Embryonic Development in Corkwing Wrasse, Symphodus melops

Enrique Blanco Gonzalez[1,2,3], Ingrid Torstensnes[1], Mana Naito[4]

[1)] Department of Natural Sciences, University of Agder, N-4604 Kristiansand, Norway

[2)] Center for Coastal Research, University of Agder, N-4604 Kristiansand, Norway

[3)] Norwegian College of Fishery Science, UiT The Arctic University of Norway, N-9037, Tromsø, Norway

[4)] NAV Arendal, N-4836 Arendal, Norway

Corresponding author

Enrique Blanco Gonzalez

Telephone/Fax: (+47) 37059000 / (+47) 45496531

e-mail: enrique.blanco@uit.no

Ingrid Torstensnes

e-mail: ingrid.torstensnes@gmail.com

Mana Naito

e-mail: mananaito922@gmail.com

Abstract

Corkwing wrasse, Symphodus melops, is one of the main species used as cleaner fish to combat sea lice infestation in salmon aquaculture; however, there is little knowledge about its biology. Here, we describe the embryonic development of this species and examined the viability of the eggs under three temperature regimes. The experiments were conducted at three water temperature regimes, 12, 15 and 18 ˚C, which resemble common sea water temperatures registered during the spawning season of corkwing wrasse at different latitudes along the Norwegian coast. Corkwing wrasse spawn small spherical eggs of 0.75-0.80 mm in diameter (mean 0.78, cv = 3.6 %) with several oil droplets and go through eight developmental stages until hatching. The shortest hatching time was registered after 144 h at 18 ˚C, hatching after 222 h and 372 h at 15 and 12 ˚C, respectively. These observations provide important baseline biological information to advance the establishment of commercial rearing techniques and sustainable fishing management practices for this heavily exploited species.

Keywords: fish egg, developmental stages, cleaner fish, salmon aquaculture, hatching time, temperature

Introduction

The use of cleaner fish as biological treatment to combat sea lice infestation in Norwegian salmon farming has reached unprecedented levels (Blanco Gonzalez and de Boer 2017), with more than 54 million fish used in 2017 (Statistics Norwegian Directorate of Fisheries: http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Rensefisk). At present, five main species are used as cleaner fish, namely: corkwing Symphodus melops, goldsinny Ctenolabrus rupestris, ballan Labrus bergylta and rock cook Centrolabrus exoletus wrasses and lumpfish Cyclopterus lumpus (Deady et al. 1995; Skiftesvik et al. 2013). While most lumpfish used in salmon farms are of hatchery origin, the vast majority of wrasses are wild-caught fish, except for a small number of cultured ballan wrasse (Statistics Norwegian Directorate of Fisheries: http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Rensefisk). The abundance of wild wrasses in Norway has increased drastically over the last decades (Barceló et al. 2016), a trend that appears associated with the warmer sea water temperatures registered (Knutsen et al. 2013). As result, millions of wrasses are fished in warmer southern regions and annually translocated to salmon farms located in colder northern areas where local stocks are not large enough to support their high demand (Blanco Gonzalez and de Boer 2017). The intensive fishing pressure on wrasses raised concerns about the sustainability of the fishery and a maximum fishing quota was implemented for the first time in 2016. In 2017, approximately 20 million wild wrasses were used by the salmon industry (Statistics Norwegian Directorate of Fisheries: http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Rensefisk), despite little knowledge about their biology (Blanco Gonzalez and de Boer 2017). Additional concerns lie in the possibility that translocated wrasses with distinct genetic profiles to recipient populations (Blanco Gonzalez et al. 2016; Jansson et al. 2017)

may escape (Espeland et al. 2010), and spawn viable eggs that may survive the colder temperatures experienced in northern regions (Blanco Gonzalez and de Boer 2017; Faust et al. 2018). Eventually, they could establish a genetically distinct population and/or compromise the viability and evolutionary potential of the species (Blanco Gonzalez et al. 2016; Faust et al. 2018). The development of breeding programs to produce domesticated wrasses was proposed as an alternative to reduce the fishing pressure on wild stocks and mitigate any risk associated with fish translocations (Blanco Gonzalez and de Boer 2017).

Major constraints to the development of wrasse aquaculture are the facts that wrasses grow slowly (Costello 1991), requiring at least two years before they can be placed in the salmon nets (Helland et al. 2014), and that their metabolic and feeding activity on sea lice decrease at temperatures below 10°C (Costello et al. 1995: Sayer et al. 1996), which are common around Norwegian salmon farms during winter months. Initial attempts to produce the three most abundant wrasse species in Norway brought promising results (Skiftesvik et al. 1996; Stone 1996; Van der Meeren and Lønøy 1998). However, the small size at mouth opening in the two smallest species, goldsinny and corkwing wrasse, represented a major challenge during first feeding, and rearing techniques were directed exclusively towards the larger ballan wrasse (Helland et al. 2014).

Corkwing wrasse is the second largest wrasse used as cleaner fish in Norway and, together with ballan wrasse, it is the only species used to remove sea lice during the second year of salmon in the net pens (Skiftesvik et al. 2013). This fact makes these two species particularly interesting for the salmon aquaculture industry. Similarly to other cleaner wrasses, the metabolic activity of corkwing wrasse is correlated to sea water temperatures. At sea water temperature below 8°C, they enter a hypometabolic state (Costello et al. 1995) while high mortality rates have been reported below 4°C (Bjelland et al. 1996). The spawning season of corkwing wrasse in Norway extends from late April to August, when territorial

89 males gather and overlap several layers of different algae species to build and guard complex
90 nests where females will lay their sticky eggs (Uglem and Rosenqvist 2002). The larval
91 development of corkwing wrasse was described earlier by Quignard (1967); however, there is
92 no information about the embryonic development of the species.
93 This study describes for the first time the embryonic development of corkwing wrasse.
94 Additionally, it confirms the viability of corkwing wrasse eggs under three temperature
95 regimes commonly found along the Norwegian coast during the spawning season. This
96 manuscript provides fundamental baseline biological information on this heavily exploited
97 species and contributes to advancing the future development of rearing techniques for this
98 cleaner fish.
99
100 Materials and Methods
101 The parental stock used in this study comprised one male (total length = 18.5 cm, total
102 weight = 88.4 g) and one female (total length = 16.0 cm, total weight = 52.8 g) caught using
103 eel pots by a local fisherman in Arendal, Norway, on 24th June 2014 at 17 °C. The fish were
104 kept at approximately 10 °C following natural fluctuations in a large mesocosm basin of
105 approximately 5000 l located at the facilities of the Institute of Marine Research (IMR) at
106 Flødevigen, Arendal. The seawater was pumped up from 19 m depth and kept at an exchange
107 rate of 2-3% per day. On June 27th 2016, the parental pair were caught with eel pots from the
108 basin where the temperature was 16.7 °C, manually stripped and eggs and milt were split in
109 three small PVC cylinder collectors attached with a net of 160 microns where eggs adhered
110 and ensured good aeration. Each PVC cylinder with fertilized eggs was immersed in 450 l
111 squared tanks at 12.0±0.4, 15.0±0.4 and 18.0±0.3 ˚C, respectively, to follow the embryonic
112 development under the three temperature regimes. The election of these values of temperature
113 is based on the fact that they are common sea water temperatures registered during the

spawning season of corkwing wrasse at different latitudes along the Norwegian coast. The lighting regime in the tanks simulated natural light (18L:6D). Egg inspections were performed on batches of 10-20 eggs every 15 min for the first 8 h and subsequently every 6 h until 50% of the eggs hatched. Their developmental stages were examined under a binocular microscope, key features annotated and photographed under a Leica MZ16a stereomicroscope (Leica, http://www.leica-microsystems.com) fitted with a Tucsen CMOS IS1000 camera (Tucsen, http://www.tucsen.com/). We also recorded timing of the developmental stages and hatching times; however, this information should be considered orientative, as only one single breeding pair was used in our experiments. The images were then analyzed with the software Fiji (Schindelin et al. 2012). In order to determine the size of spawned eggs and newly hatched larvae, a total of 20 eggs and 20 newly hatched larvae were measured and their average values and coefficient of variation, cv = sd / mean x 100, determined. The details of the embryonic development of corkwing wrasse were monitored after the gelatinous layer that cause debris and contaminants to attach to the sticky eggs was carefully removed using dissecting needles. The criteria to consider a new stage of development was set as where 50% of the batch of eggs sampled had reached the subsequent stage. Each batch of eggs was used only once and they were preserved in 95% ethanol after examination in case they were needed to create the illustrations.

Results

Corkwing wrasse spawned small spherical eggs of 0.75-0.80 mm in diameter (mean 0.78, cv = 3.6 %). The eggs were attached by mucus to the bottom of the PVC cylinder collectors and it was necessary to remove the gelatinous layer with dissecting needles to examine the unique features during the embryonic development. The eggs presented several small oil droplets embedded in the yolk which remained through the whole embryonic

development until hatching. The embryonic development of corkwing wrasse is documented in VIII stages by the progressive occurrence of specific developmental stages through cleavage, blastulation, gastrulation and segmentation to hatch (see illustrations in Fig. 1 and Fig. 2 and detailed description in Table 1). In short, at 15 ˚C, the first cleavage takes place after 2 h and 15 min with subsequent cell divisions recorded at regular intervals of 15-30 min (Fig. 2a-2i). Initially, multiple oil droplets are located at the vegetal pole. Later on, they spread gradually through the animal pole. After the blastulation (Fig. 1c) and gastrulation (Fig. 1d) stages, the appearance of the somites marks the beginning of the segmentation stage where the embryo starts developing progressively. After 90 h, the tail detaches from the yolk, the presence of otoliths becomes apparent and the embryo starts acquiring some pigmentations. At stage VII, after 174 h, the tail of the embryo reaches the head, the presence of melanophores and xanthophores darkens the embryo gradually and its movement and heartbeat become more frequent. After 222 h, 9 days and 6 h, the chorion is broken and a newly hatched larva of 2-2.5 mm in total length starts swimming freely.

Timing of the developmental stages presented in Table 1 correspond to a temperature of 15.0±0.4 ˚C, a common value during the spawning season of corkwing wrasse in the proximities of the facilities where these experiments were conducted. In parallel to the observations and characterization conducted at 15 ˚C, our experiments confirmed that corkwing wrasse eggs can survive and hatch at 12 ˚C and 18 ˚C. At 18±0.3 ˚C, the embryonic development of corkwing wrasse sped up and hatching time shortened to 144 h, 6 days and 6h. In contrast, those eggs exposed to the colder sea water temperatures commonly found in northern regions, 12±0.4 ˚C, delayed their rate of development and hatching occurred after 372 h, 15 days and 12 h.

Discussion

This study describes for the first time the embryonic development of corkwing wrasse, one of the main cleaner fish species used against sea lice infestation in European salmon aquaculture. The embryonic development of corkwing wrasse encompasses VIII stages by the progressive occurrence through cleavage, blastulation, gastrulation and segmentation to hatch.

Corkwing wrasse spawn small spherical eggs ranging 0.75-0.80 mm in diameter (mean 0.78, cv = 3.6 %), in the lower limit of those previously recorded by other authors (Quignard 1967; Stone 1996). The presence of a gelatinous layer of mucus attached to the surface, the occurrence of several small oil droplets embedded in the yolk, and the progressive succession of developmental stages observed in corkwing wrasse eggs resemble common features among several demersal marine labrids (Dulcic et al. 1999; D'Arcy et al. 2012).

We registered large variation in hatching times among corkwing wrasse eggs exposed to different temperature regimes, ranging between 144 h (6 days) at 18 ˚C and 372 h (15 days and 12 h) at 12 ˚C. Previously, at 14-15 ˚C, Stone (1996) recorded hatching times ranging between 9 to 15 days while Torstensnes (2016) reported that the first eggs hatched after only 144 h (6 days). Interindividual variation and sea water temperature are known to play an important role in in the rate of development of marine fish eggs (Pepin 1991; Chambers and Leggett 1996; Brooks et al. 1997). Using a single breeding pair, we followed the embryonic development of corkwing wrasse eggs; however, the lack of inter-individual variance refrains from driving comparisons of egg sizes or hatching times to previously published work. Future studies rearing a larger broodstock are encouraged as fluctuation in hatching times may have major implications on egg production, survival, connectivity, and eventually on population viability (Laurel and Bradbury 2006; Houde 2008; Laurel and Blood 2011).

Our results confirmed that corkwing wrasse eggs produced by a breeding pair from southern Norway could hatch at 12 ˚C, a common summer temperature at higher latitudes in

Norway where wrasses are commonly translocated to. These observations support the results of a recent study conducted by Faust et al. (2018) who reported successful reproduction of translocated individuals of southern origin neighboring salmon farms in mid Norway. It should be noted, however, that corkwing wrasse may be translocated to northern areas at the limit of their temperature tolerance (Costello et al. 1995; Bjelland et al. 1996; Maroni and Andersen 1996), where sea water temperatures above 12.0 ˚C may be restricted to very short periods. Further investigations should help to clarify to what extent translocated wrasses may colonize new areas further north and how low temperatures compromise the viability of corkwing wrasse eggs and larvae.

Newly hatched corkwing wrasse larvae in this study were 2-2.5 mm in total length, again in the lower limit of the sizes previously reported by Quignard (1967) and Stone (1996). They are smaller than those of most labrid species found in European waters (see Kožul et al. 2011). One exception is goldsinny wrasse, the other main cleaner wrasse species used in Norwegian salmon farms, whose larvae are smaller and less developed (Stone 1996). Indeed, the small size of the larvae at the time of mouth opening in corkwing and goldsinny wrasses has been a major constraint to develop the rearing techniques for these species. Instead, efforts have been directed towards the larger ballan wrasse which is the only wrasse species farmed commercially (Blanco Gonzalez and de Boer 2017). The development of aquaculture techniques to produce large numbers of domesticated wrasses may open new opportunities to mitigate the intensive fishing pressure on wild wrasses as well as the ecological and genetic risks associated with fish translocated populations (Blanco Gonzalez and de Boer 2017). The production of offspring from wild specimens with regular broodstock replacement may also help to reduce the risk of inbreeding in case of escapees; although it may bring some of the threats reported to hatchery-released practices (Blanco Gonzalez and Umino 2012). This manuscript provides important baseline biological information on this heavily exploited

species and contributes to advances in the future development of commercial rearing techniques for this cleaner fish.

Acknowledgments

This work was funded under the Havkyst program of the Norwegian Research Council (Project #234328/MO “Adaptation or plasticity as response to large scale translocations and harvesting over a climatic gradient in the marine ecosystem?”). We would like to thank Stian Stiansen for his assistance during the experimental set up. We would also like to thank two anonymous reviewer for their valuable comments on earlier versions of the manuscript.

References

Barceló, C., Ciannelli, L., Olsen, E. M., Johannesen, T., and H. Knutsen. 2016. Eight decades of sampling reveal a contemporary novel fish assemblage in coastal nursery habitat. Global Change Biology 22:1155–1167.

Bjelland, R., L. Simensen, and P. G. Kvenseth.1996. Successful survival of wrasse through winter in submersible netcages in a fjord in western Norway. Pages 265-271 in M. D. J. Sayer, J. W. Treasurer, and M. J. Costello, editors. Wrasse: biology and use in aquaculture. Fishing New Books, Oxford.

Blanco Gonzalez, E., and F. de Boer. 2017. The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fisheries Science 83:651–660.

Blanco Gonzalez, E., H. Knutsen, and P. E. Jorde. 2016. Habitat discontinuities separate genetically divergent populations of a rocky shore marine fish. PLoS ONE 11(10):e0163052.

Blanco Gonzalez, E., and T. Umino. 2012. Managing the genetic resources in the intensive stock enhancement program carried out on black sea bream in Hiroshima Bay. Pages 217-230 in M. Caliskan, editor. Analysis of genetic variation in animals. InTech, Rijeka, Croatia.

Brooks, S., C. R. Tyler, and T. R. Sumpter. 1997. Quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7:387–416.

Chambers, R. C., W. C. Leggett. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. American Zoologist 36:180–196.

Costello, M. J. 1991. Review of the biology of wrasse (Labridae: Pisces) in northern Europe. Progress in Underwater Science 16:29–51.

247 Costello, M. J., W. R. T. Darwall, and S. Lysaght 1995. Activity patterns of North European
248 wrasse (Pisces, Labridae) species and precision of diver survey techniques, Pages 343-
249 350 in A. Eleftheriou, A. D. Ansell, and C. J. Smith, editors. Biology and ecology of
250 shallow coastal waters. Proceedings of the 28th European Marine Biological Symposium,
251 Crete, Greece, 23-28 September 1993. International Symposium Series. Olsen & Olsen,
252 Copenhagen.
253 Deady, S., S. J. A. Varian, and J. M. Fives. 1995. The use of cleaner-fish to control sea lice on
254 two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in
255 salmon cages. Aquaculture 131:73–90.
256 Dulcic, J., V. Kozul, M. Kraljevic, B. Skaramuca, B. Glamuzina, and P. Ré. 1999. Embryonic
257 and larval development of the brown wrasse *Labrus merula* (Pisces: Labridae). Journal of
258 the Marine Biology Assocoation of the UK 79:327–332.
259 D'Arcy, J., E. Dunaevskaya, J. W. Treasurer, O. Ottesen, J. Maguire, N. Zhuravleva, A.
260 Karlsen, C. Rebours, and R. D. Fitzgerald. 2012. Embryonic development in ballan wrasse
261 *Labrus bergylta*. Journal of Fish Biology 81:1101–1110.
262 Espeland, S. H., K. Nedreaas, S. Mortensen, A. B. Skiftesvik, A. -L. Agnalt, C. Durif, L.
263 Harkestad, E. Karlsbakk, H. Knutsen, T. Thangstad, K. Jørstad, Å. Bjordal, and J.
264 Gjøsæter. 2010. Current knowledge on wrasse—challenges in an increasing fishery. Fisken
265 og havet 7:1–38 (in Norwegian).
266 Faust, E., K. T. Halvorsen, P. Andersen, H. Knutsen, and C. André. 2018. Cleaner fish escape
267 salmon farms and hybridize with local wrasse populations. Royal Society Open Science
268 5:171752.
269 Helland, S., S. W. Dahle, C. Hough, and J. Borthen. 2014. Production of ballan wrasse
270 (*Labrus bergylta*). Science and practice. The Norwegian Seafood Research Fund (FHF),
271 Norway.

Houde, E. D. 2008. Emerging from Hjort's shadow. Journal of the Northwest Atlantic Fishery Science 41:53–70.

Jansson, E., Quintela, M., Dahle, G., Albretsen, J., Knutsen, H., André, C., Strand, Å., Mortensen, S., Taggart, J. B., Karlsbakk, E., Kvamme, B. O., and K. A. Glover. 2017. Genetic analysis of goldsinny wrasse reveals evolutionary insights into population connectivity and potential evidence of inadvertent translocation via aquaculture. ICES Journal of Marine Science 74: 2135-2147.

Knutsen, H., P. E. Jorde, E. Blanco Gonzalez, J. I. Robalo, J. Albretsen, and V. Almada. 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(6):e67492.

Kožul, V., N. Glavić, P. Tutman, J. Bolotin, and V. Onofri. 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.

Laurel, B. J., and I. R. Bradbury. 2006. "Big" concerns with high latitude marine protected areas (MPAs): trends in connectivity and MPA size. Canadian Journal of Fisheries and Aquatic Sciences 63:2603–2607.

Laurel, B. J., and D. M. Blood. 2011. The effects of temperature on hatching and survival of northern rock sole larvae (*Lepidopsetta polyxystra*). Fishery Bulletin 109: 282–291.

Maroni, P., and P. A. Andersen. 1996. Distribution and abundance of wrasse in an area of northern Norway. Pages 70-73 in M. D. J. Sayer, J. W. Treasurer, and M. J. Costello, editors. Wrasse: biology and use in aquaculture. Fishing New Books, Oxford.

Pepin, P. 1991. The effect of temperature and size on development and mortality rates of the pelagic early life history stages of marine fish. Canadian Journal of Fisheries and Aquatic Sciences 48:503–518.

297 Quignard, J. -P. 1967. L'oeuf et la larve du lab ride *Symphodus* (*Crenilabrus*) *melops* (Linne,
298 1758): influence de differents facteurs physiocochimiques sur la duree du developpement
299 embryonnaire. Revue Travail de I'Institut Peches Maritime 31:355–358 (in French).
300 Sayer, M. D. J., and J. P. Reader. 1996. Exposure of goldsinny, rock cook and corkwing
301 wrasse to low temperature and low salinity: survival, blood physiology and seasonal
302 variation. Journal of Fish Biology 49:41–63.
303 Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch, S.,
304 Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri,
305 K., Tomancak, P., and A. Cardona. 2012. Fiji: an open-source platform for biological-
306 image analysis. Nature Methods 9:676–682.
307 Skiftesvik, A. B., R. M. Bjelland, C. M. F. Durif, I. S. Johansen, and H. I. Browman. 2013.
308 Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus*
309 *bergylta*). Aquaculture 402:113–118.
310 Skiftesvik, A. B., K. Boxaspen, and A. Parsons. 1996. Preliminary breeding trials of wrasse
311 in an intensive system. Pages 136–141 in M. D. J. Sayer, J. W. Treasurer, and M. J.
312 Costello, editors. Wrasse: biology and use in aquaculture. Fishing New Books, Oxford.
313 Stone, J. 1996. Preliminary trials on the culture of goldsinny and corkwing wrasse. Pages
314 142–167 in M. D. J. Sayer, J. W. Treasurer, and M. J. Costello, editors. Wrasse: biology
315 and use in aquaculture. Fishing New Books, Oxford.
316 Torstensnes, I. 2016. Embryonic development of corkwing wrasse, *Symphodus melops*. M.Sc.
317 Thesis, University of Agder, Kristiansand, Norway.
318 Uglem, I., and G. Rosenqvist. 2002. Nest building and mating in relation to male size in
319 corkwing wrasse, *Symphodus melops*. Environmental Biology of Fishes 63:17–25.

320 Van der Meeren, T., and T. Lønøy 1998. Use of mesocosms in larval rearing of saithe
321 [*Pollachius virens* (L.)], goldsinny [*Ctenolabrus rupestris* (L.)], and corkwing
322 [*Crenilabrus melops* (L.)]. Aquaculture Engineering 17:253–260.
323

Tables

Table 1. Developmental stages of corkwing wrasse, Symphodus melops, eggs at 15±0.4 ˚C. The time where 50% of the batch of eggs sampled had reached the stage, a description of the morphological features and illustrations at each stage are also provided.

330 Figure Legends

331

332 Figure 1. Embryonic development in corkwing wrasse, Symphodus melops, from fertilization
333 until hatching. a) Stage I: fertilized egg gastrula, b) Stage II: first cleavage, c) Stage III:
334 blastula d) Stage IV: gastrula, e) Stage V: Segmentation: somites visible. Embryo covers
335 approximately 1/2 of the yolk circumference, f) Stage VI: Segmentation: tail detaches from
336 yolk. Embryo covers approximately 3/4 of yolk circumference, g) Stage VII: Segmentation:
337 embryo reaches the head. Beginning of the stage, h) Stage VII: Segmentation: embryo reaches
338 the head. Tail starts overlaps the head of the embryo, i) Stage VII: Segmentation: embryo
339 reaches the head. Before hatching, the tail of the embryo overlaps approximately 1/4 of its
340 body. j) Stage VIII: Hatching. Newly hatched larva. A: anal opening, AP: animal pole, B:
341 brain, BD: blastodisc, BE: beak-like mass, BL: blastula, BM: blastomere, CH: chorion, EY:
342 rudimentary eye, GA: gastrula, GR: germ ring, H: heart, KV: Kupffer's vesicle, L: lens, MF:
343 membranous fin, O: Olfactory apparaturs, OD: oil droplet, OT: otolith, OV: otic vesicle, PF:
344 pectoral fin, PI: pigments, PS: perivitelline space, SM: somite, VP: vegetal pole; Y: yolk; YS:
345 yolk-sac.

346

347 Figure 2. Development of the cleavage stage in corkwing wrasse, Symphodus melops, a) first
348 cleavage: 2-cells lateral view, b) second cleavage: 4-cells apical view, c) third cleavage: 8-
349 cells apical view, d) fourth cleavage: 16-cells lateral view, e) fifth cleavage: 32-cells apical
350 view, f) sixth cleavage: 64-cells lateral view, g) seventh cleavage: 128-cells apical view, h)
351 eight cleavage: 256-cells lateral view, i) 512-cells apical view.

Table 1. Developmental stages of corkwing wrasse, *Symphodus melops*, eggs at 15±0.4 ˚C. The time where 50% of the batch of eggs sampled had reached the stage, a description of the morphological features and illustrations at each stage are also provided.

Stage	Name	Time	Description of morphological features	Illustration
I	Fertilised egg	15 min	The newly fertilised egg has a cloudy yellowish core with the blastodisc situated at the animal pole and the yolk at the vegetal pole. It presents a narrow perivitteline space which widens gradually to occupy approximately 10 % of the egg diameter and the blastodisc and yolk become more uniform in colour (Figure 1a).	Figure 1a
II	Cleavage	2 h 15 min	The first cleavage occurred after 2 h 15 min and subsequent cell divisions took place at regular intervals of 15-30 min (Table 1 and Figure 2a-2i). Initially, the blastodisc is cleaved into two even blastomeres that occupies approximately 1/3 of the egg while the yolk occupies about 2/3. At the site of the cleavage the blastodisc narrows and gives the blastodisc an ellipsoidal shape when seen from an apical view (Figure 2a). At the vegetal pole, the yolk presents multiple small circular oil droplets that will spread gradually. 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 2b). After the third cleavage, blastodisc divided into 8 cells (Figure 2c), the oil droplets gradually spread through the yolk towards the animal pole and after the next cell division they appear evenly dispersed throughout the yolk (Figure 2d). At the end of this stage, the blastodisc is divided into 512 small blastomeres and adopts a circular shape with lightly bulging edges (Figure 2i).	Figure 1b and Figure 2a-2i
III	Blastula	6 h	At this stage, it becomes difficult to distinguish individual blastomeres (Figure 1c). Instead, a homogenous half-moon shaped clump of cells is seen at the animal pole, it is called blastula. In the blastula stage the border between the blastodisc and the yolk is smooth, running in a continuous line which envelops the yolk.	Figure 1c
IV	Gastrula	15 h	At this stage, the blastula has flattened outwards into a dome shaped gastrula at the animal pole. The germ ring starts migrating over the yolk towards the vegetal pole which starts narrowing and becomes more elongated and	Figure 1d

			ellipsoidal in shape (Figure 1d). 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 future embryo starts becoming distinguishable. At the end of this stage, the germ ring envelops the yolk almost completely and the embryo covers about half of the circumference of the yolk. At this point, oil droplets appear concentrated in the opposite side to where the body of the embryo is developing.	
V	Segmentation: somites	42 h	The appearance of somites in the central part of the embryo marks the transition from gastrulation 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 and Kupffer's vesicle become apparent (Figure 1e). 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, the Kupffer's vesicle enlarges and more somites are seen posteriorly along the body of the embryo. At the end of this stage oil droplets tend to concentrate in the area close to the head and tail of the embryo which is still fully attached to the yolk.	Figure 1e
VI	Segmentation: tail detaches from yolk	90 h	At this stage the Kupffer's vesicle shrinks and disappears, while the tail of the embryo starts to detach from the yolk (Figure 1f). As the embryo elongates the brain and heart become clearly visible, the otic vesicles enlarges and the otoliths are evident. The embryo starts displaying a membranous fin, more somites are apparent and a few melanophores reveal signs of pigmentation which become more evident in the next stage.	Figure 1f
VII	Segmentation: embryo reaches the head	174 h	The tail of the embryo reaches the head and continues growing, overlapping the body of the embryo (Figure 1g-1i). At this stage, the embryo darkens gradually and show denser pigmentation. Melanophores and xanthophores appear aligned along the dorsal and ventral side of the body of the embryo covering approximately the upper 2/3 of it (Figure 1h). A few melanophores are also evident are visible in the yolk-sac. The pectoral fin becomes visible posteriorly to the otoliths. The yolk presents some pigmentation and oil droplets appear again evenly dispersed. Eyes are more developed and the	Figure 1g-1i

			movement and heartbeat of the embryo become more frequent. At the end of this stage, the tip of the tail encircles approximately 1/4 of the body of the embryo (Figure 1i).	
VIII	Hatching	222 h	The chorion of the embryo is broken and the newly hatched larva of approximately 2-2.5 mm in total length swims freely (Figure 1j). A few melanophores and oil droplets are still observed in the yolk-sac. The head presents an enlarged brain and well developed eyes, while the olfactory apparatus becomes evident. The heart and a more developed small pair of pectoral fins are now visible and the anal opening is recognized (Figure 1j).	Figure 1j

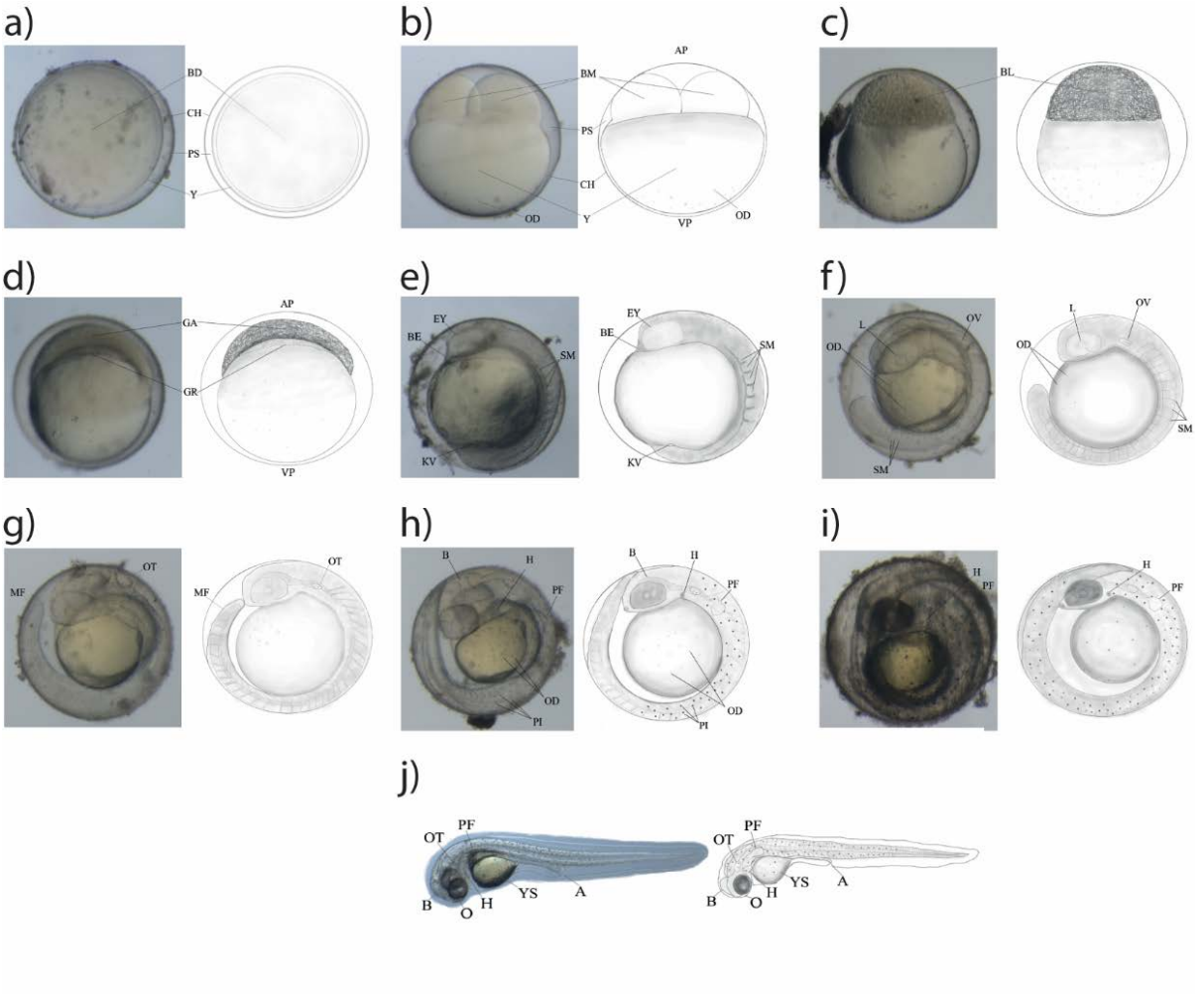

Figure 1. Embryonic development in corkwing wrasse, *Symphodus melops*, from fertilization until hatching. a) Stage I: fertilized egg gastrula, b) Stage II: first cleavage, c) Stage III: blastula d) Stage IV: gastrula, e) Stage V: Segmentation: somites visible. Embryo covers approximately 1/2 of the yolk circumference, f) Stage VI: Segmentation: tail detaches from yolk. Embryo covers approximately 3/4 of yolk circumference, g) Stage VII: Segmentation: embryo reaches the head. Beginning of the stage, h) Stage VII: Segmentation: embryo reaches the head. Tail starts overlaps the head of the embryo, i) Stage VII: Segmentation: embryo reaches the head. Before hatching, the tail of the embryo overlaps approximately 1/4 of its body. j) Stage VIII: Hatching. Newly hatched larva. A: anal opening, AP: animal pole, B: brain, BD: blastodisc, BE: beak-like mass, BL: blastula, BM: blastomere, CH: chorion, EY: rudimentary eye, GA: gastrula, GR: germ ring, H: heart, KV: Kupffer's vesicle, L: lens, MF: membranous fin, O: Olfactory apparaturs, OD: oil droplet, OT: otolith, OV: otic vesicle, PF: pectoral fin, PI: pigments, PS: perivitelline space, SM: somite, VP: vegetal pole; Y: yolk; YS: yolk-sac.

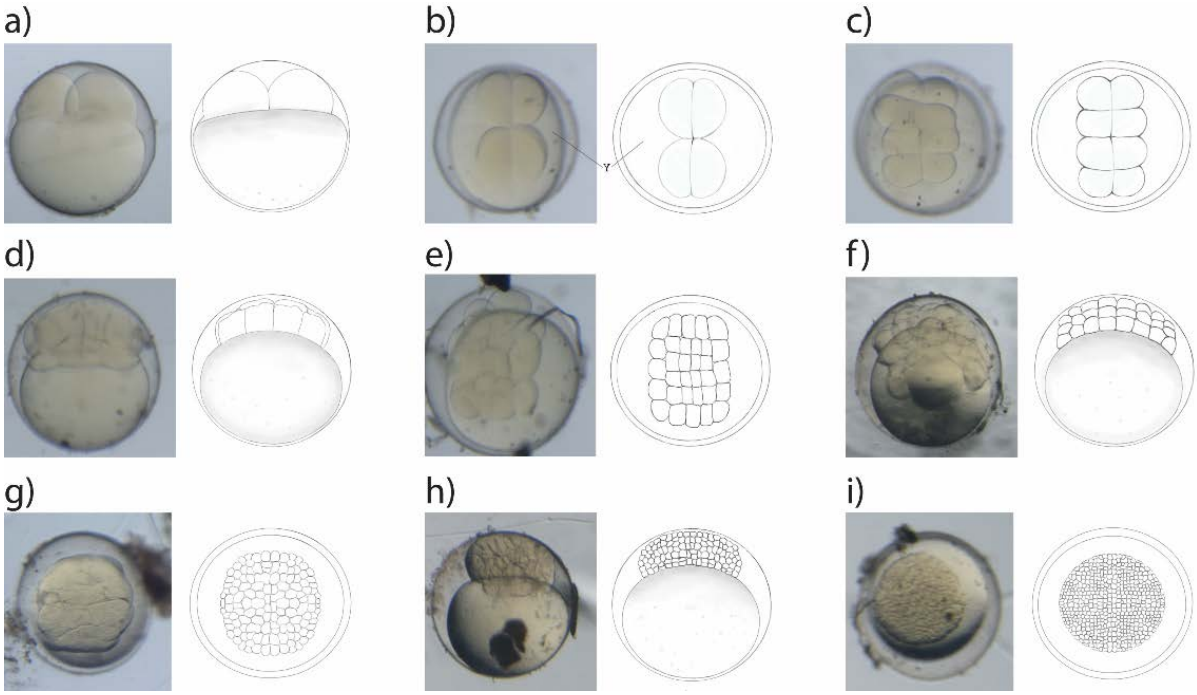

Figure 2: Development of the cleavage stage in corkwing wrasse, *Symphodus melops* a) first cleavage: 2-cells lateral view, b) second cleavage: 4-cells apical view, c) third cleavage: 8-cells apical view, d) fourth cleavage: 16-cells lateral view, e) fifth cleavage: 32-cells apical view, f) sixth cleavage: 64-cells lateral view, g) seventh cleavage: 128-cells apical view, h) eight cleavage: 256-cells lateral view, i) 512-cells apical view.