An investigation into the parasitic barnacle, *Anelasma squalicola*; prevalence, infection behaviour and effects on its host, *Etmopterus spina*x, in Lusterfjord, Norway.

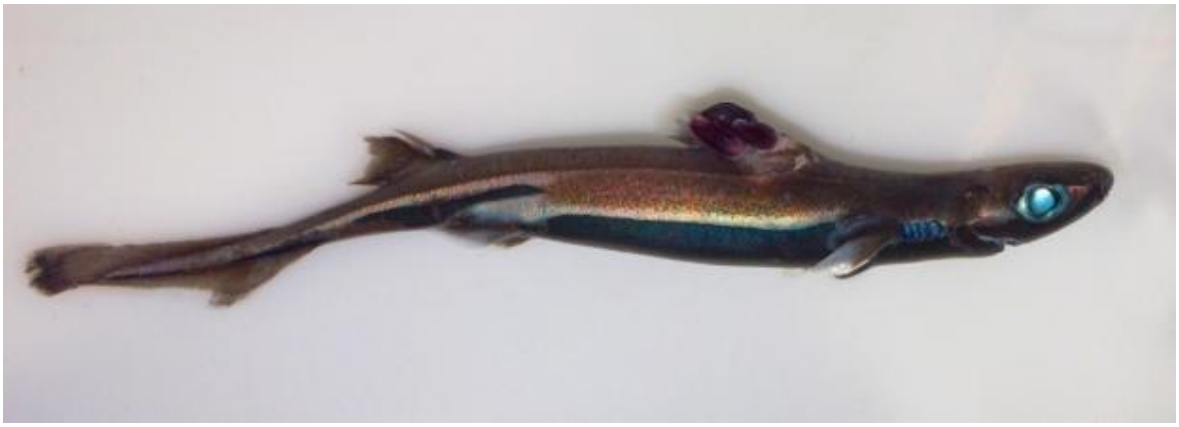

Photo by Rees et al., 2014

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Abstract

Anelasma squalicola is a recently evolved parasite. Very little is known of its biology due to its normally low prevalence in deep sea lantern sharks (family: Etmopteridae), in which it is found embedded in the skin.

A population of more heavily infected sharks (*Etmopterus spinax*) in Lusterfjord, Norway, has allowed for sampling by trawl, and subsequent observations and measurements of the parasite and host.

The study shows that *E. spinax* has a heterogeneous population structure and potentially narrow home range, which likely affects prevalence and dispersal of *A. squalicola.*

A. squalicola appears capable of infecting hosts regardless of size. It has high site specificity, which may be due to areas of the shark where the skin is easier to penetrate. It is most commonly found in pairs, which gives it an atypical intensity distribution.

The data suggests the first individual to settle can attract partners to the same site, but this attraction does not result in more infections at other sites on the host. Attracting more than one partner appears to severely reduce their fecundity, which may imply a crowding effect.

Infection did not affect liver mass or condition of hosts, but appears to prevent maturation in males.

This study provides the most extensive parasitological description of *A. squalicola* to date, and reveals both a complex host population as well as a highly distinctive infection behaviour, which combined shape this parasite-host interaction.

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1. Introduction

1.1. Description and history of *A. squalicola*

A. squalicola is a monophyletic, stalked barnacle found on certain deep sea sharks of the family Etmopteridae. It is found partially embedded in the tissue of the shark, into which it extends a system of rootlets from its peduncle; an organ normally used for attaching to the substratum.

The curious morphology of *A. squalicola* has puzzled scientists, including Charles Darwin (Darwin, 1851). Conventional barnacles are suspension feeders; they rely on filtering minute planktonic organisms. This has led to the evolution of cirri ('legs') with very fine setae (hairs) used to catch food. As follows, they also have a mouth and a digestive system used to masticate and process their food. In contrast to conventional barnacles, the filter-feeding organs, and indeed the general morphology of A. squalicola, do not appear to allow for suspension feeding: the cirri, which Darwin describes as "shapeless and rudimentary" (Darwin, 1851), are devoid of setae and appear unable to catch food items, and the mouth is reduced in size. How it obtains nutrients has therefore been a key question.

Despite the unusual morphology of *A. squalicola*, it has rarely been studied. The first person to write about it was the Norwegian naturalist Gunnerus (1758), who correctly identified it as a crustacean. However, his focus at the time was on the shark and not the attached barnacle, so no further effort was made to explore its biology. Because Gunnerus published his findings in a rather obscure journal, his work remained unnoticed for decades (Broch 1919). Nearly a century later, the Swedish zoologist Lovén (1844) identified the organism as a barnacle and

described it as *Alepas squalicola*. Later, working on his Monograph on Cirripedia, Darwin realised that Lovén had placed it in the wrong genus and re-described the species into a new monotypic genus, *A. squalicola* (Darwin 1851).

In his Mongraph on Cirripedia (Darwin, 1851), Darwin hypothesised that the rootlets are mainly a means of anchoring to the host, and concluded that *"[A. squalicola] can reach minute animals crawling by on the surface of the shark's body*". In fact, he quite extensively described A. squalicola as a predator, including how the mouthparts are "*beautifully adapted to catch and force down any small living creature into the muscular oesophagus".* Despite not finding any food present in the gut of the specimen, Darwin did not consider that this barnacle could have a completely different means of obtaining nutrients. In his defence, Darwin only had one specimen available to him, and at the time did not know of the existence of parasitic barnacles, which he was introduced to later through the work on rhizocephalan barnacles by German zoologist Müller (1862). If he had known of the root system in rhizocephalan barnacles, Darwin would probably have made the link between those roots and the rootlets in *A. squalicola*, and seen its potential as a parasite. Today, the rootlets are believed to have evolved in order to increase the surface area of the peduncle, which at a pivotal point in time evolved the ability to absorb nutrients from the shark's tissue.

Although there has been some uncertainty about the extent to which *A.* squalicola is a true parasite or not, with some describing it as a facultative "meso" parasite (Yano & Musick, 2000), a recent study by Ommundsen et al. (2016) strongly support the obligatory parasitic nature of the species. From their investigation they found that none of the inspected specimens had food in their guts; their mouthparts were highly reduced, with severe left-to-right asymmetry; and the cirri were highly

abnormal and lacking the setae necessary for filtering. The degenerate nature of the organs associated with filter-feeding thus suggests a lack of stabilising selection, implying that the precise morphology needed in order to filter feed is no longer of importance to the survival of *A. squalicola*. Isotopic δ^{13}Carbon and δ^{15}Nitrogen analyses further revealed that the trophic level was more similar to that of a parasite than a conventional barnacle (Ommundsen et al., 2016).

The continuing presence of the conventional filter-feeding traits alongside the rather limited parasitic root system, are difficult to explain unless viewed in the light of evolution. This overlapping presence of traits for two different modes of feeding suggests that the evolutionary transition to parasitism occurred very recently. This assumption is further backed up by the limited genetic differentiation between the Cytochrome Oxidase 1 genes of individuals from distant regions of the world (New Zealand, South Africa, the southern and western Atlantic and Sognefjorden) (Rees et al., 20014).

Phoresy, the act of hitching a ride on another organism, is thought to facilitate the evolution of parasitic relationships (Poulin, 2007). Phoresy is common within Cirripedia, with barnacles found on various marine mammals, such as sea cows (Manatees) and whales, as well as on reptiles like turtles or sea snakes. Given *A. squalicola*'s parasitic association with a vertebrate host, one might postulate that it came from a lineage of epibionts, especially on vertebrates, such as whale barnacles (Balanomorpha: Coronulidae). However, Darwin (1851) dismissed this relationship due to *A. squalicola*'s morphology, in particular the peduncle, which is not found in balanomorphs.

Recent phylogenetic work by Rees et al. (2014) revealed the evolutionary history of *A. squalicola*, placing it as a sister species of the East-Asian *Capitulum mitella* and a close relative of *Pollicipes*, which are both suspension feeders from the intertidal zone, and not related to epibionts. *C. mitella* is the last surviving member of a large group of Capitulum-like barnacles that had a large radiation roughly 100 mya ago, with members experimenting with a range of different substrates (Rees et al., 2004*).* The analysis further estimated the divergence between *C. mitella* and *A. squalicola* at roughly 120mya ago. This suggests that *A. squalicola* may be the only species left of what was once a more speciose clade of suspension feeding barnacles that were capable of utilising a wide range of substrates, from which it recently diverged into parasitism.

Despite many epibionts within the Thoracica, there is only one other type of parasite, the *Rhizolepas*, containing only two known species, which parasitizes polychaete hosts using a similar system of roots as *A. squalicola (*Rees et al., 2014). All other parasitic thoracican barnacles use their mouths to parasitise. In comparison with *A. squalicola*, Rhizolepas are much more profusely branched, and their obsolete filter-feeding morphology is even more vestigial. The rarity of parasitism in Thoracica highlights the uniqueness of the evolutionary journey of *A. squalicola*, as the only barnacle to have evolved a parasitic peduncle capable of infecting a vertebrate host.

We will likely never get the full picture of how an intertidal barnacle found its way to parasitizing deep sea sharks; however, A. squalicola does give us the rare chance of studying the first evolutionary steps of a parasite. Organisms undergoing such a drastic change in niche - where traits of both niches are still present - are not expected to remain in their morphological state of limbo for long, as vestigial traits

are costly to maintain and the morphology associated with the new niche is expected to evolve rapidly due to strong selection. As such, *A. squalicola* gives us a rare glimpse into a fleeting event in nature and the possibility of better understanding evolutionary transitions; particularly towards parasitism, which are exceedingly rare to observe in both the fossil record and in extant species.

1.2. Aims

For an understanding of the ongoing evolutionary process in *A. squalicola*, the animal's biology must be better understood. Very little is known of *A. squalicola* due to low prevalence, which has made strategic sampling difficult. Therefore, most collected specimens have been bycatch of studies on sharks. In one study (Hickling, 1963), it took piecing together of material from 4000 sharks collected over a time period of 17 years to obtain only 79 specimens.

During an exploration of the fauna in the Sognefjord, a minor fjord, the Lusterfjord, within the greater Sognefjord, yielded a prevalence in the population of velvet belly lanternshark, *Etmopterus spinax,* high enough for strategic sampling. It is mainly data gathered from Sognefjorden which forms the basis for this thesis. This sampling programme is part of the project "mapping and characterising benthic fauna communities and nature types in Sognefjorden – Norway's longest and deepest fjord" funded by the Norwegian Biodiversity Centre.

The theoretical, maximum geographical distribution of *A. squalicola* is the same as the distribution of all its hosts. *A. squalicola* has been reported on multiple species in the family Etmopteridae across the globe (Yano & Musick, 2000). Despite its cosmopolitan distribution, its prevalence remains low, except in certain areas such

as the Lusterfjord. The varying prevalence suggests certain conditions, abiotic or biotic, may provide better conditions for higher prevalence. The aim of this section is to describe and discuss the population structure of *E. spinax* which may explain the variation in prevalence and distribution.

A. squalicola has to successfully penetrate the skin of its host, which may differ in susceptibility on and between hosts. Because they are embedded in the body of the shark, there may be a varying amount of space and nutrition at different sites. They also have to make sure they settle near a partner in order to be capable of reproducing. The aim of this section is to describe and discuss the infection behaviour, and assess whether there are differences in variables such as size or fecundity between different sites and group sizes, as well as whether infection is limited to particular host qualities.

Barnacles show diverse patterns of sexuality (Darwin, 1851). Individuals of most species are simultaneous hermaphrodites, though under certain conditions other patterns have arisen, such as coexistence of males and females (dioecy), where male barnacles tend to be smaller ("dwarf males") and live on or inside the female (Yamaguchi et al., 2012). Group size is thought to be a main contributor to sexual strategy (Ghiselin, 1974; Yamaguchi et al., 2012). In highly gregarious species, reproduction is guaranteed and so *maximising* reproduction becomes more important, which has led to very long penises capable of fertilising multiple mates. If chances of reproduction are lower, such as in deep sea, solitary species where the likelihood of encountering a conspecific is low, separate sexes (dioecy) may evolve. As a descendent of a gregarious barnacle, *A. squalicola* has a very short penis unsuitable for mate competition. The aim of this section is to describe and

investigate the reproduction of *A. squalicola* given its new niche as a deep-sea parasite.

As parasites obtain nutrients from their host, and therefore exploit resources which the host could have used, we expect to see differences between infected and uninfected hosts. Differences could be qualitative, such as reduced growth or fecundity, or qualitative such as castration. The aim of this section is to test for differences in the condition, liver mass and maturity between infected and uninfected hosts.

Finally, given the seemingly vestigial morphology, one aim was to quantify the variation in different traits to investigate whether, or to which degree, they are under selective pressure. Based on the results, an appropriate trait was to be assigned as the measurement of size. Due to unforeseen problems with the data collection, the initial plan had to be discarded. The consequences of this is discussed.

The aims described above are by no means mutually exclusive, and the structure of the discussion will not necessarily reflect that of the introduction.

2. Methods

2.1. Species taxonomy

Anelasma *squalicola* (from ITIS.gov (2016))

Class	Maxillopoda
Subclass	Thecostraca
Infraclass	Cirripedia
Order	Pedunculata
Family	Anelasmatidae
Genus	*Anelasma*
Species	*Anelasma squalicola*

Etmopterus *spinax* (from ITIS.gov (2016))

Class	Chondrichthyes
Subclass	Elasmobranchii
Superorder	Euselachi
Order	Squaliformes
Family	Etmopteridae
Genus	*Etmopterus*
Species	*Etmopterus spinax*

2.2. Trawling

The trawling events took place at different locations in Sognefjorden between 2011 and 2015 (Figure 1). In addition, a separate trawling even took place in Masfjorden 2015, as part of the BIO310 Marine Methods course by the University of Bergen.

The stations sampled were chosen based on an attempt to map the fauna of different parts of Sognefjorden. After the discovery of *A. squalicola* in the Lusterfjord, additional sampling events took place with the aim of collecting as many specimens as possible.

In total, 950 specimens of *E. spinax* have been inspected from 12 different stations in the time period between 2011 an 2015. These have taken place during different times of the year and at different locations in Sognefjorden (Table 1).

153 specimens of *A. squalicola* were collected from the different stations (table 1). The specimens were only found inside Lusterfjorden, and only within the central part of the fjord, except for two individuals found on a shark in the innermost part of the fjord (figure 2).

The majority of specimens were dissected out, although some were kept in-situ. The samples were preserved in 4 % formalin and later transferred to 70 % ethanol for storage, except for some that were preserved only in ethanol for DNA analyses.

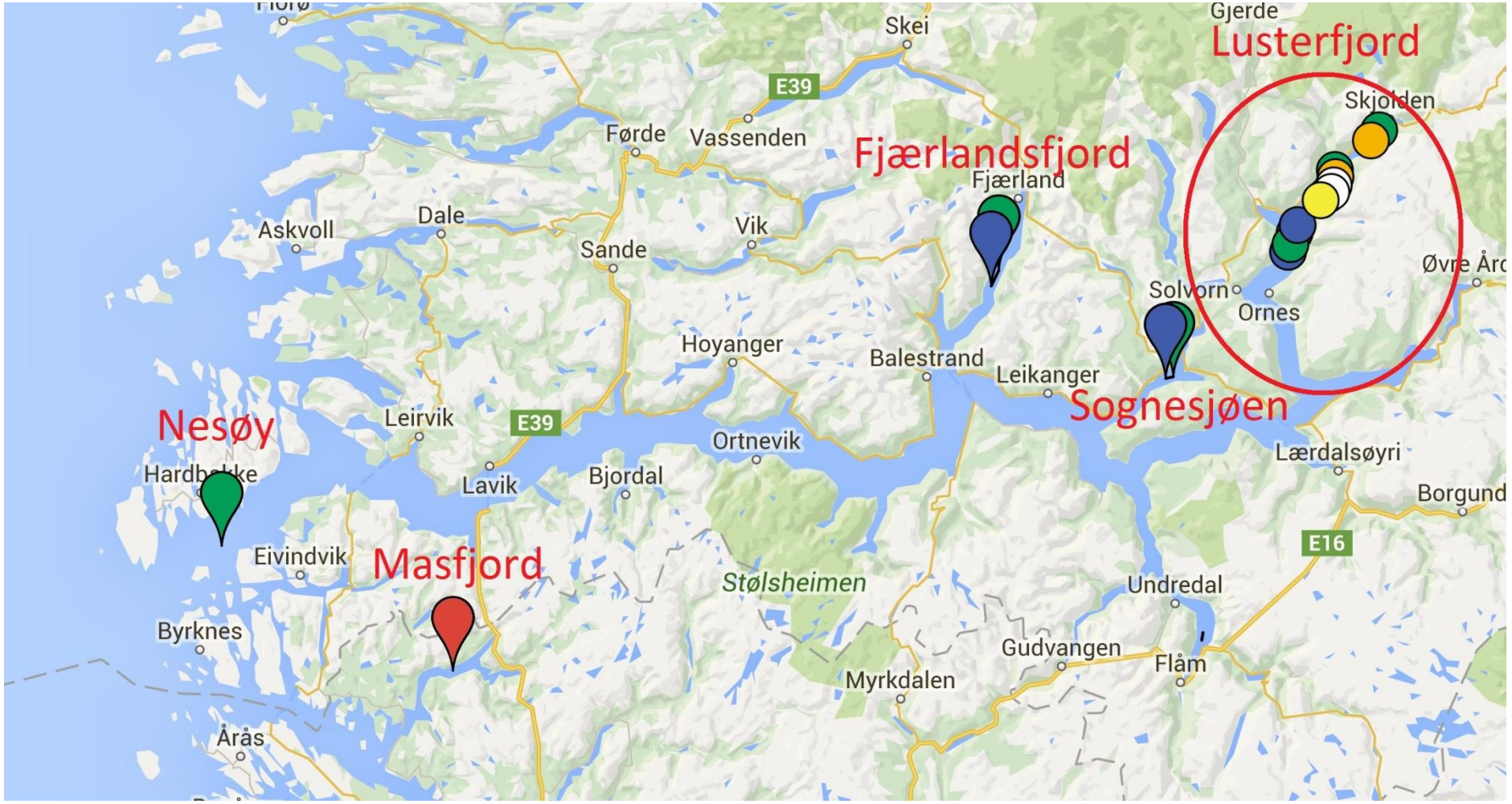

Figure 1: the different locations sampled. A. squalicola was only found within the lusterfjord (red circle). Map from Google maps.

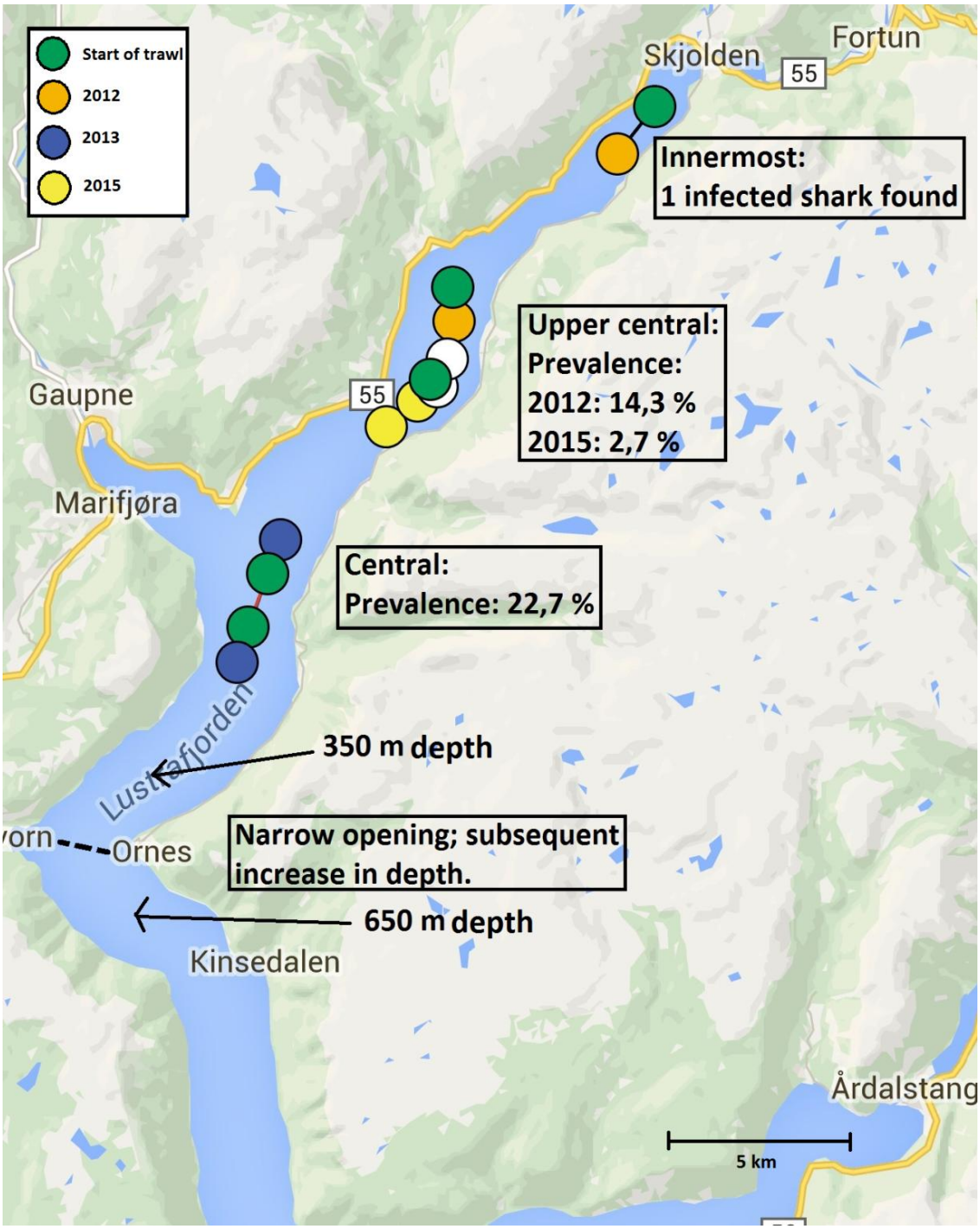

Figure 2: map of Lusterfjorden, with the different stations sampled. The colours indicate the different sampling events (2012, 2013 and 2015). The green colour indicates the starting point of the trawl. The prevalence was highest in the central part of the fjord, as well as at the upper central station, during 2012. At Ornes, there is a is a slightly narrow opening (still more than 1 km wide at the surface), where the depth increases from roughly 350 to 650 meters. A map showing the toplogy can be found in the appendix (figure 9.1), as well as a link to the interactive google map. Map from google maps.

Table 1: the stations, with information on location, time of year, year, depth, the number of sharks inspected and the number of specimens of *A. squalicola* obtained.

Station code	Station name	Location	Time	Mean Depth(m)	Sharks inspected	No. of specimens
2012-11-18RT	Innermost	Lusterfjord, Innermost	Nov, 12	115,5	72	2
2012-11-21RT	Upper Central 1	Nattropefjord	Nov, 12	340	140	44
2015a	Upper central 2	Nattropefjord	May, 15	340	69	5
2015b	Upper central 3	Nattropefjord	May, 15	340	146	5
2015c	Upper central 4	Nattropefjord	May, 15	340	41	1
2013-05-02RT	Central 1	Lusterfjorden	May, 13	375	94	55
2013-05-01RT	Central 2	Lusterfjorden	May, 13	373	47	34
2013-05-09RT	Sognesjøen	Sognesjøen	May, 13	259,5	89	0
2011-unknown	Nesøy	South of Nesøy	NA, 11	360	25	0
2013-05-10RT	Fjærland	Fjærlandsfjorden	May, 13	204,5	53	0
MAS15	Masfjord	Masfjorden	Sept, 15	410	121	0
HB – unknown	NA	NA	NA	NA	53	7
				Total	950	153

2.3. Observations and measurements

Note: because of previous handling of the parasite specimens, it was often not possible to obtain a measurement.

Parasite data

The site of *A. squalicola* on the host, the number of specimens sharing the same site ("cluster size") and the number of specimens in total on the host were recorded. Measurements (in millimetres) were taken of the total length, mantle length, peduncle length, thorax length and the width at the interface between the mantle and peduncle (referred to simply as 'width') using an electronic caliper (Cockraft Vernier Digital Caliper, accuracy: 0,03 mm.). Figure 1 illustrates the different measurements taken. The presence of eggs in the mantle cavity, or lack thereof, was noted as either present or absent.

Measurements (figure 3).

- **Total length** (blue line) was measured from the bottom of the peduncle (pd) to the top of the mantle (ma).
- **Mantle length** (blue line, from top of mantle to mantle-peduncle interface) was measured from the interface between mantle and peduncle (red line) to the top of the mantle.
- **Mantle-peduncle interface (width)** was measured at its slimmest/shortest distance at the junction between the mantle and peduncle (red line).
- **Thorax length** (white line) was measured from the space between the mouth and the first pair of cirri, to just behind the last pair of cirri, before the penis.
- **Peduncle length** (blue line, from bottom of peduncle to mantle-peduncle interface) was measured from the mantle-peduncle interface to the end of the peduncle.

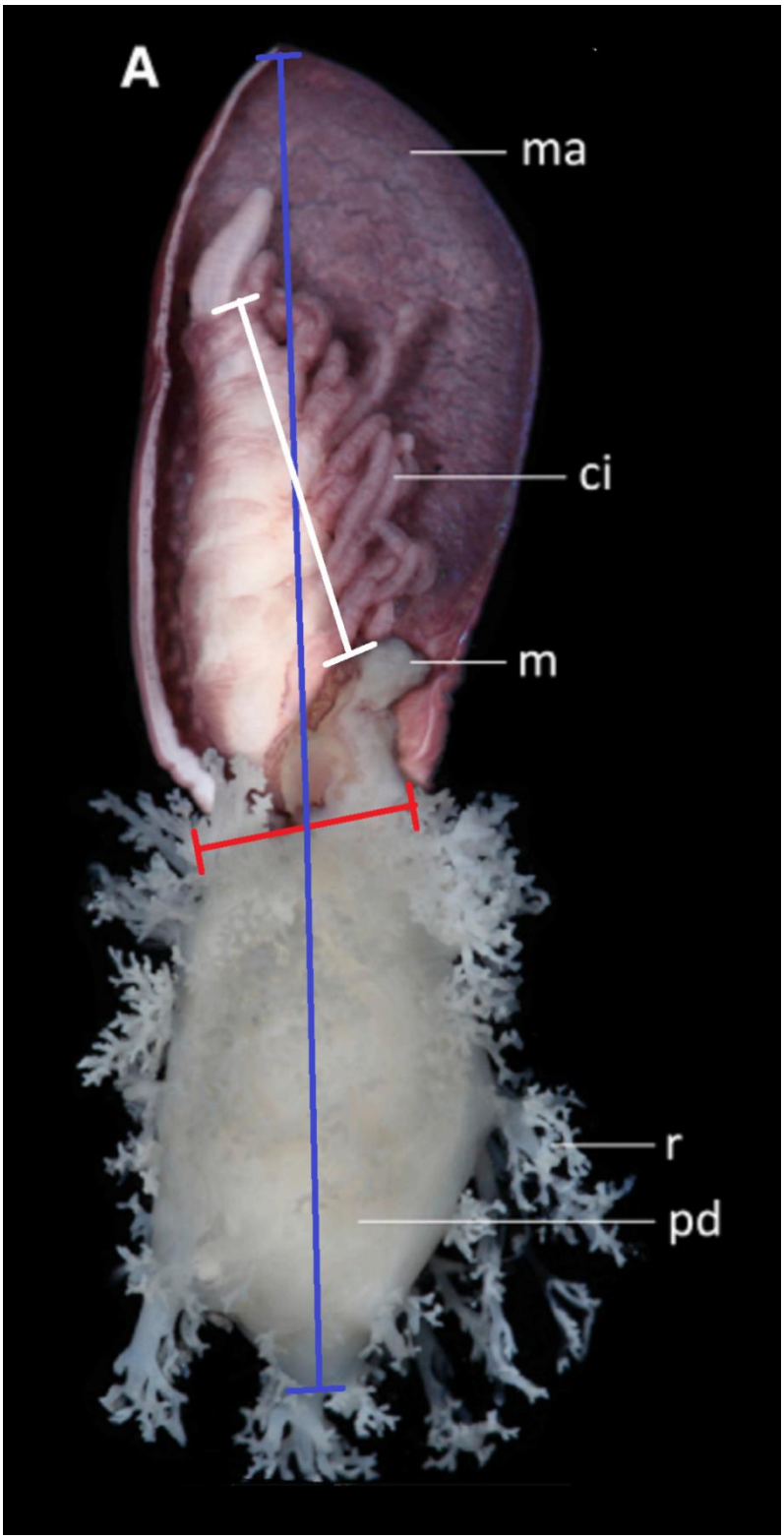

Figure 3: a specimen of *A. squalicola*, showing the different traits measured and the morphology. ma = mantle, ci = cirri, m= mouth, r = roots, pd= peduncle. Picture modified from Rees et al. 2014.

The intention was to assess the morphological variation and evaluate the strength of selection on different traits. Following this, we wanted to choose an appropriate trait to use as a measurement of size which was under selection, in order to be able to detect fitness differences between individuals due to competition and/or site specificity. This was to be assessed by performing model selection on the traits.

Unfortunately, the different traits were often not possible to measure, due to the condition of many of the specimens. Because of the many missing observations, models with multiple terms had very low sample sizes (sometimes only 20).

This approach was therefore abandoned and thorax length was chosen as the measurement of size, due to its high sample size and normal distribution, as well as the ease of measurement (the consequences of this choice are dealt with in the discussion).

Table 2: the different traits and the number of observations possible to extract from the 164 specimens collected.

Trait	Number of observations (max = 146)
Thorax length (mm)	114
Peduncle length (mm)	73
Mantle length (mm)	57
Peduncle-mantle width (mm)	55
Total length (mm)	53

Host data:

The expeditions varied in the data that was collected. Both parasitised and unparasitised sharks were measured. All expeditions recorded the length and sex of the sharks, however, weight was only measured for the Sognefjord 2013, 2015 and the Masfjord 2015 expeditions. The maximum total length of the sharks was measured using a 1 metre fish measuring board with 0,5 cm increments, with the shark positioned laterally. Weight was measured using an electronic scale (Sartorius TE612), to the nearest gram.

Liver weight and maturity were recorded during the Sognefjord 2013 expedition: the liver was dissected out and weighed on an electronic scale (Sartorius TE612), to the nearest gram. The Hepatosomatic index (HSI) was calculated based on the formula:

$$HSI = 10\ x\ \frac{Liver\ weight\ (g)}{Total\ weight\ (g)}$$

Maturity was assessed by inspection of the gonads, and a maturity stage was assigned based on the maturity scale developed for aplacental and placental viviparous sharks, by Stehmann (2002). To maximise sample size, maturity was later simplified to two categories: mature and immature.

The condition factor, K, developed by Fulton (1902), was calculated from the weight and length data for each shark, according to the formula:

$$K = 100\ x\ \frac{Weight\ (g)}{Length\ (cm)^3}$$

2.4. Statistical procedures:

All statistical analyses and production of figures were executed in R (R Development Core Team, 2016).

Because of the multitude of tests performed, a small introduction and description of the statistical procedure is given before every result in order to aid the reader, rather than presenting all the statistical procedures here.

To maximise sample size, separate models including only one term at a time were created to test for correlations between observations. The models were either simple linear regression models (lm, in R), Generalised Linear models (GLM) or Generalised Linear Mixed-Effect Models (GLMM). A binomial distribution was chosen when the response variable was binary/proportional, such as in the egg-presence analyses, otherwise, a Gaussian distribution was chosen for normally distributed data, such as in the thorax-size analyses. The R-output can be found in the appendix.

In order to control for variation between stations, where relevant, the analyses are restricted only to stations that can be treated as one. Furthermore, sharing the same host was included as a random term in relevant analyses to control for nested effects between hosts.

Finally, binomial tests were used to compare expected proportional observations with those observed.

Results

3.1 stations

Table 3: the stations and their prevalence, total number of specimens, mean and median intensity

Station code	Station name	Location	No. of sharks	No. of inf. sharks and prevalence	No. of specimens	Mean intensity	Median intensity
2012-11-18RT	Innermost	Lusterfjord, Innermost	72	1 - 1,4 %	2	2	2
2012-11-21RT	Upper Central 1	Nattropefjord	140	20 - 14,3 % CI [0.089, 0.212]	44	2,2	2
2015a	Upper central 2	Nattropefjord	69	3 – 4,3 %	5	1,67	2
2015b	Upper central 3	Nattropefjord	146	3 – 2,0 %	5	1,67	2
2015c	Upper central 4	Nattropefjord	41	1 – 2,4 %	1	1	1
2013-05-02RT	Central 1	Lusterfjorden	94	20 - 21,3 % CI [0.135, 0.309]	55	2,75	2
2013-05-01RT	Central 2	Lusterfjorden	47	12 - 25,5 % CI [0.139, 0.403]	34	2,83	2
2013-05-09RT	Sognesjøen	Sognesjøen	89	0	NA	NA	NA
2011-unkn.	Nesøy	South of Nesøy	25	0	NA	NA	NA
2013-05-10RT	Fjærland	Fjærlandsfjorden	53	0	NA	NA	NA
MAS15	Masfjord	Masfjorden	121	0	NA	NA	NA
HB (unknown)	NA	NA	53	NA	7	NA	NA

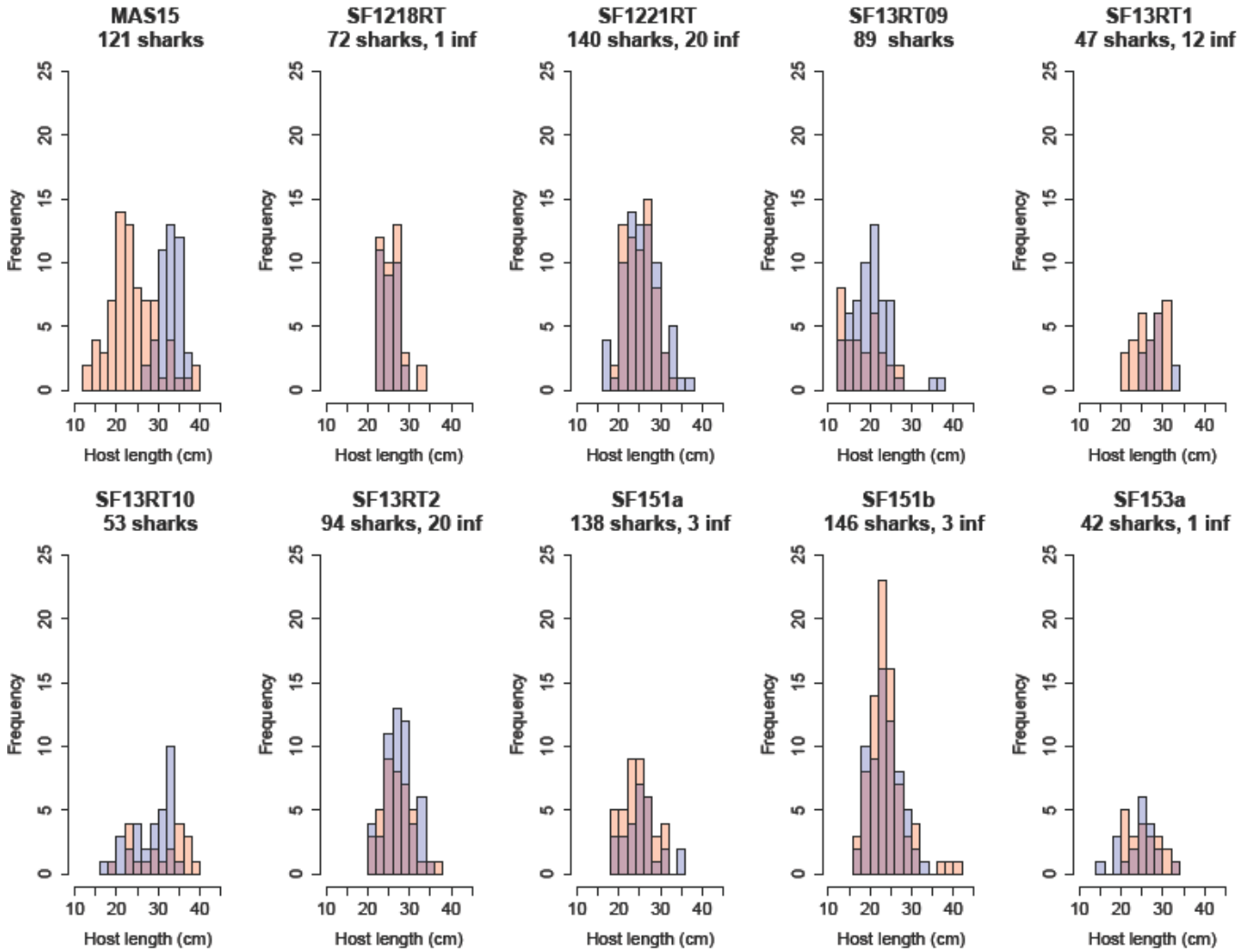

Figure 4: Overlapping female and male size histograms for each station. Orange colour bars are females, blue are male and purple bars indicate the overlap between them.

3.2. Parasite and host size distributions

Size measurements were obtained for 114 specimens of *A. squalicola*. Specimens ranged in size from 1,7 to 16 mm, with a mean size of 5,2 ± 1,9 SD.

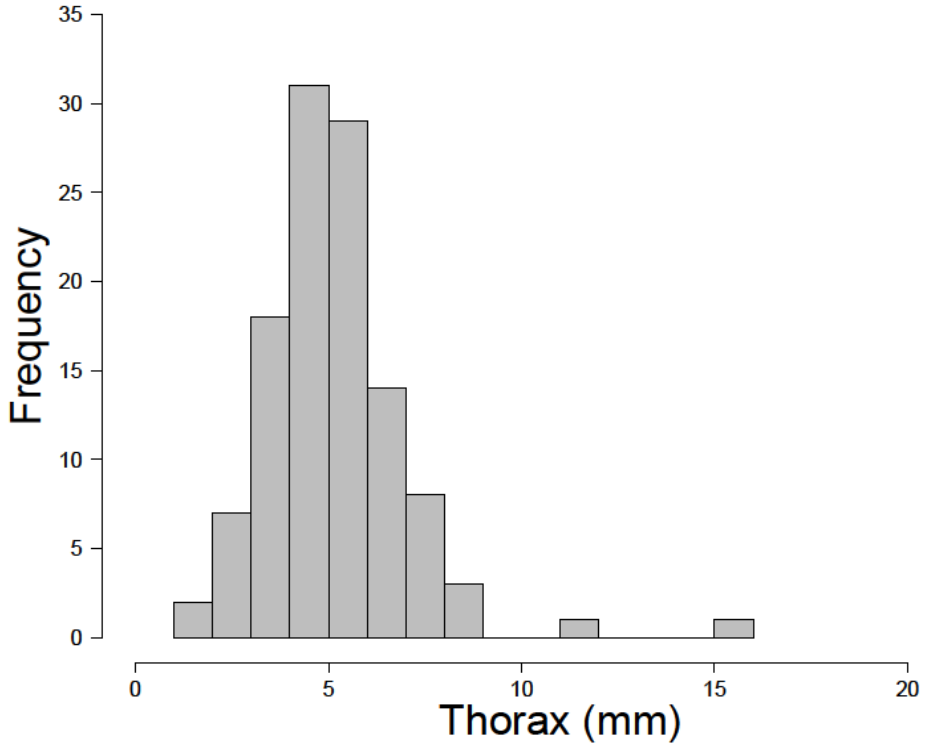

Figure 5: The frequency distribution of the size (thorax) of *A. squalicola*.

The maximum size range of *E. spinax* was 12 to 43 cm, with a mean size of 25,4 cm ± 5,2 SD. The host population where specimens of *A. squalicola* was found ranged in size from 13-41 cm, with a mean size of 25,5 cm ± 4,0 SD. Infected individuals ranged in size from 18 to 34 cm, with a mean size of 27,4 cm ± 3,3 SD. In addition, there were no differences in infection between the host sexes.

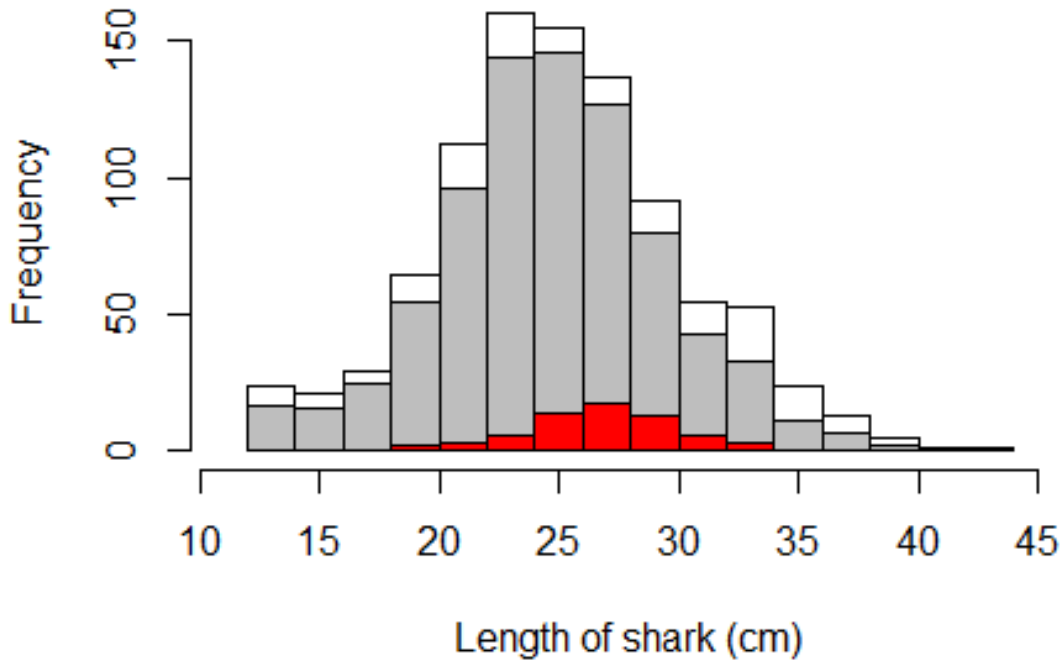

Figure 6: the size distribution all sharks (white), of sharks at stations with *A. squalicola* (grey) and sharks infected with *A. squalicola* (red).

3.3. Observations: intensity, site and egg presence[1]

A. squalicola is found embedded in the tissue of the shark, where they share the same lesion in the skin. The total number of specimens on the host, the number of individuals in each cluster, the site of the cluster on the host and the presence of eggs in their mantle cavity were recorded.

Intensity and cluster size

The number of parasites per host was recorded for 65 infected hosts. Hosts were found with one to eight parasites, although two parasites per host is the most common occurrence.

Table 4: Intensity distribution of *A. squalicola*

Total number of parasites on host	**1**	**2**	**3**	**4**	**5**	**8**	**Total**
Number of infections	11	33	11	4	5	1	65

1: Some presentations of the data may be confusing to the reader, as values do not always add up; this is due to missing specimens or data points.

Cluster size, defined as the number of individuals sharing the same site, was recorded for 91 parasites from 40 different clusters. Some sharks were found with more than one cluster of parasites, although the occurrence of an additional cluster is rare: out of 65 hosts, only 7 (10%) had more than one cluster and only one host had three clusters. This latter host was unique in having a total of 8 parasites. 11 instances were found where a shark had only a single parasite, and two parasites per cluster is by far the most common occurrence.

The occurrence of two clusters of 4 and one cluster of 5 individuals warrants some caution, as it was not possible to discern whether these are made up of sub-groups (2+2, 2+3) or whether they are to be considered as whole units. Due to this ambiguity and the low sample sizes for cluster size 4 and 5, they have been excluded from subsequent analysis.

Table 5: the number of individuals in the different cluster sizes.

Cluster size	One	Two	Three	Four	Five	Total
No. of clusters	11	23	11	2	1	40

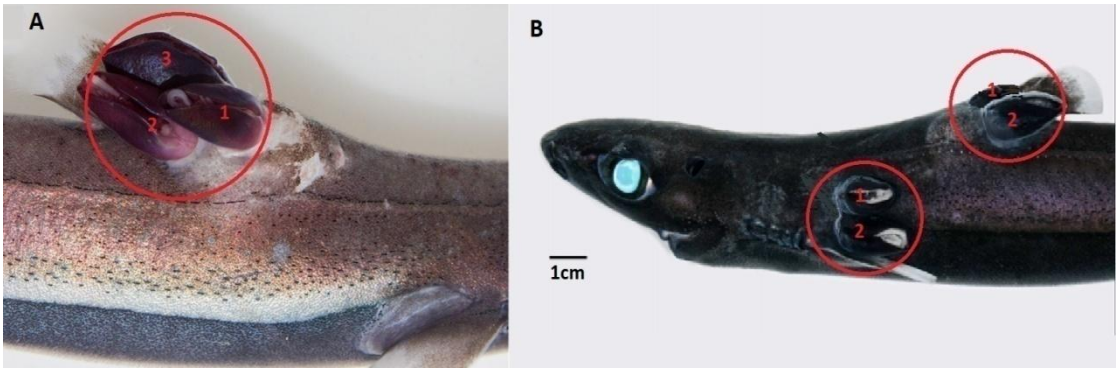

Figure 7: (A) A cluster (red circle) of three specimens, embedded near the dorsal fin. (B) Two separate clusters, one at the dorsal and one near the pectoral fin, with two specimens per cluster. Pictures modified from Rees et al., 2014.

Site on host

A. squalicola is found on specific sites on the host, although there are some outliers. The original sites were first and second dorsal, left and right pectoral, and a few instances such as the anal fin, mouth and eyes. These sites have been clustered into three categories; dorsal, pectoral and other. In total, site on host was obtained for 106 specimens. *A. squalicola* is most commonly found on the dorsal fins.

Table 6: frequency of the sites of specimens of *A. squalicola* on the host.

Position	Dorsal	Pectoral	Other	Total
No. specimens	63	28	15	106

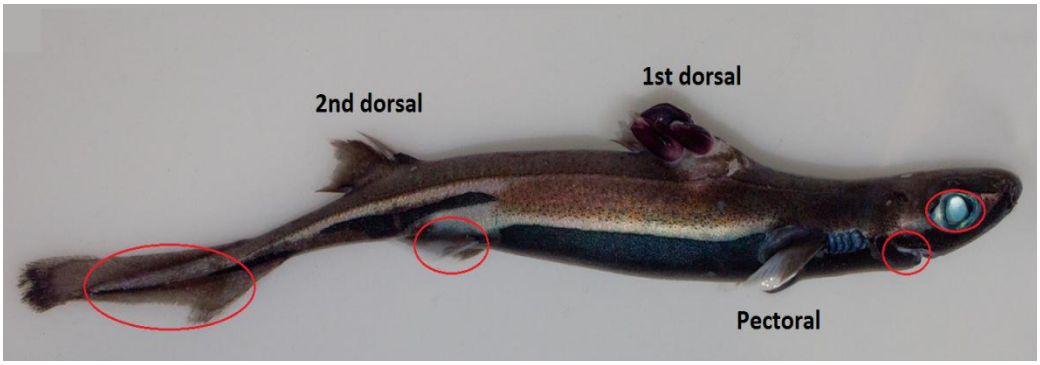

Figure 8: the most common sites were the 1st and 2nd dorsal fins, and the pectoral fins. There were instances of specimens found on the eyes, mouth, anal and caudal fins, grouped together as "Other" (red circles). Picture modified from Rees et al., 2014.

Egg presence

63 out of 103 (61,2%) specimens had eggs present in their mantle cavity, and egg-bearing specimens were found at all stations, both spring and autumn. Egg-bearing individuals ranged in size from 3,5 to 16 mm.

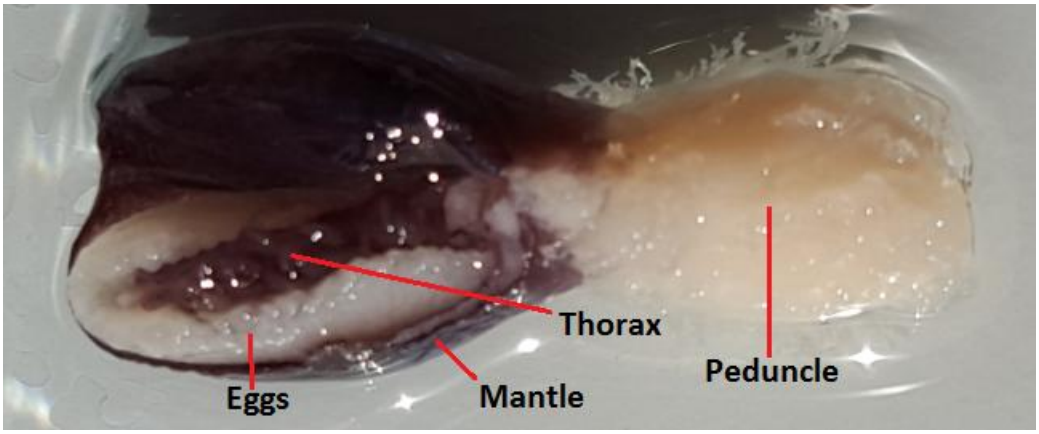

Figure 9: a dissected specimen with an egg sheath (white mass) surrounding the thorax in the mantle cavity. Picture by Lasse Eliassen.

3.4 Various Analyses

3.4.1 Is *A. squalicola* able to infect hosts regardless of host size?

As an ectoparasite which depends on penetrating the skin, hosts may be more susceptible to infection at an early age, when the skin may be less developed. If this is the case, we will not expect to see small parasites on larger hosts, as a small parasite is indicative of a recent infection.

The relationship between the size of the parasite and the length of the host was analysed using an ANOVA. No significant trend between the size of parasite and host was found for thorax ($F(1,69)=0,4314$, $p=0.5122$, $R^2=0$) . As can be seen, relatively small and large parasites are found on both small and large hosts, suggesting *A. squalicola* can infect hosts regardless of their size.

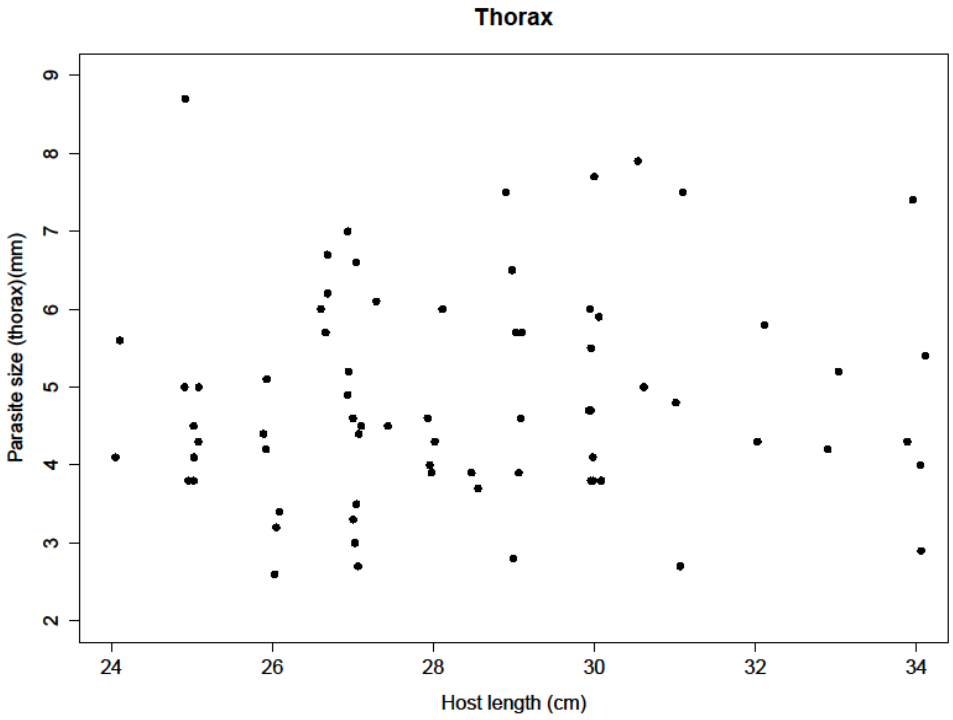

Figure 10: the relationship between the size of *A. squalicola* and its host. There is no clear correlation between size of host and parasite, suggesting infection is not limited to young hosts.

3.4.2. Are partners similar in size?

Choosing an appropriate site is of utmost importance to barnacles, as the choice is permanent. Feeding, space for growth and competition for mates are all governed by this initial choice of site. As a sessile, sexually reproducing organism, it is important to settle near a partner in order to ensure reproduction, otherwise their fitness will be null. Following this, the hypothesis is that cyprids likely settle on a host and signal for a partner. We therefore expect to see the individuals being more similar in size to their partner than they are to other individuals in the population.

Alternatively, we may also expect them to match the size of their partner as they grow, as their short penises may be unable to reach the mantle cavity of a partner, if very dissimilar in size.

The analysis is based on all paired specimens and their thorax size (26 specimens, 13 pairs), and compares the variation within the pairs to the variation between pairs (figure 11). A significant difference was found between within-pair variation and between-pair variation, meaning individuals are more similar to their partner than to the general population ($F(12, 13) = 2{,}633$, $p=0{,}04809$, $R^2 = 0{,}4395$).

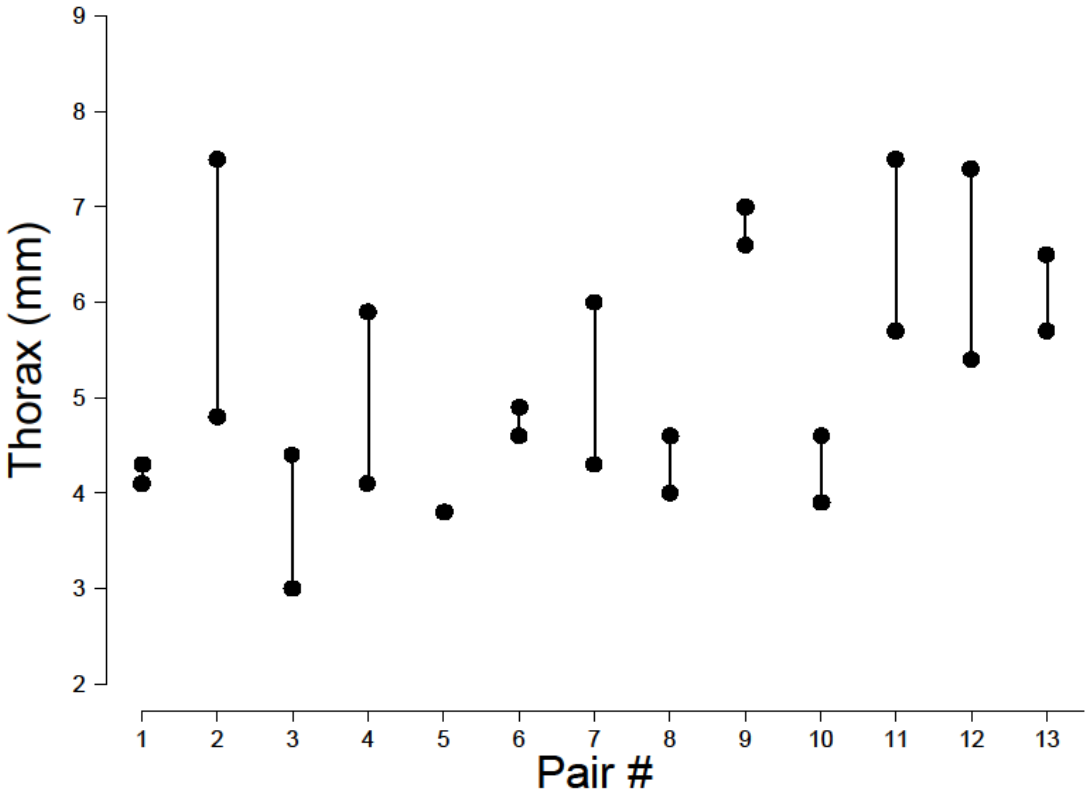

Figure 11: 12 different pairs, their size (dots) and the size difference between them (line).

3.4.3. Maturity in *A. squalicola*

A. squalicola carries its eggs inside the mantle cavity, where they form a semi-solid sheath around the thorax. Eggs were present both in spring and autumn expeditions,. There were 63 specimens with eggs, and 40 without. Lack of eggs does not exclude the individual from having previously had them.

As there is a difference in egg presence between cluster size 2 and 3 (see next analysis), the analysis is limited to 21 individuals known to be in pairs, with accompanying data on thorax size and egg presence. Due to the limited sample size, initial attempts at creating a model to predict egg presence given size was discarded, and the observations are simply presented as is.

Table 7: counts of eggs present/absent in different size classes from 2,7 to 7,7 mm, with 1 mm increments.

Eggs\ Size class (mm)	2,7-3,7	3,7-4,7	4,7-5,7	5,7-6,7	6,7-7,7	Total
Absent	2	3	1			6
Present		9	1	3	2	15
Grand Total	**2**	**12**	**2**	**3**	**2**	**21**

The majority of individuals in pairs had eggs (71,4%). Individuals without eggs were found in the size range 2,7 – 5,7 mm. Individuals with eggs were found in the 3,7 - 7,7 mm size range. The smallest individual with eggs was 3,8 mm. The majority of specimens in the 3,7 – 4,7 mm size class have eggs. This suggests the population likely reaches maturity around a size of 3,7-4,7 mm.

3.4.4. Which factors affect egg presence in *A. squalicola*?

The position of the parasite, sex of host, cluster size, host length and condition factor K were analysed using GLMMs to test for any patterns between them and egg presence. Sharing the same host was controlled for. A highly significant difference in egg presence was found between individuals in cluster sizes 2 and 3 (p=0,002). No other correlations were found, suggesting that the position on the host, the size of the host (length), the sex and condition are not correlated with egg presence (models and R-output in appendix).

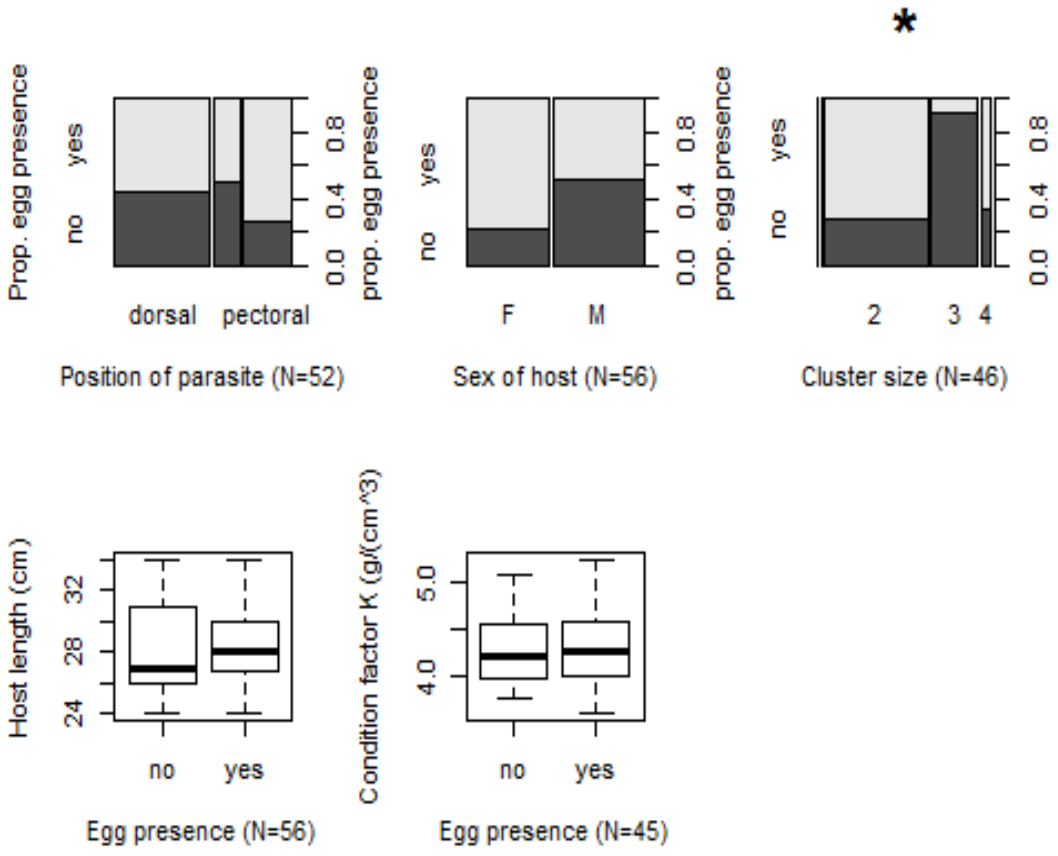

Figure 12: Egg presence given position, sex of host, cluster size, host length and host condition. A trend was found for cluster size, where specimens in pairs have a higher proportion of eggs than triplets, The X/Y-axes of condition and host length were inversed for plotting purposes. Asterix indicates significant difference.

3.4.5. Site specificity: does site affect size or fecundity of *A. squalicola*?

A. squalicola shows very high site specificity on the host, with a clear preference for the dorsal and pectoral fins. Hickling (1963) suggests that the preference for the dorsal fin is due to a small scar caused by the dorsal spine, which penetrates the skin *after* the shark is born. There is no such scar on the pectoral fin, which implies that a more universal explanation may be the need for thin or abnormal skin, through which the parasite can penetrate.

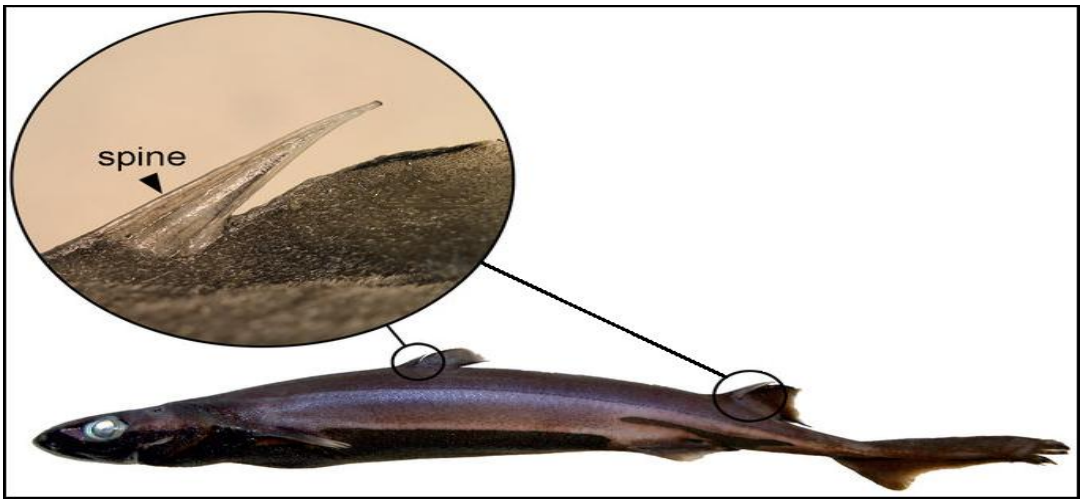

Figure 13: the dorsal spines protruding from the skin of *E. spinax*. Picture modified from Claes et al., 2013.

An alternative explanation is that there can be differences in the quality between sites. *A. squalicola* can become relatively large in comparison to its host (figure 14), and the choice of site may reflect a need for sufficient space in which to grow, or that some sites provide more nutrition due to their proximity to the central body.

Figure 14: three specimens on the dorsal fin. Picture from Rees et al., 20014.

If such limitations exist, we might expect different sites to yield individuals of different size, or different proportions of egg-laying individuals. However, no difference in size was found between sites (ANOVA: $F(2,64)=1.971$, $p=0.1477$)[2] (figure 15), or for the proportion of specimens carrying eggs (see GLMM in appendix).

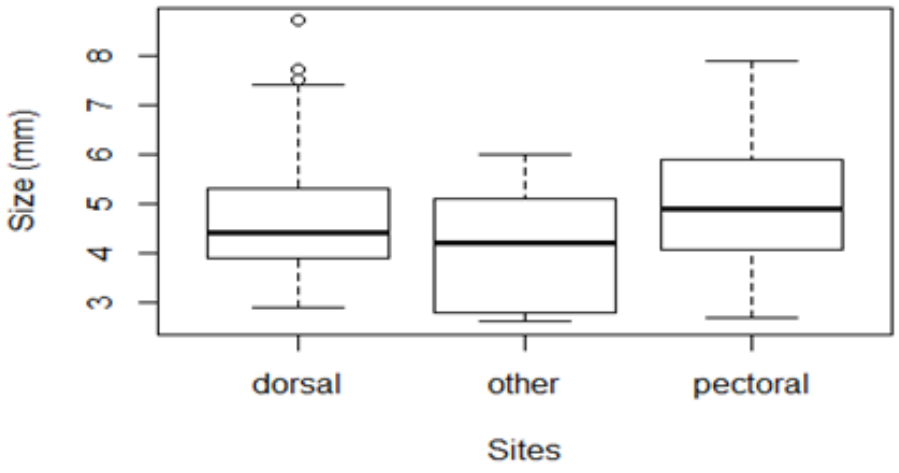

Figure 15: boxplot of the size of individuals at the different locations.

2. The ANOVA reported here has not controlled for sharing the same host. A more appropriate GLMM model has been created (see appendix), but this does not provide a p-value. Regardless, visual inspection suggests there is no considerable difference (figure 15).

3.4.6. Intraspecific competition in *A. squalicola*

As previously shown (figure 12), the number of parasites in a cluster may have an effect on egg presence, as specimens in pairs had eggs present more often than specimens in triplets.

However, no effect of cluster size, or the total number of individuals, was found on the size of *A. squalicola*, when controlling for nested effects of sharing the same host (see GLMM models in appendix). In the graph below, all available data has been included, however, the analyses were limited to the difference between cluster size 2 and 3, because of the low sample size and uncertainty of whether cluster size 4 and 5 in reality were separate clusters 2+2 and 2+3, they were not included in the analysis.

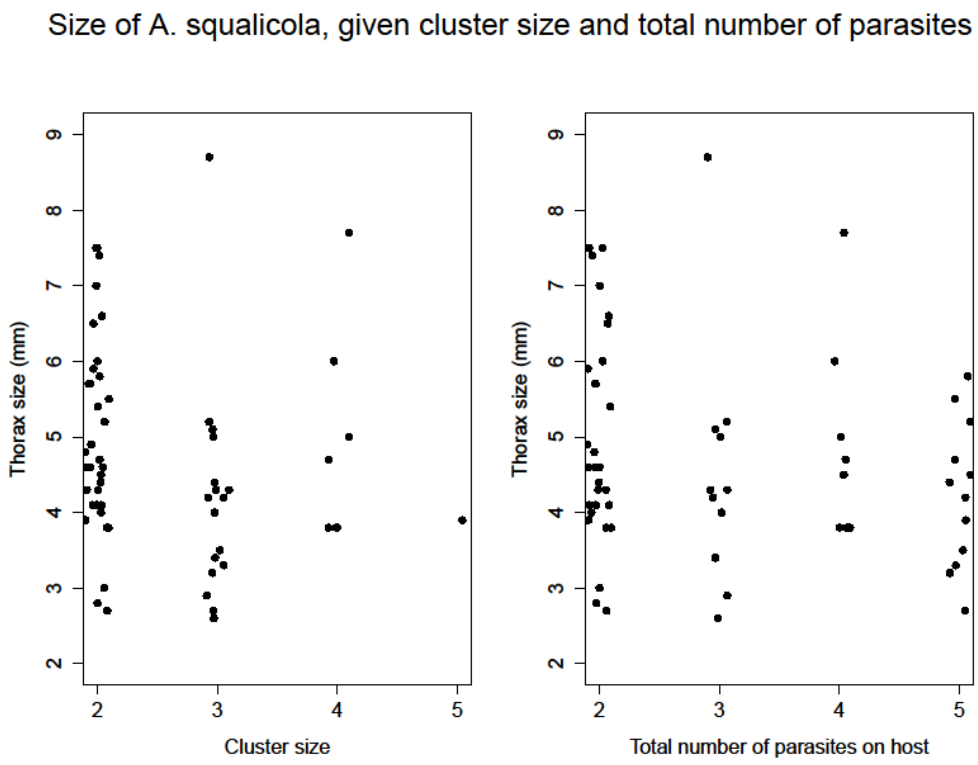

Figure 16: there is no difference in size between the different cluster sizes, or the number of parasites in total on the host.

3.5. Effects on host

3.5.1 Does infection with *A. squalicola* affect host susceptibility?

From parasitology we know that a small proportion of hosts normally carry the majority of parasites (Bush et al., 1997). This suggests that some hosts may be predisposed to infection, or that the presence of a parasite makes subsequent infections more likely.

A. squalicola are rarely found alone; usually they have a partner. As such, the presence of one parasite most definitely attracts more; however, these are attracted to the same site, right next to the pioneering parasite. This analysis does not look at the attraction of a partner into the same site, but rather the attraction of a secondary cluster, which may be located elsewhere on the shark.

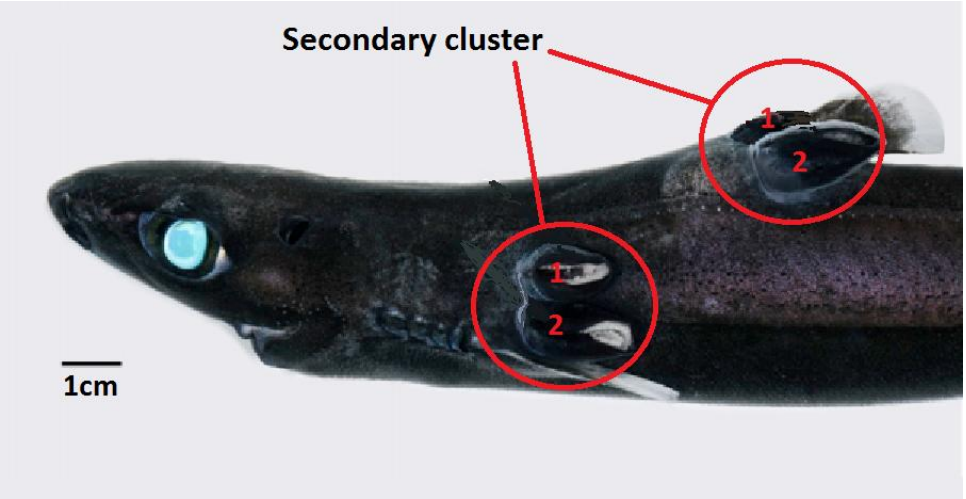

Figure 17: a host with two separate clusters, each with one pair. Picture from Rees et al., 20014.

The analysis is limited to the three stations, 2013-05-02RT, 2013-05-01RT and 2012-11-21RT, which have high sample size (281), high mean prevalence (18,5%) and close geographic proximity, and can therefore be treated as one.

If infection of a host by *A. squalicola* is random, and the probability of having *one* infection is p, then the probability of having a secondary infection is p^2. Based on infection rate in *A. squalicola*, this equates to a 0.185 probability of one infection, and a 0.034 probability of a secondary infection.

Being infected does not affect the probability of a secondary infection. 2,1% (6 individuals) had a secondary infection, and a binomial test indicate that the proportion of the population having a secondary infection is not significantly different to the expected proportion if infection is random, p=0,3216 (two-sided).

3.5.2. Hepatosomatic index

Liver weight was measured for 141 individuals during the 2013-05-RT01/012, of which 22,7 % (32) of the individuals were parasitised. Liver weight was divided by total weight to control for the size of the host (hepatosomatic Index (HIS)).

Infection of *A. squalicola* does not have an effect on the liver weight of *E. spinax* (ANOVA: F(1,137)=0.1542, p=0.6952)

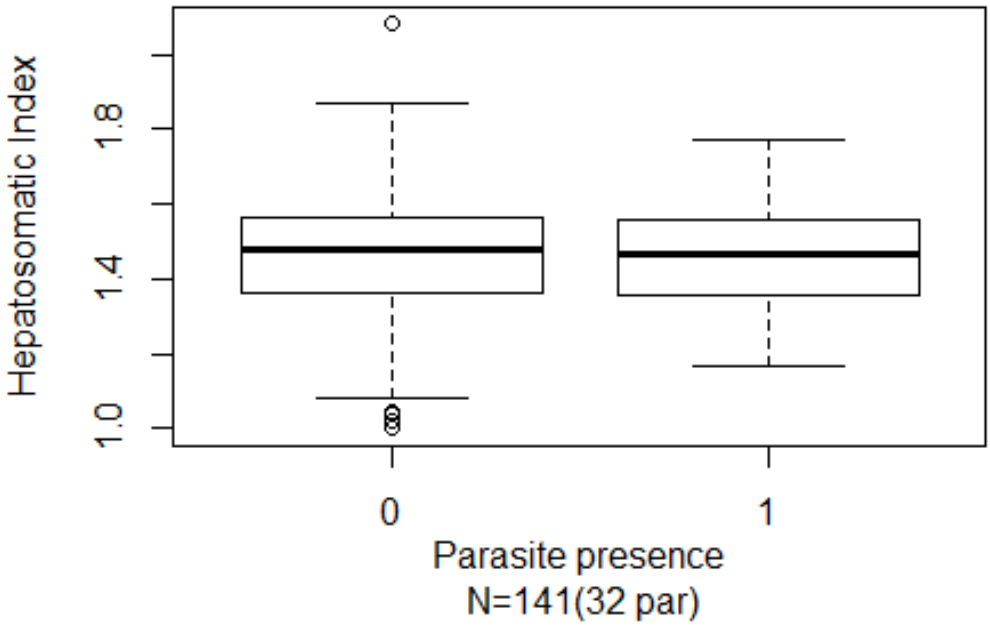

Figure 18: box plots showing the HSI for uninfected and infected individuals. 141 sharks were included in the analysis, of which 32 were parasitised.

3.5.3. Fulton's condition factor

The analysis is limited to the two central stations (2013-05-1/2RT) in order to control for temporal or spatial variation in condition. 139 sharks, where 32 were infected, were included in the analysis.

There was no significant difference in the condition factor K between infected and uninfected sharks (ANOVA: F(1,137)=2,995, p=0,086), in fact, the data suggests parasitised individuals may have a higher condition factor, although the difference is small regardless of potential trend.

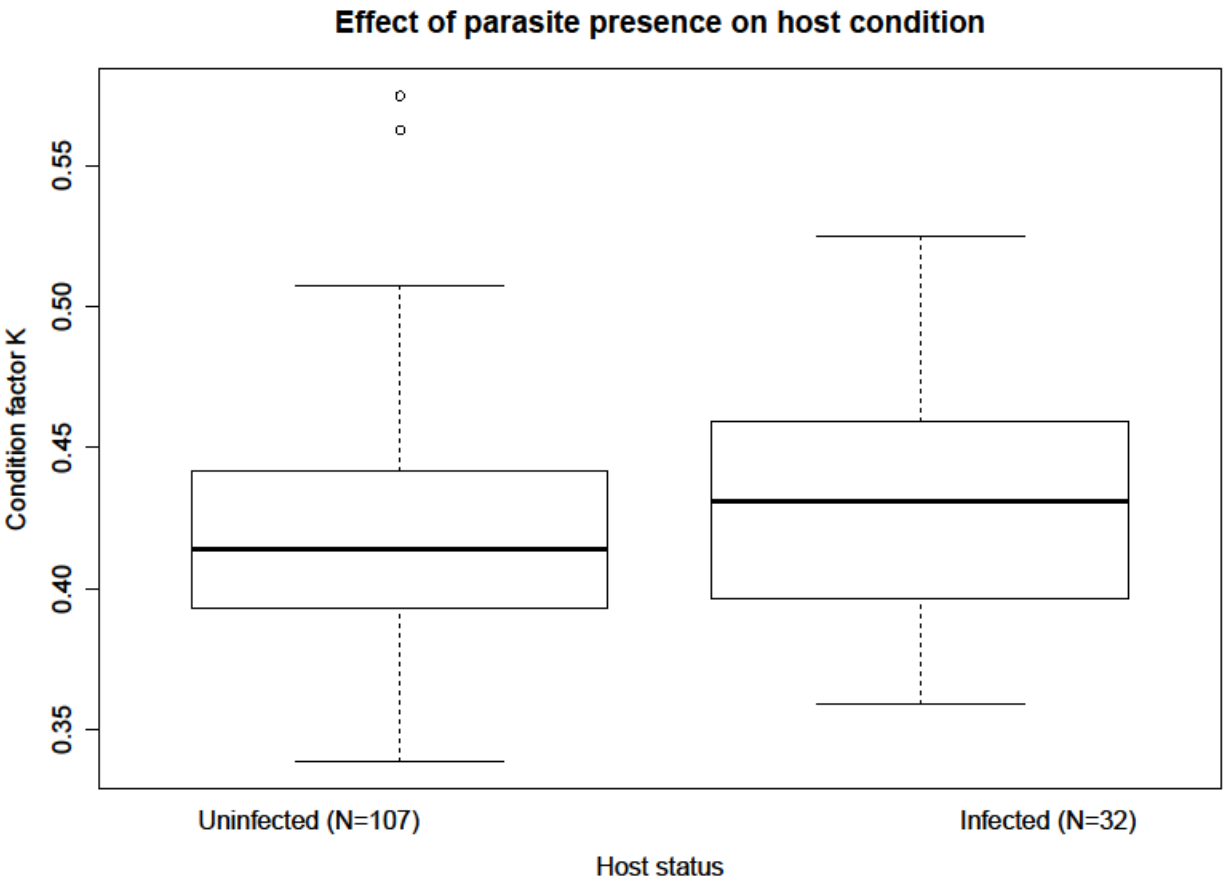

Figure 19: the condition factor K for infected and uninfected sharks.

3.5.4. Does *A. squalicola* impact host reproduction?

283 individuals were inspected for maturity stages, according to the maturity stage by Stehmann (2002). The analysis was limited to stations SF13RT1 and SF13RT2, as these sampled the same location during the same time of year. Of the 283 individuals, 32 were infected. The smallest female shark showing signs of maturation (stage 4) was 32 cm in length, and smallest male was 30 cm (stage 2). The female however, being at stage 4, suggest that the onset of maturation can occur at a size smaller than 32 cm. To avoid inclusion of sharks that would be immature regardless of parasite presence, the analysis was limited to sharks longer than 30 cm. This provided 50 sharks, of which 12 were infected.

None of the infected sharks showed any signs of maturation, whilst 10 out of 38 uninfected sharks showed some sign of maturation.

Table 8: the maturity stages of parasitised and unparasitised individuals

Maturity stage	Unparasitised	Parasitised
Mature	10	0
Immature	38	12

This was further separated into males and females to account for potential differences.

Table 9: the population separated according to infection, maturity and sex, with the expected number of infected mature individuals, based on the maturity ratio in uninfected hosts.

	Females		**Males**	
	Uninfected	Infected	Uninfected	Infected
Mature	3	**0**	7	**0**
Immature	19	4	9	8
proportion	15,8 %	0	77,8 %	0
Expected number of mature infected individuals		**1 (0,6)**		**6 (6,2)**

The proportion of mature uninfected males was much higher than the proportion of mature uninfected females. Infection by *A. squalicola* did not have a significant effect on maturation in females, binomial test (0.4, expected = 3/9, p=0,3086: two sided), but did have an effect on males, binomial test (0.8, expected= 7/9, p<0,001: two sided).

4. Discussion

4.1. Summary of results

The results show that *E.* spinax has a limited geographical distribution (figure 1, figure 2 and table 3), and is capable of forming groups of different sex and size compositions (figure 4). *A. squalicola* has a normal size distribution (figure 5), and a normal-shaped distribution within the size distribution of its host (figure 6). It is an atypical intensity distribution (table 4), is most commonly found in pairs (table 5), and most commonly on the dorsal fins (table 6). More than one infection is rare.

Most individuals had eggs, and eggs were found in both spring and autumn, suggesting reproduction may be continuous. Maturity is reached around a size of 3,7-4,7 cm (table 7).

No correlation between size of parasite and host suggests hosts remain susceptible to infection throughout their lifespan (figure 10). Individuals are similar to their partner, which supports the hypothesis that they arrive within a similar timeframe (figure 11).

Fecundity (egg presence) of *A. squalicola* is not affected by the size, sex or condition of the host, nor the site in which it is embedded, but appears to be affected by the cluster size (figure 12).

Size of *A. squalicola* appears unaffected by site (figure 15), the number of partners in the cluster or the total number of parasites on the host (figure 16).

Infection with *A. squalicola* does not impact susceptibility to additional infections, nor does it result in a change in HSI (figure 18) or condition (figure 19). It does however appear to affect maturity, but this was only found in male hosts (table 9).

4.2. Prevalence and geographic distribution of *A. squalicola*

A range of studies, both theoretical and empirical, show that infection levels (prevalence and intensity) are dependent on the right environmental and host conditions (Anderson & May, 1978). *A. squalicola* has been reported in most of the major oceans, and on multiple hosts within the *Etmopteridae* family (Yano & Musick, 2000). Genetic analyses reveal that specimens are highly similar, despite vast geographical distances (Rees et al., 2014). It is puzzling that populations so far away, e.g. New Zealand and Sognefjord, remain so genetically similar. It suggests that the populations recently diverged, which although may have taken several thousand years, suggests that *A. squalicola* is capable of dispersing great distances. In addition, the population in central Lusterfjorden proves that the species is capable of reaching a high prevalence (figure 2 and table 3). Combined, these properties show that *A. squalicola* has the potential to be ubiquitous in most host populations, yet the results of this study show that prevalence is highly variable, even over relatively short distances within Lusterfjord (figure 2). This suggests the variation observed reflects the extent to which specific sites satisfy the niche requirements of a species (Brown, Mehlman and Stevens, 1995), which implies variation in host biology, host environment and the environment during the larval stage of *A. squalicola* shape the prevalence and distribution.

The Environment

Salinity and temperature are two important factors controlling ectoparasite prevalence and distribution (Bush, 2001). Although there is variation and complexity in the physical oceanography of fjords, the deeper parts, where *E. spinax* was sampled, remains fairly stable (Farmer and Freeland, 1983; Storesund et al., 2015). If salinity and temperature are in fact uniform throughout the stations sampled, they may be at suitable levels (evident by the high prevalence), but unable to explain the variation.

Fjords have many physical boundaries and obstacles that can reduce migration between populations (Olsen et al., 2004). These may explain why the high prevalence is restricted to the Lusterfjord, although to our knowledge, there are no major obstacles other than a relatively steep increase in depth between the central part of the fjord and the greater Sognefjord (Figure 2, Appendix 9.1.), the impact of which this has on the host and larval dispersal of *A. squalicola* is unknown.

Interestingly, Lusterfjord is deeper than any of the other side fjords (arms) and is known for its dark water (Henrik Glenner, *pers. comm*.). The population in Lusterfjord also contains more juveniles than in Masfjord (Henrik Glenner, *pers. comm*.), and it may be that the combination of depth with darkness provides conditions suitable for juveniles and young adults (i.e. nursing ground or breeding ground) for a photosensitive species such as *E. spinax*. Our own sampling of the Masfjorden may support this, as the station in Masfjord has a very different population structure than Lusterfjord, with a distinctively older population of males (Figure 4).

An alternative explanation is that the home range, the area in which an animal lives and moves on a daily or periodic basis, of *E. spinax* is limited. There are no apparent obstacles preventing movement further into the fjord (Appendix 9.1.), which should

result in a homogenous prevalence if they moved and interacted freely with other individuals within the fjord. This suggests the biology of the host, in particular the behaviour, may be more influential on the dispersal of *A. squalicola* than environmental factors.

An exception is during the larval stage of *A. squalicola.* The movement and dispersal once settled, is the same as its host. However, during its larval stage it is exposed to both the influence of currents (De Wolf, 1973) and predation (Turner et al., 2001), which can affect its ability to disperse. De Wolf (1973) also found that the retention of larvae in the water column was dependent on the amount of suspended matter, and there is indication that this amount is particularly high in the Lusterfjord (Aksnes, D; *Pers comm*). The unique combination of currents, predation and run-off from rivers in the fjord may thus shape the distribution of both *A. squalicola* as well as its host. Unfortunately, too little is known of the biology of its dispersal stage and the abiotic factors in the fjord in order to explore this avenue further.

Regional variation

Despite E. spinax being a relatively common species, information on its biology is limited (Coelho & Erzini, 2008). Most of the more extensive work has been done on populations off the coast of Portugal, by Coelho & Erzini, which may cause some degree of bias, as there is considerable variation in the biology of populations in different regions (Coelho, 2007). This is not surprising given its wide-ranging distribution in different climates and at different depths. Variations in their biology, such as growth, maturity, feeding and social behaviour may therefore impact the prevalence of *A. squalicola*. However, if the prevalence is solely affected by regional differences, a similar prevalence is expected throughout the fjord, which is not the case

(figure 2). This suggests regional differences alone cannot explain the variation in prevalence.

Population dynamics within the fjord

The level of engagement in social behaviours changes throughout the lifetime of many organisms (ontogeny). Since the transmission of contagious parasites is often facilitated by close contact, the choice of shoalmates as well as sexual partners may influence the exposure of individual sharks (Magnhagen, 2008). The high prevalence may therefore coincide with involvement in specific behaviours that change throughout the lifetime. This was found by Anderson (1976), where the age distribution of the host population, as well as its feeding behaviour, had a significant impact on the population biology of the parasite, *Caryophyllaeus laticeps.*

In the case of *A. squalicola*, mating may be influencing prevalence. The existence of one station containing mostly juveniles (Figure 4: station SF13RT09) suggests *E. spinax* has nursery/spawning grounds, implying that individuals migrate (and segregate) to other areas as they age. These areas may be feeding or breeding grounds. Based on the conversion table of Coelho & Erzini (2008), the populations in which *A. squalicola* was highly prevalent consisted of sharks roughly 4 years old (figure 4). *E. spinax* is estimated to reach maturity around 4 years for males and 4.7 years for females (Coelho & Erzini, 2008), although it differs between populations (Coelho, 2007). Many individuals in the population may therefore be juveniles soon about to, or young adults, engaged in reproduction and/or increased social activity, which can increase transmission of parasites (Magnhagen, 2008), especially as copulation involves direct physical contact.

Additional evidence that may indicate engagement in reproduction (or of reproductive

age) comes from their distribution in the water column. *E. spinax* is known to exhibit a vertical sex distribution, with the proportion of females increasing with depth (Coelho 2007, Hickling, 1963), although it has not been observed in all studies (Coelho, 2007). The trend is thought to be the result of females growing bigger than males, and bigger sharks can feed on larger prey found deeper (intraspecific niche partitioning). This separation was not observed at the populations exhibiting high prevalence in Lusterfjorden (figure 4), where males and females of similar size were mixed. This may be indicative of the sharks deviating from their normal vertical distribution in order to mate.

However, the life history and behaviour of a species are known to change depending on the environment. Therefore, the differences seen may not be due to mating, but rather local adaptation to the fjord environment. In this case, it may simply be that the vertical sex distribution does not exist in the fjord due to its maximum depth of roughly 500 metres (appendix 9.1). Support for this was found by Coehlo (2007), where the sex ratio of populations in southern Portugal remained similar until below a depth of 600 metres, at which point females begin to dominate.

All populations of *E. spinax* have individuals that mate, and thus the existence of a population that may be engaged in mating obviously cannot solely explain the high prevalence. It may be that the stations sampled serve as breeding grounds, where close interactions, in combination with other variables, such as currents, food availability (for the host) and population density can provide favourable conditions for *A. squalicola* to achieve high prevalence.

A. squalicola as a biological tag

Parasites have been extensively used as biological tags, in order to identify the range, migration and existence of resident and migratory populations in many marine species (Mackenzie, 2012). No information on the home range of *E. spinax* was found in the available literature. As a biological tag, *A. squalicola* shows that its host may have a limited home range, or that they have a social structure that reduces mixing, despite movement within the fjord population.

If the distribution of its host is spatially homogenous and individuals mix freely, *A. squalicola* should be able to disperse throughout the fjord, leading to a similarly homogenous prevalence. This is not observed (figure 2), which suggests that *E. spinax* may exhibit some degree of shoaling behaviour, or patchy distribution, which reduces migration between populations, and can also explain the temporal variation observed at the upper central stations (Figure 2), as the shoals change location. Other squaloid sharks, such as the spiny dogfish, *Squalus acanthias*, are found in shoals segregated by sex and/or size (Stenberg, 2005). Additional evidence of shoaling behaviour / patchy distribution comes from the occurrence of both bountiful and empty hauls of *E. spinax* in Sognefjorden, and the differences in size and sex ratios between the stations (figure 4).

This study provides a shallow glance into the depths of a fjord which likely houses considerably complex interactions between environmental and biological factors that govern the prevalence and distribution of *A. squalicola*. The nature of the original expedition was to map the fauna of Sognefjorden, and the later cruises in Lusterfjorden were undertaken mainly to collect as many samples of *A. squalicola* as possible. Therefore, the sampling methodology was not designed with the intent of

exploring the distribution and prevalence of *A. squalicola*, and the ability to answer these questions is affected by this. Nevertheless, the data provides evidence to suggest that the population dynamics and behaviour of *E. spinax*, and the physical properties of the fjord, likely have interactive effects on the prevalence and distribution of *A. squalicola.*

Future studies should aim to strategically sample more stations in Lusterfjord, in order to obtain a higher resolution of the distribution. These should be random transects, as well as transects radiating from stations with high prevalence. The stations should ideally be repeated over time to assess temporal variation. The sample sizes also need to be larger in order to get more accurate estimates of the prevalence, and the density of sharks should be calculated from each station, due to the known effect of density on parasite transmission.

Tagging and tracking sharks is likely the method that will give the most definite answers about the movement of individuals, and group structure, within the fjord. Ex-situ tagging using trawls is difficult because the sharks do not survive the rapid ascension. However, archival satellite pop-up tags have been successfully used on Greenland sharks (*Somniosus microcephalus)* caught on long lines (Campana, Fisk and Peter Klimley, 2015). Acoustic telemetry is more expensive and labour intensive, but has been successfully tested on elasmobranchs to estimate home range and movements, and is capable of tracking multiple individuals simultaneously for up to several years (Espinoza et al., 2011). Although requiring highly specific equipment, in-situ electronic tagging of deep sea fish has been successfully done on saithe (*Pollachius virens)* and redfish (*Sebastes mentella)* (Sigurdsson, Thorsteinsson and Gustafsson, 2006), and could probably be adapted to *E. spinax*.

Currents can have a major influence on the dispersal of invertebrates during their larval stages (Palmer, Allan and Butman, 1996), and so information on the currents within the fjord may help explain the dispersal of *A. squalicola*. It may also explain the distribution of *E. spinax* as they (particularly as juveniles) mainly feed on planktonic crustaceans (Klimpel et al., 2002), which are affected by the same currents and thus there may be a two-fold effect by currents as it can affect both the location of larval *A. squalicola* as well as potential hosts that follow the abundance of current-driven prey. Data on currents, as well as salinity and temperature from CTDs should therefore be collected from each station due to their known influence on ectoparasites (Bush et al., 2001).

4.3. The infection biology of *A. squalicola*

Most parasite populations normally exhibit a right-skewed (aggregated) intensity distribution, where the majority of hosts have few or no parasites, whilst some harbour many (Bush et al., 1997). *A. squalicola* is similarly restricted to a few host individuals in the population, yet the intensity distribution differs in having a peak in frequency of two individuals per host (Table 4), and fewer single individuals per host than expected based on the right-skew trend normally seen. Furthermore, the cluster size distribution reveals that most individuals are situated in pairs (Table 5), and at very specific sites (table 6). These observations open up some interesting hypotheses about the biology of *A. squalicola*.

Limited number of cyprids, initially difficult to locate a host

The first scenario for explaining the intensity pattern (table 4) is that locating a host may be difficult, but once achieved, the parasite could increase the susceptibility of its host. The 'coordinated settlement' analysis suggests this (figure 11). Such an increase is commonly observed and can be achieved through several pathways, including especially the behaviour of the host (Bush, 2001). This can explain why there are few hosts with single individuals (table 4), because the altered behaviour increases the likelihood of more individuals settling. It may also be that *A. squalicola* itself produces cues, such as chemical compounds, which signal its presence. This has been reported in many cases for barnacles, whose gregarious nature necessitates an ability to attract and locate conspecifics (Pawlikj, 1991). Either way, *A. squalicola* may be capable of altering the overall susceptibility of *E. spinax*.

Individuals likely discontinue the altered susceptibility once it has acquired the necessary partner to ensure cross-fertilisation. Otherwise the host would end up with a heavy parasite load, which is not observed (table 4 and section 3.4.1.).

Abundance of cyprids, easy to locate a host

The second scenario is that locating hosts is fairly easy, but only a few of these are successfully infected. In this example, there may be an abundance of cyprids already present on the shark, or in the shark's immediate environment, and successful penetration of the skin by one cyprid leads to a quick scramble by other cyprids to join the lesion. Support from this is found in the similarity in size between individuals sharing a cluster, which may suggest they settle within a similar time frame (Figure 11). Because there are *normally* only two individuals in a cluster (figure 5), they appear to be capable of controlling the number of individuals that are allowed into the lesion. This could be through chemical cues or through spatial constraints due to the size of the lesion. Once they begin growing, it seems additional cyprids are unable to join.

Both scenarios can explain the intensity distribution; however, a third explanation may be a compromise between the two. In this scenario, there may be an intermediate number of cyprids in the environment, and they encounter hosts on an intermediate frequency. However, most hosts are not susceptible to infection. The few susceptible ones eventually get infected by a cyprid, which may affect its host's susceptibility, and additional cyprids join the lesion.

It is not easy to draw any conclusions regarding the eligibility of these hypotheses. The study could be improved by estimating the population density of the host based on the volume of water trawled, to better understand host availability.

The sharks were only inspected by eye, and the data is therefore likely biased due to overlooking the smaller, recently settled individuals. They grow to be several magnitudes larger than they were when they settled, and observing these younger individuals will provide data on a much larger distribution of developmental stages, as well as the exact site of the infection, which is obscured by their subsequent growth.

A whole avenue of options would open up if it were possible to raise cyprids and lanternsharks under experimental conditions (experimental infections). This would allow for detailed monitoring of the infection behaviour, and it would be possible to test all of the abovementioned hypotheses.

Site specificity

Most parasites are highly predictable in their distribution on a host (Bush, 2001), and many parasites are often considered to be site-specific first and secondarily host-specific (Adamson and Caira, 1994). This implies that despite occurring in multiple host species, they are always found associated with a specific body part. This appears to be the case for *A. squalicola*, which is found on several different hosts, but normally in the same sites (Table 6, pictured in figure 8).

Site specificity is often the result of an adaptive advantage of specialising on one body part in relation to others (Adamson and Caira, 1994). More importantly, it implies that the parasite has a 'choice' between different sites. This should result in reduced fitness when the ideal site is not obtained. However, no such variations were observed in fecundity or size of individuals between the different sites (figure 12 and figure 15 respectively).

This suggests the specific sites on the host are not chosen for their quality; instead they may be the only sites where individuals are capable of penetrating the skin.

Furthermore, the normally low prevalence can imply that the sharks' skin in general provides good resistance against *A. squalicola*. Given the sites' association with protruding body parts, it may be that the skin is thinner in certain areas, such as the junction between the fins and the main body. Support for this is found in infection biology of the rhizocephalan barnacle, *Loxothylacus panopaei,* which always settles at the thinnest/softest-skinned area of its host (Glenner, 2001).

Alternatively, the site specificity may be explained by unequal encounter rates with different body parts of the host. In this case, it may be that the site specificity is a result of increased contact with particular sites due to the turbulence caused by certain body parts, such as the fins. A study by Carrillo et al. (2015) assessed the occurrence, distribution, abundance, orientation and size of the whale barnacle, *Xenobalanus globicipites*, on its striped dolphin vehicle, *Stenella coeruleoalba.* Their results indicate that the barnacles are likely able to chemically detect the dolphin's skin/presence, but their distribution on the different body parts, such as flukes and fins, are passively selected due to vortices created which increases contact of the cyprids with the skin. They further suggest that the barnacles can actively move to the trailing edge, and orientate themselves according to the flow of water.

It may therefore be that the site specificity has less to do with areas where it is easier to penetrate the skin, but rather that the protruding body parts of the shark may serve as points of entry for *A. squalicola* (pictured in figure 8). Considering it requires ample tissue in which to develop its peduncle and rootlets, it may migrate down from the fin to the more suitable main body, where it proceeds to penetrate the skin adjacent to the fins. Therefore, the site specificity may not be due to where the skin is thin enough to penetrate, but rather that this is the first suitable body surface they encounter, although the hypotheses are not mutually exclusive.

It is important to note that the velvet belly lanternshark is a much slower and smaller animal than the striped dolphin, and migration from the point of entry to a different site may therefore be much easier for *A. squalicola* than *X. globicipites,* due to less drag and shorter distances. Therefore, the factors affecting the distribution of *X. globicipites* may not be equally influential on the distribution of *A. squalicola*.

It may also be that the site specificity is the result of a need to maintain intraspecific contact in low density populations. This was explored by Rohde (1979), who argued that many potential niches for fish ectoparasites are empty, which suggests intrinsic factors may be largely responsible for site distributions. Considering their requirement of a partner for reproduction and tendency to appear in pairs, this argument is clearly relevant for *A. squalicola*. It may therefore be that cyprids arrive and move to specific locations on the host whereby they increase the likelihood of finding a mate, with which they subsequently settle.

A final hypothesis is that the site specificity is a vestigial phenomenon. In relatively recent evolutionary time, *A. squalicola* must have sustained itself by filter-feeding whilst attached to *E. spinax.* Considering that many whale barnacles are associated with specific body parts, it may well be that these sites provide more favourable conditions for a filter-feeding lifestyle.

Because *A. squalicola* has vestigial morphology, it may also have vestigial behaviour. Some nematodes, such as *Ascaris* and *Strongylus*, undergo migrations through the tissue of their host that begin and end in the same site (Read & Skorping, 1995). This has been suggested as vestigial behaviour following the evolutionary loss of skin penetration or intermediate hosts. However, the behaviour may be selected for due to benefits in size achieved during the migratory phase, and consequently provide a

reproductive advantage (Read & Skorping, 1995). The fascination with *A. squalicola* lies in its recent divergence to parasitism, and the remnants of this lifestyle should not be ignored. Considering its morphologic resemblance to filter-feeding barnacles, one may wonder whether there is a similar behavioural vestige in its choice of site, due to better conditions for filter-feeding near the fins.

In the end, indirect evidence may favour the 'thin-skin hypothesis' the most. Epibionts are associated with many taxa, but not commonly sharks. Therefore, the shark's skin, which ismade up of so-called 'skin teeth', is believed to provide excellent protection against epibionts and ectoparasites. If the specific sites that *A. squalicola* is found on were simply the result of where they are more likely to arrive, rather than the need for a penetrable integument, we would expect to see many other epibionts associated with sharks, which we do not. Therefore, the most parsimonious explanation may be that *A. squalicola* is uniquely adapted to locating and penetrating the weaker areas of the shark's skin.

These hypotheses could be tested to some degree by more accurately describing the microhabitat of *A. squalicola* on the host. The study only described individuals as being associated with a general area such as '1st dorsal fin' and 'left pectoral fin'. By more precisely describing their position, at least the validity of whether the skin is somehow easier to penetrate at the junction between the protruding parts and main body can be tested. It would also likely help to find more recently settled individuals, as their position more closely mirrors the original site of penetration.

4.4. Intraspecific competition.

Two individuals per cluster is the most common configuration, although there were many instances of three individuals as well (table 5). One partner is needed for cross-fertilisation; however, more individuals in the cluster may severely reduce the fitness of individuals through intraspecific competition for nutrients and space. The addition of a third cyprid is therefore likely not a benefit to the pioneering individual. Rather, it is likely the result of two additional individuals joining within a similar time frame, which this study supports their ability to do (table 5 and figure 11). Considering the intimate, limited space they share, and their dependence on their immediate tissue for nutrition (shown in figure 14), competition is likely an important factor.

There are a few caveats regarding the data that the reader should be aware of. Firstly, the sample sizes for the analyses (figure 16) are small, and so the insignificance in size variation may be due to statistical weakness rather than absence of any differences. Furthermore, it was not possible to control for the variation in size of the individuals; comparisons were therefore made between individuals without knowledge (control) of how long the parasites had been on the shark, which in junction with the small sample size offers limited credibility to the analyses.

There is also the question of whether the use of the thorax as a measurement of size (figure 5) is appropriate for measuring competition in an organism that now relies on its peduncle and rootlets for nutrients. A better measurement may have been the peduncle length, or ideally the peduncle girth (which was never measured) (figure 3). Unfortunately, not enough peduncle length measurements could be obtained from the collection in order to be used in the analysis (table 2).

The fecundity analysis is simply based on whether eggs were present or not (figure 12). This does not exclude the possibility that eggs have been successfully produced and released in the past. *A. squalicola* is most likely capable of reproducing continuously (Hickling, 1963) which this study supports (section 3.4.3). Inspection of the ovaries may therefore shed light on their development and potential previous reproductive cycles.

A common result of competition between parasites is stunted growth, reduced fecundity or both, due to what has been coined the 'crowding effect' (Read, 1951). In this study we found no reduction in growth from being three individuals versus two (figure 16). Instead, we found a stark contrast in egg presence between individuals in pairs and triplets (figure 12).

Reduced fecundity is a recognised phenomenon (Read, 1951), and has been observed in the liver fluke, *Fasciola hepatica,* in sheep (Boray, 1969) and the nematode, *Haemonchus contortus,* in lambs (Flemming, 1988). Crowding has traditionally been viewed as causing a carbohydrate shortage, although there is also evidence of crowding due to spatial constraints (Bush & Lotz, 2000). The peduncle of *A. squalicola*, which houses the ovaries, swells with the onset of maturity (Hickling, 1963). Our data suggests maturity occurs around a size of 3,7-4,7 mm (table 7). From observations, space in the host's tissue may be limited (figure 14). This may explain the lack of egg-bearing individuals in groups of three, as the ovaries may be unable to fully develop due to spatial constraints.

An alternative explanation is that the finite resources of the shark, such as carbohydrates, are unable to support the egg-production of more than two individuals. However, this is likely not the case because sharks with multiple infections, where the

total number of parasites exceeded three, had individuals with eggs (raw data). This suggests local competition for space (at the 'cluster level', rather than 'host level') may indeed be affecting the fecundity, and that the effect is qualitative, as eggs were completely absent.

A final option is that the presence of a third individual may result in them being pushed away from each other, or somehow ending up in a physical configuration in which they are unable to fertilise each other.

Although the mechanisms causing it are not understood, the result of this analysis reveals a potential tremendous cost to individuals that end up in a cluster size of more than two individuals. If this is the case, there will be strong selective pressure on *A. squalicola* to evolve methods of ensuring a cluster size of two individuals, and is maybe one of the main reasons why this is indeed the most frequent cluster size observed (table 5).

4.5. The potential for evolution of male dwarfism

The short penis of *A. squalicola* shows that the selective pressure on reproduction has undergone major changes. In comparison, its closest sister species, *Capitulum mitella,* has a conventional, highly sophisticated and elongated penis associated with fierce mating competition (appendix 9.2). The short penis makes sense givens its new group size, where there is normally no mate competition.

The new niche could pave way for further evolution towards dioecy, and ultimately male dwarfism, a common occurrence in species where the likelihood of finding a mate is low, which is often the case for deep sea species (Yamaguchi et al., 2012). It may especially be the case, considering the many similarities between *A. squalicola* and Rhizocephalan barnacles, which indeed have dwarf males (Yamaguchi et al., 2012), and the multiple independent evolutions of dwarf males in thoracican barnacles, all associated with invasions into new habitats (Lin et al., 2015).

Despite this hypothesis, the normally low prevalence and the lack of single specimens on hosts, but relative abundance of pairs and triplets, suggest that locating a mate may not be that difficult for *A. squalicola*, at least not in the Lusterfjord (table 5). If most individuals are capable of finding a partner, then there is little selective pressure to sacrifice the benefits of cross-fertilising for the security of being able to reproduce.

4.6. Effects on host

Hosts have a finite amount of resources, which they allocate to a range of functions, such as growth and reproduction. Parasites therefore have several pathways to exploit in order to obtain nutrition. This can make assessment of the impact of parasites very challenging, as the effects can manifest in different and potentially inconspicuous ways.

4.6.1 Fulton's Condition factor K and the Hepatosomatic index (HSI)

Fish store energy in muscle tissue or in the liver during periods of high food and energy intake (Harrison, Gault and Dick, 2006). Because of this, both condition factor (Fulton's K) and the relative size of the liver (hepatosomatic index, HSI) are recommended as indirect indicators of the energy status, or 'health', of fish (Harrison, Gault and Dick, 2006). Neither measurement differed between infected and uninfected individuals (figure 18 and 19). This may suggest either of three assumptions: (1) that the test was not appropriate for assessing an impact, (2) that *A. squalicola* does not have a large/detectable impact on its host's energy reserves or (3) that the impact unfolds in a different part of the host.

Although recommended for assessing the energy reserves of fish, Fulton's condition factor may not be reliable when assessing parasitic impacts (Morton & Routledge, 2006). Morton & Routledge (2006) assessed the use of Fulton's condition factor (FCF) on indicating the impact of sea lice infestations, *Lepeophtheirus salmonis*, on juvenile pink, *Oncorhynchus gorbuscha,* and chum salmon, *O. keta*. Their results showed that the condition factor will remain high until the very end of the infection, and concluded that FCF is not a reliable indicator. Similarly, FCF may be unsuitable for assessing the energetic toll of a parasite in velvet belly lanternsharks.

Liver mass (HSI) is commonly used as a measure of the energy content and thus well-being of individuals (Wootton, 1984), and it is known to quickly respond to changing environmental conditions, such as food availability (Allen & Wootton, 1982). As such, parasites have been shown to reduce the HSI in many fish species (Malek, 2001). In the case of squaloid sharks, such as *E. spinax*, the HIS may unfortunately not be a good indicator of health. The content of deep-sea shark's livers is mostly the hydrocarbon squalene, which is not a conventional material to use as a metabolic reserve (Corner, Denton and Forster, 1969). Instead, it is particularly suited for providing lift, in order to establish buoyancy (Corner, Denton and Forster, 1969). Consequently, if the liver does not function primarily as a metabolic reserve, then any energetic cost by a parasite may not lead to a reduction in liver size.

4.6.2 Reduced fecundity of host

A common phenomenon in parasitism, especially for crustacean parasites, is reduced fecundity or castration of hosts (Bush, 2001). Previous work by Hickling (1963), and more recently by Yano & Musick (2000), showed that *A. squalicola* retards the development of reproductive organs of host sharks (although in the latter study the sharks were not *E. spinax,* but still from the *Etmopteridae* family). Our study found a similar effect, but only for males (table 9).

Despite the effect *A. squalicola* appears have on host reproduction, and the knowledge that many crustacean parasites exhibit castration of their hosts (Bush, 2001), it is not possible to rule out whether the retarded reproduction is actively caused in order to free up resources for the parasite, or whether the parasite's competition for resources prevents the host from investing in reproduction.

The low sample size prevented attempts at more complex statistics, where one could potentially have controlled for variation in host qualities and reproductive cycle. In addition, maturity was assessed based on the method by Stehnmann (2002), which involved visual inspection of the gonads according to their set of criteria. This was not the same approach used by Yano & Musick (2000) and Hickling (1963), which may explain the lack of an observed effect on female reproduction (table 9). By applying the same methodology as Yano & Musick, who arguably performed the most extensive analysis, a similar result may be found. Moreover, the gonadosomatic index (GSI) is an alternative that is found to be more reliable in some cases than visual inspection (but cheaper and less laborious than histology), and should be included in future studies (Flores et al. 2014).

Current views in parasitology indicate that the outcome of host-parasite interactions can vary from antagonism to mutualism, and that it depends on the natural history of the organisms involved, in particular the mode of parasite transmission and reproduction (Anderson & May, 1982; Ewald, 1995). Unlike many other parasites that have multiple hosts in their lifecycle, *A. squalicola* only has one. In addition, the relationship with the host is permanent. This means that *A. squalicola* is dependent on the movement of its host for dispersal to new areas (except for the potential dispersal during the larval stage), and survival. Consequently, the fitness of *A. squalicola* is intimately linked with that of its host.

In order to maximise reproduction, one option for *A. squalicola* could involve extracting nutrients from the host much faster than the host can replenish them, which quickly kills the host, but involves a high rate of offspring production, but the host dies very prematurely (high virulence approach). This is unlikely to be the optimal strategy considering the low prevalence and dependency on the host for survival,

which is a slow growing species capable of living for up to 11 years (females) (Coehlo, 2007). Therefore, *A. squalicola* is expected to balance its virulence (egg production and growth) with the survival of its host, in order to maximise its lifetime reproductive success. This involves maintaining a host which is capable of feeding and escaping predation.

In order to maintain a host with a high chance of survival, yet maximise reproduction, many parasites exploit a 'loop hole' in their host's biology. Whilst maintaining the survival of its host is very important, there is no benefit for the parasite to let the host reproduce. By diverting the resources normally partitioned for reproduction, parasites can secure the required nutrients without sacrificing the survival chances of the host. The end result can be, rather ironically, a physically fit host with zero fitness, which is in agreement with the results found in this study (figure 18 and 19, and table 9) and that of Hickling (1963) and Yano & Musick (2000). In conclusion, albeit a recently evolved parasite, *A. squalicola* may already have evolved towards an optimal level of virulence by exploiting the energy normally allocated for reproduction in *E. spinax.*

5. Conclusion

A. squalicola is the only parasitic barnacle found on a vertebrate host, as well as being the only one to use its peduncle as a trophic organ that we know of (Rees et al., 2014). These qualities shape the ecology of the organism and the result is a truly unique parasite.

This study is the first to consider *A. squalicola* as a biological tag, and the results suggest movement within the fjord, and/or migration between shoals may be limited. Furthermore, it is the first to show that partners of *A. squalicola* are similar in size, and the third to provide evidence supporting the capability of *A. squalicola* to affect the reproduction of its host.

Of particular interest is the qualitative effect that cluster size may have on egg presence, because of the profound consequence it has on the fitness and therefore selection in *A. squalicola*. If the trend observed in this study continues to be confirmed, the effect of cluster size on egg presence will be an excellent, novel example of the "crowding effect", maybe caused by spatial constraints. As such, this study is the first that I know of to report a potential qualitative response in fecundity to crowding, and for the effect to occur from the addition of only 'one extra' parasite.

This thesis will hopefully serve as a foundation upon which future studies of *A. squalicola* will draw inspiration from. It is the author's personal opinion that although a better understanding of the interaction between *A. squalicola*, the host and Lusterfjord should be obtained from continuing to study the fjord population; this is ultimately a secondary goal. The primary goal should be to describe the mode of transmission, by facilitating and observing infections under experimental conditions.

Once the mode of infection is better understood, one can begin to use this knowledge in the broader context of the natural setting of *A. squalicola*.

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8. Glossary

Prevalence: the number of hosts infected with 1 or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species (Bush et al, 1997)

Intensity of infection: the number of individuals of a particular parasite species in a single infected host, i.e. the number of individuals in an infrapopulation (Bush et al., 1997). Can be used to produce the descriptives:

Mean Intensity: the average intensity of a particular species of parasite among the infected members of a particular host species (Bush et al., 1997)

Median Intensity: the median intensity of a particular species of parasite among the infected members of a particular host species.

Cluster: A group of individuals sharing the same location on the host.

Cluster size: the number of individuals sharing the same location on the host.

9. Appendix

Table 9.1: Coordinates for the stations, including location, station code, start- and stop coordinates and distance.

Location	Station code	Start coordinate	Stop coordinate	Distance (kilometres)
Nesøy	SF11	61.0138, 4.88874	NA	NA
Masfjord	MAS15	60.8732527°, 005.4138626°	NA	NA
Fjærlandsfjord	HM2013-05-10RT	61.32331, 6.69289	61.30533, 6.67549	2,2
Sognesjøen	HM2013-05-09RT	61.20406, 7.09831	61.20219, 7.08	1,0
Central Lusterfjord	HM2013-05-01RT	61.36475, 7.37998	61.34326, 7.3646	2,5
Central Lusterfjord	HM2013-05-02RT	61.3518, 7.37011	61.37306, 7.3866	2,5
Nattropfjord, Lusterfjord	HM2012-11-21RT	61.41202, 7.46246	61.42607, 7.47444	1,7
Nattropfjord, Lusterfjord	2015a	61.43438, 7.47393	61.4067, 7.45585	3,4
Nattropfjord, Lusterfjord	2015b	61.43438, 7.47393	61.40048, 7.44011	4,51
Nattropfjord, Lusterfjord	2015c	NA	NA	NA
Innermost, Lusterfjord	HM2012-11-18RT	61.47805, 7.57594	61.46669, 7.5573	1,6

An interactive map, with all the details, can be found via one of the following links:

Short link:

https://goo.gl/t13mc2

Full link:
https://drive.google.com/open?id=1e41eUeYUXo6tM9GdPTk7ZEdB_H0&usp=sharing

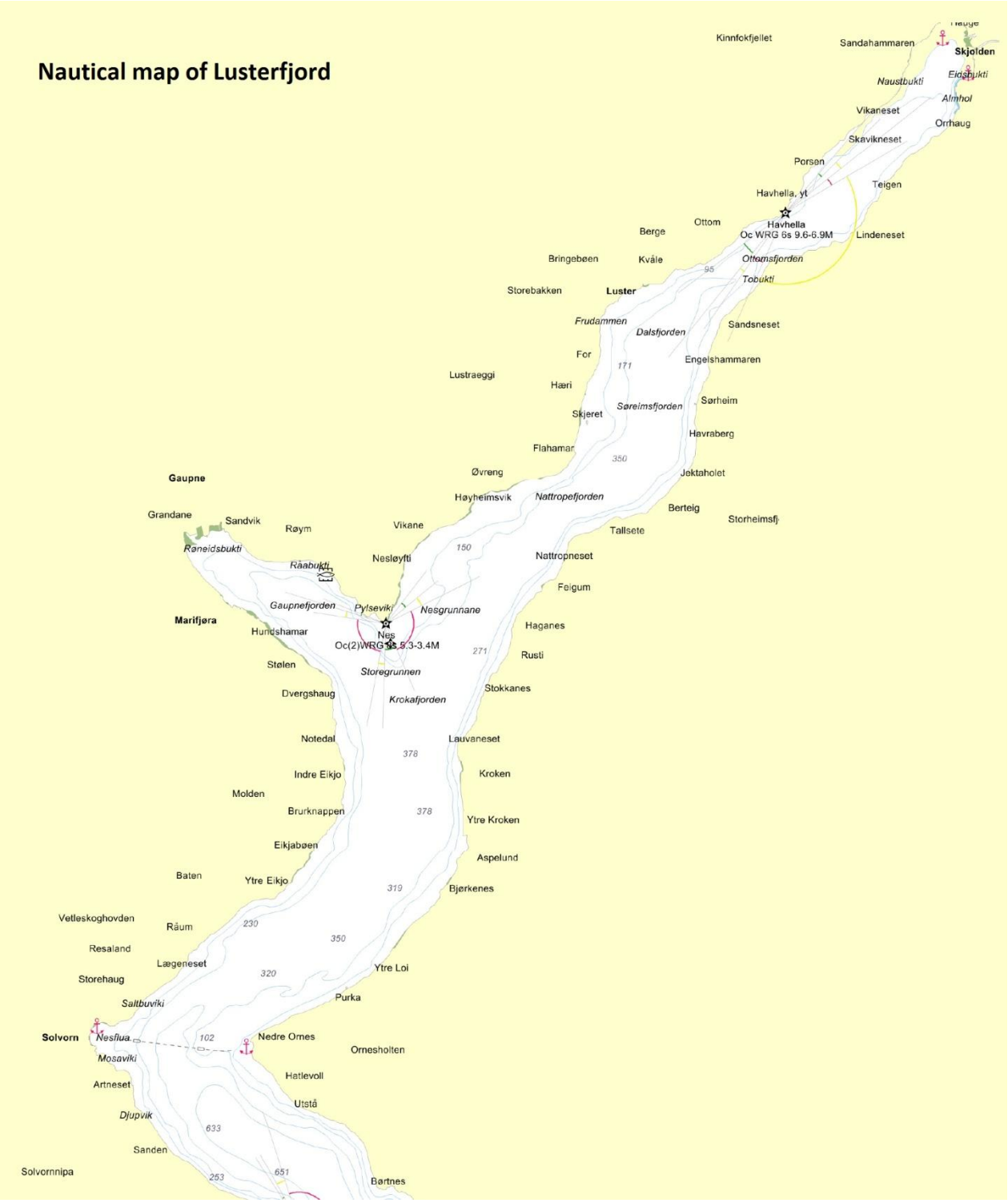

Figure 9.1: Nautical map showing the depth of the fjord. Notice the relatively narrow opening between Solvorn and Nedre Ornes, near the bottom of the map.

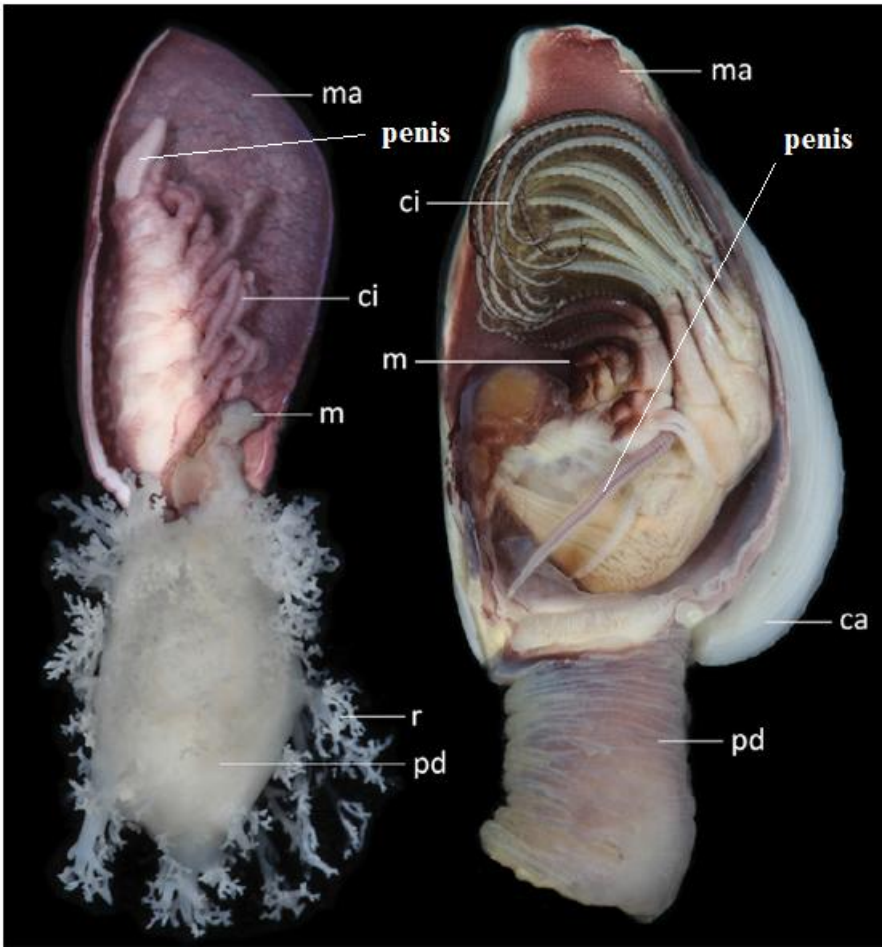

Figure 9.2. Comparison between the morphology of *C. capitulum* and *A. squalicola*. Notice the highly reduced penis. From Rees et al., 2014.

R model outputs

Egg presence

egg presence ~ number of individuals in a cluster

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
 Family: binomial  ( logit )
Formula: eggs ~ cluster size + (1 | cluster)

     AIC      BIC   logLik deviance df.resid
    47.2     52.4    -20.6     41.2       39

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.6202 -0.2887  0.6172  0.6172  3.4641

Random effects:
 Groups               Name        Variance Std.Dev.
 Cluster              (Intercept) 0        0
Number of obs: 42, groups:  cluster        , 26

Fixed effects:
                 Estimate Std. Error z value Pr(>|z|)
(Intercept)         7.865      2.426   3.242  0.00119 **
Cluster            -3.450      1.121  -3.078  0.00208 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
           (Intr)
egg.df$n.c1 -0.987
```

Egg presence ~ sex of host

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
Family: binomial (logit)
Formula: egg presence ~ sex of host + (1 | cluster)

AIC	BIC	logLik	deviance	df.resid
71.2	77.3	-32.6	65.2	53

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.5735	-0.5384	0.2782	0.5202	0.9861

Random effects:

Groups	Name	Variance	Std.Dev.
Cluster	(Intercept)	2.907	1.705

Number of obs: 56, groups: cluster, 33

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	2.141	1.083	1.976	0.0481	*
Sex of host	-2.072	1.203	-1.723	0.0848	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:

	(Intr)
eggsx.df$.M	-0.839

Egg presence ~ position on host

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
Family: binomial (logit)
Formula: egg presence ~ position on host+ (1 | cluster)
Data: eggset.df

AIC	BIC	logLik	deviance	df.resid
72.3	80.1	-32.2	64.3	48

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.5348	-0.5784	0.2908	0.4923	0.9268

Random effects:

Groups	Name	Variance	Std.Dev.
Cluster	(Intercept)	3.348	1.83

Number of obs: 52, groups: cluster, 31

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.4058	0.6921	0.586	0.558
position.of.parasite.on.hostother	-0.2943	1.5485	-0.190	0.849
position.of.parasite.on.hostpectoral	1.8034	1.4734	1.224	0.221

Correlation of Fixed Effects:

	(Intr)	pstn.f.prst.n.hstt
pstn.f.prst.n.hstt	-0.443	
pstn.f.prst.n.hstp	-0.319	0.153

Egg presence ~host length

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
 Family: binomial (logit)
Formula: egg presence ~ host length + (1 | cluster)
   Data: eggset.df

     AIC   BIC   logLik deviance df.resid
    75.2   81.2   -34.6   69.2   53

Scaled residuals:
    Min   1Q Median   3Q   Max
-1.4483 -0.5590 0.3633 0.4051 0.9584

Random effects:
 Groups   Name   Variance Std.Dev.
 Cluster   (Intercept)   3.69   1.921
Number of obs: 56, groups: cluster, 33

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   5.4722   6.3366   0.864   0.388
Host length -0.1538   0.2147 -0.717   0.474

Correlation of Fixed Effects:
            (Intr)
host.length -0.994

Egg presence ~ Condition of host

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
 Family: binomial (logit)
Formula: egg presence ~ condition + (1 | cluster)
   Data: eggset.df

     AIC   BIC   logLik deviance df.resid
    65.3   70.7   -29.7   59.3   42

Scaled residuals:
    Min   1Q Median   3Q   Max
-1.1421 -0.6879 0.4933 0.5635 0.9695

Random effects:
 Groups   Name   Variance Std.Dev.
 cluster   (Intercept) 2.331   1.527
Number of obs: 45, groups: cluster, 28

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.7555   4.7586 -0.369   0.712
condition   0.4954   1.1075   0.447   0.655

Correlation of Fixed Effects:
           (Intr)
condition2 -0.994

Size of *A. squalicola*

Size ~ position on host

Linear mixed model fit by REML ['lmerMod']

Formula: size ~ position + (1 | cluster)

REML criterion at convergence: 181.7

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.2349	-0.5060	-0.2521	0.5015	2.6512

Random effects:

Groups	Name	Variance	Std.Dev.
Cluster	(Intercept)	0.8057	0.8976
Residual		0.9917	0.9959

Number of obs: 56, groups: cluster, 28

Fixed effects:

	Estimate	Std. Error	t value
(Intercept)	4.7759	0.2914	16.392
Other	-0.6299	0.6490	-0.971
Pectoral	0.2094	0.4973	0.421

Correlation of Fixed Effects:

	(Intr)	sz.df$pstn.f.prst.n.hstt
sz.df$pstn.f.prst.n.hstt	-0.449	
sz.df$pstn.f.prst.n.hstp	-0.586	0.263

Size ~ number of parasites on host

Linear mixed model fit by REML ['lmerMod']

Formula: size ~ number of parasites + (1 | cluster)

REML criterion at convergence: 184.7

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.4275	-0.5276	-0.1585	0.5708	2.7113

Random effects:

Groups	Name	Variance	Std.Dev.
Cluster	(Intercept)	0.6879	0.8294
Residual		1.0290	1.0144

Number of obs: 56, groups: cluster, 28

Fixed effects:

	Estimate	Std. Error	t value
(Intercept)	5.3821	0.5631	9.558
Number of parasites on host	-0.2120	0.1748	-1.213

Correlation of Fixed Effects:

	(Intr)
sz.df$n....	-0.928

Size ~ cluster size

Linear mixed model fit by REML ['lmerMod']

Formula: size ~ cluster size + (1 | cluster)

REML criterion at convergence: 183.1

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.3729	-0.5270	-0.1734	0.4678	2.8094

Random effects:

Groups	Name	Variance	Std.Dev.
Cluster size	(Intercept)	0.6792	0.8241
Residual		1.0167	1.0083

Number of obs: 56, groups: cluster, 28

Fixed effects:

	Estimate	Std. Error	t value
(Intercept)	5.7663	0.7247	7.957
Cluster size	-0.4040	0.2754	-1.467

Correlation of Fixed Effects:

	(Intr)
sz.df$n.cl1	-0.958

Liver weight (relative) ~ parasite presence and sex of host.

```
Call:
lm(formula = liver weight ~ parasite presence +
    sex of host)

Residuals:
    Min      1Q  Median      3Q     Max
-47.502  -9.894   1.679  10.307  62.335

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          145.810      2.328  62.639   <2e-16 ***
Parasite presence     -1.601      3.727  -0.430    0.668
Host sex               1.692      3.138   0.539    0.591
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 18.45 on 136 degrees of freedom
  (812 observations deleted due to missingness)
Multiple R-squared:  0.003255,          Adjusted R-squared:  -0.0114
F-statistic: 0.2221 on 2 and 136 DF,  p-value: 0.8011
```

Sex removed due to insignificance:

```
Call:
lm(formula = liver weight ~ parasite presence)

Residuals:
   Min     1Q Median     3Q    Max
-46.62 -10.06   1.21  10.11  61.53

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          146.616      1.779  82.414   <2e-16 ***
Parasite presence     -1.456      3.708  -0.393    0.695
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 18.4 on 137 degrees of freedom
  (812 observations deleted due to missingness)
Multiple R-squared:  0.001124,          Adjusted R-squared:  -0.006167
F-statistic: 0.1542 on 1 and 137 DF,  p-value: 0.6952
```

Host condition ~ parasite presence.

Call:

lm(formula = condition ~ parasite presence)

Residuals:

Min	1Q	Median	3Q	Max
-0.80309	-0.26786	-0.04837	0.22779	1.55689

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.18958	0.03960	105.790	<2e-16	***
Parasite presence	0.14283	0.08254	1.731	0.0858	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4097 on 137 degrees of freedom

(812 observations deleted due to missingness)

Multiple R-squared: 0.02139, Adjusted R-squared: 0.01425

F-statistic: 2.995 on 1 and 137 DF, p-value: 0.08579

Binomial tests

Chance of additional infection cluster

Exact binomial test

data: 5 and 52

number of successes = 5, number of trials = 52, p-value = 0.1091

alternative hypothesis: true probability of success is not equal to 0.185

95 percent confidence interval:

0.03196424 0.21029747

sample estimates:

probability of success

0.09615385

Maturity in males and females

Exact binomial test

data: 0 and 4
number of successes = 0, number of trials = 4, p-value = 0.3086
alternative hypothesis: true probability of success is not equal to 0.3333333
95 percent confidence interval:
0.0000000 0.6023646
sample estimates:
probability of success
0

Exact binomial test

data: 0 and 8
number of successes = 0, number of trials = 8, p-value = 5.947e-06
alternative hypothesis: true probability of success is not equal to 0.7777778
95 percent confidence interval:
0.0000000 0.3694166
sample estimates:
probability of success
0