Reproductive biology in corkwing wrasse (*Symphodus melops)*

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Acknowledgements

The past two years have passed as fast as expected, it has been an amazing experience and something I am very proud of. Fishes has always been part of my life and working with them is nothing but fun. I remember taking breaks during my bachelor and watching the aquarium where there has been this amazing corkwing wrasse, who I was calling Muriel. Now I wrote whole thesis on that species.

I kindly thank so much to my great two supervisors. Asbjørn Vøllestad thank you for letting me talk about my thousand ideas and fairy tales long from scientific way of thinking. Kim Halvorsen thank you for always helping me with answers to all my wrasse question, and there had been many and most of them not even relevant to this master thesis. I learned so much from both of you and hope you will look back at my work with a smile.

Then I would also like to thank my family for great support and helping me to get where I am now. My father Tomasz, he would take me on fishing trips since my youngest years and my mom Dorota she has always said I can do it and told me to always go forward. Then let’s say thanks to my siblings Kacper and Wiktoria, now it’s your turn and good luck.

The last paragraph here I reserve to a special someone who has been with me through some best parts of my life and hope she is up for much longer. The one that has supported me and told me to work hard and fulfil my dreams, helped me with writing and relaxing when needed. She always helped with an extra push to read one more article or to write extra page. My biggest motivation and treasure in this world my girlfriend Jinsil :)

Abstract

There is a huge variation in the reproductive biology of fish within the family of wrasse (*Labridae*). They all come with many complex systems and characteristics. It is crucial to understand the behaviour and spawning strategies fish utilize for better understanding of the population dynamics or how future may influence the populations.

In my master project I focus on the reproductive behaviour of corkwing wrasse (Symphodus melops) during the spawning season. Territorial corkwing males build and guard the nest where they invite females to spawn, while also smaller males that exhibit female mimicry and does not build nests may visit and perform sneak spawning. For over a month I have been gathering the data around a small island called Saltskjærholmane in west Norway. I have been monitoring 11 territorial male nests with over 1,7 million-point observations; coming from 233 individually marked wrasses. I have analysed over 50 hours of film observing in detail 7 unique territorial males and every observed interaction and behaviour that took place withing its nest.

Corkwing wrasse were captured and tagged with PIT (Passive Integrated Transponder) tags and nests were monitored by cameras and RFID antennas to observe behavioural interactions. I developed methods to infer spawning visits from pit detections and analysed whether female body size affected visitation and spawning rates at nests. Smaller females visited more nests and had higher visitation rates (detections). The underlying reasons for these patterns remain unresolved, but it is possible that larger females are experienced in when and where to spawn, and therefore needs to spend less energy on visiting many nests to lay their eggs.

Sneaker males have been observed during the time when the territorial male and the female were busy with spawning, many times revealing itself with quick surprise fertilization shortly after female releases the eggs.

Many different species have been observed close to the nest of territorial corkwing male, the most common visitor being goldsinny wrasse (*Ctenolabrus rupestris*). Its time at the nest was correlated closely to the territorial corkwing that often would chase the fish away. Also, a negative interaction was observed where goldsinny would aggressively chase female corkwing away.

The work of mine summarize the reproductive behaviour of corkwing wrasse in three distinctive stages. Following this pattern, male build the nest, spawn, and take care of the eggs. The sneaker male has their own strategy that is closely correlated to spawning time of territorial male and the corresponding female at the nest. The length of females plays a role and help to predict some factor of the spawning. Through elaborating these newly learned reproductive behaviors and interactions, this thesis aims to contribute to more profound understanding of corkwing wrasse.

Contents

1 Introduction

1.1 Study background

Fish reproduction is a complex process with large variation and diversity. Different fish species have different mating systems, and these systems are generally categorized by the way the sexes interact. Mating systems can be classified as either monogamous, polygamous of various kinds, or promiscuous. In promiscuous mating systems there is no mate choice or within-sex competition for mates, and thus no strong sexual selection. In monogamous species, where one male and one female form a lasting pair bond, or in polygamous species where individuals of one sex mates with several of the other sex; sexual selection may be strong (Campbell et al., 2015). For example, cleaning gobys (*Elacatinus oceanops*) are monogamous, and even if they have been separated they can find the same partner and mate with it again, showing complex mate recognition (Whiteman & Côté, 2004). More complex mating systems can be represented by lantern bass (*Serranus baldwini*) where all individuals are initially hermaphrodites; some transform later into males that establish large harems (Petersen & Fischer, 1986). A common trait for monogamous fish is that both sexes look very alike, whereas the polygamous species are known for morphological differences between sexes (called sexual dimorphism) (Campbell et al., 2015). Sexual dimorphism is a result of the sexual selection (Breed & Moore, 2016).

Sexual selection involves two different processes, the first where one sex chooses the other sex based on specific traits (intersexual selection) and the second where there is a competition within individuals of the same sex for access to mates (intrasexual selection) (Breed & Moore, 2016). Intersexual selection leads to mate choice. As an example, in guppies (*Poecilia reticulata*) the female chooses the male based on the body coloration, and male chooses female based on the size (Dosen & Montgomerie, 2004). There are also many examples where selection have resulted in creation of alternative reproductive strategies within same sex such as territorial males competing for access to females and female mimics that sneak fertilizations. One species with two such male reproductive tactics is the corkwing wrasse (*Symphodus melops*), the topic of this study.

The corkwing wrasse is one of the six wrasse species in Norway, with exception of scale-rayed wrasse (*Acantholabrus palloni*) all Norwegian species can inhabit the same inshore area (Moen & Svensen, 2004; Costello, 1991). Corkwing together with remaining wrasse species being goldsinny (*Ctenolabrus rupestris*), ballan wrasse (*Labrus bergylta*), rock cock (*Centrolabrus exoletus*), cuckoo wrasse (*Labrus mixtus*) are territorial (Darwall et al., 1992; Moen & Svensen, 2004). Territoriality plays a major role in the spawning season, all but one species builds spawning nests; the sole exception being the goldsinny having pelagic eggs and larva (Costello, 1991; Darwall et al., 1992; Moen & Svensen, 2004). Inhabitation of the same area combined with territorial lifestyle can lead to competition both within the species (intraspecific) and between the species (interspecific) based on resources like food or territory itself (Hickman, 2014). In addition, corkwing wrasse, together with the ballan and cuckoo wrasse are displaying the parental care in the nest they build (Darwall et al., 1992).

The corkwing wrasse has a complex breeding system, with two male morphs (Halvorsen et al., 2017; Potts, 1985; Uglem et al., 2001). During recent years it has been harvested to supply cleaner fish to the Atlantic salmon aquaculture industry.

1.2 Corkwing wrasse

The corkwing matures on average between the age of 2 and 3 years, when they reach an average mean length of 100 mm (Potts, 1974; Costello, 1991; Darwall et al., 1992). The size and age at which fish mature greatly differs between populations. Based on the work of Halvorsen et al. (2016) majority of territorial males are mature at the age of 3 with the average size at around 136 mm in Norway. Also, most females mature at age 2 when they are on average around 110 mm, while sneakers become matured at age 1 (Halvorsen et al. 2016). During the maturation, the corkwing goes through morphological changes and develops its distinguish features. The coloration increases in strength during the spawning season, getting stronger in March-April and fades slightly away after the breeding season in late summer (Potts, 1974; Uglem et al., 2000). The older males can be also distinguished from the young ones based on size and strength of pigment in their body, as with age it tends to fade slightly darker (Potts, 1974).

Most commonly the corkwing reaches sizes around 200 mm, there are some bigger individuals but still less than 300 mm (Costello, 1991; Sayer et al., 1996). It is closely connected to shallow coastal areas and are usually found on substrate filled with rocks and eelgrass (Potts, 1985; Costello, 1991). Algae are very important in the areas inhabited by corkwing wrasse since some individuals use it as a building material for their nests. The other reason that algae are crucial in habitat of corkwing wrasse is that it provides habitat to small organisms including crustaceans, gastropods and bivalves, which corkwing is feeding upon (Potts, 1974; Sayer et al., 1996; Costello, 1991).

The spawning season starts in May, the most active month is June and it follows with ending of the season latest in July (Costello, 1991; Skiftesvik et al., 2015). The territorial male starts by building a nest out of algae and then later invites the female to spawn in the nest (Potts, 1974; Costello, 1991). The sneaker male will try to sneak in and fertilize the eggs (Halvorsen et al., 2016). The fertilized eggs are later guarded by the territorial male until they hatch. The time it takes for eggs to hatch is mostly influenced by water temperature, ranging from 7 to 16 day after the spawning (Potts, 1974; Uglem et al., 2001). There is competition for egg fertilization between the male morphs, and the ratio between sneakers and territorial males varies. Based on the studies of Uglem et al. (2001) less than 20% of the mature males are sneakers.

Figure 1. A mature territorial male corkwing wrasse with building material in the mouth for nest building or repair. Photo: Maciej Karaszkiewicz

The mature male can follow two alternative reproductive strategies (often called two morphs). The first strategy is to be a territorial male who builds and defends its nest where he spawns with female (Halvorsen et al., 2016; Uglem et al., 2001). The territorial morph is on average larger than the other male morph and is more colourful (figure 1). There is large individual variation in colours and patterns, with blue, green and purple/red colours dominating. On the other hand, the second male morph can be classified as a sneaker (having a sneaker breeding tactic). As an alternative reproductive strategy, they exhibit female mimicry and are often impossible to distinguish from females (Uglem et al., 2001). The only time one can see a difference is during the spawning season based on presence of milt or eggs. Both sneaker males and females are darker in colour than the territorial male, with colours mostly consisting of brown, grey and charcoal (figure 2).

Figure 2. A mature female corkwing wrasse during the spawning session. Note the enlarged abdomen and external part of genitalia (genital papilla). Photo: Maciej Karaszkiewicz.

The possibility of aggressive behaviour has been observed, such as the territorial males tending heavily to chase away all intruders beside females (Potts, 1974). At the same time both male morphs have been found with flesh wounds and missing scales (Halvorsen et al., 2017). The agonistic activities may force the immature to swim away far from nesting grounds (Potts, 1974). Those observation show strong intraspecific selection for the egg fertilization within the males. Unfortunately, there is no knowledge of the intraspecific interactions within females.

1.3 Fish size

Fisheries often target the largest, fastest growing fish rather than the small, slow growing ones (Browman, 2000; Rowe & Hutchings, 2003). In addition, fishing is more likely to capture more active individuals; the higher moving activity the higher chance to be caught by fishing gear (Rowe & Hutchings, 2003). The study of Halvorsen et al., (2017) found a tendency for male-selective harvesting, a tendency that is mention later in this paper.

Corkwing wrasse is known to feed upon salmon louse (*Lepeophtheirus salmonis*) and is harvested for that characteristic to be utilized in aquaculture. In 2019, 19 million wrasse individuals were caught in Norway (Appendix; table 1), where around 40% was corkwing alone. The minimum size for corkwing wrasse harvesting was in 2019 set to 120 mm (Appendix; table 2), meaning that every individual longer than 120 mm was in danger of being harvested.

1.4 Aim of study

My thesis is a part of a larger research project on the population dynamics and life history strategies of wrasses (Halvorsen et al. 2017). During four years a specific area has been chosen where different studies based on wrasse biology have been performed. For example, that includes projects that investigate the wrasse movement (Aasen, 2019) and growth among the fish (Vik, 2019).

My part of the project was to investigate in some detail the reproductive behaviour of the corkwing wrasse. I started with capture recapture methods and tagged the fish with Passive Integrated Transponder (PIT) tags. Then for weeks I monitored the nests of territorial male corkwing wrasse using RFID antennas and video cameras. The area I have been working on have been closed for commercial fishing and I was thus observing the fish in their undisturbed habitat. The aim was to study the intraspecific and interspecific interaction within the nests, as well as quantify unique activities performed by each observed territorial male. At the same time, I was investigating the importance of female length on the number of visited nests. I was also interested in finding any strategies sneaker males utilize. Lastly, I was observing if any other wrasse species would approach the nest belonging to territorial male.

2 Material and Methods

2.1 Field work

2.1.1 Study site

All the field work was conducted at Austevoll, south of Bergen, western Norway. The area is an archipelago consisting of hundreds of islands. I was working at the Austevoll research station, owned by the Institute of Marine Research. Gathering of the data happened around a relatively small island called Saltskjærholmane (figure 3). The area is protected from commercial fishing and up to five species of wrasse have been PIT-tagged in a capture-recapture survey established in 2017. I studied reproductive behaviour of corkwing wrasse in the bay, which is in the middle of Saltskjærholmane, where the density of corkwing nests is particularly high (see the red ring of figure 3).

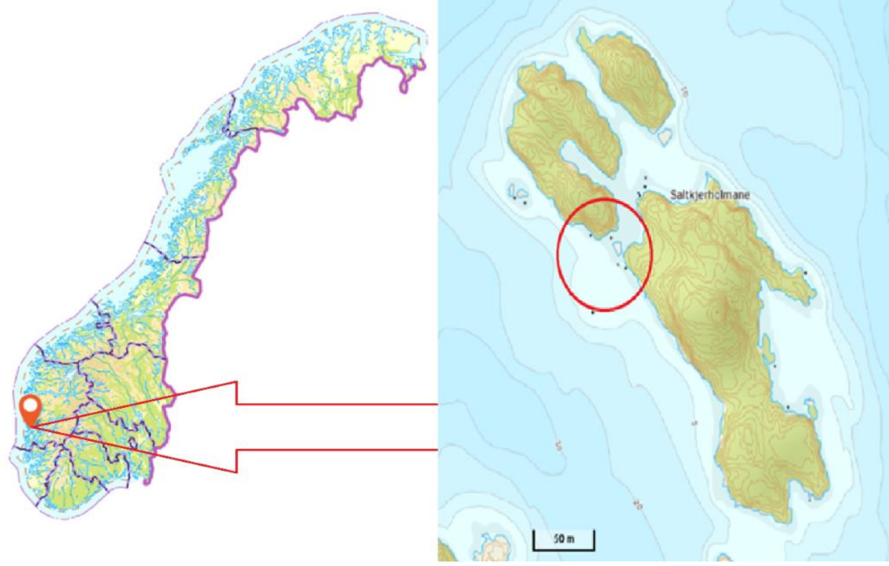

Figure 3. On the left site there is a map of Norway with marked point that is enlarged to the right showing the island Saltskjærholmane I was working on. Notice the red ring showing the sector where monitoring and filming have been conducted. ©kartverket/norgeskart

2.1.2 Fish sampling and tagging

As explained in the introduction, this master thesis is a part of a larger project (Halvorsen et al. 2017). The project started in the fall of 2017 when researchers started with fish sampling and tagging; the fish were identified, measured, weighed and marked with a tag unless it was already tagged before. Then in 2018 from May through June and September sampling and tagging took its place again, just like in the previous year. Capture-recapture study was happening upon three protected islands in Austevoll, one of which is the same one I have been working on in 2019. The previous research and all the methods are well described in the two master theses published in 2019 (Aasen, 2019; Vik, 2019).

During one week in May 2019 an intensive scientific sampling was performed in order to tag as many fish as possible in the study area. The fish were captured using fyke nets, that were placed in specific areas around the island for no longer than 12 hours. After the time in water the nets would be taken back on boat one by one, each time the caught individuals would be segregated and treated accordingly to the species. All non-target animals were noted, measured to the nearest mm and then released on the spot. The wrasse which was caught was moved to the temporary tank. First every individual was checked if it was captured before. If positive, they were only measured, checked and released. If negative, the wrasse was anesthetized in a 5 L tank containing a sea-water solution with 50–100mg l-1 Finquel (Ethyl-3-aminobenzoate methanesulfonate - MS-222). When possible, species where visually sexed, and during the spawning season wrasses were gently massaged on the abdomen and backwards to the anal area to assess the presence of eggs or milt. This allowed us to assess whether an individual was ready to spawn, and if so, precisely distinguish females from sneaker males (Halvorsen et al 2016).

Fish larger than 100 mm were tagged with passive integrated transponder (PIT) tags; a small glass capsule with measurements of 12 mm x 2.12 mm and weight of 0,1 g, that reacts with compatible antenna (monitoring system together with PIT tags from RFID solutions). The tag was inserted into the abdominal cavity with marking pistol. After tagging, the fish was moved into a recovery tank. The fish were released when it showed normal swimming behaviour. The whole procedure is described in detail in the work of Halvorsen et al., (2017). The application for the process of sampling and tagging was granted before the field work took place based on the FOTS-ID 15307.

2.1.3 Nests monitoring

The north-east part of Saltskjærholmane was used for monitoring of corkwing nests (see figure 3). The nests were all associated with some hard substratum on bed rock or under a larger rock, often together with dense thread algae with kelp here and there. There all the monitoring devices were deployed. The area was investigated by snorkelling, and all visible territorial corkwing nests were identified and marked with a rock, a rope and a buoy. After mapping the nests, the RFID-monitoring system was deployed to enable in-situ detections of PIT-tagged fish in the proximity of the nests. The system consisted of logger units; plastic boxes which each contained two RFID readers, operated by a Raspberry PI computer. The system was powered by Lithium batteries that was changed every day for continuous monitoring throughout the study. Four RFID antennas were connected to each box. Each antenna was circle-formed with diameter of 50 cm and was equipped with a 20-meter cable, that was the maximum length from the shoreline to the antenna. The antennae were placed around the nests (see figure 4 as an example) using snorkelling. The range of detection was tested in an artificial pool, for the detection to be registered the PIT-tag had to cross the ring radius. Outside of the ring, the detection was also registered to check if the PIT-tag was near the antenna, within no more than two cm from any ring part.

The detections from the antennas were saved on a SD card in the logger unit. At the same time the information was available online through a 4G internet connection. I was thus able to monitor detections in real time. It was extremely useful as I could see if the territorial male was present and if it had visits. Every nest and its territorial male were checked if it was tagged (by observing the live detections sent over 4G to a mobile device). If it were, the antenna would stay there. If not, I would move it to the next candidate nest. In a few cases, the male would disappear, leaving the nest abandoned. When that situation occurred, the antenna was moved to the new nest.

2.1.4 Nest filming

The camera rigs were built using a flat plastic platform with weight blocks attached to the bottom. A camera holder was mounted on the top of the platform and connected to a rope for balanced submerging. Every rig was attached to a buoy for identification and later retrieval. Cameras used in the experiment were standard GoPro 6 and 7 hero black. I used two different camera set ups. One set-up contained a GoPro with its own battery that allowed filming for one and half hour. The other set up was equipped with an external power-bank that made it possible to film up to 15 hr continuously. Nests were filmed during eight days in the period 6th - 23rd of June 2019.

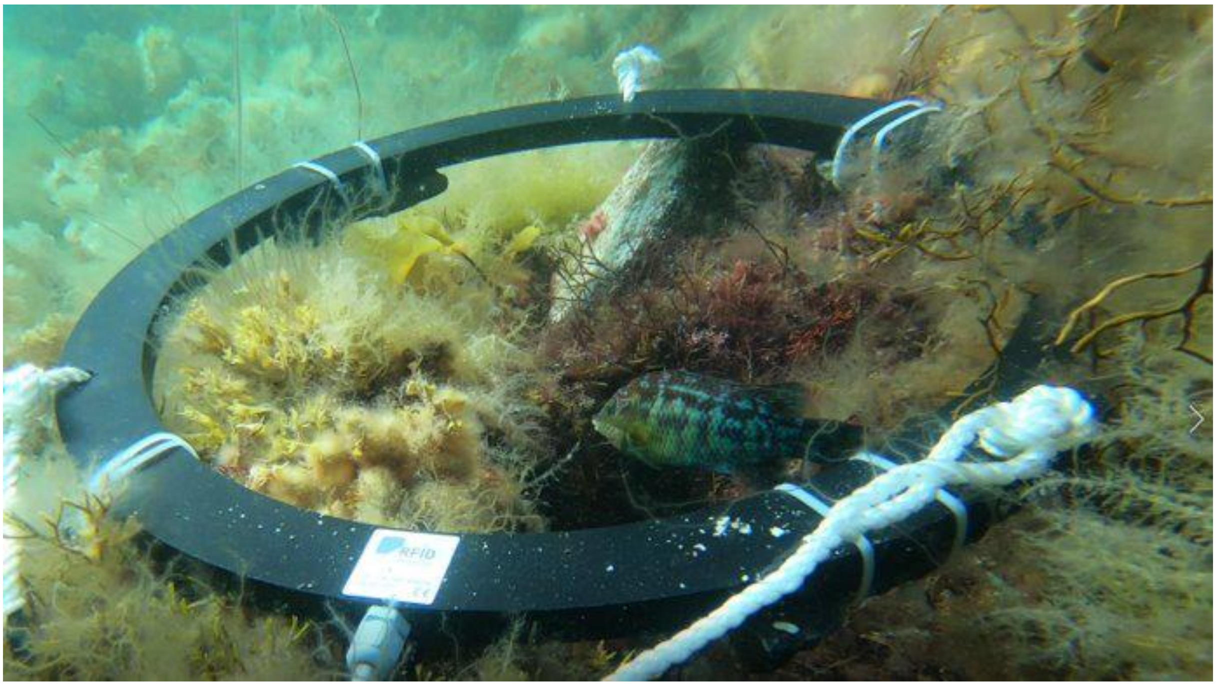

Figure 4. The underwater set up where the nest is in the middle, protected by a territorial male shown in the picture. The black circular ring is the antenna itself; every time a marked fish crosses it the detection would be registered. Photo: Maciej Karaszkiewicz.

For later synchronization of the video recording together with information retrieved from PIT-data a reference PIT-number (432091) was glued to the end of the stick (called “reference stick”). When a camera was placed underwater, I would put the top of the stick in the middle of the nest. Making it clearly visible in the camera, at the same time the reference PIT-number was also registered and saved by the antenna. After synchronization it allowed me to read the information's of the tagged individuals seen in the video.

2.2 Analysis

2.2.1 Video data

All the movies from the cameras have been sorted on a hard disk based on the nest id, the day when it was filmed and camera number. To analyse behaviour in those videos, I used a computer program called Behavioral Observation Research Interactive Software - Boris (Friard & Gamba, 2016). First, I had to check every video for specific factors and qualities. The selection system I created have been based on those three categories:

- Video quality (grade from 1 to 3) - the grade 1 means the nest was not visible. The grade 2 means the nest was partially visible and the grade 3 means that whole nest entrance was included in the film. Only the grade 2 or 3 videos have been analysed.
- The territorial male was tagged with PIT-tag.
- There was at least one visitor beside the territorial male that was tagged with PIT-tag during the whole movie.

After the selection I ended up with 22 videos. Before analysis I had to program the events I was interested into Boris, here are how I defined them:

- Male present or not – the territorial who build the nest is within the antenna ring included in the shot.
- Female visit – female corkwing wrasse cross the antenna ring and is inside the nest. Only used if the data from PIT-detections said that it was in fact a female or based on the spawning behaviour, without the confirmation I would note non-territorial corkwing visit.
- Other fish present – when other fish visit the nest. This might include a territorial male who is not the resident of the nest, a sneaker male, a goldsinny and any other species like pollock or ballan wrasse. The program was set up in a way that I could use under-categories and write specific categories based on species or gender.
- Building – defined when the territorial male leaves the nest and comes back with algae in its mouth that he incorporates into the nest structure.
- Spawning – defined here as a point event, where the female swim into the nest entrance abdomen first and supposedly releasing the eggs, followed immediately with the territorial male performing the same behaviour supposedly releasing sperm. Since it was not possible to see the release of sperm or eggs directly due to its small size, the behaviours are inferred. In practise I assume that not every interaction of this sort ends up in release of eggs and sperm.
- Sneaking- a sneaking male trying to release sperm and fertilize the eggs in the nest belonging to the territorial male.
- Chase – when the territorial male chase something, but not always possible to see what he is chasing. Sometimes it is happening within the ring and sometimes outside. This event starts with reorientations of the body into a specific direction followed by swimming with high acceleration rate.

- Fanning – the wave like motion of the territorial male who is taking care of the fertilized eggs in the nest.
- Maintenance - when the territorial male moves algae within its nest and rearrange the structure or fixes small supposedly damages.
- Egg predation - an individual swimming into the nest entrance headfirst and trying to consume the eggs that the territorial male is taking care of.

After all selection processes were over, I could prepare every video for analysis. To do that I had to synchronize each video with PIT-data individually. Based on the "reference stick" I knew exactly when each video started. Knowing that I could extract the PIT-detections from PIT-data based on the nest-ID, the day it was filmed and unique reference time. The file was then saved in a table format and incorporated into the video through Boris. As a result, I was able to put real time into the video (in format hh:mm:ss) and get information of every tagged fish as they were appearing in the film.

When all those steps were done, I analysed every video in Boris. Saving each summary with information of what happened, when and how often. The extracted data were saved as time budget, observation lists and plots visualizing summary of what happened in every analysed video.

For the investigation of the spawning length I extracted observation lists form Boris. I selected only for the events called "female visit" and "spawning", further selecting only female visit that resulted in spawning (spawning visit). The start of spawning visit is defined as female entering the nest and the end with female leaving the nest. I will also calculate the time between female approach and the first spawning cycle. Then I will measure the length between the spawning cycles for the females with more than one cycle. Last measure will be the time it takes for the female to leave the nest after last spawning cycle.

2.2.2 Estimate of corkwing wrasse length

Of the 210 individuals detected at the antenna system, 89 had been captured in 2019, just prior to deployment and therefore had observations of size in 2019. 121 corkwings were seen last time in 2017 or 2018, and I had to predict the expected length in 2019 based on capture-recapture data (based on May 2019). I used data that were provided to me from the earlier mentioned project of Halvorsen et.al (2017), to calculate the fish growth estimate. Gathered 720 (405 females, 276 territorial males and 39 sneaker males) corkwing wrasse were recaptured and had been calculated with its growth based on the length difference between periods. I plotted the length at the recapture against the previous measurement at given past period (p-value <0.001).

To check how the estimate was predicting the length, a test was conducted. 155 (98 females, 41 territorial males and 16 sneaker males) corkwing wrasse have been recaptured in the period of summer 2019 and the observed length at the time was compared to predicted estimated length (see figure 5). The estimate predicts the lengths well and was used to update length of all detected corkwing wrasse from the period of summer 2019. It is worth noticing the estimated length for small males appears to be overestimated, but this is not a major problem since nesting males are much larger and small males are rarely detected by the antenna system.

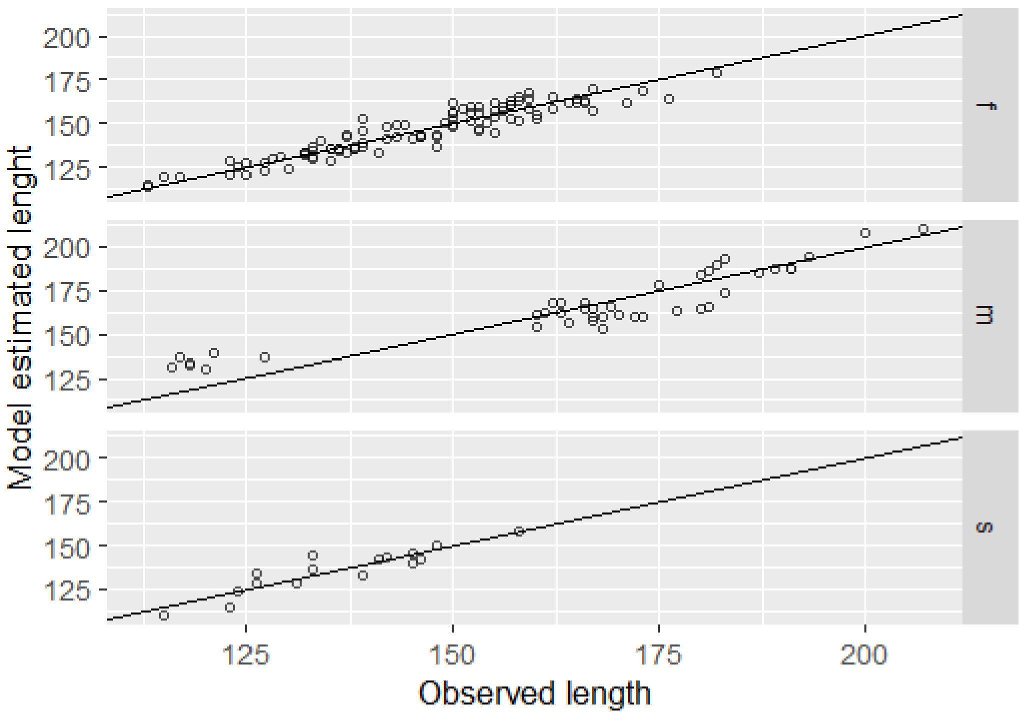

Figure 5. Comparison of the observed length against predicted length, the line symbolizes the growth estimate and the points belong to recaptured individuals with length measured. The total number of fish in the graph are 155 corkwing wrasse, consisting of 98 females (f), 41 territorial males (m) and 16 sneaker males (s).

2.2.3 PIT Data

I gathered all PIT detections from the outside computers into a hard disc. To use all the information from the antennas, there was a need to create a data set with all detections of marked fish. The program used for that job was R software version 3.6.0 (R Core Team, 2018) and RStudio (version 1.1.463 (Rstudio, 2016)), where all data were sorted. For the creation of graphs, I have chosen to use ggplot2-package (Wickham, 2016) and MASS-package (Venables & Ripley, 2002). For analysis of summary statistics (comparing chosen data by variables) I used doBy-package (Højsgaard & Halekoh, 2018).

As a rule of thumb when there is a detection of a PIT number belonging to specific marked territorial male, I know he is in the nest at that moment. When I see a new PIT number belonging to a marked female, I know someone is visiting the nest at the moment. The antenna can register only one PIT-tag at once. If there are more tags in the field, it will record the tag with the strongest signal based on the orientation and distance of the tag relative to the detection field (strongest inside the antenna ring). When two or more tagged fish is moving around the antenna (e.g., during spawning), we expect an alternating sequence of detection of different tags. As I described before, the centre of the ring was the nest entrance (see figure 4) and the spawning event starts when a female swim into it and immediately after the activity is repeated by the territorial male.

With that understanding, I programmed R to seek for estimated spawning, where the male tag is followed by female tag and then male tag one more time (within 4 seconds). I classified it as an assumed spawning cycle called “PIT-spawn”. Every repetition of that sort would be noted as additional spawning cycle.

I have performed a test to check if the decided spawning “rule” was correctly identifying spawning events. I manually analysed the spawning events from the videos (called Video-spawns) and based on the synchronized time I was able to compare those values together with corresponding PIT-spawns. The last step was to calculate the percentage of correct predictions and update the algorithm accordingly to it. That information may help with understanding how the estimate work and may be used to add more cycles to define an interaction as a spawning.

Length was either measured in May of 2019 or estimated accordingly to growth model described in the previous subchapter. My observations are what is called count data, referring to variables that cannot be less than zero and are well tested with linear models. Every model has been checked for the fit for the residuals with Residuals vs Fit visualization of the model. When the residuals from the model are scattered relatively evenly around the level 0, it is possible to assume homogenous variance and normally distributed error; being a good indicator to use simple liner model test. When the residuals from the model are scattered in different forms representing heterogeneous variance and non-normally distributed error, that indicates need for generalized linear model (glm) test. For the data tested with glm function I decided on choosing negative binomial regression as it works much better with the overdispersion data.

To investigate the influence on female length on nests visits, I first tested whether the total number of different nests visited could be predicted by female body size using a simple linear model. Secondly, I used a glm to test the effect of female body size on the total number of detections.

Based on the spawning estimate I was able to investigate the relationship between number of PIT-spawns and the female length. I used a simple linear model test to the relationship between the number of spawning cycles and fish length. The goal was to see if the cycles were evenly distributed among the length groups. Lastly, I checked for the relationship between the number of estimated spawning and the female length by using the glm.

3 Results

3.1 Data overview

Out of 18 checked nests only 11 were defended by a marked territorial male and could be used in the analyses. Three males (ID 424801, 423562, 430918) built two nests each. The transition between the nests happened in the middle of June. I gained a large dataset containing 1745506 point observations based on PIT-tag detections from 11 nests belonging to 8 territorial males (see table 1). The average number of PIT-tag detections per day was estimated to be 7780 detections. Five of the nests had an average of between 5000 to 6000 detections per day, while three nests had under 2500 detections and three nested over 10000 detections. In addition, 7 nests were observed with the underwater camera system, both as a control to the monitoring with antennas and to analyse visible behaviour and observations. From now on when I refer to "PIT-data" I mean the data I gathered from the antennas. And when I refer to "Video-data" I refer to video data analysed using Boris.

Table 1. Summary of PIT-data based on the PIT-detections gathered throughout the summer of 2019. Every observed nest has its own specific Nest-ID and identity of the territorial male (Male PIT-ID). Every male has its own length in mm, either checked or calculated accordingly to growth estimate. The dates that each antenna was working, the total number of detections and the average number of detections per day is given. The total time a nest was filmed is also included.

Nest-ID	Male PIT-ID	Length (mm)	Antenna working from	Antenna working to	Total PIT data obs.	Average PIT/day	Video (hh:mm:ss)
1	423723	164.6	06.06	16.06	51674	5167	01:29:04
2	424801	201.4	06.06	15.06	51817	5757	-
4	423562	182.0	07.06	17.06	17084	1708	-
6	430918	187.0	06.06	17.06	114020	10365	-
8	424556	165.0	06.06	10.07	624410	18365	04:14:08
9	209104	183.7	19.06	10.07	51349	2445	06:26:40
10	430918	187.0	17.06	10.07	543254	23620	09:35:22
11	67765	205.2	17.06	10.07	36685	1595	-
12	423562	182.0	17.06	22.06	27674	5535	03:20:24
13	424801	201.4	17.06	10.07	133637	5810	30:53:08
17	210333	190.6	22.06	10.07	93902	5217	06:42:45

All-together, based on the 11 analysed nests, a total of 233 individually marked (including the 8 territorial males) fish have been detected, the majority being corkwing wrasse (see table 2). The majority of the corkwing wrasses were females (166 individuals), followed by territorial males (32 individuals) and sneaker males (12 individuals). Sneaker males thus constituted 27 % of the observed males.

Around 9 % of all observed corkwing wrasse were smaller than 120mm, which is the minimum size limit used in the commercial wrasse fishery. All territorial males and approximately 66 % and 90 % of sneakers and females respectively would have been exposed for harvesting.

Table 2. Number of different individually tagged wrasse species detected by the PIT-detectors with mean length (mm) and standard deviation is also given.

Species	Mean length	Standard deviation	Nr. of individuals
Ballan	196.9	110.5	7
Corkwing	141.3	17.4	210
Cuckoo	109.5	4.9	2
Goldsinny	109.6	10.6	9
Rockcook	163.6	11.9	5

A total of 23 tagged individuals of other wrasse species visited the nests of territorial corkwing wrasse (see table 2). It could be also confirmed that all five wrasse species were present at least once in the Video-data. Beside wrasse, some other species were also observed in the videos. The only species that clearly interacted with the corkwing wrasse were the goldsinny. There have been occasions where the female corkwing would approach the nest and would be chased away not by the territorial male but by a goldsinny. Five out of 19 tagged females that approached the nest were chased from it by a goldsinny. All five were chased by goldsinny while the territorial male was taking care of the eggs. The female visitor would be chased away very aggressively by the goldsinny.

3.2 Reproductive behaviour of the territorial males

The reproductive behaviour of the territorial males could be described based on video observations from seven individual nests. The plan was to start monitoring and filming by the end of May, however the water was very unclear due to the strong algal bloom. The project has been postponed with almost two weeks until water visibility stabilized. With water improvement the project restarted on 6th June, then however many fish were already spawning or even taking care of the eggs (see figure 6 and 7 as examples of two time-budgets). Based on the observations of all the videos, I suggest three stages of behaviour of a territorial male:

Building - when the territorial male gathers algae from surrounding areas and builds its nest in a specific place. During this time the male is not ready to spawn and chases every female away. In practice the state can be observed only after the territorial male starts building and there is visible nest structure. The structure varies from individual to individual, mostly in size and where the nest is placed.

Spawning - after the nest is built the territorial male goes into the most active stage. The male waits for a female to approach the nest and start spawning. There is some sort of interaction before spawning itself (here meaning releasing eggs and sperm) that the two fish perform, to some degree it looks like a dance. After the "dance", the female enters the nest entrance with the abdomen and release her eggs into the nest. If that happens, the territorial male follows with the same behaviour to release sperm. Some fish do it once, others repeatedly. The female may come and go, sometimes there is spawning and sometimes only visit. The other part in this stage are sneaker males trying to fertilize eggs in the territorial male nest. It takes a few days until the territorial male goes into the next and final stage.

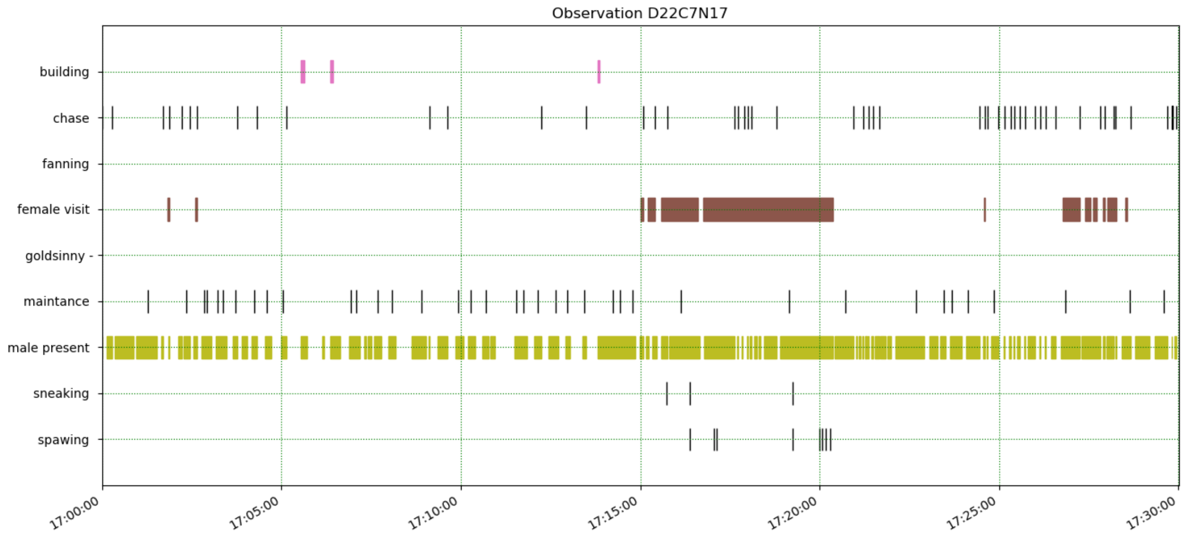

Figure 6. Time budget of a territorial male. Chosen as example 30 min cut of time budget from the territorial male with ID21033 and length 190,6 mm in the spawning stage, video was filmed 22 June 2019. The x-axis is the synchronized time ranging from 17:00:00 to 17:30:00 in the format of hh:mm:ss. The y-axis is the categorized events.

Fanning - the wave like motion the territorial male performs in front of the nest most of the times, guarding the eggs and sustaining them with fresh oxygen-rich water. This stage often includes other minor activities like maintenance (and the building) of the nest, while also potentially pushing the eggs deeper into the nest for protective reasons. Other common activities are observation of the surroundings, most often the male lifts its head up and looks; then comes back to fanning (unless a threat is detected). At this stage, the male is chasing every female away to signalize (hypothetically) he is done with spawning and takes care of the eggs until they hatch.

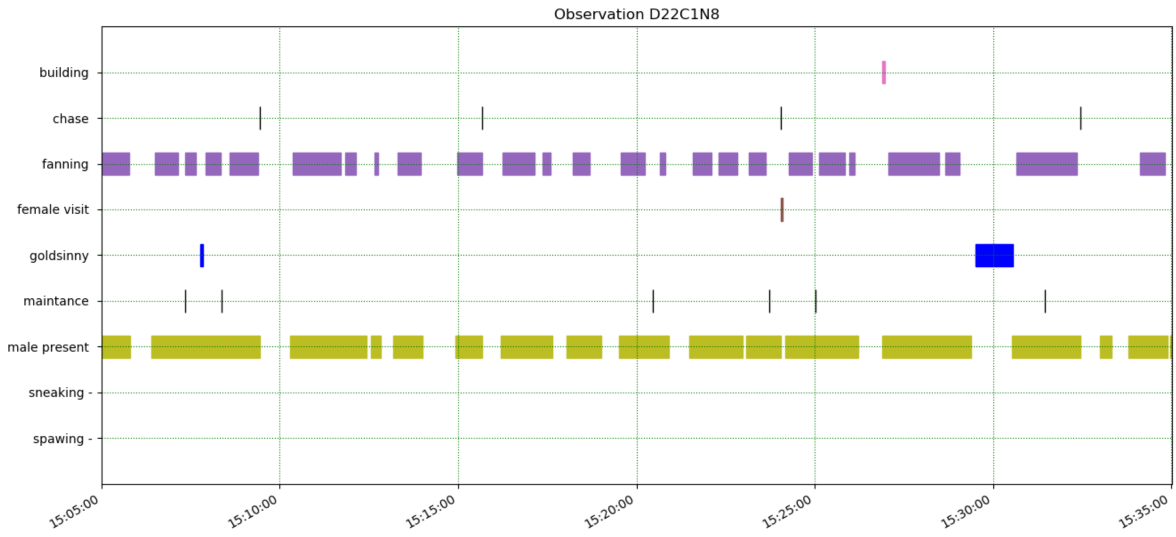

Figure 6. Time budget of a territorial male. Chosen as example 30 min cut of time budget from the territorial male with ID424556 and length 165,0 mm in fanning stage, video was filmed 22 June 2019. The x-axis is the synchronized time ranging from 15:05:00 to 15:35:00 in the format of hh:mm:ss. The y-axis is the categorized events.

To present numbers of observed behaviours and interactions I gathered all observations that were analysed and then quantified using Boris. I have chosen only videos with best quality (quality 3 presented in table 3), making a summary of events that occurred during spawning and fanning stages; based on 15 independent video-analyses. I have chosen to present the data as average, where total number of each event have been divided by the number of videos. In both stages, territorial males have been at the nest most of the time; slightly longer in the fanning stage. Main activity of the fanning stage is taking care of the eggs (event “fanning”), and the territorial males spend most of their time performing it. The spawning stage is however rich in interaction with females visit and chases of other fish away including sneakers, unwanted females, and other species.

Table 3. Observations of spawning and fanning stages, results are shown as a mean of total number of the event divided by the number of video observations.

Territorial stage	**Spawning**	**Fanning**
No. of videos analysed	2	13
Total length of all videos	1:57:00 (hh:mm:ss)	18:33:29 (hh:mm:ss)
Male present at nest (%)	73.35	81.2

Male fanning (%)	2.85	53.8
Male building (%)	1.1	0.1
Maintenance	65.5	47.2
Chase	64.5	16.3
Female visits	45	3.7
No. of tagged females	4.5	1.8
Spawning cycles	32	0
Sneaking events	2	0
Egg predation events	0.5	0
Goldsinny visits	0	4.5

3.3 Reproductive behaviour of sneaker males

None of the tagged sneaker males was observed in any of the selected videos. During the analysis I observed two videos that included the behavioural definition of the sneaker: a non-territorial corkwing approaching the nest and try to sneak in, when the territorial male is spawning with a female. Eight out of ten observed sneaker events happened shortly after spawning between the territorial male and the female (see table 4). The average time between spawning and sneaking was 1.75 seconds.

Table 4. First column shows the time of spawning between territorial male and female, the second column shows the corresponding time of sneaker male attack. Here are eight out of ten total sneaker attack observations, when the sneaker tries to fertilize the eggs shortly after spawning. The last column refers to Video-ID with the information of the day it was filmed, the camera number and the Nest-ID

Time of spawning event	Time of sneaking event	Video-ID
19:32:17	19:32:18	D17C1N10
19:33:50	19:33:50	D17C1N10
19:34:46	19:34:50	D17C1N10
19:53:47	19:55:48	D17C1N10
19:55:18	19:55:24	D17C1N10
19:56:03	19:56:03	D17C1N10
17:16:22	17:16:23	D22C7N17
17:19:15	17:19:16	D22C7N17

3.4 Analysis of female visitations

Firstly, I analysed the length of the visit based on the Video-data. I have observed 17 independent spawning visits with the mean length of 74 seconds in total. The shortest visit was only 1 second and the longest 266 seconds (see figure 7). Few of observations were over 100 seconds and really skewed the mean (around 70 seconds), the median for all observations was around 45 seconds.

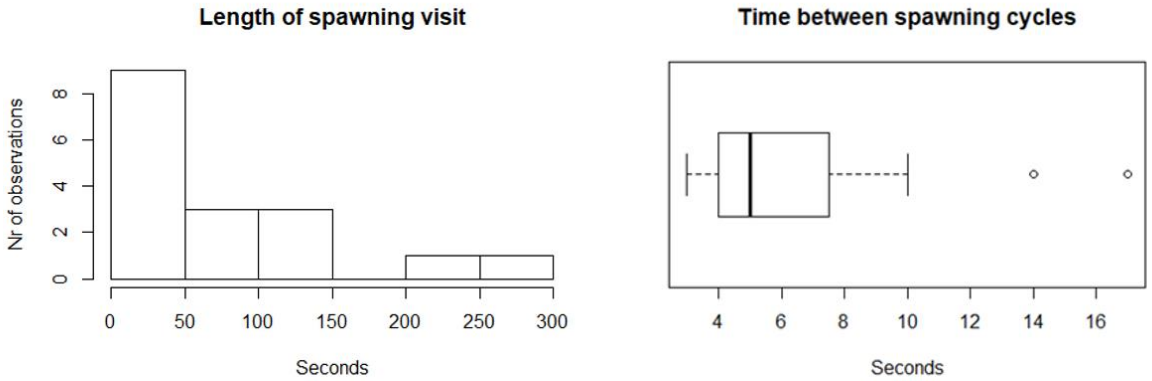

Figure 7. The histogram to the left shows the variation in the time for 17 observations of individual spawning visit, here grouped in the 50 seconds interval. The boxplot to the right shows the observation between the spawning cycles, here included 39 observations from the group where all intervals have been under 20 seconds.

Out of 17 spawning visits, 11 had more than one spawning cycle per visit: making up the total of 47 cycles. The time between the cycles have been calculated, the majority (39 observations) happened with less than 20 seconds between the cycles (see figure 7). The shortest time was 3 seconds and longest 17 seconds, with the median at 5 seconds and the total mean in that group was 6 seconds between each spawning cycle. The majority of the females started spawning after a short interaction with the territorial male and they left shortly after the spawning (see figure 8). The median for the start of spawning is at 13 seconds and the mean at 20 seconds, the minimum being 1 second and the maximum 74 seconds. The median for the leaving of the nest after last spawning is at 5 seconds and the mean at 6 seconds, the minimum being 0 seconds and the maximum 15 seconds.

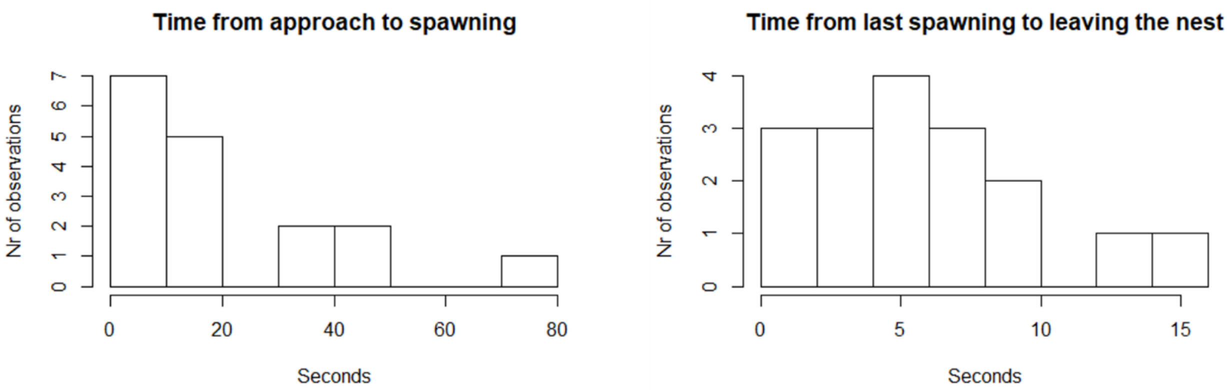

Figure 8. How quickly the female starts spawning after approaching the nest is visualized in the histogram to the left. How long it takes for a female to leave the nest after last spawning cycle is visualized in the histogram to the right. The total number of observations for each histogram is 17 independent observations.

The relationship between female body length and nest visitations

A total of 166 individually tagged females were observed visiting one or more nests. I tested if female size influenced nest visitation rate (see figure 9). The maximum number of visited nests was 11, and only two females visited all the nests. Most visited from three to five nests with the mean being around 3.5 nest per female. There was a significant, decrease in the number of nests visited by the larger females (see table 5 and figure 9).

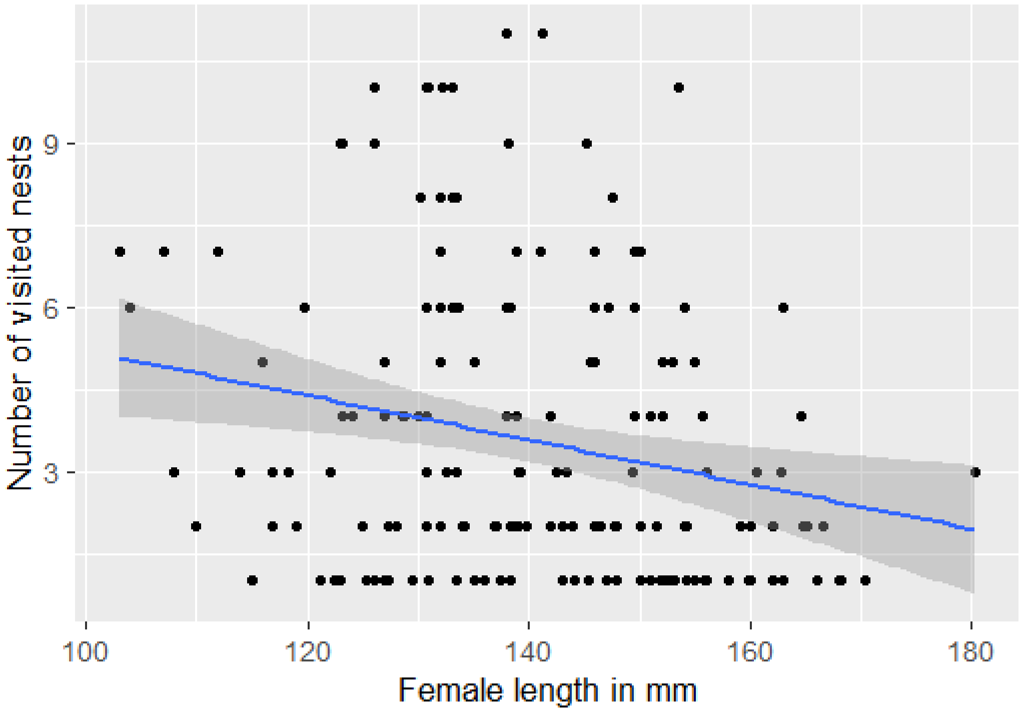

Figure 9. The number of visited nests plotted against female length (mm) (n=166). The regression line shows a declining pattern with the female length. The confidence interval is shown as well. Statistical data included in table 5.

Table 5. Summary of coefficients from the linear model between the female length and number visited nests (n=166). Visualization of the variables in figure 9.

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	9.27	1.93	4.80	<0.001
Length	-0.04	0.01	-2.96	0.0036

The relationship between female body length and nest visitations total number of reads

Using every detected female (166) I have tested if the body size and the total number of reads could have been correlated. Testing the hypothesis using the generalized linear model with negative binomial structure, resulting in significant relationship between the variables (see table 6 and figure 10)

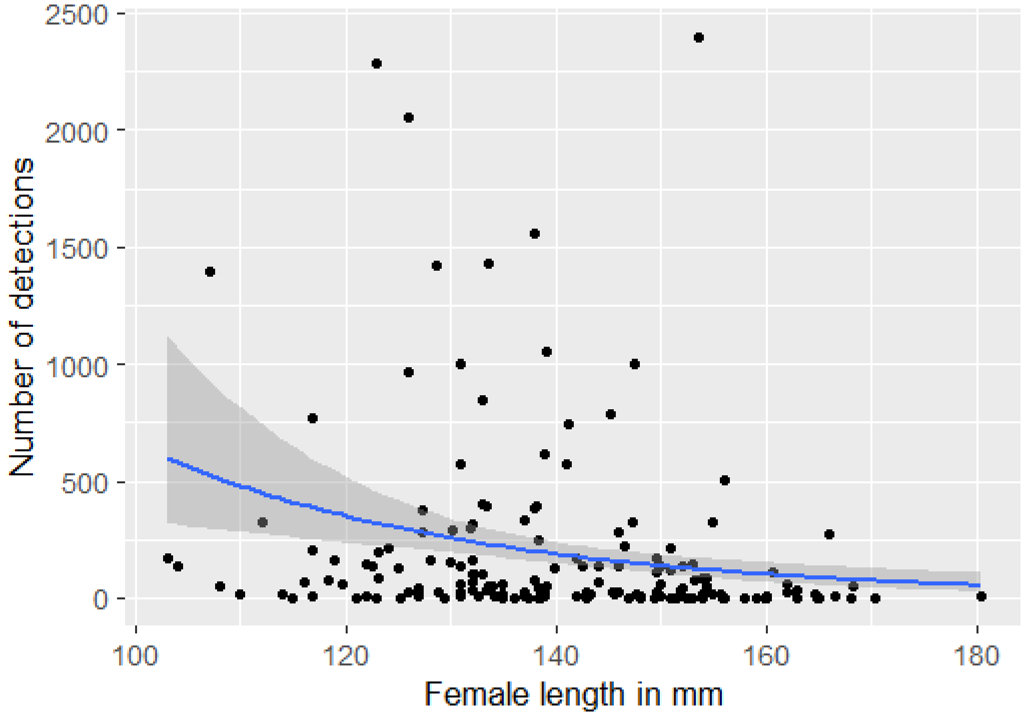

Figure 10. The total number of detections plotted against female length (mm) (n=166). The regression line shows declining pattern with the female length. The confidence interval is shown as well. Statistical data included in table 6.

Table 6. Summary of coefficients from the generalized linear model with negative binomial structure between female length and total amount of visited nests (n=166). Visualization of the pattern in figure 10.

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	9.60	1.14	8.39	<0.0001
Length	-0.03	0.01	-3.82	0.0001

3.5 Inferring spawning events from pit-telemetry

With the aim to train the program R how to recognize spawning behaviour based only on the data from PIT detections, I created an algorithm. Based on the exchange of PIT-tag numbers between territorial male and a female during short interval I predicted spawning. To check how the algorithm worked, I compared PIT-spawns (predicted spawning) and the Video-spawns (observed spawning). In this context, a “spawn” refers to one spawning cycle. In total, it was possible to link and compare 58 predicted spawning events from the PIT-data and the corresponding Video-spawns based on the synchronized time. Out of 58 PIT-spawns, only 23 were classified as correct (figure 11).

Most of PIT-spawns in category 1 and 2 were false positives, meaning that the algorithm categorized an interaction as a spawning whereas there was no observed spawning in the

Video-data. On the other hand, most of PIT-spawns in category 3 and every PIT-spawns higher that category 3 were true positives, where predicted spawning matched observed spawning in the Video-data. In addition, there was no false negatives. Every single observed spawning was predicted by the estimate. To increase the strength of the algorithm I decided to include only observations with 3 or more PIT-spawns.

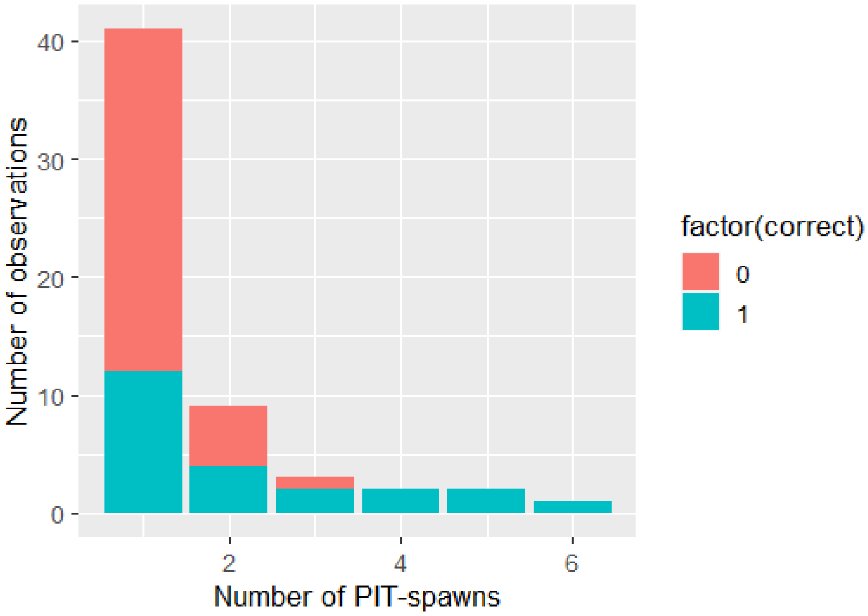

Figure 11. Histogram visualizing the comparison of PIT-spawns (estimated number of cycles) to Video-spawns. The y-axis shows the number of observations and the x-axis the number of estimated PIT-spawns. Factor 1 represent true positives and the factor 0 represent false positives. Out of 58 predicted spawning, only 23 were classified as correct. The PIT-spawns under the value of 3 (under 3 estimated cycles) is comprised of mostly false positives.

Before moving further, I tested if there was a relationship between the mean number of PIT-spawns and female length (see table 9). There was no relationship between number of PIT-spawn and female length.

Table 9 Summary of coefficients from the linear model between number of spawning cycles and female length (n=61)

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.017	0.91	5.51	<0.001
Length	-0.01	0.01	-1.20	0.235

Using the more restricted version of the algorithm (3 or more cycles) during the monitoring period I classified 61 spawning females. Figure 12 shows the relationship between number of spawning and female length. I used a generalized linear model with negative binomial structure to test for a relationship between female length and number of spawning (see table 10). There was slight, but marginally non-significant, decrease in the number of total spawning for the larger females.

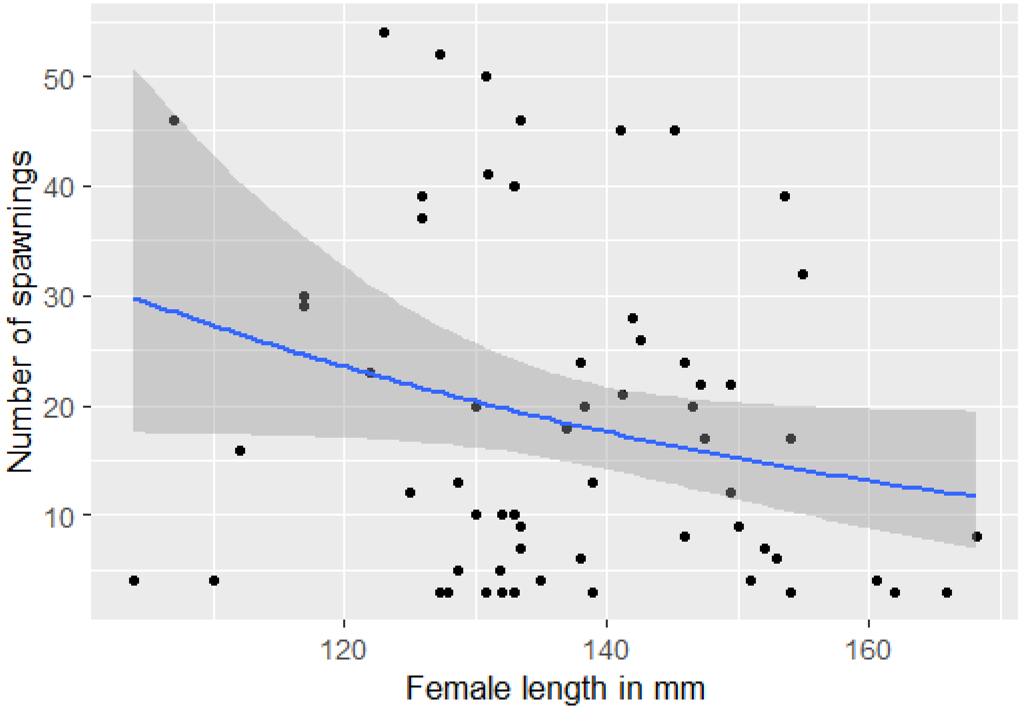

Figure 12 The total number estimated spawning per female plotted against female length (mm) (n=61). The regression line shows small declining pattern with the female length. The confidence interval is shown as well. Statistical data included in table 10.

Table 10 Summary of coefficients from the generalized linear model with negative binomial structure between total number of spawning and female length (mm) (n=61). Visualization of the variables in figure 12.

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	4.92	1.05	4.68	<0.001
Length	-0.01	0.01	-1.91	0.056

Last I wanted to extract the data showing when and how much estimated spawning was occurring throughout the whole monitoring period based on the updated algorithm. Figure 13 visualize all observations of estimated spawning, here showed as total spawning estimate per day; clearly showing three peaks. There is 18 days between the first and second peak, and 14 days between the second and third peak.

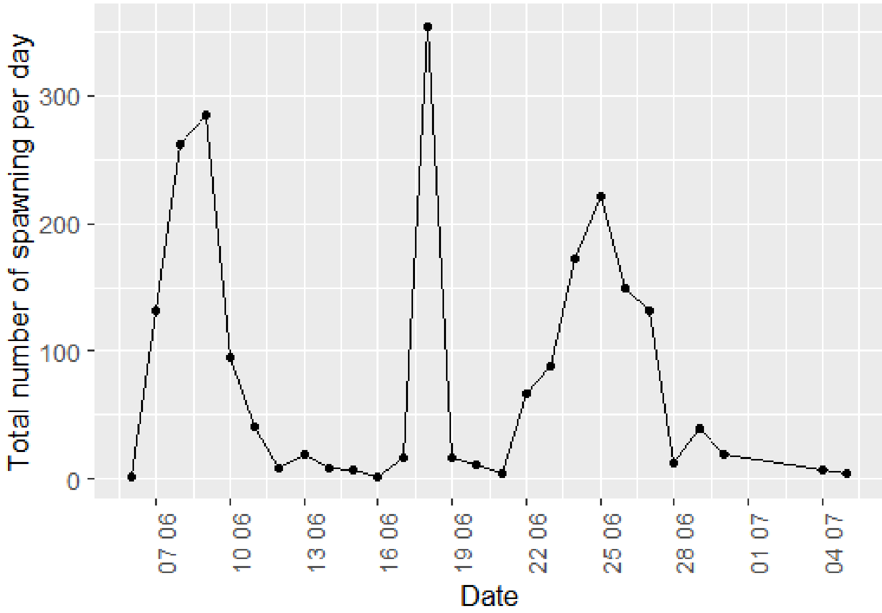

Figure 13. Visualization of the total number estimated spawning per day from beginning of June to beginning of July 2019 (n=61).

4 Discussion

For over a month 11 unique nests have been monitored with RFID antennas, detecting in total over 1.7 million detections coming from 233 PIT tagged wrasse. At the same time, I was able to analyze the reproductive behavior in detail thanks to over 50 hours of film. Seven territorial males have been analysed in detail, showing the activity rich spawning season with possibility to be divided in three stages.

One of the main questions of this dissertation is if any pattern could be found regarding the body size of the female fishes in reproduction of corkwing. I found a pattern showing that the larger females visit a smaller number of nests, at the same time they visit those nest fewer times than the smaller fish.

Also, sneaker males have been observed attending to fertilizing the eggs when the territorial male was spawning with the female. Only 27 % of total male detection belonged to sneaker males, which correlates closely to the observations of Uglem et al. (2001) where the sneaker ratio has been up to 20 % of all observed males. The main reason of the observed ratio is that the sneakers are minorities and the only reason for them to show up at the nest is to fertilize the eggs (Uglem et al., 2001).

4.1 Reproductive behaviour of corkwing wrasse

This search for the reproductive behaviour started early June and many territorial males were in fanning or spawning stage. I establish that assumptions by defining the end of building stage when the nest structure is visible, and the territorial male does not chase approaching females. It is worth noting that after the main structure is done, the territorial male maintains the nest and brings new algae throughout the whole spawning session. Such behaviour of nest caring corresponds with observations of Potts (1985). The same study also concludes that the main nest structure is built within 4 to 7 days (Potts, 1985). Based on these studies it can be assumed that the initial spawning session in Saltskjærholmane started in the second half of May. I have observed many nests being placed either in the crevice’s or being surrounded by dense algae or rock structures. The placing of the nest is very important for later eggs survival. The main goal is to sustain water turbulence and be protected from ichthyovores and graziers (Potts, 1985).

Spawning stage is often chaotic and full of activities and interactions. The main goal of that stage is the spawning between the resident who build the nest and females. I have observed the territorial male spawning with many females; and later taking care of several egg clutches, observed as well by Potts (1985) and Costello (1991). The spawning happens mostly one by one, in few occasions observed two females spawning with the male at the same time. Mating starts with the territorial being either inside of the nest or close to it. The first interaction is decided by territorial. The territorial shows if its ready to spawn or not, it is worth noticing that it does not spawn with every visited female. The way the territorial male displays the interest or lack off it has been described by Potts (1974). If male is interested in courtship, he approaches the female from her side, if it is not interested it chases female away swimming straight at her (Potts, 1974).

The second decision is up to the female, declaring if it wants to spawn with the certain male or not. The female would enter the nest, by that I mean entering the area surrounded with antenna. The ring had diameter of 50 cm with the centre being the nest, based on Costello (1991) the average nest is between 20 to 30 cm in diameter. As both fish are inside of the nest, it is clear to say that the potential mating pair display to each other some sort of mating ritual. Often the fish would be swimming together and around each other, almost like checking the other mate out. Similar observations made by Potts (1974). During that time female either start spawning or swims off. For many individuals it takes only few seconds between the initial approach and the start of spawning (observations based on figure 8). Female releases the eggs in the inner part of the nest first, male follows while releasing sperm on top of the eggs. Same observations of fertilization have been observed by Potts (1985). The spawning ends with female swimming away short after last spawning cycle (see figure 8). Most of the observed spawning visits that were analysed happened within approximately 50 seconds, with a few exceptional visits that took longer or shorter time (see figure 7). Those longer interactions were usually disturbed by intruder or when male would spawn with two females at the same time.

In my observations every time female releases the eggs, without a single exceptional case male always follows releasing the sperm. The eggs have been reported to be spherical and ranging from 0.7 to 0.8 mm in diameter (Costello, 1991; Darwall et al., 1992). The release of egg and sperm is only assumed based on the behaviour since the eggs and sperm are too small to be seen by the naked eye through the camera. Based on Darwall et al. (1992) it has been calculated that the fecundity for corkwing females is 50 000 eggs a year.

While the territorial male and the female are busy with their interaction, the alternative morph (sneaker male) lures in the background in some of the observations of mine. Most of the sneakers attack happened short after a cycle of spawning between territorial male and the female (average length after spawning 1.75 seconds). Only two out of ten total separate observations sneaker attacked outside of the pattern. In these occasions, it is possible that those two sneakers miscalculated their attack a second or two before spawning.

The alternative strategy of sneakers is hypothesised to be based on two main factors, first of which is female mimicry as mentioned above. The second factor which was suggested by Uglem et al. (2000) is that the sneaker also imitates the female behaviour. This makes territorial male believe that the sneaker is female, which results in toleration of the close presence of the sneaker and lowered danger intuition (Uglem et al., 2000). Such conditions make it possible for the sneaker to approach relatively close and to strike fast after the female releases its eggs. It is very important to attack fast as once the territorial male recognises the sneaker male, it very aggressively chased it away; the bites are visible in the videos. The male competition is a form of fight for fertilization, fish have been observed displaying aggressive behaviour and have been seen wounded and damaged (Halvorsen et al., 2017; Potts, 1974).

Territorial male would react to the sneaker attack in different ways, the most common of which was to chase it away. However, when there was more than one present fish, the chasing would be not efficient. In this case, the territorial male sets itself in alternative defending mode and try to prevent the fish from entering the nest. I have observed few territorial males lying down on the nest covering the eggs with its whole body, closing the distance between intruders and the eggs. After all fish would leave the nest, the territorial would slowly rise and go back to normal behaviour.

The only interaction I have observed between female and sneaker were the occasions when the female shows aggressive behaviour towards the sneaker (after sneaker exposes itself). The reason could be summed up by the response the sneaker has on the territorial male. While the territorial male is surprised by the sneaker it shows symptoms of getting paranoid and aggressive, resulting in interruption of whole spawning. In such circumstance, female swims away while still having the eggs in her. Based on this observation perhaps by helping to chase the sneaker away the female could prevent the territorial from stopping their spawning session.

After few days of spawning the territorial male goes into its final fanning stage. The main aim of the stage is providing optimal care to increase egg survival (Potts, 1985). Male supply the eggs with oxygen by moving in wave-like motion and does not allow any fish to enter; clearly notifying he ceased to spawn. Similar observations of corkwing parental care observed by Costello (1991) and Potts (1985). The fish spends most of its times by being in the nest, unless approached by visitor whom he chases away. Occasionally the male leaves the nest, coming back with new algae (that later is incorporated into the nest). In addition, there have been times when fish moves sea stars and other unidentified objects out of the nest. I have programmed an event in Boris called maintenance, when fish uses its mouth to push or move the desired object.

Main maintenance should refer to the behaviour occurring throughout all stages, defined as fixing of the nest. I hypothesise some possibilities where the nest structure could have been damaged. The reason could range from the algae decomposition, water turbulent or attack from other individuals (personal observation). The other behaviour that starts in the same way is hypothetical feeding. I have observed several times fish gulping in parts of sand and/or algae, then swimming slightly up releasing all stuff from its mouth. It ends its behaviour by picking some parts and swallowing them. Work of Sayer et al. (1996) found sand grains in over 20 % of investigated corkwing guts, which could explain the feeding strategies where fish engulfs food particles together with sand.

The last version that looks like maintenance happens only in the last stage. The differentiation here is that the behaviour is enclosed to territorial bashing its mouth into the nest entrance, often more than once in the same time span. It pushes the eggs further into the nest, hypothetically for its protection. During that stage I also hypothesised to seek for egg predation as there are many fishes. However, this event was observed only once which limits the possibility to analyse it. The one observed predation happened by non-territorial corkwing.

Many territorial males build the nests in observed area and on few occasions, I have noticed other territorial males approaching nests that did not belong to them. When this happens, the resident would always start a fight. The agonistic behaviour between territorials have been observed as well by Potts (1974). He divided the interactions between the territorials in two groups. The first is when the intruder does not fight back and escape within 1 to 2 seconds, while the other is to fight back and usually last up to 10 sec (Potts. 1974). Personally, I would apply his statement towards all intraspecific interactions. I have observed the fights within same morphs and between the morph, where the opponent would either escape or in fact fight back

4.2 The influence of body size on female spawning behaviour

The first two test were based on 166 tagged females. Based on the first test, it can be argued that the larger females were on average visiting less nests. The number of visited nests suggests that instead of interacting with many territorial males, the large females prefer to interact with smaller number of males. This observation can be supported by Bateman gradient that states at female fecundity does not increase if it mates with many males (Bateman, 1948; Östlund-Nilsson, 2000). Based on the observation, it can be argued that perhaps older thus larger females are using different strategy than the younger ones. The result of the second test showed in addition that the larger individuals had a lower number of total detections. This suggests that either the larger females visit the nest less often or perhaps they spend less times at their spawning visits. I have observed in my videos that sometimes smaller individuals would interact with the territorial male for longer period and they would come to visit without spawning multiple times. Perhaps an experienced older fish is more efficient with the time it spends in spawning season.

I have also created a spawning estimate, which enables investigating if length of the female could be corelated with the total number of spawning events. Out of 166 tagged females that interacted with territorial males, 61 have been classified with spawning. There was a slight decrease in the number of spawning for larger females, however marginally not significant.

The smallest detected female that was interacting in the nest have been 103 mm and the smallest female that was categorized as spawning was 104 mm. Showing that at the length females were mature correspond with observation from Potts (1974), Costello (1991) and Darwall et al. (1992) and close to the average mature female found in Austevoll (Halvorsen et al., 2016). However, it is worth remembering that the minimum length the fish could have been tagged was 100 mm, so there is in fact possibility that there are some individuals maturing at smaller size. The largest female that interacted in the nest was 180 mm and the largest female categorized as spawning was 168 mm. The mean length of spawning female was 137 mm and the mean length of interacting female was 140 mm.

The main outcome is that the larger female of corkwing wrasse interacts with a smaller number of territorial males and either visit them less often or for shorter time. The reason behind it could be based on the male size, however my data variety for territorial males have been too small to work with. The other explanation could be hypothesised with the nest itself. There have been bigger and smaller nests, some covered in dense algae and some more accessible. For the future it would be reasonable to test if "attractive nest" or more accessible nest would have the higher number of female visits.

In the study of Uglem & Rosenqvist (2002) the territorial males with eggs in their nest did not vary in size from the territorial males without eggs in their nests. It suggests that the female choice is not based on the territorial male size. Also, Uglem & Rosenqvist (2002) found that the larger territorial corkwing wrasse builds larger nests. Many other species show the same positive correlation between the nest size and male size (Uglem & Rosenqvist, 2002). The correlation has been reported in rock bass (*Ambloplites rupestris)* (Noltie & Keenleyside 1987), pumpkinseed sunfish (*Lepomis gibbosus)* (Danylchuk & Fox 1996) and sand goby (*Pomatoschistus minutus*) (Kvarnemo 1995) among the others. The large size can be favorable for both sex reproduction as it is able to hold more eggs than smaller nests (Lindström, 1992; Uglem & Rosenqvist, 2002).

In the study of Östlund-Nilsson (2000) the species of fifteen-spined stickleback (*Spinachia spinachia*) have been studied. The stickleback is similar to corkwing wrasse in two important ways, the males build nests and display parental care (Östlund-Nilsson, 2000). The outcome of the project showed that the male behaviour and the nest ornaments attract females (Östlund-Nilsson, 2000). The proposed explanation is that the more attractive nests are built by males putting more energy into building (Östlund-Nilsson, 2000). The extra used energy is metabolically costly and is often correlated with great nutrition status, hypothetically showing females that the male with attractive nest will have more than enough of energy to take care of the eggs (Östlund-Nilsson, 2000). To summarize Östlund-Nilsson (2000) suggest that the males of fifteen-spined stickleback by building and displaying their nest signalize to females high parental quality as a direct benefit; that results in higher reproductive rate for both sexes.

The ocellated wrasse (*Symphodus ocellatus*) is a species closely related to corkwing. Where nesting males mating success is driven by female choice for nest high mating rates (Alonzo, 2008). The main drive of that statement is that the males with successful nest are more likely to provide care (Alonzo. 2008). In the work of Alonzo & Heckman (2010) it is suggested that the indicator of male care is recent mating success based on presence of other females and new eggs, as well as sneakers.

Based on the studies on other species I could suggest the possibility that the partner selection of female chose in corkwing wrasse is perhaps based not directly on the male size but indirectly on the nest that he builds. Based on visual observations of the nest I noticed that the nests range in size, structure and where they are placed. My data was too small to show the variety of the nest however it is described in the work of Potts (1985) and Costello (1991).

4.3 Intra-specific interactions

I have observed different species coming into the nest, both in my PIT-data and my videos. Every species would either swim randomly or come and stay for a while and then swim away with no clear interaction with the resident of the nest. The only exception has been the goldsinny, often seen in the backgrounds in most of videos as well as approaching the nest from time to time. The only thing it did there beside just entering would be picking some stuff here and there (possibly feeding). The corkwing would sometimes respond positively meaning not chasing goldsinny away, sometimes negatively meaning chasing it away.

In the work of Hilldén (1981) it has been observed that the goldsinny rarely leaves its territory, however the fish have been observed to leave it in purposes of feeding or interactions with conspecifics. I have observed that the goldsinny is not afraid to bite the intruders trespassing its boundaries, and the same behavior was observed by Hilldén (1981) in addition to frontal and boundary display. In the same study fish have been observed fighting with the species as well as observed attacking rock cock (Hilldén, 1981). Based on the work of Hilldén (1981) the goldsinny spawns in May and June and its territory can be up to 2 square meters. It is possible that the territory of observed corkwing could overlap with the territory of goldsinny, thus resulting in certain interactions.

During my video analysis five out of 19 tagged females that have approached the nest have been chased away by goldsinny wrasse (the rest by territorial corkwing). The goldsinny were on average smaller, however would appear very aggressive towards some corkwing females. As mentioned before the goldsinny is also territorial fish and I hypothesize that the corkwing female could disturb its territory. I also hypothesize that the territorial corkwing male is too big and could easily chase the goldsinny, making it smart for goldsinny not to attack the territorial corkwing male.

4.4 Implications for management and harvesting

The fish inhabiting the area were protected from commercial harvesting. In the year of 2019, every fish longer than 120 mm has been exposed for the harvesting in the wild (Appendix table 2). I was thus able to calculate the amount of fish from my dataset that would have been exposed to the harvesting if not being in the protected area; only fish longer than 100 mm could have been tagged. As mentioned in previous sections based on Halvorsen et al. (2016) in Norway female and sneaker male corkwing can reach maturity around that size. The territorial males mature later and grow bigger maturing around the size of 136 mm (Halvorsen et al. 2016). Approximately 90 % of all observed corkwing wrasse were longer that the minimum harvesting length, making them endangered in the area open for harvesting.

Majority of females were over the threshold, it is very dangerous as fecundity increases with size (Conover & Munch, 2002). In theory the large females are producing more eggs, making reduction of number within large females play important role on the whole population structure. At the same time the most jeopardized by harvesting would have been territorial males, as every one of 32 observed individuals have been over the minimum length.

The study of Halvorsen et al., (2017) mentions a tendency for male-selective harvesting, further stating that the territorial ones were the morph with higher capture vulnerability, due to their higher capture probability; not due to their larger body size. The reason behind it is hypothesized to be caused by higher growth rate, the explanation was drawn to more active and risk-taking behaviour caused by higher feed rate (Halvorsen et al., 2017). Based on my observations I could not agree more with that statement. The territorial males are clearly very active during the spawning season. The high amount of the activities could have increased the risk of being harvested, just like in the work of Halvorsen et al. (2017). Higher growth together with demand of high activity normally is correlated with the higher feeding rate. Higher feeding rate leads to more spatial movement in the purpose of finding the food. I described in the fanning stage that the male puts lots of energy into caring the eggs, which includes supplying them with oxygen and chasing of the intruders that trying to approach. The work of Darwall et al. (1992) states that removing of the territorial male and leaving the eggs in the nest for itself, would greatly impact the egg survival stages. The same article states that the fishery impact might reduce fish population to spawning individuals under the minimum length threshold (Darwall at al., 1992). Most of the observed fish have been over the minimum length threshold, the futuristic influence by the harvesting seems to be in some degree inevitable. Thus, more studies and focus are required to prevent the population from dramatic outcomes in the form of either size reduction in general or influencing the faster maturation.

4.5 Improvements and impact

There were several difficulties in the post field work analysing. Along the way I have learned how difficult it was to manage the video camera, and it was essential to make sure that the nest entrance is in the centre of the shot. The camera with power bank was bigger, heavier, and lower, those factors made it more difficult to handle under water end were less adjustable resulting in films of less quality on average. Logically the longer film the higher probability that the tagged fish will visit. However, when there was no visit the movie was monotony and had not as much value to my analysis. The camera with external power banks should be placed more carefully and used for the nest where are lots of actions, optimally to the nest where the territorial is in the spawning stage. In conclusion minority of my films were with the territorial male in spawning stage but that is due to harsh spring bloom and technical problems. There have been two weeks delay in monitoring and filming; it was caused by a bloom of the coccolithophore *Emiliania huxleyi*. During the time the water was filled with it and affecting my work.

While watching the videos I recognized that there was some impact to some of the individuals I was filming. In one case the antenna was covering the nest slightly so that the entrance was partially blocked, in few others the rope connecting the antenna to the water buoy would fall around the nest itself, making the territorial swimming around it with extra difficulties. First and last minutes of every video showed fish with disturbed behaviour, caused by the person snorkelling. Sometimes the fish would swim away and come back after a certain amount of time, sometimes the fish stopped its activities and look in the direction of the snorkel or the sound that was coming from him. Often the fish would lose camera interest and go back to its normal behaviour very quickly. It is possible to argue that the effect camera had one the territorials were present, however relatively small.

5 Conclusion

Using cameras and Pit-telemetry, I studied reproductive behaviour of corkwing wrasse during several weeks of their nesting period in Austevoll, western Norway. Based on the observations I defined three stages of territorial male, each named after the main activity in the stage, respectively building, spawning, and fanning. The study also revealed that the larger females visit less nest and visit not as often as the smaller females. The explanation behind it is unknown and need more research, I could hypothesize that it is based either on the experience or the visited nest. In addition, I have found that the sneaker males try to fertilize the eggs short after the female releases them in the inner part of the nest. I have observed intraspecific aggression within territorial males and between them and the sneaker males. In some occasions, females would show active aggression towards exposed sneaker male.

Many different species visit the nest; however the only clear interaction is the negative one between female corkwing wrasse and goldsinny. Where the goldsinny would protect its territory and chase other fish away. Goldsinny do not chase territorial corkwing away, most possibly because of the size difference.

From the point of view of the nests belonging to territorial corkwing male, the spawning session is a period rich in unique behaviours, interactions and strategies from late May, throughout the June and early July.

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Appendix

Table 1. Reported landing of each species of wrasse in Norway based on catch from 2019. Information derived from Norwegian Directorate of fisheries at 14th June 2020 (https://www.fiskeridir.no/Yrkesfiske/Tall-og-analyse/Fangst-og-kvoter/Fangst-av-leppefisk)

Species	Number
Cuckoo wrasse	12 860
Corkwing wrasse	8 073 998
Rock cook	451 807
Goldsinny wrasse	8 519 557
Ballan wrasse	1 949 448

Table 2 The minimum length required for harvesting in Norway, while corkwing and ballan wrasse have their own defined minimum size, the other species have one common minimum length. Information derived from Norwegian Directorate of fisheries at 14th June 2020 (https://www.fiskeridir.no/Yrkesfiske/Tema/Leppefisk/Minstemaal)

Species	Minimum size
Corkwing wrasse	120 mm
Ballan wrasse	140 mm
Other wrasse	110 mm