pathogen. The host can be resistant or have different levels of susceptibility to the pathogen. Then the environment, which can be favourable for the host or unfavourable, in which case it commonly makes the host more susceptible to diseases. With regards to parasites, there can be a good parasite/host homeostasis or this homeostasis can, for some known or unknown reasons, be disrupted. Indeed, knowing these unidentified conditions would be good. However, in an environment like the ocean, there are numerous environmental parameters which could disrupt this homeostasis which in many cases are hard to determine. In fact, one can almost never state that a change in one (or several) particular environmental factor(s) is to blame. In some cases the likeliness is high but statements like these will always be speculative to a certain extent. Even in aquaculture, factors causing subclinical infections (bacteria, viruses, parasites) to develop into a disease outbreak aren´t always known, although these are controlled conditions.

Our findings strongly suggest that the apicomplexan parasite played a major role in the collapse of the Iceland scallop stock in Breidafjordur. Furthermore, compelling evidence exists that indicate that it affects other scallop populations, as similar macroscopic changes and the parasite itself have been identified or observed in association with other mass mortality events in several different scallop species and commercial stocks. However, it seems that these parasite infections can stay at low levels without causing the host any harm. That indicates that some factors, biotic (e.g. host density, presumable intermediate host, different host resistance) or abiotic factors (e.g. temperature, salinity, acidity) cause the parasite to extensively proliferate and cause an epidemic. Which factors could drive such a process are hard to currently ascertain.

Although the recovery of the scallop stock in Breidafjordur has been slow, research has shown clear signs of recovery the last few years.

Supporting Information

S1 Statistics.
(DOCX)

S1 Table. Sampling times and–sites of all 1493 scallops examined from 2003–2014 (see Fig 1). Abbreviations: AU = autumn; SP = spring; SU = summer.
(DOCX)

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Author Contributions

Conceived and designed the experiments: ÁK. Performed the experiments: ÁK MAF. Analyzed the data: ÁK ÁE MAF. Contributed reagents/materials/analysis tools: ÁK MAF. Wrote the paper: ÁK MAF.

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Paper III - Supporting data.

S1-Statistical Analyses

Overall

Terms and indices

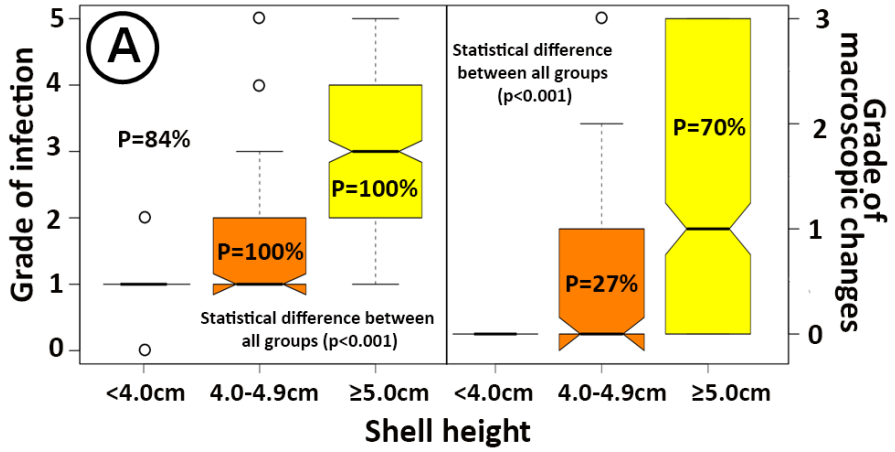
Ecological terms are according to previous definitions [19]. All statistical tests and plots were performed using RStudio (version 0.98.1062). The Gonad index (GI) and the Muscle Index (MI) are defined in the manuscript.

On plots and tests

Notches on boxplots provide an approximate 95% test of the null hypothesis that the true medians are equal: if the notches do not overlap, the medians could be described as statistically significant. However, this is an insufficient way to formally test a hypothesis. Therefore, after determining the normality of the data with the Shapiro-Wilk normality test, multiple groups were compared using ANOVA or the non-parametric Kruskal-Wallis test. When comparing pairs, the non-parametric Wilcoxon test was used.

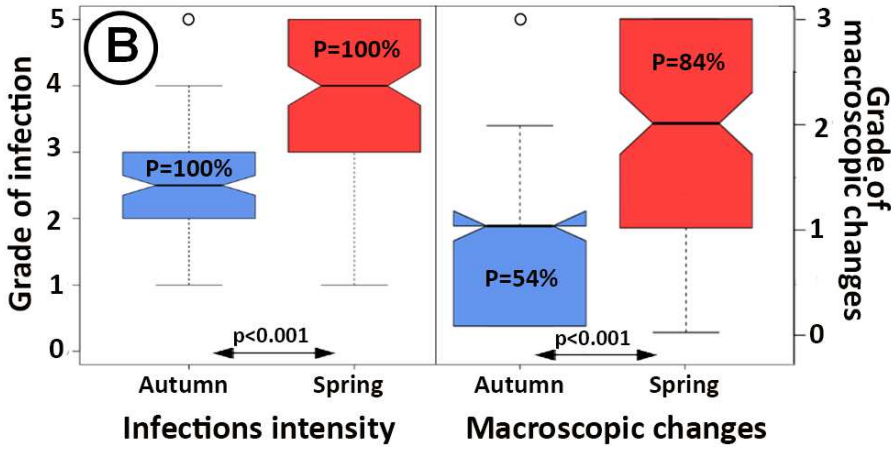
Detailed analyses for relevant figures

Fig 2A.



Normality of the data was determined with the Shapiro-Wilk test of normality. In cases of non-normality a non-parametric test was applied. Grade of infection and microscopic signs were compared between three shell size groups using the non-parametric Kruskal-Wallis test. Results showed a significant difference ($p < 0.001$) between all size groups in both cases.

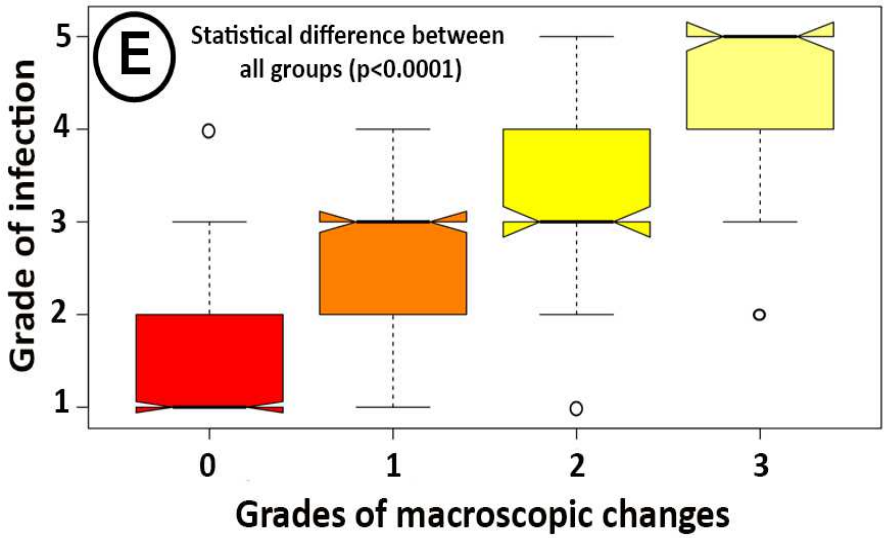
Fig 2B



Seasonal differences of macroscopic changes and the grades of infection were examined separately with the non-parametric Wilcoxon test. The left

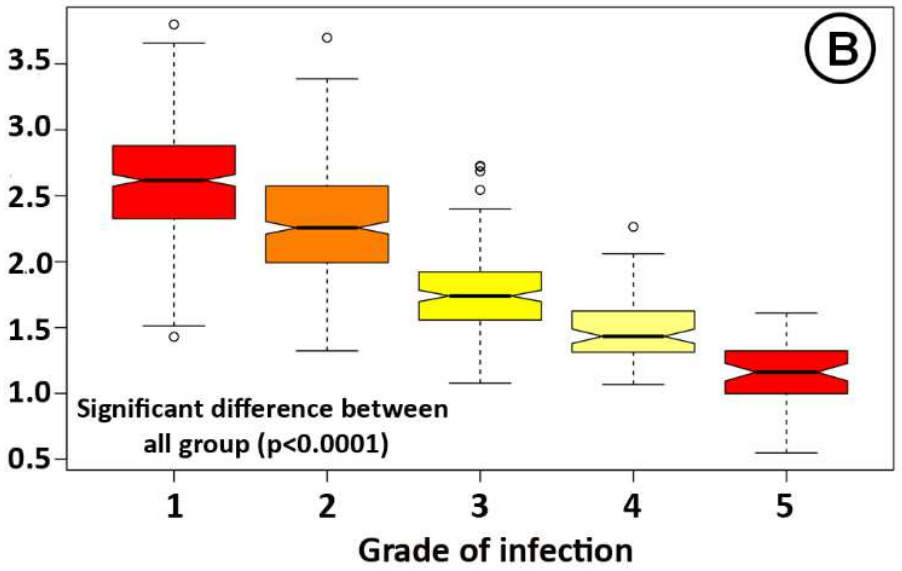
side of the figure shows a significant difference in grades of infection between spring and autumn during the years 2005 – 2006 ($p < 0.001$), where the grade of infection is higher in spring than in autumn. The outcome is the same regarding macroscopic changes as significant differences were found between spring and autumn over the years 2005 – 2006 ($p < 0.001$), where the macroscopic changes were greater during spring.

Fig 2E



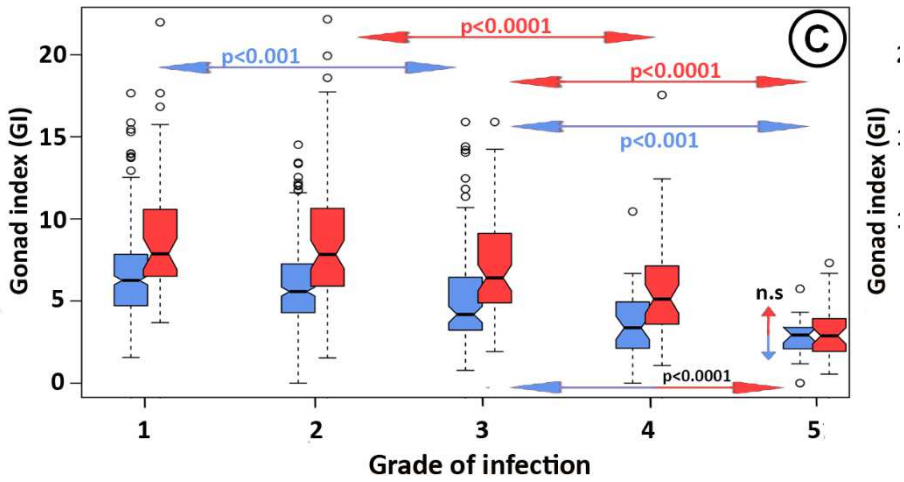
Grades of infection was compared between grades of macroscopic changes with the non-parametric Kruskal-Wallis test. Significant differences were observed between all four groups ($p < 0.0001$), where the grade of infection was higher with greater macroscopic changes.

Fig 3B



Muscle index was compared between grades of infection, using ANOVA and subsequently, Tukey's test. Significant differences were found between all five groups ($p < 0.0001$), where the muscle index was significantly lower in groups with a higher grade of infection.

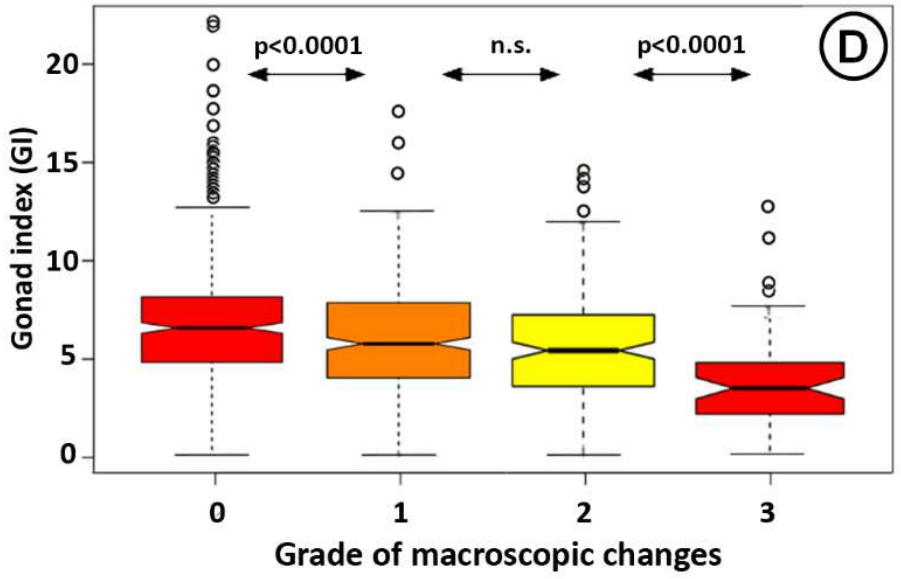
Fig 3C



Gonad index was compared between grades of infection for each season, spring (red) and autumn (blue), using the non-parametric Kruskal-Wallis test. Results for autumn showed significant differences between all five groups ($p < 0.001$), except for groups; 1-2, 3-4 and 4-5 ($p > 0.05$), which do not significantly differ. Results for spring showed no significant difference between groups; 1-2, 1-3, 2-3 and 3-4 ($p > 0.05$). Other comparisons were significantly different ($p < 0.0001$).

When examining each grade of infection between seasons the non-parametric Wilcoxon test was applied. A significant difference was found between seasons for grades 1-4 ($p < 0.001$). However, the gonad index showed no significant difference between spring and autumn at grade 5 of infection ($p > 0.05$). In addition, a significant difference was observed between group 5 in spring and groups 1, 2 and 3 ($p < 0.0001$) in autumn.

Fig 3D



Gonad index was compared between four groups of macroscopic changes using the non-parametric Kruskal-Wallis test. A significant difference was found between all groups ($p < 0.0001$), except between groups 1-2 ($p > 0.05$).

S1. Sampling times and –sites of all 1493 scallops examined from 2003 – 2014 (see Fig. 1). Abbreviations: AU = autumn; SP = spring; SU = summer.

	2003	2004	2005		2006		2007		2008		2009	2010	2011		2012	2013	2014	
Sites	AU	AU	SP	AU	SP	AU	SP	AU	SU	AU	AU	AU	SU	AU	AU	AU	SP	Total / site
11		74	50	57	61	60	50	50	60	57	46	31	24	30	30		30	718
12.1		83	50	54	31	56	25											298
12.2						26	40	55		59	14	30		30	30			281
2										45								45
32.2	10		22	5												38		75
31												20						20
33.2									60									60
Total/ year	10	157	122	116	92	142	115	105	120	161	60	81	24	60	60	38	30	1493
Of those mature	10	96	68	56	64	77	112	105	120	158	60	81	24	60	59	38	30	1218

Paper IV

Gray meat in the Atlantic sea scallop, *Placopecten magellanicus*, and the identification of a known pathogenic scallop apicomplexan.

Susan D. Inglis, Árni Kristmundsson, Mark Andrew Freeman, Megan Levesque and Kevin Stokesbury (2016).

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Author contributions: SDI and ÁK conceived and designed the experiments. SDI, ML and KS performed the field studies and the proximate and amino acid analysis. ÁK performed the histological analysis and MAF the molecular work: ÁK, SDI and MAF analysed the data. SDI, ÁK and MAF contributed reagents/materials/analysis tools. SDI, ÁK and MAF wrote the paper.

All authors read and approved the final manuscript.

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Gray meat in the Atlantic sea scallop, *Placopecten magellanicus*, and the identification of a known pathogenic scallop apicomplexan

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ABSTRACT

Atlantic sea scallop (*Placopecten magellanicus*) meats are normally firm and creamy white. However, scallops with small, darkened and stringy adductor muscle (gray meat) episodically occur along the Eastern Seaboard, most recently in the rotational management areas of Georges Bank after extended fishing closures. These gray meat scallops are associated with reduced harvestable biomass and mass mortality events. We tested age, nutritional stress and disease as causative agents for this condition. Adult scallops of different shell heights (SH) ranging from (90–145 mm) were collected from Georges Bank and analyzed for meat quality and the presence of pathogens using biochemical, histopathological and molecular methods. Gray meat occurrence was weakly correlated with shell height only explaining 8.49% of the variance in a generalized additive model (GAMS). Gray meat weights were lower than white meat ($p < 0.001$) and there was a dramatic reduction in protein content ($p < 0.05$) in gray meat scallops associated with extensive myodegeneration. Amino acid profiles confirmed the breakdown of muscle tissue with an increase in free hydroxyproline in gray meat scallops. Infection by an apicomplexan parasite was detected in the muscle tissue of all gray meat scallops tested. An intermediate pathology stage (brown meat) was also identified. As the parasitic infection increased, meat quality decreased. Numerous developmental stages of the parasite were present in various organs of the scallops. This apicomplexan has an identical SSU rDNA sequence to a novel parasite occurring in the Iceland scallop during a recent mass mortality event. The range of this parasite in Atlantic sea scallops and the effect of abiotic/biotic stressors on pathogenicity are currently unknown. Results from this study link an apicomplexan species, known to be highly pathogenic in scallops, to gray meat occurrence with a potentially high impact on the fishery.

1. Introduction

The Atlantic sea scallop, *Placopecten magellanicus*, supports one of the highest valued commercial fisheries in the United States and Canada and is found along the western North Atlantic continental shelf from Newfoundland to North Carolina (Hart and Rago, 2006; Stokesbury, 2012). Adult scallops range from 80 to 170 mm in shell height (SH) when measured from the umbo to the shell margin and are recruited into the commercial fishery at approximately 100 mm SH or 3–4 years of age (Serchuck and Wigley, 1986; Hart and Chute, 2004).

Atlantic sea scallop meat is normally firm and creamy white. However, commercial size scallops with small, darkened and stringy adductor muscles (gray meat) occur episodically along the Eastern Seaboard. Darkened gray, stringy meat was first observed in Atlantic sea scallops from the Bay of Fundy in 1936 and linked to senescence (Stevenson, 1936). Medcof (1949) described a "darkened meat" condition in sea scallops off Digby, Nova Scotia, attributing the condition to chronic infestation of older scallops by boring sponges (*Cliona* sp.). He found that the meat yield continued to decrease as the meat became darker in color and suggested that this may be due to energy being transferred from growth to repairing the shell structure. Gray meat has also been reported as symptomatic of prokaryotic infestation in sea scallops and was associated with a mass mortality in 1979–80 in Narragansett Bay, Rhode Island (Gulka et al., 1983). Stokesbury et al. (2007) reported gray meat in adult scallops during a mass mortality in Nantucket Lightship Closed Area (NLCA) between 2004 and 2005. In this event, the cause was attributed to a synergistic effect of senescence and parasitism by shell borers, and prokaryotic infections.

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Similar "gray meat" descriptions have been documented in other species of scallops. Wild stocks of the Iceland scallop, *Chlamys islandica,* in Icelandic waters and the queen scallop, *Aequipecten opercularis* from the Faroe Islands exhibit gray meat quality. For the Iceland scallop, this condition was associated with a heavy apicomplexan infection which caused a complete collapse in the stock (Kristmundsson et al., 2015) and subsequent total fishing ban (Eiriksson et al., 2010; Kristmundsson et al., 2011a).

In 2011, large numbers of discolored gray and brown meat scallops were observed in the Closed Area 1 (CA1) rotational scallop management area of Georges Bank after a three year fishing closure (Fig. 1). These scallops were discarded by fishermen due to low meat yield, and the low market value of the discolored and stringy meat (Fig. 2). In 2013, fishermen were only able to collect 32% of their total allowable catch (TAC) in the CA1 access fishing area due to the high number of "gray meat" scallops landed (SAW 59, 2014).

Poor quality meat in Atlantic sea scallops has impacts beyond market value. The adductor muscle (meat) in this scallop species represents 33% of the somatic tissue and 10–18% of the total weight (Mottet, 1979). It is comprised of a large phasic (striated or quick) muscle that is capable of rapid contractions for locomotion and removal of feces and pseudofeces from the mantle cavity (Gould, 1971). A smaller smooth or catch muscle is used for closing the valves tightly for long periods for protection from predation (Thompson et al., 1980). It is also an important energy storage site for glycogen and protein (Barber and Blake, 1981). Thus, deleterious impacts on adductor muscle function could have serious implications to scallop health and survival.

Based on published descriptions of "gray meat' in Atlantic sea scallops, we tested the following causative agents for gray meat quality; old age, nutritional stress, and/or disease. This study presents the discovery of a newly identified and pathogenic apicomplexan parasite linked to the gray meat condition observed in Atlantic sea scallops.

2. Materials and methods

2.1. Sample collection

In 2013 and 2014, adult Atlantic sea scallops ranging from 90 to 155 mm in shell height (SH) exhibiting normal (white), brown and gray adductor meat color (Fig. 3) were collected from commercial scallop fishing vessels and seasonal research scallop dredge surveys conducted in Georges Bank (Fig. 1). The sample collections included live (n = 2159), whole frozen (n = 88) and preserved tissues (n = 80). The samples were assessed for reproductive stage (n = 2088), shell height meat weight ratio (n = 1530), gonad and somatic tissue weight (n = 71) (live samples), proximate composition and amino acid profiles of the adductor muscle (frozen

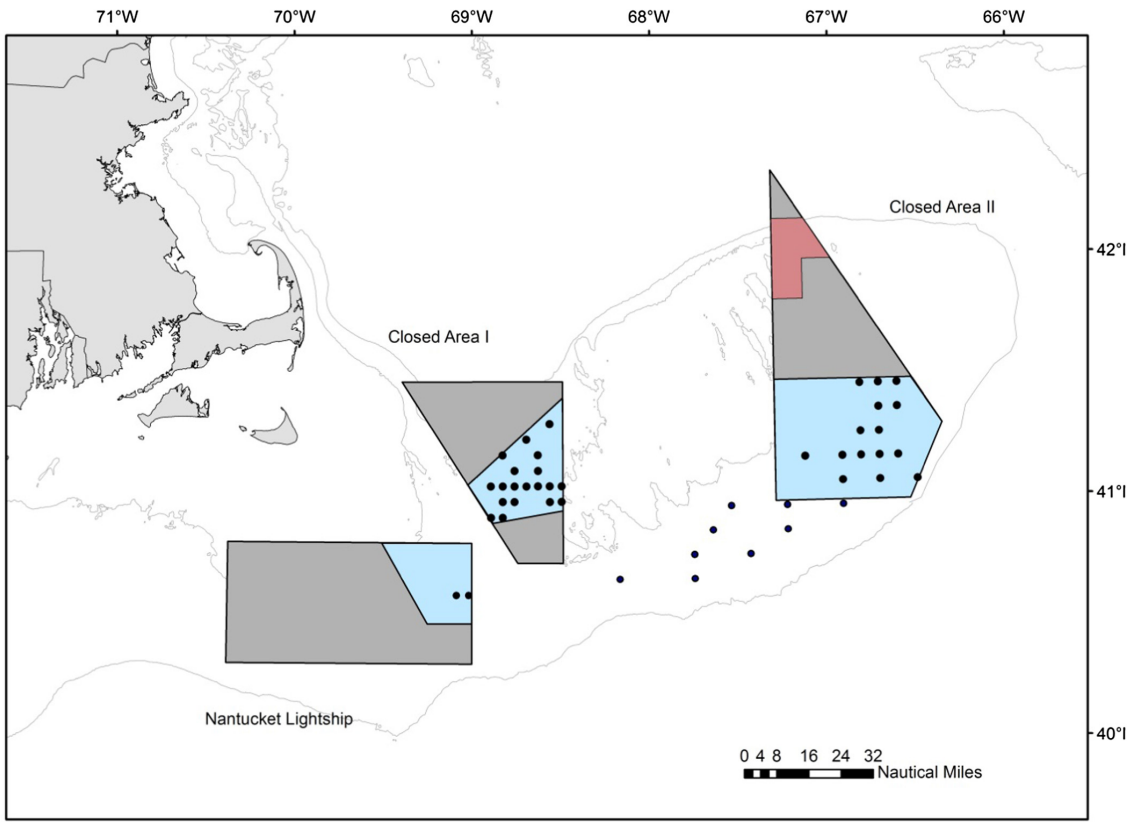

Fig. 1. Map of study site and Atlantic sea scallop sample collection locations (black points) on Georges Bank for shell height, meat weight, reproductive condition and histological analysis. The light blue shaded areas represent the rotational management access areas Closed Area 1 (CA1), Closed Area 2 (CA2) and Nantucket Lightship (NLCA). The area shaded in red represents the general area for samples collected for adductor muscle proximate analysis and amino acid composition.

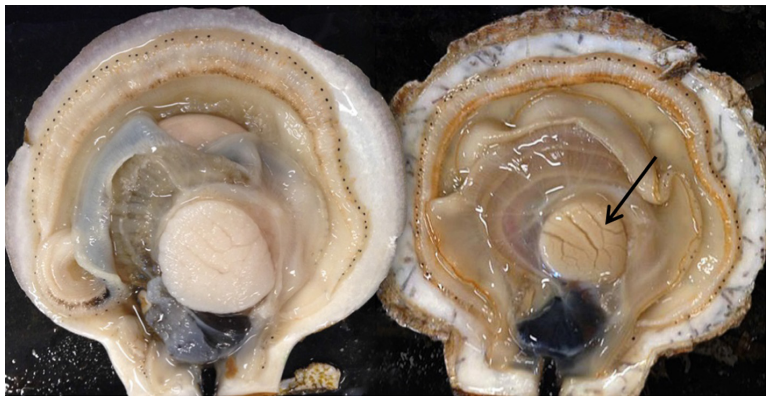

Fig. 2. Photograph of white (left) and gray meat scallops (right). Note that the scallop shell heights are similar but the gray meat is smaller in size and less compact. The arrow points to large gaps in meat texture. The gray meat scallop also shows signs of shell infestation by boring polychaetes that were identified as *Polydora* sp.

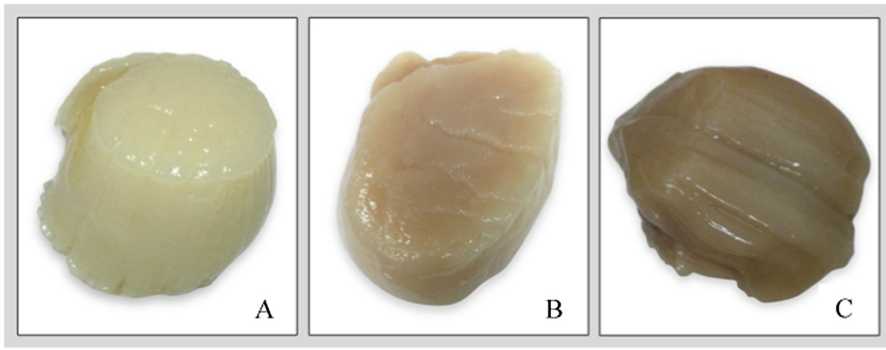

Fig. 3. Scale for meat color assessment used in the analyses. The white color A represents normal meat quality and the other colorations (brown, B and gray, C) are considered poor quality scallops.

samples) and the presence of pathogens (preserved tissues) by histopathological and molecular methods.

2.2. Field studies

2.2.1. Age, meat weight and reproductive condition

Scallop shell growth is incremental and models have been developed that associate shell height with age (Mackenzie, 1979; Packer et al., 1999). We examined the relationship between shell height and meat color (white, gray) of live scallops collected randomly from survey (n = 1864) and commercial dredge tows (n = 224) within Georges Bank access areas (CA1, Closed Area 2 (CA2) and open fishing areas south of CA2) (Fig. 1). Three collection areas were analyzed separately to assess if there were any location specific variables in the results and brown and gray meat scallops were pooled as poor quality meat for this analysis. Shell heights were measured to the nearest millimeter dorso-ventrally from the umbo to the shell edge. The adductor was then removed and the quality recorded based on color (Fig. 3). We also collected data on the reproductive status of these scallops to investigate any impact of observed meat color on reproductive potential. The reproductive stages (developing (D), ripe (R), spent (S), partially spent (PS) or latent (L)) were recorded from these samples according to Naidu (1970).

Following the methodology of Sarro and Stokesbury (2009), the shell height/meat weight ratios (SH/MW) were calculated for white, brown and gray meat quality scallops from the same samples described above from CA1 (n = 663) and CA2 (n = 867) locations.

Season greatly affects gonadal weight in scallops (Shumway and Parsons, 2012). Thus, the effect of meat quality on the gonadal somatic index (GSI) was investigated using samples (n = 71) collected from a single survey area in the Nantucket Lightship Closed Area (NLCA) during the month of August.

2.3. Laboratory procedures

2.3.1. Proximate and amino acid analysis

Scallops frozen whole at sea (n = 88) were dissected, the adductor muscle removed and the meat quality (color) recorded. Samples were shipped to the New Jersey Feed Laboratory Inc, Ewing, New Jersey for tissue analysis. Proximate analysis was conducted on the adductor muscle to provide the percent water, lipid, ash, protein and carbohydrate content of the tissue. Protein content was determined through laboratory nitrogen analysis. The nitrogen value was then converted to crude protein by multiplying the nitrogen by 6.25 based on the assumption that protein is 16% nitrogen (100/16 = 6.25) (AOAC, 1990). Carbohydrate content was

determined by subtraction: Percent carbohydrate = 100 − (Percent moisture + Percent protein + Percent lipid + Percent ash).

Amino acid profiles (includes: lysine, phenylalanine, leucine, isoleucine, threonine, valine, histidine, arginine, glycine, aspartic acid, serine, glutamic acid, proline, hydroxyproline, alanine and tyrosine) were also determined to elucidate changes in protein content. All analyses were conducted using the methods of the Association of Official Analytical Chemists (AOAC) (1990).

2.3.2. Histological and molecular analysis

Tissue samples from scallops exhibiting white, brown and gray adductor muscle coloration were collected and preserved from CA1 and CA2 sample locations on Georges Bank. Photographs were taken of each scallop to document the adductor muscle, gonad and shell condition. The shell height of the scallop (mm), date and location were recorded.

A small piece from each adductor muscle ~0.5 × 0.5 cm was removed and preserved in 2 ml Eppendorf tubes containing 95% ethanol for DNA analysis. The remaining adductor muscle as well as all other major organs were fixed in 10% formalin (in seawater) for histopathological analysis and the presence of any pathogens. A subset of the formalin fixed samples (n = 30) were shipped to the Kennebec River Biosciences Laboratory in Maine, USA for histopathological analysis. The remaining formalin samples (n = 50) and DNA samples (n = 50), were shipped to the Fish Disease Laboratory at the Institute for Experimental Pathology at Keldur, University of Iceland, for both histopathological and molecular analysis.

All formalin fixed samples were processed for histopathological examination according to Kristmundsson et al. (2015), i.e. embedded in paraffin wax, sectioned (4 μm thick), stained with Giemsa and mounted in resin based medium. All histological slides were thoroughly examined for the presence of pathogens and associated histopathological changes using a compound microscope at 100×–1250× magnifications.

For molecular examination, the total DNA was extracted from all 50 ethanol-fixed adductor muscles, using a GeneMATRIX kit (EURx Poland) following the tissue protocol. Apicomplexan small subunit ribosomal DNA (SSU rDNA) was amplified using the diagnostic primers, 18e-Mer 5′ ctgccagtagttatacgt 3′ and Mer-790r 5′acacscttgaagcaccctac, and PCR methodology described by Kristmundsson et al. (2015), that amplifies a 772 bp section on the 18 s rDNA including the variable regions v1-v4. Amplicons of the expected size were recovered from the PCR products using a GeneMATRIX PCR extraction kit (EURx Poland). DNA sequencing was performed using BigDyeTM Terminator Cycle Sequencing chemistry and the same primers that were used for the original PCR. DNA sequencing was performed in both forward and reverse directions and contiguous sequences compared with those available in GenBank.

2.4. Statistical analysis

The relationship between scallop meat color and shell height was statistically analyzed using a generalized additive model system (GAMS) with meat quality (color) as the dependent variable and SH and location (CA1, CA2, Open) as the explanatory factors. The shell height: meat weight (SH:MW) data from two Georges Bank locations were log transformed and tested for significance using an analysis of covariance (ANCOVA) with MW as the dependent variable; color as the factor and SH as the covariate (Zar, 2010). A one way analysis of variance (ANOVA) was used to explain the relationship between meat color and reproductive stage.

Data from nutritional analyses (proximate composition and amino acid profiles) were analyzed statistically by initially performing a Shapiro-Wilk test for normality. If the data were found to be normal the data were analyzed using analysis of variance (ANOVA) followed by Tukey's post hoc tests on treatment means. Kruskal-Wallis and Mann-Whitney tests were used on results requiring nonparametric analysis (Zar, 2010).

All statistical tests were performed using R statistical software (R Core Team, 2013). Significance level was set to the alpha level of 0.05.

3. Results

3.1. Age, meat weight and reproductive condition

A generalized additive model system (GAMS) found only a weak correlation of shell height (mm) with meat color in CA1, CA2 and the Open Area of Georges Bank (Fig. 4). Based on the akaike information criteria (AIC) values, the best fit model of quality with both shell height and location only explained 13.7% of the deviance. Shell height alone only accounted for 8.49% of the expected deviance. As scallop age is associated with shell height (Mackenzie, 1979; Packer et al., 1999) these finding suggest that the gray meat condition is not directly caused by old age and that there may be a weak location specific interaction.

The reproductive stages observed in scallops from (CA1, CA2 and NLCA) with white, brown and gray adductor muscles through a yearly cycle are reported in Table 1. These qualitative finding suggest that scallops with gray and brown meat follow white meat or "normal" scallop reproductive cycles. However, although we did not see any correlation between the reproductive stage and gray meat condition (Fig. 5), samples collected from the NLCA during the month of August and analyzed for gonadal somatic index (GSI) found a significant ($p < 0.05$) reduction in GSI between gray and white meat scallops. This finding was not observed in the GSI between brown and white meat scallops ($p > 0.05$). The mean GSI for white scallops was 16.2 ± 2.27 SD, 15.8 ± 1.72 SD for brown meat, and 11.4 ± 2.16 SD in gray meat scallops.

Shell height: meat weight analyses from Georges Bank CA1 and CA2 showed a significant reduction ($p < 0.001$) in meat weight in gray, brown and white meat scallops (Fig. 6) The ANCOVA analysis found there was a significant effect for SH and color (CA1 SH: $F_{1,721} = 1571.6$, $p < 0.001$; CA1 color: $F_{1,721} = 339.3$, $p < 0.001$; CA2 SH: $F_{1,656} = 1526.2$, $p < 0.001$; CA2 color: $F_{1,656} = 95.5$, $p < 0.001$), but not for the interaction term, for both CA1 and CA2. Thus, an ANOVA was used to test for differences in the slope without the

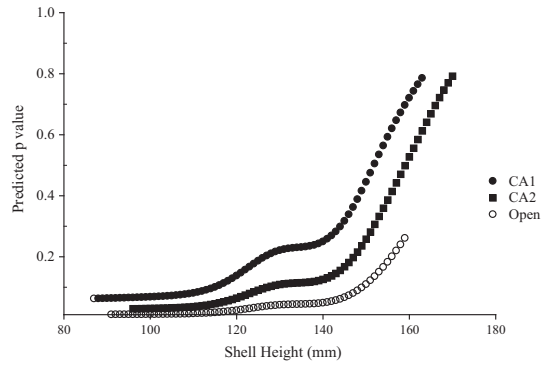

Fig. 4. The predicted probability of a scallop of certain shell height (mm) being of poor quality (gray) in three areas sampled on Georges Bank (Closed Area 1 (CA1), Closed Area 2 (CA2) and Open) using a general additive modeling system (GAMS). The best fit model of quality with both shell height and location only explains 13.7% of the deviance.

Table 1
The percent (%) of different reproductive stages (Naidu, 1970) observed through a yearly cycle for scallops with white, brown and gray adductor muscle.

Reproductive stage	White meat n = 1753	Brown meat n = 287	Gray meat n = 48
Developing	23.56	12.89	22.92
Ripe	25	16.03	12.16
Spent	4.28	13.59	8.33
Partially spent	6.37	8.71	10.42
Latent	40.10	48.78	46.17

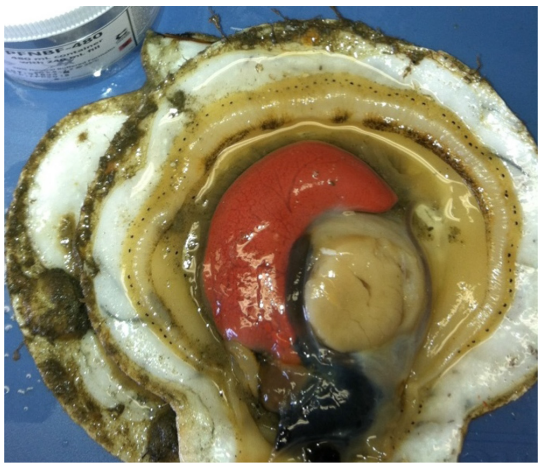

Fig. 5. A female gray meat scallop exhibiting a ripe gonad stage. Gray and brown meat scallops followed normal (white meat) reproductive staging.

interaction term. Linear regression was used to test for differences in intercepts. A significant effect of SH and color on MW was observed in both CA1 and CA2 (CA1 SH: $F_{1,722} = 1573.5$, $p < 0.001$; CA1 color: $F_{1,722} = 339.7$, $p < 0.001$; CA2 SH: $F_{1,657} = 1521.3$, $p < 0.001$; CA2 color: $F_{1,657} = 92.2$, $p < 0.001$). The analysis found the following meat weight relationship; white meat > brown meat > gray meat.

3.2. Adductor muscle composition

Results on the proximate composition of white, brown and gray meat colored adductor muscle (n = 88) found a significant decrease (ANOVA; $p < 0.05$) in the protein content of scallops with discolored meat; the darker the meat color (brown to gray) the greater the decrease in protein content. The percentage carbohydrate content were also reduced from 2.56 (±0.87) in white meat to 0.62 (±0.92) and 0.08 (±0.76) in brown and gray meat respectively (Table 2). The % lipid content in all scallop meat tested was l% or lower but consistent with other studies (Webb et al., 1969; Krzeczkowski et al., 1972; Naidu and Botta, 1978; Robinson et al., 1981; Shumway and Parsons, 2012).

The moisture: protein ratios for each meat quality category (white, brown, gray) were calculated to compare the discolored meat moisture content with industry standards natural moisture content in scallop meat (Codex, 2003). In sea scallops, the normal moisture:protein ratio is considered to be 4.0–4.9:1.0 (Lampilla, 1993). The adductor muscle of gray meat scallops exhibited a significant reduction in protein and increase in moisture content (Table 2).

Differences in amino acid concentration between the three adductor meat colors (white, brown, gray) were compared using a Kruskal-Wallis test because the data were not normally distributed even after log transformation (Sokal and Rohlf, 1995). All total amino acid concentrations decreased in gray and brown meat scallops which was associated with the overall reduction in protein content. However, when the data were transformed to % mole values there was a significant increase observed in the amino acid hydroxyproline in the gray meat scallops compared to white and brown meat scallops ($df = 2$, $p < 0.05$) (Table 3).

3.3. Histopathology

Infections with an apicomplexan parasite were found in all brown and gray meat scallops and occasionally in scallops exhibiting normal (white) adductor muscles. The infections were characterized by several distinctive forms; the most dominant forms were clusters of apicomplexan zoites found both intracellular, in muscle fibers or hemocytes, and extracellular and associated with necrotic muscle cell debris. Furthermore, dark stained spherical or oval bodies, were commonly found in great numbers, most often in association with the apicomplexan zoites. These forms, which presumably are apicomplexan trophozoites, are fairly consistent in size (approximately 4–5 μm in diameter) and are found both intra- and extra-cellular (Fig. 7). Other life forms, i.e. apicomplexan cysts, were also observed less frequently in the tissues. Apicomplexan life forms were observed in muscular and connective tissues in several different organs including the gonad, the digestive gland and the heart, but were most concentrated in the striated adductor muscle tissue.

In normal, white scallop meat the infections were either absent or very mild, in which case only occasional apicomplexan zoites were detected in the adductor muscle. Conversely, moderate to severe infections were generally detected in brown and gray meat scallops measured by the number of apicomplexan forms present per tissue (Kristmundsson et al., 2015). Histopathological changes were restricted to the presence of the apicomplexan parasite. In mild infections, of normal looking meat, the muscle structure was normal apart from some focal necrosis in close vicinity of the apicomplexan parasite. In the more heavily infected gray meat scallops, histopathological changes were observed in most organs; the most extensive ones being in the adductor muscle which were characterized by degeneration of muscle fibers (myodegeneration) at 'moderate to extensive' level (per Gulka et al., 1983) with extensive thinning of muscle fibers, loss of striations, muscular fragmentation and hyalinization. In the most severe cases a total necrosis of extensive areas of the adductor muscle were observed (Fig. 8). Different degrees of pathological condition were also observed in other organs, e.g. necrosis in muscular connective tissues in the inter-acinal area of the gonads (Fig. 9A) and the connective tissues adjacent to the epithelial layer of the gastrointestinal tract. Infiltration of hemocytes was commonly observed in association with the apicomplexan infections, especially in the digestive gland and the adductor muscle and commonly associated with sporoblast-like apicomplexan forms and sporozoites, respectively. Occasionally, fibroblast like hemocytes surrounded apicomplexans forming a granuloma like structure (Fig. 9B). Furthermore, in some cases an aggregation of abnormal neoplastic-like hemocytes were observed in connective tissues in the digestive gland and inter-acinal areas, which seemed anaplastic and abnormally large. However, this condition was apparently unrelated to both to the extent of apicomplexan infections as well as the abnormal discoloration of the adductor muscles as it was equally detected in white, brown and graymeat scallops.

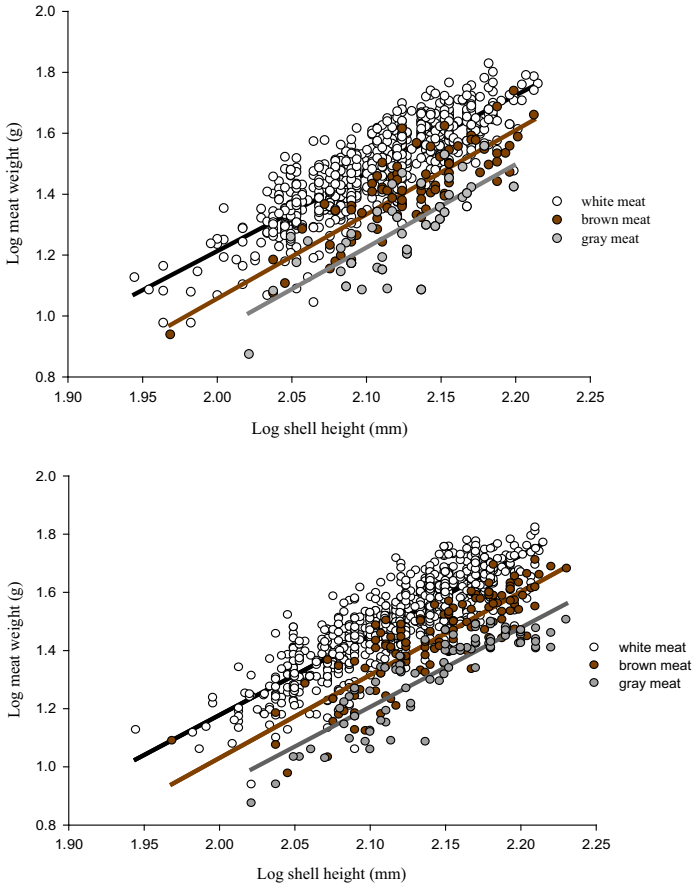

Fig. 6. The log-transformed shell height meat weight relationship between gray, brown and white meat scallops in Closed area 1 (CA1), n = 663 (top) and Closed Area 2 (CA2), n = 867 (bottom) from September 2013-March 2014. There was a significant reduction in meat weight in discolored adductor meat (brown and gray)(ANOVA $p < 0.001$) in both areas.

Table 2
Proximate composition (mean percent wet weight ± SD) of adductor muscle in Atlantic sea scallops from Georges Bank. There was a significant reduction in% protein and carbohydrate and inverse increase in moisture content in brown and gray meat compared to white meat scallops n = 88; (ANOVA, $p < 0.05$).

Analysis	White (n = 33)	Brown (n = 26)	Gray (n = 29)
Moisture	77.86 ± 2.56	80.82 ± 2.33	90.33 ± 3.05
Protein	17.68 ± 1.68	14.81 ± 2.57	6.97 ± 1.01
Carbohydrate	2.56 ± 0.87	0.62 ± 0.92	0.08 ± 0.76
Ash	2.90 ± 0.24	3.67 ± 0.14	2.77 ± 0.14
Lipid	0.08 ± 0.02	0.08 ± 0.01	0.03 ± 0.01
M:P	4.40 ± 0.89	6.46 ± 1.36	12.96 ± 4.20

M:P, moisture: protein ratio.

3.4. Molecular analysis

Using a diagnostic PCR developed for identifying the apicomplexan observed in the Iceland scallop infection; all Atlantic sea scallop gray meat samples produced a PCR band at the expected size. DNA sequencing revealed that all the scallops tested had the same apicomplexan sequence, which was 100% identical to the sequence obtained from Icelandic scallops (Kristmundsson et al., 2015).

4. Discussion

The apicomplexan parasite identified in Atlantic sea scallops has been shown to be highly pathogenic to at least two scallop species, i.e. Iceland scallop and queen scallop, and is believed to be responsible for the total collapse in a population of the former species in Icelandic waters (Kristmundsson et al., 2015). Clinical signs associated with these infections are similar in all these scallop species. It therefore seems quite plausible that the parasite plays a role in the abnormal gray meat condition and mass mortality events experienced in the Atlantic sea scallop. Like in the Iceland scallop (Kristmundsson et al., 2015), the apicomplexan was found in most organs of the Atlantic sea scallop and frequently in great numbers in the adductor muscle of gray meat scallops, while infections were in all cases very light in normal white meat. Extensive myodegeneration was associated with the infection causing a reduction in overall muscle mass and structural integrity. Similar to observations in the Iceland scallop (Kristmundsson et al., 2015), the adductor muscle became more discolored with increasing apicomplexan infection, with meat color changing from white to brown to gray. The intermediate "brown" stage of the infection was confirmed in the biochemical and histopathological results. Parasite zoites were also found inside hemocytes, due to phagocytosis or active

Table 3
The mean % amino acid content of adductor muscle in normal (white), brown and gray meat scallops ± SD. Values are presented as the mole percentage of total amino acids. An asterisk (∗) denotes significant differences ($p < 0.05$) between the meat color values (a,b,c) from Post-hoc comparisons.

Sample (n) Meat color	33 White a Mean ± SD	26 Brown b Mean ± SD	29 Gray c Mean ± SD	Kruskal-Wallis Ranked sum test (H value)
Lysine	8.09 ± 0.18	7.96 ± 0.20	8.04 ± 0.36	3.21
Phenylalanine	3.71 ± 0.09	3.71 ± 0.08	3.73 ± 0.09	2.84
Leucine	7.41 ± 0.23	7.33 ± 0.21	7.23 ± 0.27	3.12
Isoleucine	3.82 ± 0.32	3.67 ± 0.30	3.71 ± 0.35	3.61
Threonine	4.22 ± 0.08	4.24 ± 0.10	4.36 ± 0.16	2.97
Valine	3.65 ± 0.23	3.53 ± 0.25	3.58 ± 0.26	2.18
Histidine	1.87 ± 0.04	1.84 ± 0.05	1.87 ± 0.06	2.30
Arginine	10.56 ± 0.78	10.50 ± 0.54	10.64 ± 1.05	3.16
Glycine	10.82 ± 0.86	11.34 ± 1.01	10.40 ± 1.18	2.42
Aspartic Acid	10.84 ± 0.40	10.79 ± 0.39	10.84 ± 0.45	2.36
Serine	4.63 ± 0.18	4.66 ± 0.14	4.86 ± 0.19	2.51
Glutamic Acid	17.77 ± 0.52	17.66 ± 0.39	17.58 ± 0.58	2.39
Proline	3.18 ± 0.09	3.25 ± 0.16	3.32 ± 0.43	3.12
Hydroxyproline∗ (a,c); (b,c)	0.31 ± 0.07	0.40 ± 0.11	0.97 ± 0.05	12.82
Alanine	5.79 ± 0.23	5.77 ± 0.17	5.67 ± 0.25	2.60
Tyrosine	3.34 ± 0.13	3.34 ± 0.07	3.43 ± 0.10	2.07

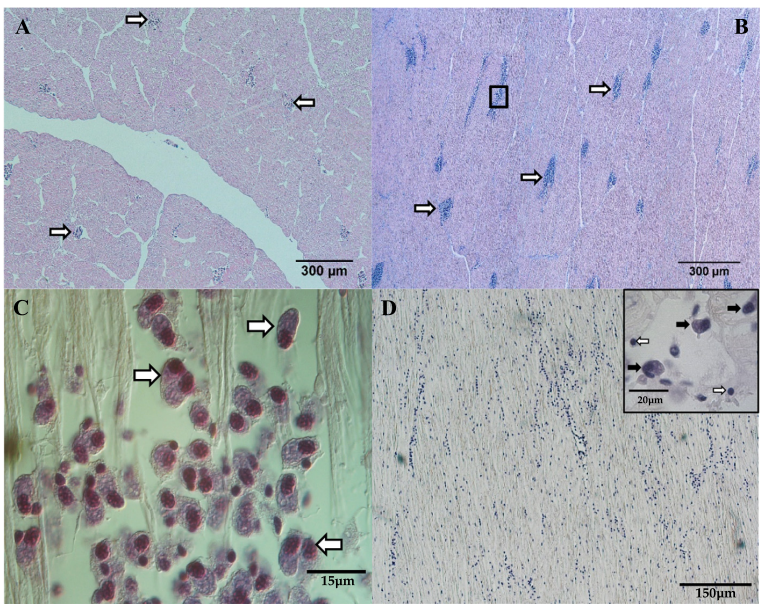

Fig. 7. (A and B) Histological section from a moderately infected brown meat adductor muscle (A) and a heavily infected gray meat adductor muscle (B) showing numerous clusters of apicomplexan zoites (arrows). (C) Higher magnification of a cluster of apicomplexan zoites (arrows) within the square from figure (B) showing necrotic muscle cell debris and deteriorated surrounding muscle fibers associated with the infections. (D) Histological section of gray meat adductor muscle severely infected with a mixture of apicomplexan zoites and presumable trophozoites. Inserted picture: Higher magnification showing apicomplexan zoites (black arrow) and trophozoites (white arrow) in association with necrotic muscle fibers.

infection by the parasites, either which would negatively affect the immune function of the scallop.

The pathological effects of this parasite on the adductor muscle tissue were observed in the other analyses for gray meat condition. We observed a significant reduction in meat yield between normal, brown and gray meat scallops from Georges Bank. This finding is consistent with the muscle necrosis associated with the parasite activity. The higher the intensity of infection the more reduced the adductor muscle mass. This could be due to a direct effect of the apicomplexan infections in the adductor muscle, as well as an indirect one, as a consequence of reduced function of other vital organs due to infections, e.g. the digestive gland.

The primary sites for energy storage in scallops are the adductor muscle and digestive gland. The digestive gland is the storage site for lipid reserves while the adductor muscle stores carbohydrate, in the form of glycogen, and protein (Barber and Blake, 1981). The low lipid levels observed in our results are consistent with the adductor muscle not being a primary lipid storage site. However, the dramatic decrease in protein content observed in the adductor muscle in gray meat scallops (~7%) compared to white meat (~18%), along with the lower carbohydrate levels indicates a significant energetic deficit. Although these findings are generally consistent with an animal in nutritional stress (Castellini and Rea, 1992), the cause of the nutritional deficit in this case is more

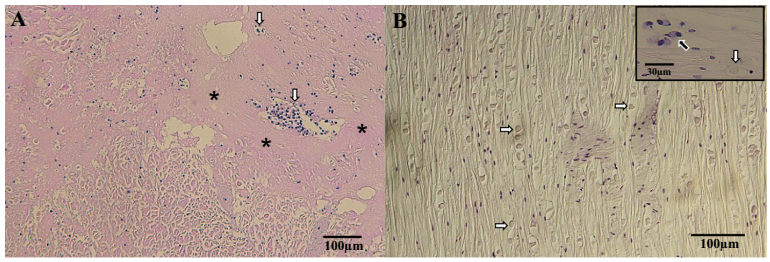

Fig. 8. (A) Histological section from gray meat adductor muscle showing liquefactive necrosis (asterisk) associated with apicomplexan zoites (arrow) and (B) hyalinization (arrows) of muscle tissue associated with severe infection by apicomplexan parasite. Inserted picture: Higher magnification of hyalinization of the muscle (white arrow) and apicomplexan zoites (black arrow).

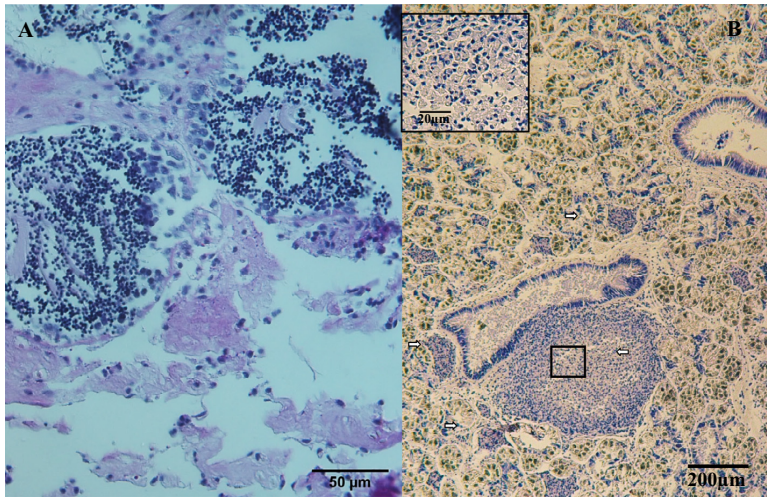

Fig. 9. (A) Necrotized inter-acinal muscular connective tissue in the gonad (testis) of a gray meat Atlantic sea scallop, causing destruction of the wall enclosing the acini. (B) Digestive gland from a gray meat Atlantic sea scallop showing aggregation of hemocytes as a reaction to the apicomplexan infection (arrows). Note the large granuloma like structure where heamocytes are surrounding a group of apicomplexan forms, presumably sporoblasts (arrowhead). Inserted picture represents a higher magnification of the area inside the rectangle.

consistent with a disease process than a lack of food resources. Energy reserves are utilized at a rate that is not sustainable and the animal is unable to restore the energy supply (Shumway and Parsons, 2012). In the case of the apicomplexan infection, this is possibly due to the energetic costs of the immune response to the infection and the pathological changes to the physical structure of both the energy storage area and feeding muscle through parasitic activity.

The amino acid profile of the adductor muscle in gray meat scallops confirmed the breakdown of protein associated with high intensity infections. Hydroxyproline is an amino acid associated with the production of collagen which provides structure to muscle tissue. It plays a key role in collagen stability (Nelson and Cox, 2005). An increase in free hydroxyproline is an indication of muscle tissue being chemically broken down into its base components. This increase was not observed in the intermediate "brown" stage of the disease and only found in highly infected scallop meat and biochemically is descriptive of hyalinization of the muscle tissue.

Molecular analysis confirmed that the apicomplexan observed in the Atlantic sea scallop has an identical SSU rDNA sequence to the parasite that caused a mass mortality in the Iceland scallop from Icelandic waters and in queen scallop in Faroese waters (Kristmundsson et al., 2011a, 2015). Furthermore, this parasite has also been identified in the king scallop off the coast of Scotland, but did not cause clinical signs (Kristmundsson et al., 2011a). Based on similar biochemical (Brenner et al., 2012) and histopathological (Ryan Burt pers. com.) findings reported in the weathervane scallop, *Patinopecten caurinus* in Alaska, it is possible that this apicomplexan is also responsible for the "weak" meat condition reported in that species. This suggests a large geographic range of infection for this apicomplexan parasite.

Apicomplexans are very diverse group of protozoan parasites that infect a wide range of taxa. With the exception of clams (Kristmundsson et al., 2011b), they have been poorly studied in bivalves, particularly in wild stocks. The bay scallop *Argopecten irradians* has been the most studied scallop associated with apicomplexan infections (Karlsson, 1991; Cawthorn et al., 1992; Whyte et al., 1994). The apicomplexan (*Pseudoklassia pectinis*) was identified in the king scallop, *Pecten maximus* off the French coast (Léger and Duboscq, 1917) and *Margolisiella islandica* was identified in the Iceland scallop in Icelandic waters, (Kristmundsson et al., 2011b). The host tissues targeted by apicomplexans is species specific but generally includes the kidney and other organs. This newly identified apicomplexan that infects Iceland, queen, king (Kristmundsson et al., 2011a, 2015) and now

Atlantic sea scallops is the only known bivalve apicomplexan that targets muscle tissues.

Fishermen have anecdotally associated the gray meat condition in Atlantic sea scallops with large, old scallops. The occurrence of gray meat in areas that have been closed to fishing for extended periods appears to support this finding. However, our analysis found that gray meat scallops were distributed throughout the commercial scallop size classes and that shell height as a proxy for age was not a good indicator of gray meat condition. The shells of older scallops often show signs of infection by boring organisms such as *Polydora* and *Cliona* species. These stressors, concurrent with the apicomplexan infection could make the pathological effects of the apicomplexan activity more virulent in these older scallops. This scenario could explain the published findings of gray meat quality associated with large, old scallops.

In the Iceland scallop, apicomplexan infection was only found in mature scallops. As Atlantic sea scallop samples were collected from commercial dredges, non-commercial size scallops were not included in our analysis and we recommend that the incidence of gray and/or brown meat condition in juvenile and sub-commercial size classes be investigated to determine if the infection is impacted by scallop maturity.

We also found that gray meat scallops did not show signs of reproductive senescence and appear to remain reproductively viable in samples collected from three areas on Georges Bank. However, the apicomplexan infection was also observed in supporting tissue (stroma) in gonadal tissue from gray meat scallops (Fig. 9). The reduction in the gonadal somatic index observed in gray meat scallops during the month of August in the NLCA suggests that the condition may impact fecundity in highly infected individuals. In the case of the Iceland scallop, heavy infection of this apicomplexan severely affected normal gonad development and the intensity of the infection and the pathological effects on the host were more severe in the spring than autumn; indicating a seasonal effect (Kristmundsson et al., 2015). Thus, further analysis is required on the impact of the apicomplexan infection on the reproductive potential in Atlantic sea scallops and any seasonal or spawning influences on the pathogenicity.

Hemocytes form the immune system of scallops. Infiltration of hemocytes was commonly observed associated with the apicomplexan parasite, both in lightly and more heavily infected scallops, indicating an immune response to the infection. As in the case of the Iceland scallop (Kristmundsson et al., 2011a), the apicomplexan parasite was also found to infect hemocytes in the Atlantic sea scallop. It therefore seems plausible that the apicomplexan parasite would negatively affect the ability of the scallop to fight the infection. The presence of neoplastic hemocytes in some of the scallops should not be ignored, although observations in this study suggested that this condition was in the initial phases and neither enhancing the apicomplexan infections nor directly causing the gray meat condition in sea scallops. Disseminated neoplasia (also termed hemic-, hemocytic- and hematopoietic neoplasia) is known from numerous species of mussels, clams, cockles and oysters and considered a serious condition in some species. The progress of the disease is commonly split into four stages; at the more advanced stages it can cause a marked reduction in the number of normal hemocytes which affects the host's ability to fight infection (Barber, 2004; Días et al., 2016). According to studies, disseminated neoplasia of bivalves follows a seasonal cycle with regards to prevalence and severity (e.g. Días et al., 2016). Consequently, to determine its progress and presumable effect on Atlantic sea scallops, a routine sampling over a whole year would be needed.

The range and prevalence of this parasite in Atlantic sea scallops, and the effect of abiotic and biotic stressors on pathogenicity are unknown. In the Iceland scallop, the apicomplexan infection had a significant effect on the reproductive potential of the scallop that was not observed in either the queen or the Atlantic sea scallop infections. However, the reduced GSI observed in highly infected Atlantic sea scallops suggests that, although reproductively viable, they may have lower fecundity. It is unknown whether the different pathological impacts of this parasite on its host are due to the physiology or specific environment of the different scallop species it infects. According to Kristmundsson et al. (2015), infections can stay at low levels in Iceland scallops without causing harm to the host. This finding indicates that in Iceland scallops some biotic factors, such as host density, host resistance, other disease agents, or abiotic factors, e.g. temperature can cause the parasite to proliferate and cause an epidemic (Kristmundsson et al., 2015). In Atlantic sea scallops, gray meat outbreaks are temporally episodic and spatially distinct, with higher prevalence in Georges Bank scallop populations compared to mid-Atlantic sea scallops. The increase in gray meat scallops following the opening of CA1 on Georges Bank, after three years of fishing closure, also suggests an environmental component to the infection. Thus, specific environmental conditions may provide the best indication of the pathogenic potential of this parasite in Atlantic sea scallop populations.

This paper identifies an apicomplexan parasite in the Atlantic sea scallop which is thought to be the cause of a collapse in a scallop population in Iceland (Kristmundsson et al., 2015). The similar clinical symptoms associated with the Iceland scallop infection, and the histopathological changes associated with the infections, suggest that this apicomplexan is likely a contributing factor in the gray meat condition in the Atlantic sea scallop. This parasite infects most all muscular and connective tissues of the host where it causes massive histopathology and has shown the potential to have significant impacts on the health of scallop populations. Further studies are needed to fully understand the role of biotic and abiotic stressors (host parasite balance) on the apicomplexan infection. Future work should include sampling at different times of the year to look at the seasonal effects, as well as focusing on the infection status of juvenile sea scallops. Furthermore, the possible combined effect of the apicomplexan infections and neoplastic disease should be examined. Subsequently, we can begin to address questions on the transmission of the parasite and possible preventive measures to manage the propagation of the infection in Atlantic sea scallops.

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Paper V

Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere

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Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere

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Apicomplexans comprise a group of unicellular, often highly pathogenic, obligate parasites exploiting either one or two hosts to complete a full reproductive cycle. For decades, various scallop populations have suffered cyclical mass mortality events, several of which shown to be caused by apicomplexan infections. We report the first dual mollusc life cycle for an apicomplexan: a species highly pathogenic in various pectinid bivalve species, but apathogenic when infecting the common whelk as *Merocystis kathae*. The sympatric distribution of the common whelk and scallops in the North Atlantic makes transmission extremely effective, occurring via the gastrointestinal tract, by scavenging and predation in whelks and unselective filter feeding in scallops. Infective sporozoites from whelks utilize scallops´ haemocytes to reach muscular tissue, where asexual reproduction occurs. Phylogenetically, this apicomplexan is robustly placed within the Aggregatidae and its inclusion in analyses supports a common ancestry with other basal invertebrate apicomplexans. Scallops seem able to regulate low-level infections of *M. kathae* as they exist in normal populations while epizootics occur during high levels of exposure from locally infected whelks. A targeted removal of whelks from valuable scallop grounds would be advantageous to minimize the occurrence of *M. kathae* epizootics and prevent damaging economic losses.

Phylum Apicomplexa forms a group of unicellular spore forming parasites sharing a defining feature, the apical complex that comprises structural and secretory elements that facilitates interaction with the host cell[1]. They are obligate parasites which develop mostly inside the host cell, but degrees of epi- and extra-cellular development are known[2]. Their life cycle is either monoxenous (one host) or heteroxenous (two hosts) and includes many and diverse developmental forms representing asexual (merogony) and sexual reproduction (gamogony) with the formation of infective sporozoites (sporogony)[3]. Both monoxenous and heteroxenous species are described from marine molluscs. Despite reports of apicomplexans infecting molluscs dating back to the 19th century[4], they are poorly understood, compared to those infecting vertebrate hosts.

The pathogenicity of apicomplexans varies considerably between species and/or their hosts. As most species are obligate intracellular parasites they cause a level of pathology. Some are considered to have low pathogenicity while others are highly pathogenic, such as those causing malaria, toxoplasmosis and cryptosporidiosis in humans. Highly pathogenic mollusc-infecting apicomplexans are known, for example the one infecting scallops (herein referred to as scallop apicomplexan or SAP). SAP has been shown to be largely responsible for the total collapse of a population of Iceland scallop *Chlamys islandica* in Iceland[5], and an unidentified apicomplexan negatively impacting oyster populations in New Zealand[6]. Furthermore, strong indications exist that SAP causes regular mass mortalities in a number of commercial scallop species inhabiting different geographic areas[5,7,8]. Kristmundsson *et al.*[7], observed a great number of developmental forms, in infected scallops, suggesting that it could potentially have a monoxenous life cycle, but could not exclude the requirement of an obligate alternate host.

Merocystis kathae, is an apicomplexan infecting renal tissues of the common whelk, *Buccinum undatum*, in northern Europe. *M. kathae* originally described by Dakin[9] more than 100 years ago, subsequently the life history

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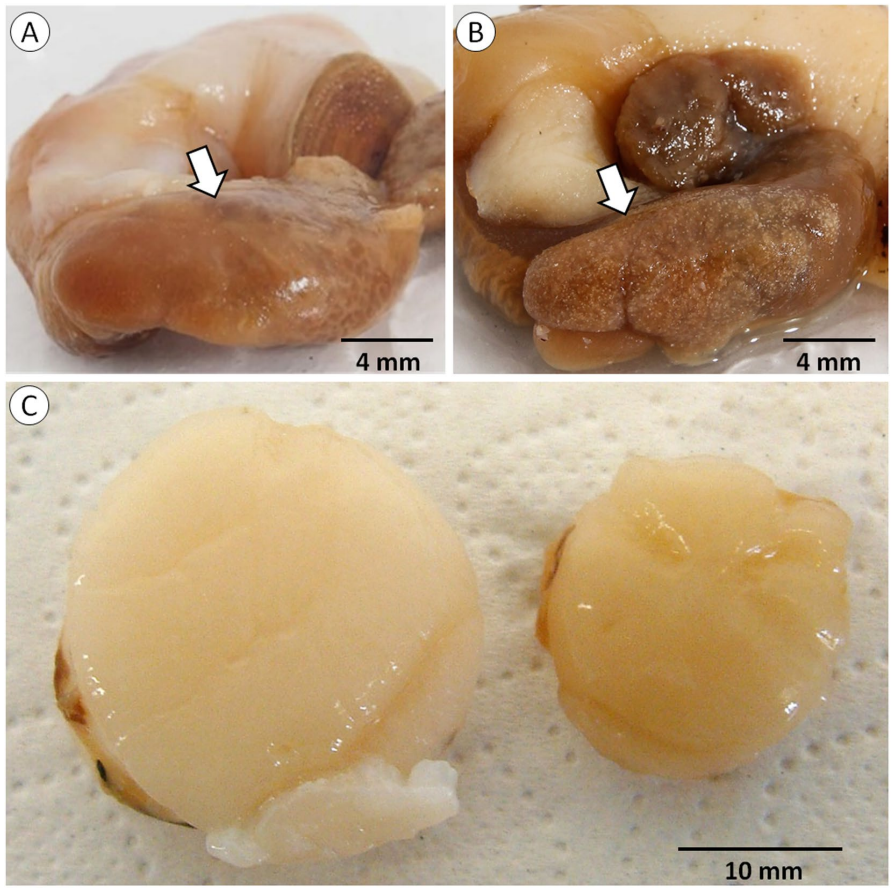

Figure 1. Macroscopic signs *Merocystis kathae* infection. (**A**) Normal, uninfected kidney of a common whelk. (**B**) Whelk kidney heavily infected with *M. kathae* characterized by numerous small white cysts visible to the naked eye. (**C**) Healthy and abnormal (right) scallop adductor muscles from equally sized shells. The muscles of scallops heavily infected with *M. kathae* are greatly reduced in size and have abnormal brown colouration.

of the parasite was described in more details[10,11]. Gamogony and sporogony was found to occur in the whelk but merogony was absent and thought to develop in an unknown alternate host. No molecular data exist for this genus but based on its morphological features it has been classified within the Family Aggregatidae[10,11].

The aim of the present study was to assess whether SAP in scallops is conspecific to *M. kathae* in whelks, and to better understand the life cycle and transmission of the parasite between the two different mollusc hosts if conspecificity is confirmed. In addition, we place *M. kathae* in a phylogenetic context within the Phylum Apicomplexa.

Results

Microscopic examination. Developmental stages of *Merocystis kathae* and the SAP were observed in all whelks and Iceland scallops examined, respectively. Gross clinical signs of infections were common, in whelks as small white cysts in the kidney (Fig. 1A and B) and in scallops as reduced abnormally brown-coloured adductor muscles (Fig. 1C). None of the king scallops, *Pecten maximus*, sampled from a scallop ranch in Scotland, where whelks are virtually absent, had clinical signs of disease and only few SAP life forms were detected in one of 20 scallops examined. Neither SAP nor *M. kathae* were observed in the other five species of gastropods and bivalves examined.

Most whelks were extensively infected (Fig. 2A) and all developmental stages, representing gamogony and sporogony, were observed in histological sections (Fig. 2B–I); the smallest forms detected were trophozoites around 10 µm, intracellular in renal cells.

In scallops, initial infections were sporoblasts and sporozoites in the gastrointestinal epithelium and later in the adjacent connective tissues associated with the digestive gland and gonads (Fig. 3A and B). These forms appear identical to sporoblasts and sporozoites which develop in the whelk (Figs 2F–I and 3D). The sporoblasts

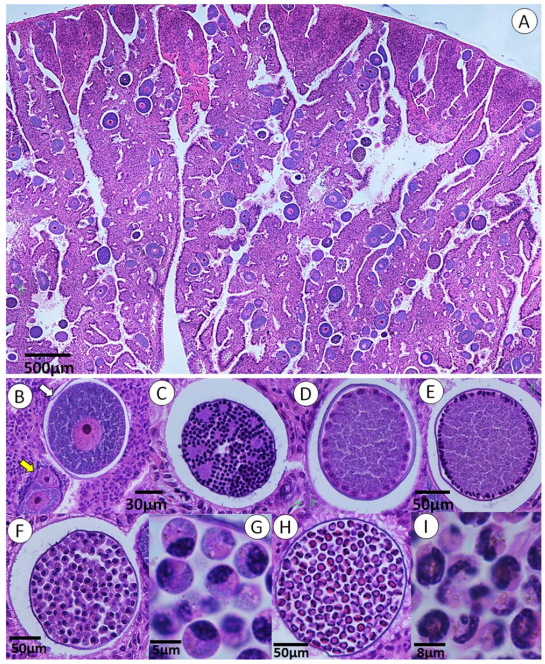

Figure 2. Histological sections of a whelk kidney infected with *Merocystis kathae*. (**A**) Kidney section of a common whelk heavily infected with *M. kathae* showing all gamogonic and sporogonic stages. (**B**–**I**) Higher magnification of the life forms; (**B**) mature macrogamont (white arrow) and two growing intracellular trophozoites inside renal cells (yellow arrow). (**C**) Mature microgamont with numerous microgametes. (**D** and **E**) Immature oocysts with peripherally located nuclei. (**F**) Premature oocyst filled with numerous sporoblasts. (**G**) Higher magnification of sporoblasts. (**H**) A mature oocyst filled with sporocysts, each containing two sporozoites. (**I**) Higher magnification of disporous sporocysts All sections are stained with H&E.

commonly sporulate in the connective tissues, resulting in a sporocyst with two sporozoites. At these sites, the sporozoites are often seen inside haemocytes (Fig. 3C). In addition, sporoblasts are occasionally seen in the adductor muscle (Fig. 3D). The target organ of the infective sporozoites, which develop in whelks and to some extent in scallops, is muscular tissue, especially the adductor muscle (Fig 3E). The sporozoites actively invade muscle cells which become hypertrophied and eventually rupture. It appears that only a fraction of the sporozoites develop further in the scallops, i.e. enter the merogonic phase. The first indication of merogony is the presence of 15–20 µm trophozoites inside muscle cells (Fig. 3F). They significantly increase in size and develop into early meronts (Fig. 4A). Subsequent development involves recurrent nuclear cleavage giving rise to multinucleated premature meronts with regularly arranged nuclei (Fig. 4B–D) which eventually become mature meronts containing numerous merozoites (Fig. 4E–H). Two generation of merozoites are present (Fig. 4F and H) originating from two types of meronts (Fig. 4E and G) with morphologically different merozoites; type I being shorter and with both ends somewhat pointed (Fig. 4F) while type II is convex, more slender and sausage-shaped (Fig. 4H).

SAP causes severe histopathological changes in the Iceland scallop which was comprehensively described by Kristmundsson *et al.*[5]. The histopathology of *M. kathae* in the whelks is minor, even in those with extensive infections. Regardless of infection status the whelks appear to be in good condition. As an intracellular parasite it causes some focal pathological changes in affected cells, i.e. the renal epithelial cells, which increase in size as the parasite grows and hence projects into the renal cavity or the underlying connective tissue. The host cell retains its position in the renal epithelium with no signs of penetration of the parasite into other host cells.

PCR, DNA sequencing and *in situ* hybridization (ISH). All Iceland scallops and whelks tested positive using a diagnostic PCR, initially developed for the SAP[5]. Furthermore, DNA sequencing showed that the SSU rDNA of *Merocystis kathae* and SAP was 100% identical.

ISH further confirmed the conspecificity of *Merocystis kathae* and SAP as all the developmental forms detected in both the whelks and the scallops gave strong positive reactions to the specific probes (Fig. 5A–I). Furthermore, small (5–6 µm) intracellular forms in the intestinal tract of the whelks showed a positive reaction for the parasite DNA in ISH (Fig. 5A), indicating that the transmission of the parasite, from scallops to whelk, occurs via the gastrointestinal tract.

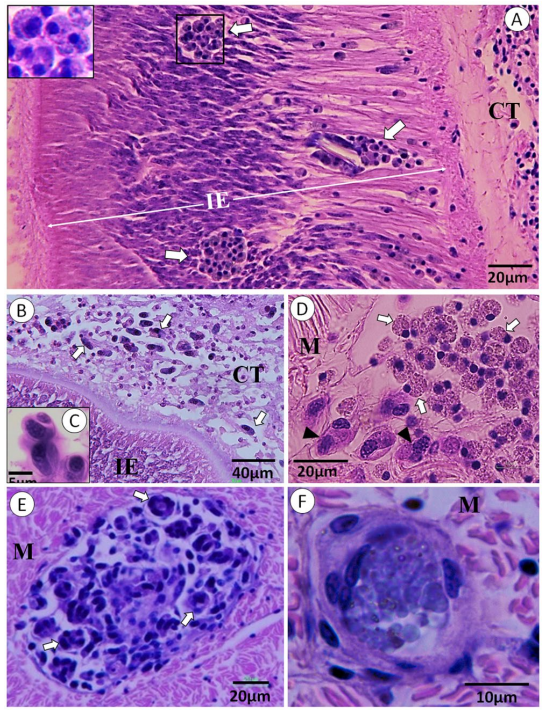

Figure 3. Histological sections of Iceland scallop infected with *Merocystis kathae*. (**A**) Histological section through the intestinal epithelium (IE) of Iceland scallop showing clusters of sporoblasts (arrows) of *M. kathae* entering the connective tissue (CT) adjacent to the epithelial lining. Inserted picture: Higher magnification of the sporoblasts. (**B**) Numerous sporozoites (arrows) which have entered the connective tissue between the intestinal epithelium (IE) and the digestive gland of Iceland scallop. (**C**) High magnification of two sporozoites inside haemocytes. (**D**) Cluster of sporoblasts (white arrows) and sporozoites (black arrowheads) in the adductor muscle of Iceland scallop. (**E**) Sporulated sporocysts (arrows) in the adductor muscle of Iceland scallop. (**F**) Initiation of merogony; young trophozoite developing inside an adductor muscle cell. All sections are stained with H&E.

Proposed life cycle of *Merocystis kathae*. The life cycle of *Merocystis kathae* is proposed in Fig. 6. In brief, whelks acquire infections via the gastrointestinal tract. The apicomplexan merozoites migrate to the kidney where gamogony is initiated by active invasion of the parasite into renal cells. Micro- and macrogamonts develop, leading to fertilization and the formation of a zygote. Subsequently, the sporogonic phase is initiated when the zygote nucleus divides followed by a series of further nuclear divisions, the end product being an oocyst with numerous sporocysts, each containing two sporozoites. In scallops, the transmission of *M. kathae* occurs via the gastrointestinal route; i.e. an active invasion, by either mature sporozoites or immature ones (sporoblasts), into connective tissues through the gastrointestinal epithelium. The final location for the parasites are muscular tissues where merogony takes place. Two generations of merozoites are produced; the latter one being infective to whelks.

Phylogeny of *Merocystis kathae*. SSU rDNA sequences obtained from infected scallops have been confirmed as identical to DNA sequences obtained from whelks infected with *Merocystis kathae*. Phylogenetic analyses consistently and robustly place *M. kathae* in a clade with other members of the Aggregatidae (Fig. 7). This aggregatid clade is weakly, but consistently, associated with a sister clade containing *Filipodium phascolosomae* and *Platyproteum vivax* (Archigregarinorida (Squirmida)) from sipunculids. Other sequenced scallop-infecting apicomplexans, *Pseudoklossia pectinis* and *Margolisiella islandica* do not form part of this clade, but form a group of apicomplexans that infect marine bivalves and polychaetes (Rhytidocystidae). This entire group forms a weakly-supported clade of apicomplexans that are found in marine invertebrates (Fig. 7).

Discussion

Merocystis kathae is the first apicomplexan parasite known to require two mollusc hosts to complete its life cycle, the common whelk and pectinid bivalves (scallops). It is the type species for the genus and was originally described by Dakin in 1911[9], from the renal organ of common whelks from Port-Erin, Isle of Man.

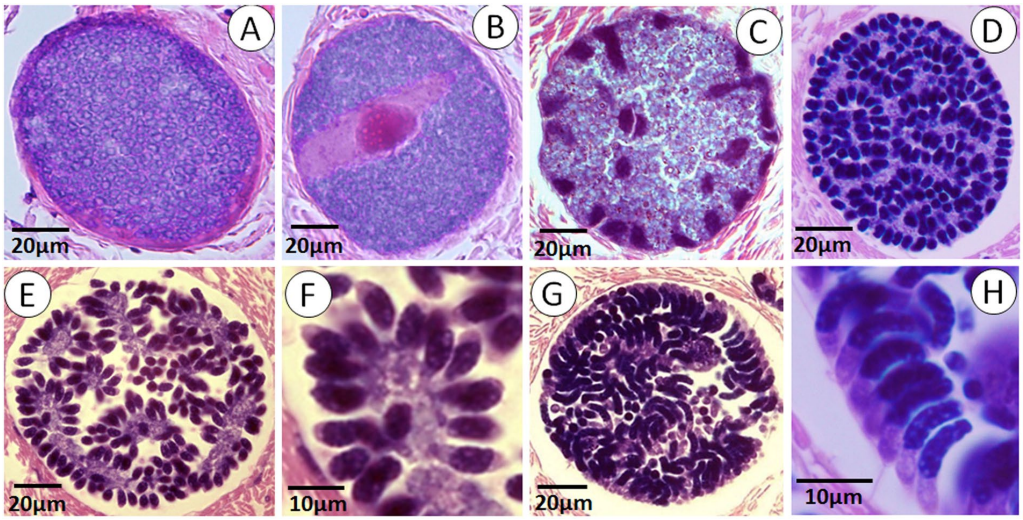

Figure 4. Merogonic stages of *Merocystis kathae* in the adductor muscle of Iceland scallop. (**A**) Premature meront. (**B**) A meront with a spindle-like apparatus indicating the initiation of nuclear cleavage. (**C** and **D**) Further development of a meronts characterized by further divisions. (**E**–**H**) Two different types of meronts representing two generation of merozoites. (**E**) Mature type I meront with numerous merozoites arranged in a rosette like fashion. (**F**) Higher magnification of merozoites from image (**E**). (**G**) Mature type II meront with numerous merozoites, more convex and slender in appearance and differently arranged compared to type I meronts. (**H**) Higher magnification of merozoites from image (**G**). All sections are stained with H&E.

Dakin[9] placed *M. kathae* within the Family Polysporocystidae but later Foulon[10], who described all life stages of the parasite in whelks in details, transferred it to the Family Aggregatidae and suggested it should be moved to the genus *Aggregata*. Patten[11], who studied the seasonal life cycle of the parasite supported the suggestion that *M. kathae* should be placed within the Aggregatidae, but believed it was different enough from *Aggregata* species to retain the genus *Merocystis*. In these original descriptions[9–11], only gamogonic and sporogonic stages were observed and it was hypothesised that merogonic stages must exist in an unknown host. Patten[11] pointed out the similarity of the life histories of *M. kathae* and *Aggregata* species and the possibility that the missing host could be a crustacean, as was already known for *Aggregata eberthi*[12,13].

Species within the Family Aggregatidae, are defined as parasites infecting marine invertebrates, especially molluscs and annelids, having all conventional apicomplexan life stages, mostly heteroxenous with merogony in one host, gamogony and sporogony in another and typically having oocysts with numerous sporocysts[14]. Therefore, with regard to morphology and life cycle, *M. kathae* conforms well to the family description. Currently the most speciose genus of this family is *Aggregata* with more than 20 nominal species, all of which are found in cephalopods, where the gamogonic and sporogonic stages are found[15]. In cases where the life cycle has been resolved, the merogonic stages have been observed in crustaceans, from which the cephalopods acquire infections by consuming infected individuals[12,14]. Therefore, the definitive hosts for both *Aggregata* spp. and *M. kathae* are molluscs. However, merogony in *M. kathae* occurs in pectinid bivalves not crustaceans as in *Aggregata* spp. Not much is known for species of other genera presently classified within the family Aggregatidae, except that the sexual development occurs in various marine invertebrates while the asexual ones are presumed to occur in unknown hosts[16,17]. The genera *Pseudoklossia* (Aggregatidae) and *Margolisiella* (Eimeriidae) are the exception for this including a number of known species, some recently described[18]. The genus *Margolisiella* was created to accommodate *Pseudoklossia* species known to be monoxenous and hence placed in the Family Eimeriidae. The remaining and expected heteroxenous species were left within the genus[19] and retained in the Family Aggregatidae. The current classification of the genus *Pseudoklossia* within this family is questionable as many, and possibly all species, are monoxenous, and our current phylogenetic analyses support this theory placing the genera *Pseudoklossia* with *Margolisiella* away from the true Aggregatidae.

The phylogenetic placement of *M. kathae* in a clade with *Aggregata* spp. is fully resolved and creates a monophyletic grouping for the family, assuming *Pseudoklossia* species are not aggregatids. This is further supported by comparable studies of life cycles and developmental stages in other members of the family. However, support for the larger clade of apicomplexans infecting marine invertebrates (Marine Invertebrate Clade) is poor, yet consistent. These poor support values increase significantly, if the analyses are run without the Archigregarinorida (Squirmida) taxa (data not shown). However, when included, these taxa are consistently grouped within the Marine Invertebrate Clade as a sister to the Aggregatidae. *Filipodium phascolosomae* and *Platyproteum vivax* were both considered to be marine archigregarines in the family Selenidiidae[20], but have been recently reclassified in a new taxonomic order, the Squirmida[21]. In previous phylogenetic analyses[20] these taxa were found to be related to

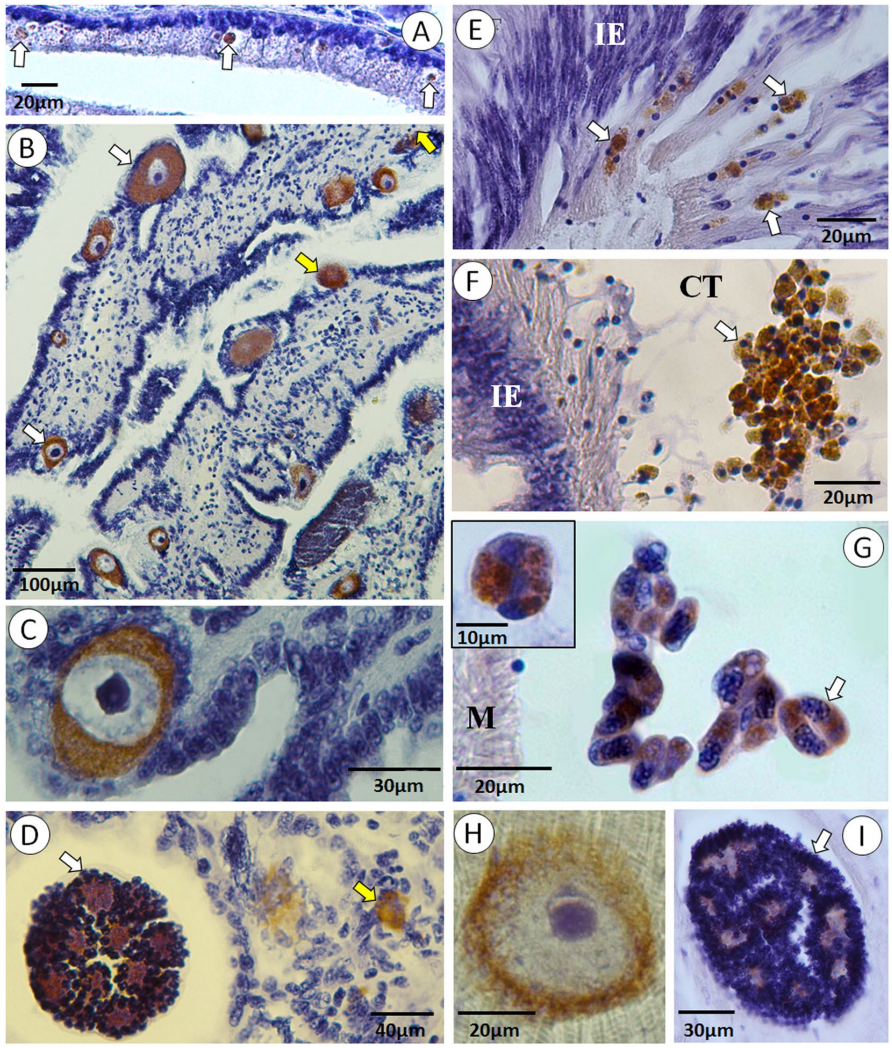

Figure 5. *In situ* hybridization of *Merocystis kathae* from whelks and scallops. Developmental stages in the common whelk (**A**–**D**) and the Iceland scallop (**E**–**I**). (**A**) A positive reaction to *M. kathae* in the intestinal epithelium of a whelk (white arrows). (**B**) Kidney section showing numerous developing gamonts (white arrows) and trophozoites (yellow arrows). (**C**) Higher magnification of a mature macrogamont. (**D**) Mature microgamont (white arrow) and a trophozoite (yellow arrow) inside renal cell. (**E**) A section through the intestinal epithelium (IE) of an Iceland scallop showing *M. kathae* sporoblasts (white arrows) entering the host. (**F**) A cluster of sporoblasts in the connective tissue (CT) adjacent to the intestinal epithelium (IE) of an Iceland scallop. (**G**) A group of sporozoites in the scallop's adductor muscle (**M**). Insert shows a sporocyst with two sporozoites. (**H**) Developing meront in muscular tissue of a scallop with a large nucleus and a prominent nucleolus. (**I**) A mature meront with numerous merozoites in the adductor muscle of Iceland scallop.

the archigregarines, whilst later ones[21] found no clear phylogenetic placement within the Apicomplexa, rather a weak association with the Dinozoa.

In the current study, we find a consistent phylogenetic placement of *F. phascolosomae* and *P. vivax* in the marine invertebrate clade, with no reliable association with the Dinophyceae or the perkinsids as suggested by Cavalier-Smith[21]. Currently little sequence data are available for this diverse group of apicomplexans infecting marine invertebrates and their relationship with other basal apicomplexans such as the marine archigregarines, will without doubt become more apparent when more data is available for this very under-sampled group of apicomplexans.

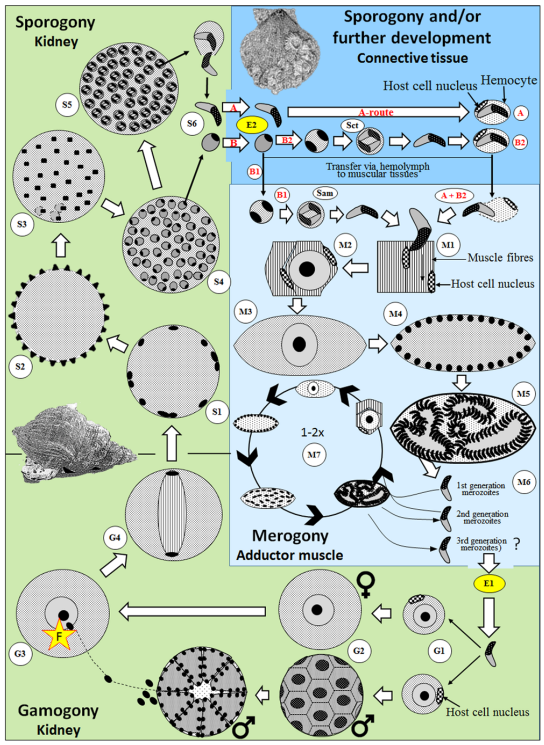

Figure 6. Schematic drawing of the proposed life cycle of *Merocystis kathae*. Merozoites invade the whelk through the intestinal tract (E1) and migrate to the kidney where they infect renal cells and gamogony **(G)** starts (G1). Some develop into macrogamonts (♀) while others become microgamonts (♂) (G2). The gamonts mature, eventually leading to fertilization (F) (G3) and the formation of a zygote which starts nuclear division (G4) initiating the sporogony process **(S)**. Subsequent recurrent nuclear cleavage occurs at the periphery of the zygote (S1) resulting in a cyst with regularly arranged nuclei at the periphery (S2). With further development the nuclei migrate into the cyst and start forming uninucleate sporoblasts, each containing cytoplasm (S3 and S4). The sporoblasts divide to form an oocyst with numerous sporocysts, each containing two sporozoites (S5). Sporogonic stages are released from the common whelk, either in the form of mature sporozoites (route A) or sporoblasts (route B), and enter the Iceland scallop via the gastrointestinal tract to invade the host via the intestinal epithelium (E2) and into adjacent connective tissues. For route A, the sporozoites are transmitted via haemolymph, commonly inside haemocytes, to muscular tissues. For route B, the sporoblasts are either transmitted directly to muscular tissues (Sam) where they sporulate (B1) or they sporulate in the connective tissues (Sct) surrounding the gastrointestinal tract prior to transportation to muscular tissues (B2). The merogonic phase (M) starts when the sporozoites invade muscle cells (M1). The muscle cells become hypertrophied as the pre-meront increases in size (M2), eventually leading to rupture of the muscle cell (M3). Further development of the meront is characterized by recurrent nuclei cleavage resulting in a multi-nucleated cyst (M4) which forms into a mature meront containing numerous merozoites (M5). Free merozoites, which are released from the meronts (M6), then infect new muscle cells starting a new merogonic cycle in the scallop's muscle. After the formation of 2–3 generations of merozoites (M7), the last generation merozoites infect the whelk (E1) where the gamogonic phase starts again (G1).

An apicomplexan life cycle involving two different mollusc species is previously unreported, which may reflect the limited examination of invertebrate apicomplexans rather than it being an unusual occurrence. The present knowledge of the geographical distribution of *M. kathae* in the definitive host is also poorly understood, mostly due to limited research on parasites of the common whelk and related species. To date, this parasite has been reported from the Irish Sea[9–11], the Belgian part of the North Sea[22], in Øresund and Gullmarfjord in Danish- and Swedish waters[23], and now Iceland in the present study. This novel discovery of the conspecificity of *M. kathae* and the SAP has extended the known distribution of this parasite. The presence of *M. kathae* in the intermediate scallop host, was first reported in 2011, from three different scallop species, *Chlamys islandica*, *Aequipecten opercularis* (queen scallop) and *Pecten maximus* (king scallop), in Icelandic-, Faroese- and UK waters, respectively[7]. Recently, it was also reported from *Placopecten magellanicus* (sea scallop), on the western side of the Atlantic; off the east coast of the USA and Canada[8]. All the species found infected with *M. kathae* were collected within the

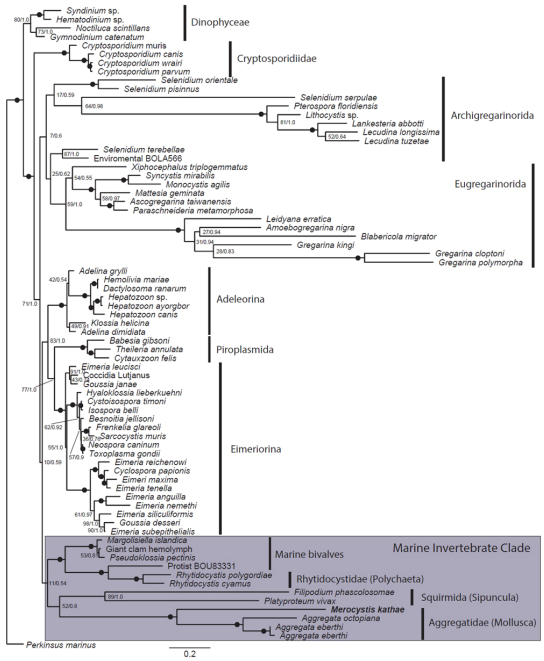

Figure 7. Phylogenetic tree. Maximum Likelihood (ML) topology of alveolate taxa focused on apicomplexans and rooted to *Perkinsus marinus*. The phylogenetic tree was inferred using the GTR + G + I model of substitution on an alignment of 74 small subunit (SSU) rDNA sequences and 1,916 sites. Numbers at the branches denote ML bootstrap percentages and Bayesian posterior probabilities; black dots on branches denote bootstrap and Bayesian posterior probabilities support of 95% and higher. *Merocystis* is fully supported in a clade with taxa from the genus *Aggregata*, in the marine invertebrate clade.

known distribution of the common whelk. In fact the distribution of these bivalve species is almost identical to that of the common whelk (Supplementary Information Fig. S1). The presence of whelks in more than 75% of tows during scallop fisheries also confirms the coexistence of these two mollusc species[24].

Our present knowledge of host specificity of *M. kathae* is limited. However, the fact that it was not found in other non-pectinid bivalve species examined from same sites as infected whelks, suggests that it is not a generalist parasite infecting many unrelated bivalve species. It however seems quite possible that other species of the genus *Buccinum*, which includes close to 70 species[25], could serve as hosts. The host range with regard to intermediate hosts, seems limited to pectinid bivalves. The two *Aggregata* species with known life cycles, seem more specific with respect to their cephalopod definitive hosts than their intermediate hosts which include various crustacean species of different families[26,27]. Therefore, it might be reasonable to think the same might be the case for *M. kathae*, being perhaps limited to certain whelk species but numerous pectinid hosts.

The transmission of *M. kathae* occurs via the gastrointestinal route in both the definitive and intermediate hosts. As unselective filter feeders, scallops consume a range of particle sizes from their surroundings by movement of ciliated cells in the gills; particles become entangled in mucus and are subsequently transferred along rejection tracts to the mouth palps, where they enter the digestive tract[28].

M. kathae follows a seasonal pattern in whelks[11], with the earliest developmental stages appearing between March and June while the first mature spores form in January and become increasingly common up to May. Consequently, the scallops are most extensively exposed to infective spores in late winter and spring. During an epizootic in the Iceland scallop population in Bay Breidafjördur in Iceland in the 2000s, the scallops caught in the spring were significantly more infected with SAP and associated macroscopic signs, than those caught in autumn[5]. The energy demanding maturation process, being close to spawning at that time of year, was suggested to make the scallops more vulnerable to infections. Although a plausible factor of influence, the extensive influx of infective sporozoites into their surroundings during this time must also play a major role. Whelks are known to be predatory, with scallops forming a regular part of their diet; they are also scavengers, feeding on moribund and dead animals[24,29]. Thus, during such mass mortality events[5], the availability of dead or moribund scallops would be plentiful, resulting in whelks intensifying their infection. Subsequently, substantial amounts of infective sporozoites are released into the surroundings which infect the remaining naive filter feeding scallops. The very high prevalence of *M. kathae* in both whelks and Iceland scallops, with many heavily infected, reflects this situation.

All available studies indicate that *M. kathae* does not negatively impact the whelk definitive host[9–11], as they are in good condition and the histopathological effect of the parasite is minor, even in extreme infections the, limited

to hypertrophy of infected cells. However, there is no doubt that *M. kathae* is a serious pathogen of scallops, playing a major role in the sudden 90% decline in populations of Iceland scallop in Breidafjördur Iceland, severely affecting the queen scallop population around the Faroe Islands[5,7] and a suspected cause of other mass mortality of sea scallops on the East coast of N-America associated with a condition called "grey meat"[8]. In addition, it has likely played a role in other unresolved mass mortality events and abnormal condition in various other Iceland scallop population, e.g. other scallop populations in Icelandic water[5,30–32], but also in Norway[33], Jan Mayen, Svalbard[34,35], Greenland[36], Russian Barents Sea[37] and North shore of Quebec eastern Canada[38]. Furthermore, the abnormal condition of adductor muscles, similar to the one caused by *M. kathae*, observed in the weathervane scallop, *Patinopecten caurinus* in the Alaska Bay NE-Pacific Ocean[39,40]. This condition is associated with an apicomplexan infection, which according to Inglis *et al*.[8] is likely to be *M. kathae*.

Although *M. kathae* has been shown to severely affect scallops, it appears that it only occurs when infections reach a high intensity, as low-level infections exist in scallop populations under normal conditions. After an almost complete collapse, the scallop population in Iceland has been slowly recovering and macroscopic disease signs are rarely detected and muscle and gonad condition appear normal. However, low-level infections still remain in high prevalence in the stock[5]. Similarly, highly prevalent but low level infections of *M. kathae* were observed in both king and queen scallops from UK waters but no abnormal clinical signs were reported[7]. This might suggest that the scallops' immune system can to some extent suppress light infections.

Historical data on stock indices and mass mortality events in scallop populations are generally poorly documented and in most the aforementioned cases, the causes cannot be verified. However, considering the wide distribution of *M. kathae* and the fact that almost all these events occurred within the known distribution of the common whelk (Supplementary Information Fig. S1), it seems plausible that it was a major factor influencing these events. The only exception is the apicomplexan found in the weathervane scallop in Alaskan waters associated with the "weak meat" phenomenon[39,40]. Whether that apicomplexan is *M. kathae* is presently unknown but the definitive host would then most likely be a different whelk species, such as the sinuous whelk *Buccinum plectrum*. Although scarce, some reports exist from scientists and fishermen showing that mass mortality events in some scallop populations are cyclical. That is the case for sea scallops on the East side of North America associated where mass mortality events associated with a condition termed "grey meat" have periodically occurred since 1936[8]. Furthermore, some indications of such periodic events exist in Icelandic waters[5]. As *M. kathae* has been reported in relation with such events in both these scallop populations, it seems possible that epizootics occur regularly as a consequence of a host-parasite relationship, a phenomenon widely recognized in nature and in many ways comparable to prey – predator systems. In both cases, severe fluctuations occur in the population size of both groups[41].

The main commercial fishing grounds for both scallops and whelks in Iceland is Breidafjördur. Until the collapse of the scallop population, commercial fisheries of that species had been reliably conducted since 1969. Whelk fisheries have a much shorter history, starting with some experimental collections in 1996 and have been quite intermittent to the present with no harvesting during some years[42]. Conversely, whelk fisheries are among the most important shellfish fisheries in the UK, dating back to the early 1900s[43]. As low-level infections do not appear to have a negative impact on the scallops, it should be possible to lower the infectious load with reasonable fisheries from both the whelk and scallop stocks. This would minimize the chance of epizootics caused be *M. kathae*, and create an optimal host - parasite equilibrium. The present study showed that infections were almost absent in king scallops from a "whelk free" area. Furthermore, only light infections were reported in both king and queen scallops from other UK locations, collected in 2007[7]. The extensive fisheries for whelks in the UK might help to explain this phenomenon.

Materials and Methods

Research material. In order to maximize the likelihood of detecting all life stages present in both host species, whelks and scallops were collected at different times during the years 2006–2016. Forty individuals of each species from different sampling times, showing gross signs of apicomplexan infections, were selected for examination. Samples were acquired by dredging in Bay Breidafjördur off the Wwest coast of Iceland from research expeditions performed by the Marine Research Institute in Iceland or from commercial whelk fisheries companies. All whelks and scallops were brought live to the laboratory and immediately dissected and examined for macroscopic/clinical signs of infections. Subsequently, the presence of *Merocystis kathae* and SAP was confirmed the by microscopic examination, for both whelks and scallops. All infected tissues were then subjected to conventional histological examination, while *in situ* hybridization (ISH) and molecular analyses were applied to the 10 most heavily infected individuals from each host species.

To check whether *M. kathae* and SAP could be generalist parasites, i.e. infecting a wide range of different host, five other mollusc species were collected from areas known to be endemic for SAP in scallops and *Merocystis kathae* in whelks, were examined for the presence of the apicomplexans. These were: rejected neptunes (*Neptunea despecta*, Gastropoda) (N = 10), dog whelks (*Nucella lapillus*, Gastropoda) (N = 10), blue mussels (*Mytilus edulis*; Bivalvia) (N = 30), northern horse mussels (*Modiolus modiolus*; Bivalvia) (N = 10) and the ocean quahogs (*Arctica islandica*; Bivalvia) (N = 30).

To further support the role of whelks in the life cycle of SAP/*M. kathae*, king scallops (*Pecten maximus*), were collected from a scallop ranching facility on the North West coast of Scotland (N = 20). Whelks are rare in the vicinity of the scallop beds and the farmers regularly remove the few individuals present during observational scuba diving inspections. All major organs of the scallops were subjected to a conventional histological examination. Furthermore, to assist with the phylogenetic placement of aggregatids from bivalves, samples of *Pseudoklossia pectinis* were taken from these scallops, the parasite identified from the kidney microscopically and samples taken for molecular analyses.

Molecular work. Kidney samples from 10 whelks, 4 king scallops, and adductor muscle samples from 10 Iceland scallops were sampled directly into a lysis buffer for genomic DNA extraction using a GeneMATRIX kit (EURx Poland) following the tissue protocol. Apicomplexan small subunit ribosomal DNA (SSU rDNA) was amplified from the parasite using the primers and PCR conditions as previously described by Kristmundsson *et al.*[5]. In addition, the primer pairs 18e/SC2–1370r 5' tccttcatatgtctggcactag 3′ and SFC-1120f 5′gaacgaaagttrg-gggmtcg3′/18 gM[44] were used following the same PCR protocol. PCR conditions were as previously described but used an annealing temperature of 64 °C with an extension time of 30 s. PCR bands of the expected sizes were recovered from the PCR products using a GeneMATRIX PCR extraction kit (EURx Poland). All PCR reactions were performed in triplicate. Sequencing reactions were performed using BigDyeTM Terminator Cycle Sequencing chemistry utilising the same oligonucleotide primers that were used for the original PCRs. DNA sequencing was performed in both forward and reverse directions for all PCR products and nucleotide BLAST searches performed for each sequence to confirm an apicomplexan origin. The contiguous sequences were obtained manually using CLUSTAL_X and BioEdit[45].

Histology and *in situ* hybridization. Tissue samples from all Iceland and king scallops (N = 60) and whelks (N = 40) were fixed for 48 h in Davidson's fixative and subsequently dehydrated through a series of ethanol and processed according to routine histological protocols.

For conventional histological examination, 3 μm thick sections were stained with haematoxylin & eosin (HE) and thoroughly screened for all developmental stages of the apicomplexans.

The ISH methodology, which was applied to 10 selected sections of each infected mollusc host species, roughly followed the procedure of Morris *et al.*[46] and Holzer *et al.*[47], with modifications which consisted of lower concentration of proteinase K. Histological sections, 7 μm thick, were hydrated and permeabilized with 10 μm/mL proteinase K in Tris-buffered saline (TBS) pH 8 for 12 minutes at 37 °C followed by a 2 × 5 min washing in PBS. Samples were then post-fixed in 0.4% paraformaldehyde in PBS for 15 min and subsequently washed for 2 × 5 min in distilled water. In order to prevent non-specific binding, sections were exposed to 10% hydrogen methanol (H_2O_2) for 10 min and then washed in distilled water for 2 × 5 min. Following that, the sections were dried in 45 °C for 10–12 min to be able to omit the time-consuming pre-hybridization step. Samples were enclosed with Frame-Seal^{TM} (Bio-Rad, Sundbyberg, Sweden) chambers and equilibrated in hybridization buffer consisting of 100 μg ml^{-1} calf-thymus DNA, 1.5 ng ml^{-1} of each of two 5′ biotin labelled oligonucleotide probes and 4x saline-sodium citrate buffer (SSC) in TBS containing 0.5% Ficoll, 0.5% polyvinylpyrrolidone, 0.5% bovine serum albumin. The following two probes were used: 790r 5′ ACACSCTTGAAGCACCCTAC 3′ and SC2-1370r 5′ TCCTTCATATGTCTGGCACTAG 3′ (5). The sections were sealed, denatured at 95 °C for 4 min followed by a 60 min hybridization at 45 °C. Hybridization was followed by non-stringent and stringent washes with 2x SSC and SSC with 0.1% Tween 20 at 42 °C, respectively. Signal detection was achieved using incubation with horseradish peroxidase-labelled streptavidin (Dako, Agilent Technologies, Glostrup, Denmark) for 20 min at room temperature followed by 3 × 5 min washing in PBS (pH 7.4) and visualized with a DAB Peroxidase Substrate (Vector Laboratories, Burlingame, USA). Haematoxylin was applied as a counterstain, after which sections were rapidly dehydrated in series of ethanol, transferred to xylol and mounted in resin based medium.

Data availability. All data needed to replicate the study are within the paper and its Supplementary Information file.

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Author Contributions

Á.K. and M.A.F. conceived and designed the study. Á.K. performed the histology and *in situ* hybridization. M.F. performed PCR, sequencing and phylogeny. Á.K. analyzed the life cycle and sketched the line drawing of the proposed life cycle. Á.K. drafted the manuscript and M.F. added data to it and improved it. Both authors read and approved the final manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-26158-1.

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Paper V – Supporting information

Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere

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Supplementary Information Fig. S1.

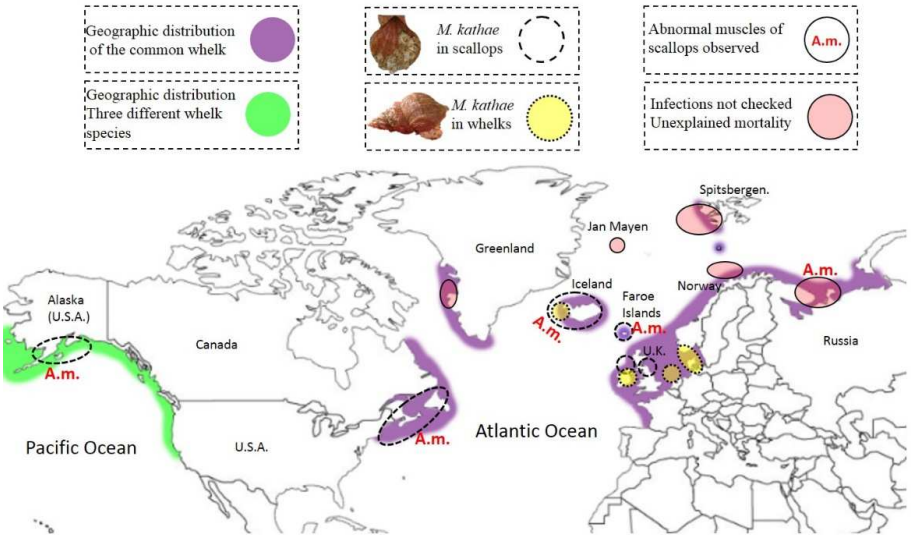

Fig. S1. Known distribution of *M. kathae* and whelks, and sites were abnormal condition of scallop populations have been experienced.
Known sites where abnormal mortality in scallops have been observed, clinical signs of disease similar to those caused by *M. kathae* and sites where the apicomplexan has been observed in scallops and whelks. Furthermore, it shows areas where unexplained mass mortalities of scallops have been observed and no examination with regard to infectious diseases performed. The shaded areas represents the geographical distribution of two different species of whelks, i.e. the common whelk, *Buccinum undatum*, and the sinuous whelk species, *B. plectrum*, a possible host for *M. kathae*.

Erratum list

Erratum list

Paper I

Árni Kristmundsson, Sigurður Helgason, Slavko Helgi Bambir, Matthías Eydal & Mark Andrew Freeman (2011). *Margolisiella islandica* sp. nov. (Apicomplexa: Eimeridae) infecting Iceland scallop *Chlamys islandica* (Müller, 1776) in Icelandic waters. *Journal of Invertebrate Pathology* 108: 139-146. DOI.org/10.1016/j.jip.2011.08.001

Page 139 (Introduction; 2nd paragraph)
Is: *Protothaca staminae*
Corrected: *Protothaca staminea*

Page 144 (Results; Table 1)
Is: *Protothaca staminae*
Corrected: *Protothaca staminea*

Page 145 (Discussion; 2nd paragraph)
Is: „Apart from the present paper and the one of Kristmundsson et al., 2011, the only paper…. sea scallops *Plagopecten magellanicus* and Iceland scallops occurring in Canadian waters“
Corrected: „Apart from the present paper and the one of Kristmundsson et al. (2011), the only paper…. sea scallops *Placopecten magellanicus* and Iceland scallops occurring in Canadian waters“

Paper II

Árni Kristmundsson, Sigurður Helgason, Slavko Helgi Bambir, Matthías Eydal & Mark Andrew Freeman (2011). Previously unknown apicomplexan species infecting Iceland scallop, *Chlamys islandica* (Müller, 1776), queen scallop, *Aequipecten opercularis* L., and king scallop, *Pecten maximus* L. *Journal of Invertebrate Pathology* 108: 147–155. DOI.org/10.1016/j.jip.2011.08.003

Page 147, Title
Is: *Aequipecten opercularis* L., and king scallop, *Pecten maximus* L.
Corrected: *Aequipecten opercularis* (Linnaeus, 1758), and king scallop, *Pecten maximus* (Linnaeus, 1758)

Pages 147-148 (Introduction; 3rd paragraph)
Is: *Protothaca staminae*
Corrected: *Protothaca staminea*

Paper III

Árni Kristmundsson, Ásthildur Erlingsdóttir & Mark Andrew Freeman (2015). Is an apicomplexan parasite responsible for the collapse of the Iceland scallop (*Chlamys islandica*) stock? *PLOS ONE* 10(12): e0144685. DOI.org/10.1371/journal.pone.0144685

Page 1, Abstract
Is: „However, the parasite only impacts mature scallops where they cause severe macroscopic changes, characterized by an extensively diminished and abnormally coloured adductor muscle“
Corrected: However, the parasite only impacts mature scallops where it causes severe macroscopic changes, characterized by an extensively diminished and abnormally coloured adductor muscle

Pages 1 (Abstract), 2 (Introduction; 2nd and 4th paragraphs), 4 (Material and Methods; legend for Fig. 1), 13 (Discussion; 1st paragraph), 18 (Discussion; 1st and 2nd paragraphs), 19 (Discussion; 3rd paragraph)
Is: Breidafjordur
Corrected: Breidafjördur

Pages 2 (Introduction; 3rd paragraph), 15 (Discussion; 3rd paragraph) and 18 (Discussion; 2nd paragraph)
Is: Hvalfjordur
Corrected: Hvalfjördur

Page 13 (Discussion; 1st paragraph)
Is: (MRI, 2007)
Corrected: [10]

Paper IV

Susan D. Inglis, Árni Kristmundsson, Mark Andrew Freeman, Megan Levesque & Kevin Stokesbury (2016). Gray meat in the Atlantic sea scallop, *Placopecten magellanicus*, and the identification of a known pathogenic scallop apicomplexan. *Journal of Invertebrate Pathology* 141: 66-75. DOI.org/10.1016/j.jip.2016.10.008

Title
Is: Gray meat in the Atlantic sea scallop, *Placopecten magellanicus*, and the identification of a known pathogenic scallop apicomplexan.
Corrected: Gray meat in the Atlantic sea scallop, *Placopecten magellanicus* (Gmelin, 1791), and the identification of a known pathogenic scallop apicomplexan.

Page 70 (Results, 3.3. Histopathology; 2nd paragraph)
Is: "However, this condition was apparently unrelated to both to the extent .....detected in white, brown and gray meat scallops".
Corrected: "However, this condition was apparently unrelated to both the extent..... detected in white, brown and gray meat scallops".

Paper V

Árni Kristmundsson & Mark Andrew Freeman (2018). Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere. *Scientific Reports* 8:7865. DOI:10.1038/s41598-018-26158-1.

Page 9 (Materials and Methods; 1st paragraph)
Is: „Samples were acquired by dredging in Bay Breidafjördur off the Wwest coast of Iceland……..from commercial whelk fisheries companies“
Corrected: „Samples were acquired by dredging in Bay Breidafjördur off the west coast of Iceland……..from commercial whelk fisheries companies“