



# Egg Production

This leaflet outlines the project's findings in relation to the sourcing of Ballan wrasse eggs as well as egg handling and egg incubation techniques.

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## PROJECT SUMMARY

**EcoFish** is a three-year transnational project financed by the European Regional Development Fund/Northern Periphery Program and national private and governmental grants. The project focuses on developing methods for culture and use of Ballan wrasse as cleaner fish.

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**EcoFish** has produced this series of summary technical leaflets on all the relevant practices covering the entire life cycle for the rearing and the production of Ballan wrasse. Readers can access and download more detailed, full-text, pdf versions of these technical leaflets at [www.eco-fish.org](http://www.eco-fish.org)

## Source of Eggs

Ballan wrasse spawn from late spring onwards, depending on latitude. Spawning commences during April in southern regions (e.g. Ireland) and as late as June in northern areas (e.g. Norway) of the North East Atlantic. Ballan wrasse are multiple spawners and may spawn several times over an extended (individual) breeding period which can extend up to two months.

At present, eggs are sourced from three primary types of adult broodstock groups held by commercial hatcheries and research units:

1. Captive broodstocks that are fully-acclimated, fully-conditioned and held over a long-term period
2. Newly-caught, wild, Ballan wrasse adults that are near/ready to spawn
3. Fish from either of the above groups which are treated with hormone analogues to achieve an induced spawning event



Manual extrusion of eggs to a glass beaker from a gravid Ballan

## Standard Examination of Broodstock Groups

Each broodstock group will need to undergo a standard examination to determine the sexual condition of the wrasse:

- Broodstock groups should be checked on a weekly basis during the lead up to the expected spawning season
- The water in the holding tanks should be lowered
- The fish should be removed with a hand net and anaesthetised in a small container using an approved treatment
- The fish should be dried at the vent using paper towel or cotton wool

- The body should be gently massaged to extrude eggs/milt
- The ovarian exudate changes in colour and volume as the ova mature with the approach of spawning season
- The final state of maturation can be confirmed by examining the distended shape of the abdomen and with ultrasound examination

### Examining established broodstocks

The male wrasse show increasing territorial behaviour and patrolling of a breeding area prior to spawning. It is critical that the fish are monitored regularly but are not stressed or disturbed too much by handling. Tanks should be checked daily for egg production on surfaces and any spawning substrates that have been added. Little information is available on the fecundity or repeat spawning habits of individuals.

### Examining wild caught wrasse

Recently acquired, wild-caught, Ballan wrasse may provide a ready source of eggs but it is necessary that they are examined carefully on arrival to determine their sex and the state of development. As with established stocks, it is necessary that they are not stressed excessively given that they have already been subject to a capture event and associated stress. In general, it is best to manually strip fish that are well advanced and if they are not ready to spawn within two weeks of capture then it is common for practitioners to attempt hormonal induction with these remaining specimens.

### Examining hormonally induced stocks

If stocks fail to spawn naturally in tanks or do not release gametes with manual stripping then hormonal induction may be attempted. From various anecdotal accounts, a variety of hormone analogues have been attempted with some success. It is normal to administer the hormonal treatment via intramuscular injection and if a succession of analogues is used then these are generally staggered over an extended period, each several days apart, allowing time for the fish to react to the sequence of treatments.

## Egg Collection and Handling

### Natural spawning and spawning substrates

In a grouping of mature fish, it is expected that the mature broodstock will utilise the available substrates within the tanks as spawning areas. To facilitate this process, spawning substrates or plates should be set on the tank bottom in the area where most of the patrolling activity is occurring. Various trials have examined potential spawning substrates, including rugs, carpet tiles, astroturf matting, terracotta tiles, and perspex sheets. There does not appear to be a preferred substrate. However, clear perspex sheeting has been used for ease of tank hygiene. These are laid on the bottom or tied to the side of the tank for easy removal.



Egg incubation tank

If no eggs have been settled then egg collection plates should be removed every 2–3 days and cleaned thoroughly to avoid bacterial build-up and contamination of eggs. During the spawning season egg plates should be checked visually for egg deposition from the surface. If eggs are present, the plates should be removed from the brood stock tank and taken to a hatchery area where they can be incubated and be still attached to the plates in the incubation units.

As some fish may still spawn on tank surfaces (sides and base), it is possible that these eggs can be recovered by scraping the surfaces of tanks and tank furniture. However, these eggs have a tendency to be mechanically damaged and this will result in overall lowered survival.

### Manual stripping of Ballan wrasse

Mature eggs can be massaged by hand from the body cavity into a clean dry receptacle and then fertilized by adding a small quantity of sperm from a convenient male.

### De-sticking Eggs

Gathering eggs while they are attached to substrates has proved to be a very effective method of acquiring and transferring viable eggs. It is preferable that eggs are not attached to each other and float freely in incubators.

With Ballan wrasse, trials to detach eggs from their settlement substrate and from each other were conducted using a urea solution at 10g/litre and 3g/litre but this was found to be ineffective. Subsequently, a tannin solution 40–50g/litre of water for 10 seconds was also tested and found to be effective. It is important to note that these are preliminary results and must be confirmed in longer-term studies.

## Egg Incubation

### Egg disinfection prior to incubation

To comply with effective husbandry procedures and to maintain quarantine conditions, the eggs should always be thoroughly disinfected before they are stocked into hatchery systems. During the EcoFish project, extensive trials were carried out by different partners on various methods for disinfecting the eggs. Eggs can be disinfected on arrival in the hatchery and before being placed in egg incubation units with 100 ppm Pyceze® for 30 minutes or 4000ppm Kickstart® (Peracetic Acid and Hydrogen Peroxide) for 30 seconds, and then rinsed in clean seawater.

### Stocking incubators with substrate-bound eggs

Egg plates can be inserted in racks or hung from the sides of standard egg incubation units that are used for marine finfish eggs. Mixed batches containing eggs of different ages should not be incubated together since they would hatch at different times in these egg incubators. It is important that the eggs, when being stocked in the incubators, are checked for standard quality criteria and fertilisation rates by detaching approximately 100 eggs and control their quality under a microscope at x10 magnification.



Incubation tank with eggs on Perspex plate



Perspex plate with layer of eggs



## ECOFISH BALLAN WRASSE PROJECT

### Incubation conditions and monitoring

Optimal rearing conditions are still unconfirmed experimentally, but a range of values have been tested and found to be adequate:

- **Temperature:** may be in range 8–18°C with an optimum between 12–14°C
- **Salinity:** full strength seawater is routinely used
- **Light:** eggs are normally incubated in the dark or very low light (about 5 lux)
- **Aeration:** a low level of aeration is provided to give 6 to 9mg/L oxygen
- **Water flow:** this is dependent on the numbers of eggs per container but a low flow rate of 200–500 ml per min appears to be optimal

Incubation units should be checked daily for temperature, salinity, oxygen and water flow. These data should be recorded on standard data sheets. Unlike floating eggs of other marine species, it is not possible to separate the 'drop out' of dead eggs and remove this sinking

non-viable aliquot each day from the incubator. However, it is recommended that the aeration and flow is switched off for a period and any patently dead eggs or clumps can be removed by pipette. Likewise, detritus should also be decanted from the incubators at this time.

### Egg development stages

It is important that the rate of embryonic development is monitored and recorded on an ongoing basis for each batch of eggs. A sample of eggs should be removed from the egg incubator at least every two days and the stage of development determined as per the stages described in the criteria confirmed in studies by the EcoFish consortium.

For detail of egg stages refer to D'Arcy *et al* (2012) Embryonic development in ballan wrasse *Labrus bergylta*. Journal of Fish Biology 81(3):1101-1110. doi: 10.1111/j.1095-8649.2012.03337.x

### The objectives of the EcoFish partnership project are:-

- To establish wrasse hatcheries with captive broodstocks in Ireland, Scotland and Norway
- To develop techniques for rearing wrasse at all life stages
- To produce eggs and larval wrasse
- To develop methods for culture and use of Ballan wrasse as cleaner fish

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