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Embryonic development of corkwing wrasse, *Symphodus melops*

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Abstract

Eggs from corkwing wrasse, *Symphodus melops* were described, photographed and illustrated to characterise the embryonic development of this species. Egg development was divided into 8 stages from fertilisation until hatching with descriptions of key features for each stage. The rate of development in corkwing wrasse eggs at temperatures commonly found along the Norwegian coast (12, 15 and 18 °C) was also investigated. The rate of development was faster at higher temperatures.

Keywords: labridae, cleaner fish, embryonic development, corkwing wrasse

1. Introduction

The corkwing wrasse, *Symhodus melops* is part of the Labridae family, a family of predominantly small, inshore marine fish (Darwall et al., 1992). It can be found in temperate-cold waters from Trondheimsfjorden (63° North) in Norway to Marocco, and in the Azores, Western Mediterranean and the Adriatic sea (Muus et al., 1999; Quignard & Pras, 1986). This species has gone through a "northward shift" becoming nearly extinct along its southern range, but increasingly more numerous in the northern areas (Knutsen et al., 2013; Robalo et al., 2012). Corkwing wrasse is a rocky shore species most commonly found in the upper (30m) part of the water column. The species presents sexual dimorphism with brownish females and larger, more colourful males (Quignard & Pras, 1986). Sneaker males (comprising about 3-20 % of the male population (Uglem et al., 2001)) are also present in this species, which are males that mimic females in appearance and sneak past the regular males to try and fertilise the

eggs. Corkwing wrasse usually range between 15-20 cm in length, but can reach a length of 28 cm. They become sexually mature at 2-3 years of age and can have a life span up to 9 years (Quignard & Pras, 1986). The spawning season for the species is from April-September (Skiftesvik et al., 2015; Darwall et al., 1992) in Norwegian waters. The females produce about 50 000 eggs per year, that are spawned in several batches (>4) throughout the spawning season (Darwall et al., 1992). Eggs are benthic and placed in seaweed nests, built among rocks and crevices on the sea floor, and guarded by the males (Quignard & Pras, 1986).

Small wrasses have not previously on a large scale, been targeted for commercial fishing, but after it was discovered that their cleaning behaviour could be applied in the control of sea lice (*Copepoda, Caligidae*) in salmon and trout farms (Costello et al., 1990), the interest in these species increased. The Norwegian Directorate of Fisheries has a time series on the use of cleaner fish to battle lice infestations in Norwegian salmon and trout farms from 1998 until 2014 (Figure 1). The use of cleaner fish decreased from 1998 to 2005, when chemotherapeutics were applied for lice control(Skiftesvik et al., 2014). In 2007-2008 it was reported that the lice were developing resistance against the chemicals (Nilsen et al., 2008), and the demand for cleaner fish rocketed. In 2008 the reported number of cleaner fish used in the farms was 1.7 million fish, and by 2014 this number increased by more than a tenfold to 24 million. Some of the cleaner fish came from hatcheries, but a vast majority; 21 million of these fish were wild caught. In 2015 Norwegian fishing boats reported a catch of almost 21 million wrasses and of these almost half (9.8 million) were corkwing wrasse (Appendix C).

Even though there is an increasing commercial interest in corkwing wrasse there is little knowledge about the ecology and life history of this species, which makes it difficult to predict the effect of the increased fishery on the wild stock. The larval development of this species was described earlier by Quignard (1967); however, there is no information about the embryonic development of the species. This paper aims at filling out this gap in the knowledge.

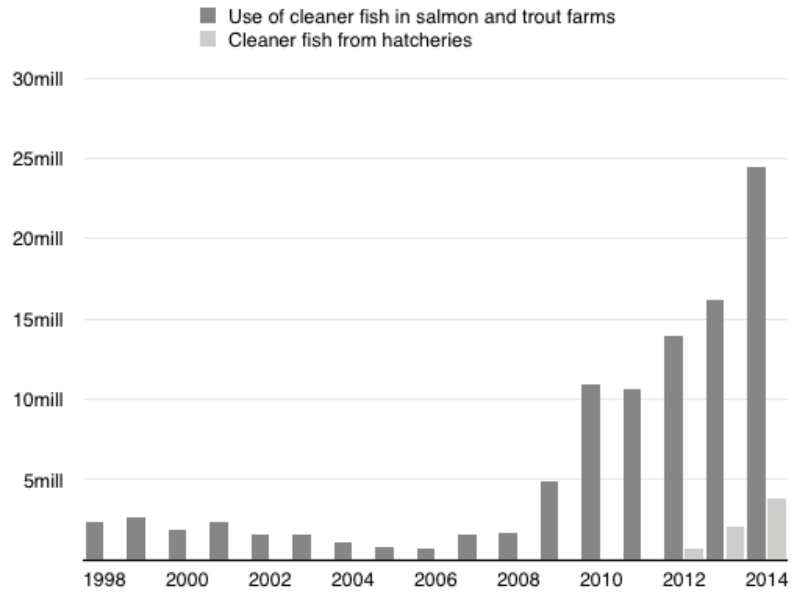


Figure 1: Reported number of cleaner fish used in Norwegian salmon and trout farms from 1998-2014, and the reported production of cleaner fish from hatcheries from 2012-2014. Source: Norwegian Directorate of Fisheries (Appendix A and Appendix B)

The main objective of this paper is to describe the embryonic development of corkwing wrasse. Establishing key morphological features and developmental criteria for this species, creates a baseline which can be an aid in determining the quality of corkwing wrasse eggs, and in recognising diseased eggs. Additionally it aims at determining how differences in the range of sea water temperatures commonly found along the Norwegian coast may affect the rate of development, to give an indication of what temperatures would be best suited for cultivating corkwing wrasse.

2. Materials and methods

The broodstock used in this study comprised five males and three females caught using eel pots by a local fisherman in Arendal, Norway, on June 24th 2014. The fish were kept in a large pool (approx. 5000l) at the Institute of

Marine Research (IMR) in Flødevigen, Arendal. On July 7th 2015, breeders were stripped manually for egg and sperm collection. Females with swollen bellies were gently stripped and their eggs were collected with a net of 160 microns attached to a small PVC cylinder to avoid dispersal of the eggs. Sperm of stripped males was collected afterwards. Eggs and sperm from each breeder were mixed and split into three PVC cylinders to follow the embryonic development under three different temperatures. PVC cylinders containing the fertilised eggs were immersed into three independent tanks of 450l each at water temperatures of 12, 15 and 18 $\pm 0,2$ °C, and provided with gentle water exchange. Eggs were kept under 24 hour constant light and continuously inspected under a binocular microscope fitted with a Nikon Coolpix camera to record changes in the development. Egg inspections were performed on batches of 20-30 eggs. The eggs had a sticky, gelatinous layer that caused debris and contaminants to stick to the eggs. This layer was carefully removed using dissecting needles under a binocular microscope to monitor the details of the egg development. If only one or two eggs presented advances in their developmental stages the process was repeated in a new batch of eggs to confirm the advancement. Key developing features were identified and characterised and it was noted when the different features appeared in the different batches of eggs. In the early stages the change in development happened quite fast and during the first 2 hours, eggs were inspected and photographed every 10 minutes in order to monitor the early cell divisions. As the development slowed down, inspection intervals were extended to every 20 minutes over the next 4 hours, and finally when the development and changes became even slower, to every 6 hours. Once the eggs had been examined and photographed they were stored in 99 % ethanol. Preserved eggs and pictures were used to complete the description of the different stages during the embryonic development and to create illustrations.

3. Results

Corkwing wrasse eggs are small (approximately 0.75-0.8mm in diameter), spherical and covered in a clear, sticky gelatinous layer.

After the eggs had been examined, they were divided into eight clearly differentiated stages with unique, distinctive characteristics. Although it would be possible to include further intermediate stages, the differences between the stages would be more subtle, and such classification might result challenging and confusing for the implementation of this methodology by other researchers in future studies. In the description the time when each of these stages is reached at 15 °C is included(see Table 1 for time comparisons between 12, 15 and 18 °C). Therefore, the description presented here focuses on eight critical stages at 15 °C, characterised by the following distinctive key features:

Stage I: Fertilised egg. The zygote or newly fertilised egg has a cloudy, opaque, yellowish core consisting of a one-celled blastodisc situated at the animal pole, and the yolk at the vegetal pole, surrounded by a narrow, clear perivitteline space. The perivitteline space gradually widens from a thin line to about 1/10 of the eggs diameter and the blastodisc and yolk become less cloudy and more uniform in colour (Figure 2a). This stage was observed 20 minutes after stripping.

Stage II: Cleavage. The first cleavage is vertically oriented and cleaves the blastodisc into two even blastomeres that occupies approximately 1/3 of the egg while the yolk occupie about 2/3. There is a distinct indent in the border between the blastodisc and the yolk where the perivitteline space is wider. At the site of the cleavage the blastodisc narrows giving the blastodisc an ellipsoidal shape when seen from an apical view. At the vegetal pole; the yolk presents multiple, small, circular oil droplets (Figure 2b). Eggs with a 2-celled blastodisc were observed 1 hour and 20 minutes after fertilisation. During this stage, cell divisions occurred at regular intervals of approximately 20 minutes.

The second cleavage is perpendicular do the first one, dividing the blastodisc along its longitudinal axis into 4 blastomeres that are similar in size and shape (Figure 2c).

The third cleavage is parallel to the first cleavage and divides the blastodisc into 8 cells. The blastomeres and blastodisc have an elongated rectangular shape

(Figure 2d). The oil droplets gradually spread through the yolk towards the animal pole and are found in a higher concentration along the outer edges of the yolk.

The fourth cleavage is perpendicular to the first cleavage dividing the blastodisc into 16 blastomeres. At this point, oil droplets are evenly dispersed throughout the yolk (Figure 2e).

The fifth cleavage is uneven on several planes running both in parallel and perpendicularly to the first cleavage. There are 32 blastomeres that are uneven in size and shape. The blastodisc has an elongated rectangular apperance (Figure 2f).

The next cleavage divides the blastodisc into 64 blastomeres. The blastodisc becomes less rectangular and more round around the edges (Figure 2g). From a lateral view the blastomeres are round and are arranged in several layers.

In the next cleavage the blastodisc is divided into 128 small blastomeres, and has a square shape with round edges (Figure 2h).

At the next cleavage the blastomeres decrease further in size and the blastodisc becomes more compact consisting of 256 cells. From an apical view the blastodisc decreases in diameter and a wider portion of the yolk can be seen. From a lateral view several layers of cells can be seen (Figure 2i).

As the blastomeres cleave again into 512 cells, the blastomeres become even smaller. At this point the blastodisc is completely circular with lightly bulging edges (Figure 2j).

Stage III: Blastula. At this stage it becomes impossible to distinguish individual blastomeres. At the animal pole, a homogenous, half-moon shaped clump of cells, called the blastula is seen. In the blastula stage the border between the blastodisc and the yolk is smooth, running in a continuous line (Figure 2k) This stage was observed after 5 hours.

Stage IV: Gastrula. At this stage the blastula flattens outwards into a dome shaped gastrula, covering about 1/3 of the yolk at the animal pole. The germ ring becomes visible and starts migrating over the yolk towards the vegetal

pole (Figure 2l). From an apical view the germ ring looks like a ring-shaped thickening of cells and consists of an external epiblast and an internal hypoblast. As the ring migrates and reaches about halfway across the yolk, the yolk narrows and becomes more elongated and ellipsoidal in shape. From a lateral view the Brachet's cleft, a visible line between the epiblast and the hypoblast can be seen, and the posterior and anterior part of the embryo can be distinguished (Figure 3a). The germ ring continues its migration towards the vegetal pole and envelops the yolk almost completely, while the embryo elongates to cover about half of the circumference of the yolk. At this point, oil droplets become more concentrated in the opposite side to where the body of the embryo is developing (Figure 3b). Eggs containing a dome shaped gastrula were observed after 12 hours.

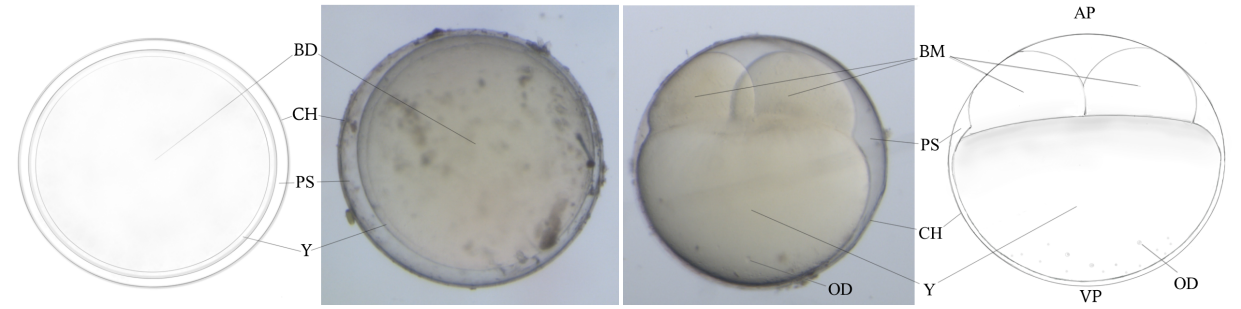
Stage V: Segmentation: somites. The appearance of somites marks the end of gastrulation and the transition to segmentation. The embryo thickens and the yolk gradually shrinks in size. A beak-like mass of cells is seen anteriorly to the head and the rudimentary eyes become visible. Somites appear in the central and posterior part of the embryo, and the Kupffer's vesicle can be seen (Figure 3c). As the embryo elongates the beak-like mass of cells disappear from the anterior part of the head and the lenses of the eyes become visible. The otic vesicles appear posteriorly to the eyes and more somites are seen posteriorly along the body of the embryo. The Kupffer's vesicle enlarges and the tail is still fully attached to the yolk. The yolk continues to shrink and the oil droplets become concentrated in the area of the yolk closest to the head and tail of the embryo (Figure 3d). Embryos with visible somites were observed after 42 hours.

Stage VI: Segmentation: tail detaches from yolk. At this stage the Kupffer's vesicle shrinks and disappears, while the tail starts to detach from the yolk (Figure: 3e) As the embryo elongates and completely encircles the yolk, the brain and heart become clearly visible, the otic vesicles enlarges and the otoliths can be seen. More somites appear towards the tail of the embryo where a membranous fin also appears. The yolk shrinks and changes to a more bean-

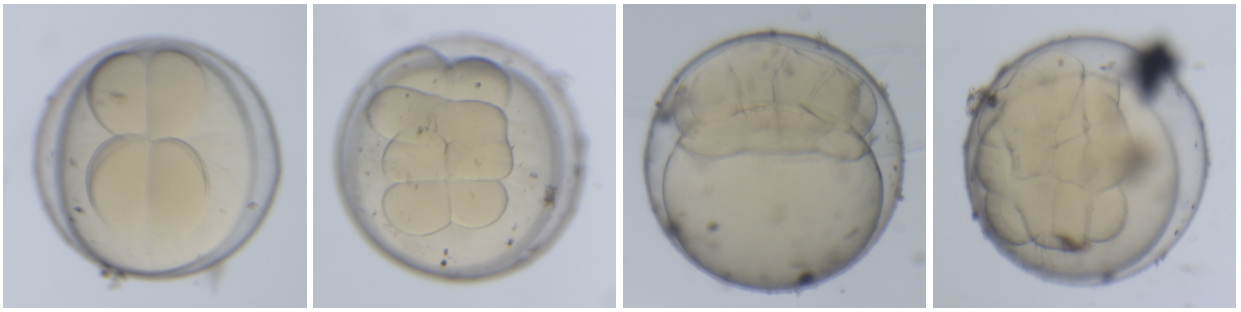
like shape, narrowing in the center where the embryo encircles it (Figure 3f). Detachment of the tail of the embryos from the yolk was observed after 90 hours.

Stage VII: Segmentation: the embryo coils and overlaps itself. The embryo gradually darkens and becomes less translucent. Black and orange pigments; melanophores appear on the body of the embryo and on the yolk (Figure 3g). The pectoral fin becomes visible posteriorly to the otoliths. The yolk shrinks even further and bulges outwards on either side of the embryo, and oil droplets are again evenly dispersed in the yolk (Figure 3h). Embryos with an overlapping tail were observed after 138 hours.

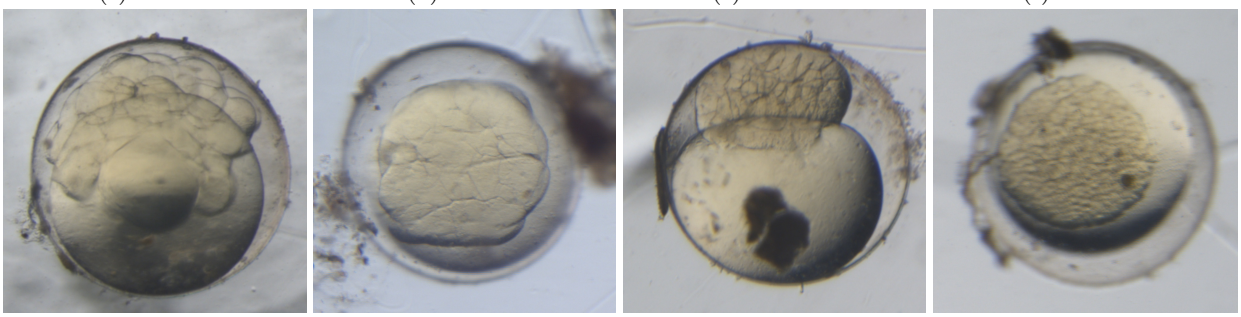
Stage VIII: Hatching. The newly hatched larvae are approximately 2-2,5mm in length, measured from the top of the head to the tip of the tail. The head and torso of the embryo curve around the yolk-sac which has a droplet shape. At the posterior part of the head the olfactory apparatus or nostrils can be seen. The brain and heart have enlarged and are clearly visible. The small pair of pectoral fins are now more developed and the anal opening is visible (Figure 4). Newly hatched larvae were observed after 144 hours.



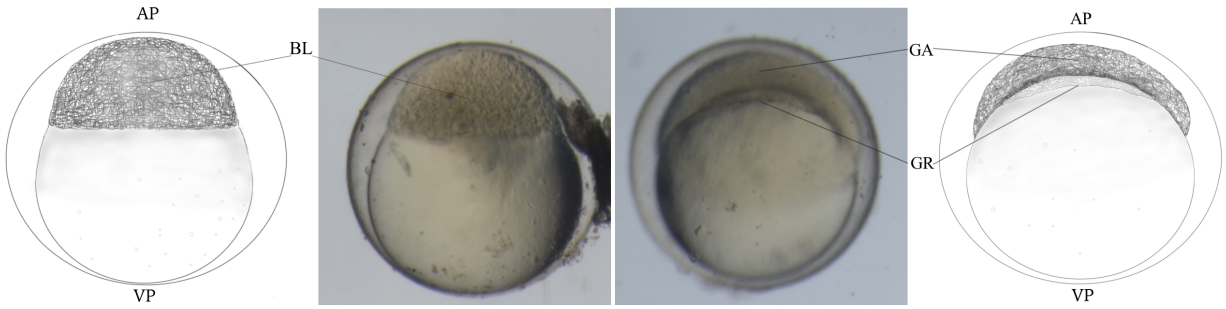
(a) Stage I: Fertilized egg (b) Stage II: 2-cells



(c) 4-cells (d) 8-cells (e) 16-cells (f) 32-cells

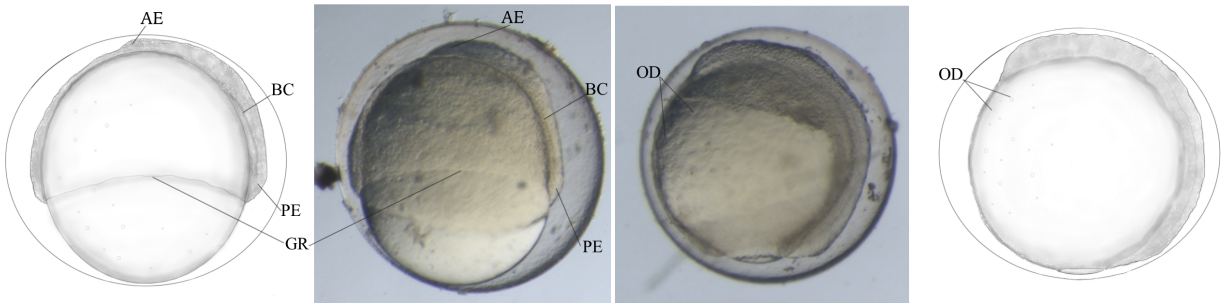


(g) 64-cells (h) 128-cells (i) 256-cells (j) 512-cells



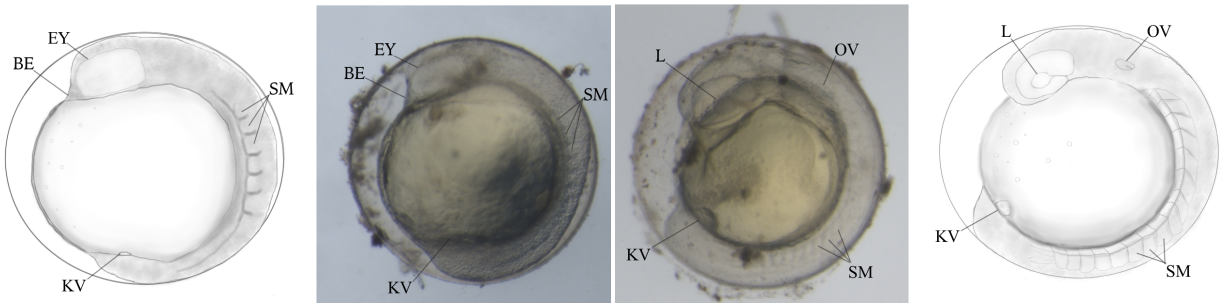
(k) Stage III: blastula (l) Stage IV: gastrula

Figure 2: Embryonic development stages I-IV in corkwing wrasse, *Symphodus melops* a) Stage I: Fertilised egg apical view, b) Stage II: Cleavage: 2-cells lateral view, c) 4-cells apical view, d) 8-cells apical view, e) 16-cells lateral view, f) 32-cells apical view, g) 64-cells lateral view, h) 128-cells apical view, i) 256-cells lateral view, j) 512-cells apical view, AP: animal pole, BD: blastodisc, BL: blastula, BM: blastomere, CH: chorion, GA: gastrula, GR: germ ring, OD: oil droplet, PS: perivitelline space, VP: vegetal pole, Y: yolk, Photos: Enrique Blanco Gonzalez



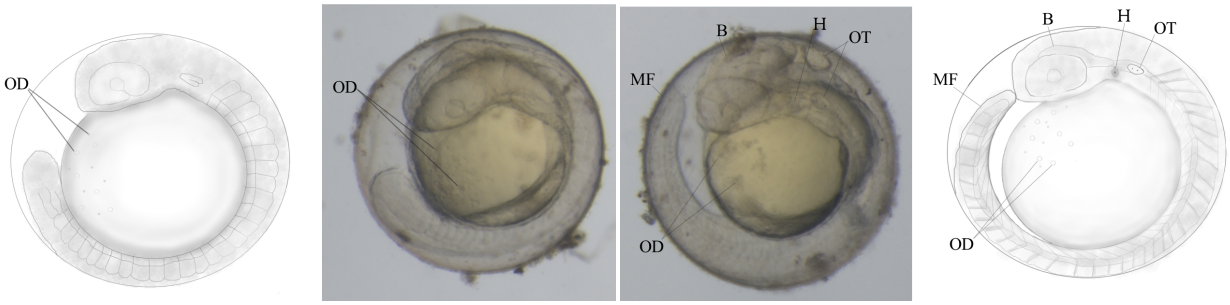
(a) Stage IV: embryo 1/4 of yolk circumference

(b) Stage IV: embryo 1/2 of yolk circumference



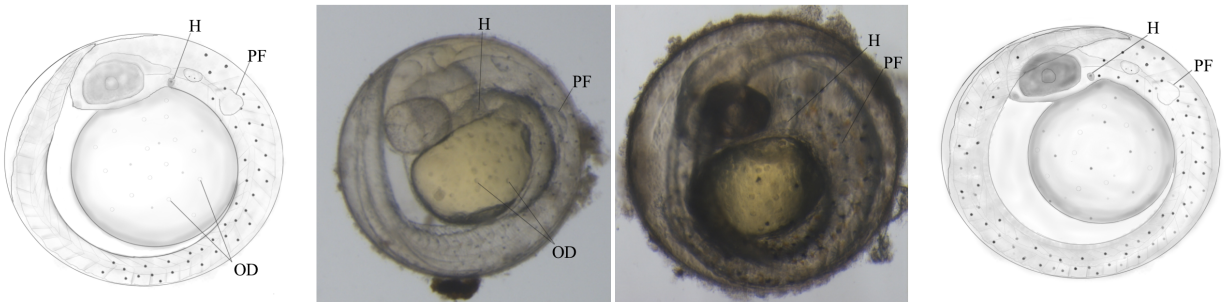
(c) Stage V: somites

(d) Stage V: embryo covers 3/4 of yolk circumference



(e) Stage VI: tail detaches from yolk

(f) Stage VI: embryo completely encircles yolk



(g) Stage VII: tail overlaps anterior to eye

(h) Stage VII: tail overlaps posterior to eye

Figure 3: Embryonic development stages IV-VII in corkwing wrasse, *Symphodus melops*, lateral view, embryos seen from the left side a) Stage IV: gastrula: embryo covers 1/4 of yolk circumference, b) embryo covers 1/2 of yolk circumference, c) Stage V: Segmentation: somites visible, d) embryo covers 3/4 of yolk circumference , e) Stage VI: tail detaches from the yolk, f) embryo completely encircles the yolk, g) Stage VII: embryo overlaps it self, tail reaching anterior to eye, h) embryo overlaps itself, tail reaching posterior to eye, AE: anterior part of embryo, AP: animal pole, BC: Brachet's cleft, BE: beak like mass BL: blastula, B: brain, EY: rudimentary eye, GA: gastrula, GR: germ ring, H: heart, KV: Kupffer's vesicle, MF: membranous fin, OD: oil droplet, OT: otolith, OV: otic vesicle, PE: posterior part of embryo, PF: pectoral fin, SM: somite, VP: vegetal pole, Photos: Enrique Blanco Gonzalez

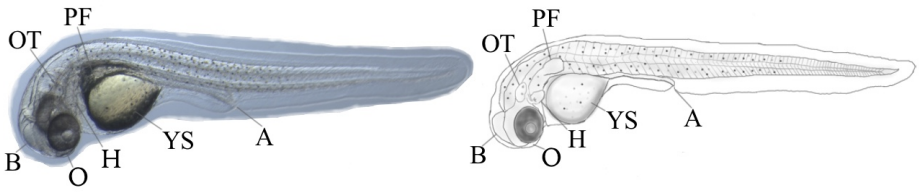


Figure 4: Newly hatched corkwing wrasse, *Symhodus melops* larvae, left, lateral view, A: anal opening, B: brain, H: heart, O: olfactory apparatus(nostrils), OT: otolith, PF: pectoral fin, Y: yolk

4. Discussion

In this paper the embryonic development of corkwing wrasse was characterised and divided into 8 clearly differentiated stages (Figure 2, 3 and 4). There is no universal or standardised method for describing the embryonic development of fish eggs, and it can be difficult to compare different studies due to the use of a various number of stages, and different and confusing stage names. The classification chosen in this study is similar to the one used by D'Arcy et al. (2012) who described the embryonic development of ballan wrasse, *Labrus bergylta*.

The eggs of corkwing wrasse are smaller in diameter (0.75-0.8mm) than ballan wrasse eggs (0.885mm (D'Arcy et al., 2012), 1-1.1mm (Darwall et al., 1992).

The eggs of corkwing wrasse goes through the same developmental steps in their embryonic development as other marine teleosts, and displays similar key features as other studied Labridae species, like the ballan wrasse (D'Arcy et al., 2012), green wrasse, *Labrus viridis* (Kožul et al., 2011) and the brown wrasse *Labrus merula* (Dulcic et al., 1999).

Newly hatched larvae of corkwing wrasse are bigger (2-2.5mm) than goldsinny wrasse, *Ctenolabrus rupestris*, larvae(1.9-2.2mm (Darwall et al., 1992)) and smaller than the ballan wrasse larvae ($<$ 3.8mm) respectively (Darwall et al., 1992)). The time from fertilisation until hatching in this study was 144hours at 15 °C in the corkwing wrasse eggs. The incubation time for the eggs of goldsinny wrasse, has been reported by Darwall et al. (1992) to be 43hours at 15 °C. D'Arcy et al.

Temperatures	12 °C	15 °C	18 °C
Stage I: Fertilized egg			
1-cell		20min	
Stage II: Cleavage			
2- cells		1h 20min	
4- cells		1h 40min	
8- cells		2h 20min	
16- cells		2h 40min	
32- cells	5h 40min	3h	
64- cells		3h 20min	
128- cells		3h 40min	
256 - cells		4h	
512- cells		4h 20min	
Stage III:			
Blastula	12h	5h	
Stage IV:			
Gastrula	18h	12h	12h
Stage V:			
Segmentation: somites	72h	42h	30h
Stage VI:			
Segmentation: tail detaches from yolk		90h	66h
Stage VII:			
Segmentation: embryo overlap itself		138h	90h
Stage VIII:			
Hatching	172h	144h	

Table 1: The time of appearance of different stages in the embryonic development of corkwing wrasse, *Symphodus melops*. Comparison of development times under 12, 15 and 18 °C.

(2012) recorded hatch times of ballan wrasse eggs at 12.9 °C of 122hours and at 16.5 °C of 96hours. These finding indicate that the incubation time of corkwing wrasse eggs are longer than for the eggs of ballan and godsinny wrasse.

The times recorded for the occurrence of each stage in the embryonic development of corkwing wrasse, are not ment to give an exact time for when each stage occurred, just give an indication of the rate of the development. The rate of development of corkwing wrasse eggs at different temperatures experiences along the Norwegian coast, increased with higher temperatures (table 1). Eggs stored at 15 and 18 °C developed in parallel with only slight differences until the gastrula stage. From Stage V, eggs stored at 18 °C developed faster, but the hatching time of these eggs could not be recorded because all the eggs died

before the completion of the experiment. Eggs stored at 12 °C developed slower, something that was already seen at the cleavage stage. There seemed to be a bigger difference in the rate of development between 12 and 15 °C than between 15 and 18 °C.

The sticky gelatinous layer covering the eggs, was removed mechanically in this study. The mortality rate of the eggs was observed to be quite high, although the actual mortality rate was not recorded, because this was outside the scope of this study. Eggs of other fish species like the Atlantic cod, *Gadus morhua* (Rollefsen, 1932) have shown sensitivity to mechanical stress, especially in the early stages of embryonic development. It is therefore suspected that the chosen method for de-sticking the eggs might have been a contributing factor for the high mortality rate in the eggs.

Corkwing and goldsinny wrasse, are the two wrasse species that are mostly used in Norwegian salmon and trout farms (Appendix A and C). The supply of these species comes exclusively from from wild caught fish. The third most used wrasse is the ballan wrasse, which comes both from wild caught and commercially cultivated stocks (Appendix B and C). Because of the limited knowledge on how the increased fisheries on the wild stocks of wrasses will affect the populations, it might be important to investigate alternative options for meeting the demand for cleaner fish from the salmon and trout farms.

This paper could be an aid to explore the possibility of commercially cultivation of corkwing wrasse as an alternative to using wild caught fish. In commercial cultivation of corkwing wrasse it would be necessary to remove the sticky layer from the eggs to be able to evaluate the development and quality of the eggs, but also to ensure an effective disinfection of the eggs between different production stages. Further investigations are needed to develop suitable methods for de-sticking the eggs of this species, that also ensure high survival of the eggs and larvae. Different methods for de-sticking eggs have been tested in ballan wrasse (Grant et al., 2016; Lein et al., 2014), that might be transferrable to corkwing wrasse.

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References

Costello, M., Bjordal, Å. et al. (1990). How good is this natural control on sea-lice? *Fish Farmer*, *13*.

D'Arcy, J., Dunaevskaya, E., Treasurer, J., Ottesen, O., Maguire, J., Zhuravleva, N., Karlsen, A., Rebours, C., & FitzGerald, R. (2012). Embryonic development in ballan wrasse labrus bergylta. *Journal of fish biology*, *81*, 1101–1110.

Darwall, W., Costello, M., Donnelly, R., & Lysaght, S. (1992). Implications of life-history strategies for a new wrasse fishery. *Journal of Fish Biology*, *41*, 111–123.

Dulcic, J., Kožul, V., Kraljevic, M., Skaramuca, B., Glamuzina, B., & Re, P. (1999). Embryonic and larval development of the brown wrasse labrus merula (pisces: Labridae). *Journal of the Marine Biological Association of the UK*, *79*, 327–332.

Grant, B., Picchi, N., Davie, A., Leclercq, E., & Migaud, H. (2016). Removal of the adhesive gum layer surrounding naturally fertilised ballan wrasse (labrus bergylta) eggs. *Aquaculture*, .

Knutsen, H., Jorde, P. E., Gonzalez, E. B., Robalo, J., Albretsen, J., & Almada, V. (2013). Climate change and genetic structure of leading edge and rear end populations in a northwards shifting marine fish species, the corkwing wrasse (symphodus melops). *PloS one*, *8*, e67492.

Kožul, V., Glavić, N., Tutman, P., Bolotin, J., & Onofri, V. (2011). The spawning, embryonic and early larval development of the green wrasse labrus viridis (linnaeus, 1758)(labridae) in controlled conditions. *Animal reproduction science*, *125*, 196–203.

Lein, I., Barr, Y., & Tveiten, H. (2014). *Eliminating egg stickiness, Stripped ballan wrasse eggs. in manual: Production of ballan wrasse, Science and practice pp.29-30*. LeppeProd project Bergen.

Muus, B. J., Nielsen, J. G., Dahlstrøm, P., & Nyström, B. O. (1999). *Sea fish*. Scandinavian Fishing Year Book.

Nilsen, F., Sevatdal, S., & Horsberg, T. (2008). Påvisning av emamectinresistente lakselus i noreg. *Norsk fiskeoppdrett*, *6*, 58–60.

Quignard, J.-P. (1967). L'oeuf et la larve de symphodus (crenilabrus) melops (linné, 1758)(poissons, téléostéens, perciformes, labridés). influence de différents facteurs physicochimiques sur la durée du développement embryonnaire. *Revue des Travaux de l'Institut des Pêches Maritimes*, *31*, 355–358.

Quignard, J. P., & Pras, A. (1986). Labridae. in 'fishes of the north-eastern atlantic and the mediterranean vol. 2'.(eds pjp whitehead, m.-l. bauchot, j.-c. hureau, j. nielsen and e. tortonese.) pp. 919-942.

Robalo, J. I., Castilho, R., Francisco, S. M., Almada, F., Knutsen, H., Jorde, P. E., Pereira, A. M., & Almada, V. C. (2012). Northern refugia and recent expansion in the north sea: the case of the wrasse symphodus melops (linnaeus, 1758). *Ecology and evolution*, *2*, 153–164.

Rollefsen, G. (1932). The susceptibility of cod eggs to external influences. *Journal du Conseil*, *7*, 367–373.

Skiftesvik, A. B., Blom, G., Agnalt, A.-L., Durif, C. M., Browman, H. I., Bjelland, R. M., Harkestad, L. S., Farestveit, E., Paulsen, O. I., Fauske, M. et al. (2014). Wrasse (labridae) as cleaner fish in salmonid aquaculture–the hardangerfjord as a case study. *Marine Biology Research*, *10*, 289–300.

Skiftesvik, A. B., Durif, C. M., Bjelland, R. M., & Browman, H. I. (2015). Distribution and habitat preferences of five species of wrasse (family labridae) in a norwegian fjord. *ICES Journal of Marine Science: Journal du Conseil*, *72*, 890–899.

Uglem, I., Galloway, T., Rosenqvist, G., & Folstad, I. (2001). Male dimorphism, sperm traits and immunology in the corkwing wrasse (symphodus melops l.). *Behavioral Ecology and Sociobiology*, *50*, 511–518.

Glossary

Animal pole: The non-yolk-containing (upper) half of the egg. During embryogenesis, cells at the animal pole divide rapidly and become actively mobile ("animated").

Blastodisc: Small region at the animal pole of telolecithal eggs of fish, containing yolk free cytoplasm where cleavage can occur and that gives rise to the embryo.

Blastula: Early-stage embryo consisting of a sphere of cells surrounding an inner fluid-filled cavity, the blastocoel.

Gastrula: A stage of the embryo following gastrulation that contains the three germ layers that will interact to generate the organs of the body.

Gastrulation: A process involving movement of the blastomeres of the embryo relative to one another resulting in the formation of the three germ layers of the embryo.

Kupffer's vesicle: Transient fluid-filled organ.

Melanophore: a pigmented connective-tissue cell containing melanin in its cytoplasm, responsible for color changes in many fishes and reptiles.

Somites: Segmented block or ball of mesoderm. Will form the axial skeleton (vertebra, ribs), all skeletal muscle, the dorsal dermis, tendons, joints and dorsal aortic cells.

Telosts: bony fish of the subclass Teleostei, having rayed fins and a swim bladder: the group contains most of the bony fishes, including the herrings, carps, eels, cod, perches, etc.

Vegetal pole: The yolk containing portion of the egg or embryo.

Appendix A. Reported use of cleaner fish in Norwegian salmon and trout farms from 1998-2014

Rensefisk

Clean fish

Oppdatert per 10.12.2015

Kilde: Fiskeridirektoratet

Source: Directorate of Fisheries

Utsett (bruk) av oppdrettet og villfanget rensefisk til lakselusbekjempelse fordelt på fylke. Antall i 1000 stk. Verdi i 1000 kroner

Use of farmed and wild clean fish in the production of Atlantic salmon and Rainbow trout by county. Number in 1000 individuals. Value in 1000 NOK

	2014		2013		2012		2011		2010	
Fylke County	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK
Finnmark og Troms	123	1493	0	0	42	495	0	0	0	0
Nordland	1 767	25 916	1 097	17 240	1 477	22 162	861	11 052	431	5 278
Nord-Trøndelag	2 050	24 526	1 183	13 962	1 386	18 187	819	8 880	1 294	12 514
Sør-Trøndelag	3 853	63 215	3 952	43 522	1 731	21 578	1 894	24 619	1 699	18 189
Møre og Romsdal	3 729	48 027	1 658	21 443	2 368	27 233	1 548	16 411	2 735	21 792
Sogn og Fjordane	4 262	10 169	921	10 382	668	6 179	847	6 816	782	5 153
Hordaland	6 236	70 637	5 167	35 560	4 696	40 542	3 209	26 884	2 462	14 501
Rogaland	2 230	27 593	2 016	17 362	1 341	11 989	1 230	12 791	1 362	11 427
Øvrige fylker	217	2 709	212	2 342	193	2 233	230	2 875	212	1 753
Totalt/Total	**24 467**	**274 285**	**16 206**	**161 812**	**13 903**	**150 598**	**10 639**	**110 327**	**10 976**	**90 606**

2009		2008		2007		2006		2005		2004	
Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK
0	0	0	0	0	0	0	0	0	0	0	0
182	1 956	101	949	30	263	7	60	71	595	131	890
237	1 613	91	624	0	0	0	0	31	197	60	400
232	2 176	14,1	130	13	120	0	0	24	177	24	173
374	1 921	30	167	92	758	70	512	169	1 096	220	1 661
175	971	0	0	1	5	0	0	26	116	13	54
2 482	14 689	933	3 772	1 003	4 272	479	1 548	203	726	480	1 701
1 046	6 691	376	2 083	300	1 129	126	120	141	689	116	558
154	1 267	152	902	124	953	0	0	115	670	90	620
4 883	**31 286**	**1 696**	**8 627**	**1 564**	**7 499**	**682**	**2 240**	**781**	**4 268**	**1 134**	**6 058**

345

2003		2002		2001		2000		1999		1998	
Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK	Antall Number	Verdi Value NOK
0	0	13	176	0	0	0	0	0	0	0	0
126	885	107	694	150	1 128	170	1 206	157	1 073	111	788
14	93	21	141	102	636	54	315	186	1 058	112	658
17	112	18	129	39	293	41	237	288	1 254	149	856
229	1 460	128	796	139	894	211	1 147	169	991	264	1 520
104	502	71	332	353	1 708	150	748	309	1 085	162	722
674	2 735	912	2 841	1 334	4 484	1 017	3 698	1 329	4 655	1 505	4 400
205	1 135	181	848	192	796	213	716	154	637	41	174
171	1 025	122	488	12	486	20	80	26	109	27	113
1 539	**7 947**	**1 573**	**6 447**	**2 321**	**10 425**	**1 876**	**8 147**	**2 619**	**10 862**	**2 369**	**9 231**

Appendix B. Reported sales of farmed cleaner fish to producers of Atlantic salmon and Rainbow trout

Andre marine fiskearter

Other marine species

Oppdatert per/updated 10.12.2015

Kilde: Fiskeridirektoratet

Source: Directorate of Fisheries

Salg av oppdrettet rensefisk til lakselusbekjempelse. Antall i 1000 stk. Verdi i 1000 kroner.

Sale of farmed clean fish to producers of Atlantic salmon and rainbow trout. Numbers in 1000 individuals. Value in 1000 NOK

	2014		2013		2012	
Art *Species*	Antall *Numbers*	Verdi *Value*	Antall *Numbers*	Verdi *Value*	Antall *Numbers*	Verdi *Value*
Berggylt/*Wrasse*	379	6 740	95	1 769	270	2 000
Rognkjeks/*Lumpfish*	3 457	52 858	1 954	27 717	431	5 175
Andre rensefiskarter/*Other species*	0	0	0	0	2	32
Totalt/*Total*	3 836	59 598	2 049	29 486	703	7 208

Appendix C. Catch data reported from Norwegian fishing vessels, sorted by species of cleaner fish

Fangstdata fordelt på art: rensefisk

Catch data sorted after species of cleaner fish

Kilde: Fiskeridirektoratet
Source: Directorate of Fisheries

Registrert fangst på leppefisk og rognkjeks for årene 2012-2015, basert på mottatte landings- og sluttseddeldata.
Registered catch of wrasses and lump fish for the years 2012-2015, based on landing data.

Antall stykk fisk er langt lavere i 2012 enn senere. Dette skyldes at informasjon om dette først ble påkrevd i 2013, og da for haiarter og leppefisk. Det mangler tall for rognkjeks siden informasjon om antall ikke er påkrevd for denne arten.
The number of fish is lower in 2012 than later. This is because it did not become compulsory to report data on species until 2013 for shark species and wrasses. Data is missing for lump fish because it is not compulsory to report on this species.

	2012		2013		2014		2015	
Art/Species	Antall stykk/ Number	Rundvekt	Antall stykk/ Number	Rundvekt	Antall stykk/ Number	Rundvekt	Antall stykk/ Number	Rundvekt
Annen leppefisk/*Other cleaner fish*	0	225			2 789	126		
Berggylt/ *Ballan*	524 778	118 387	1 207 602	155 445	1 269 565	154 223	1 530 166	193 426
Bergnebb/ *Goldsinny*	2 624 678	181 224	8 907 353	232 855	11 684 724	281 347	9 162 161	219 954
Blåstål/ Rødnebb/ *Cuckoo*							623	106
Gressgylt/ *Rock cook*	108 505	9 008	265 866	5 340	352 126	7 817	292 117	5 842
Grønngylt/ *Corkwing*	1 949 010	225 355	5 106 484	236 516	7 957 439	352 877	9 801 045	442 380
Rognkall (han)/ *Lumpfish male*	0	276		101		467	0	2 136
Rognkjeks (felles)/ Lumpfish	0	10 503		20				0
Rognkjeks (hun) / *Lumpfish female*	0	1 029 704		986 748		93 684	0	352 302
Rødnebb/ *Cuckoo*	5 147	124	1 685	40	200	5	2 138	51
Grand Total	**5 212 118**	**1 574 804**	**15 488 990**	**1 617 065**	**21 266 843**	**890 546**	**20 788 250**	**1 216 197**