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Hatchery manual for broodstock management and larval production of turbot (Psetta maxima)

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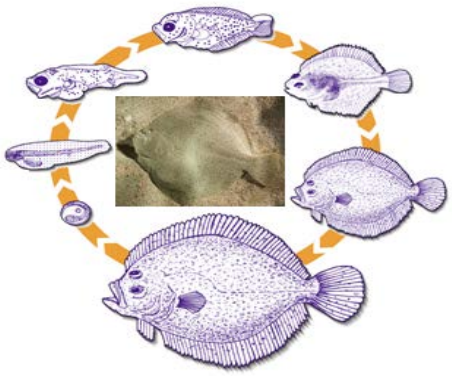

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Contents

Summary

This hatchery manual is intended to provide detailed information from available published work and grey literature on turbot broodstock management and larval production. In reviewing larviculture techniques for turbot, it is notable that the major initial zoo technical advances were made in the 1980s. Subsequent refinements have been industry-led and are subject to commercial confidentiality. Some actors in the sector that have been approached either did not provided requested information or denied access to their sources of information. This manuscript therefore considers those aspects of commercial rearing techniques that are in the public domain, together with the applied scientific literature and information collected from different experts.

With the aim to cover all aspects related to the production of turbot juveniles in Dutch farms from egg to fish of 10-15 g, the manual describes in details different steps. These have been grouped around the broodstock management, the hatchery/nursery period, the on growing and the grow out periods. A list of common diseases in turbot has been added at the end. The manual provides a link to the most update information available on live prey production and enrichment.

The manual has been released into three steps:

- First to the user group of the consortium “VIP Broedhuis” project for a first check of available information and for suggestions depending on their needs.
- Secondary it was submitted to an expert for a final evaluation of the content.
- Thirdly and finally, based on the expert comments, the manual has been revised and extra up-to-date information as asked.

1 Introduction

Turbot (*Psetta maxima* Linnaeus, 1758; *Scophthamus maximus* Raffinesque, 1870) is a benthic marine flatfish, naturally distributed in European waters from the Northeast Atlantic to the Arctic Circle (30° to 70°N; 23°W to 42°E) including the Baltic, Mediterranean and Black Seas (Danancher and Garcia-Vazquez, 2006; Busetto et al., 2008). In the Black Sea a different related species is found, *Psetta maeotica* or the Black Sea turbot *(*Froese and Pauly,2006).

Turbot farming date backs to 1970s and has shown drastic technological developments since beginning of 1990s. Due to its economical interest in aquaculture, its farming in controlled conditions has grown the last years to an aquaculture industry in different European countries: Spain (major producer worldwide), France, Denmark, Germany, Iceland, Ireland, Italy, Norway, Wales (UK), and Portugal, and on a small scale in the Netherlands. Turbot has also been introduced to other regions outside its natural distribution, notably Chile in the late 1980s and more recently China (1992).

Total turbot production from capture principally along the Atlantic coast and aquaculture during the last 15 years has changed between 10,100-14,100 tons/year in Europe. In 2008, the production reached up to 9,000 tons with Spain (7650 tons) followed with France (700-800 tons) and Portugal (540 tons) being the top producers (FEAP, 2008; Sevgili and Nezaki, 2010). However, it is indicated that the production in Portugal will be drastically increased because of a mega-Turbot farm construction in 2009, namely Pescanova (FIS). The estimated production in 2009 and 2010 were 7,760 and 7,000 tons respectively. In China, aquaculture production of turbot, native to Europe, has reached an annual level of 50 000–60 000 tons in recent years, which is about seven times the total culture production of turbot in Europe (FAO, 2010).

Turbot broodstock was initially based on wild collection of mature fish. Nowadays juveniles are produced in hatcheries, grown up to sexual maturity and used as broodstock. Females grow faster than males and sexual dimorphism manifests itself in lifetime, growth rate and size differences between sexes. Females ovulate spontaneously in captivity but eggs are of variable quality. By modifying the farming temperature and mostly the day length (photoperiod), it is possible to achieve offset spawning patterns throughout the year. Since natural spawning does not occur under hatchery conditions, artificial reproduction and hand stripping of gametes are most used to achieve a sustainable production of larvae. Turbot gametes are obtained by manual stripping and artificial fertilization (Leclerq, 1994).

The purpose of this hatchery manual is to revise available information, serve as a guide for the management of broodstock and the production of turbot larvae all-year-round. The manual focuses on broodstock conditioning, egg production and larvae rearing. Information provided is based on the review of published literature, official and unpublished reports and has been reviewed by a selected expert who contributed to the development of the turbot farming.

2 Broodstock

Initially turbot broodstock has been obtained from wild fishery captures. After several years of domestication and selective breeding, they are no longer collected from the wild and only hatchery reared juveniles are selected by farmers to be grown to adults mature fish. Broodstock populations raised in captivity are used to produce high quality eggs and larvae.

2.1. Broodstock from the wild

Initial turbot broodstock has been obtained from fish captured in the wild by beam trawlers and later the use of gill nets proved to provide good survival (Leclerq, personal communication). Before 1980, rather large fish weighing between 3 and 12 kg were used (Devauchelle et al., 1988). Operation consisted in collecting broodstock of different sizes and age classes and their replacement every 1 to 2 years by fish of the youngest class. The number of broodstock was calculated taking into account the time lag observed between the catch at sea and the first spawning in captivity, 2 years in the case of the turbot. While adult fish of 4-6 kg could be spawned within 10 to 12 months following introduction in the hatchery, the spawning of turbots collected at their immature stage was a long process and not all hatchery managers recruited juveniles from the wild. Healthy fish, not wounded or presenting any deformity were kept to start the hatchery. Combined mortality due to fishing and transportation was less than 20% for immature fish trawled for 10 to 30 min at a maximum depth of 10-20 m but could be as high as 50% or more when mature fish were trawled at 30-40 m depth.

2.1.1 Transportation

Transportation of broodstock within 3 h were successfully accomplished in oxygenated 1.5-2 m^3 tanks at a density of 10 kg/m^3 . 10-liter plastic bags containing water (fish density: 0.2 kg/l) have been used for short distance transport of less than 2 hours (Devauchelle et al., 1988). Juvenile fish were transported in fish-transport trucks. Transportation time varied from 8 to 12 h in case fish are provided with aeration from a bottom grid. The oxygen levels were kept at around 100–110% throughout the transport (Stottrup et al., 2002). Depending on fishing and transport conditions, adults could be transported over 24-48 h (Leclerq, personal communication).

2.1.2. Disinfection

Newly introduced broodstock were kept in quarantine, disinfected and regularly inspected for parasites and diseases. Disinfection baths used either dichlorovos (0.5 ppm x 30 min), neguvon (1 ppm x 24 h, + aeration), hydrogen peroxide (1500 ppm x 30 min), Benzalkonium chloride (10 ppm x 10 min), chloramine-T (2.5-10 ppm x 1h) and other preventive chemotherapeutants (Devauchelle, 1986; Athanassopoulou et al., 2009). In addition to treatments it is recommended to give an intraperitoneal injection of vitamin C (100 mg/kg fish) in diseased turbot broodstocks (Devauchelle, http://www.fao.org/docrep/field/007/af007e/AF007E08.htm). During the first weeks following introduction, it is advised to observe regularly broodstock taken in captivity for any sign of weakness or disease that may request rapid handling.

2.1.3. Adaptation

Captured broodstock are stocked in the hatchery at a density of 2-4 fish/m^2 in 15 to 40 m^3 sand-bottom tanks. Since they usually do not feed for periods lasting from 15 to 45 days with some individuals starving till death after 45 days, this delay can be shortened to 7 days by mixing previously acclimated and newly caught turbots in the same tank (Devauchelle et al., 1988). They are fed *ad libitum* 2 to 3 times a week with fresh or defrost fish at a ratio of 15% biomass. Fish are grown to maturity while acclimated to captivity. This adaptation period lasts at least 2 years. Mortality of wild broodstock kept in captivity is situated between 5 to 10 % per year.

2.2. Farm raised broodstock

In countries where the turbot industry is established, farm raised broodstock has been used for the production of larvae since 5 to 6 generations. Broodstock management programs were initiated in the 90s in France and in Spain. Hatchery reared individuals with high growth rate or specific characteristics (pigmentation and external appearance) were selected and tagged with individual tag number. Actually turbot growers in countries without access to own hatchery still rely upon importations of juveniles. In the Netherlands, turbot juveniles are still supplied from hatcheries in France and in Denmark. Broodstock recruitment from hatchery raised turbot has been supported by a selection program as an optional choice to start a turbot hatchery in the country, this measure is essential for adequate broodstock management.

2.3. Tagging

Tagging is ideal to identify, *in situ*, fish individually, by sex and age group and to trace easily their history in the hatchery logbook. Different tags have been used in the past in turbot. They include numbered T-tags and alizarin (Støttrup et al., 2002), Trovan-tags (Imsland et al., 2001), spaghetti tags (Floy, Tag). Electronic markers called passive integrated transponders or PIT tags are preferred and actually the most commonly used for their safety (Figure 1).

Figure 1. PIT tag

2.4. Sexing

Few months after fish have been adapted to the hatchery conditions, released stress and are feeding normally fish are sexed to adjust the choice of fish to the needs in both males and females. Before hand sexing using biopsy fish are anaesthetised with phenoxyethanol (5 ml per 100 L seawater). The biopsy is a technique that consists in taking samples from the gonads of immature fish to determine his sex. In female intra-ovarian oocytes are siphoned with polyethylene tubing from any region of the ovary to provide a sample representative of the maturational stage. Another method consists in the observation of the right ventral side of fish exposed to an intense light source (150 Watts) on their left dorsal side to locate the edges of the ovarian sac in females (Devauchelle, 1984). Although sexing female using this methods is accurate when performed by an experienced person, sexing immature males is not easy. The male sex is concluded from the absence of eggs by catheterization (McEvoy, 1984). During spawning season, the general appearance of the fish is also indicative.

If the abdomen of the fish is swollen, it is a female, and if it is flat and hard, it is a male. If the sex is difficult to determine, the desk light irradiation method (Figure 2) can be used for sexing.

Figure 2. Desk light irradiation of spawning turbot (left, male; right, female):

Non-invasive modern sexing methods use ultrasonography. With this ultrasound scanner method, gonads of immature males are often difficult to discern, due to their small size and similarity to adjacent organs such as the kidney and liver. Gonads of maturing males are relatively easy to measure. With female gonads easily visible in most cases, the sex of the immature males can be deduced (Mattson, 1991; Martin-Robichaud and Rommens, 2001). For Sole it is known that sexing can be done from 50 grams on, this technology should be applicable for turbot, after adequate testing.

2.5. Broodstock husbandry

2.5.1.Broodstock tanks

Broodstock tanks contain around 40 m^3 of seawater (water height: 0.7 to 1.7 m). Tanks used vary in shapes and materials used are not standardized. They are circular, square or rectangular with rounded corners; in fiberglass, in polyester, cement or treated wood lined with PVC sheeting (Devauchelle, 1980; Rodriguez, 2009). Breeders are maintained in concrete or cement squared tanks at a density of 3-6 kg/m^3 (Danancher and Garcia-Vazquez, 2006). Stocking density must not be higher than $5kg/m^2$ and dissolved oxygen concentration must be close to saturation (Rodriguez, 2009). Circular spawning tanks (5.1 m in diameter, 2.7 m deep, with a capacity of 40 m^3) constructed of glass-coated steel plates mounted on a concrete base have also been used (Bromley et al., 1986).

Tanks are supplied with seawater whose flow rate is adjusted to the biomass and the feeding level to maintain optimal water quality (dissolved oxygen, mainly TAN). Water is distributed by PVC pipes and is either rejected after use of recycled after filtration. Aeration is provided through a diffuser stone positioned beneath the water inflow. Bottom tanks contain eventually gravel as substrate or are in most cases naked. Black sheets surround the tanks and are used to modify photoperiod to control spawning season.

2.5.2. Water supply and quality

Surface or ground sea water is used. Ground water (after deironing and if needed nitrification treatment) has been adopted in some cases due to improved temperature stability and low bacterial loading (Shields, 2001). Water is filtered through a pressurized sand filter and then heated or not, depending on the temperature required. Before use, water is passed through a degassing column to avoid any problem of super saturation. A UV germicidal lamp is placed on the hatchery water inlet. In the hatchery, water temperature is regulated both by inflow water and by air conditioning.

2.5.3. Broodstock nutrition

Nutrition and performance of broodstock fish is fundamenal in hatcheries and a relationship exists between the biochemical composition of the feed (fatty acids, amino acids and vitamins) and the success of the resulting spawnings. In the past broodstock turbot has been fed on trash fish and moist diets supplemented with vitamins especially vitamin C (600 mg/kg fish) and vitamin E (80 mg/kg fish) (Leclerq, 1994; Lavens et al., 1999).

Nowadays broodstock pellets are used in some hatcheries. Vitalis broodstock feed developed by Skretting encompasses optimal nutrition and offers a specialised feeding plan suited to the specific stages of conditioning, maturation, spawning and recovery of brood fish throughout the year. **Vitalis Repro** (52% protein, 16% fat) gives good balanced nutrition optimised for marine broodstock and **Vitalis Cal (**54% protein, 18% fat), derived from high quality squid meal, is formulated specifically to build up the broodstock prior to spawning, to provide enhanced nutrition through the spawning period and to enable the broodstock to recover. Other feeds can be used, but should thoroughly be tested in collaboration with the feed manufacturer. In practice a variety of turbot hatcheries provide fresh feed as additional source.

2.6. Broodstock age at first maturity

Sexual and growth dimorphism is common in turbot. Age at first maturity of females and males is not simultaneous with females growing faster (10 to 20% higher at 800 g) but becoming mature at a later age than males. Males reach sexual maturity at 2 years (around 30 cm length) while females are sexually mature at 3 years (around 46 cm length) (Danancher and Garcia-Vazquez, 2006). When captured from the wild the time between the catch at sea and the first spawning after adaptation in captivity is 2 years when males weigh a minimum of 2.5-3 kg and females a minimum of 3.5-4 kg. Hatchery-reared F1 males weighing more than 2 kg (3-4 years old) and 2.5 kg (4-5 years) females begin spawning when placed in broodstock facilities (Devauchelle et al., 1988).

2.6.1. Effect of day length or photoperiod

By manipulating photoperiod and to a less extent temperature to which turbot broodstock are exposed in the hatchery, gametogenesis, gamete maturation and spawning can be controlled and eggs can be obtained all year around with a spawning period of 2 to 3 months (Barton, 1981; Devauchelle et al., 1988; Forés et al., 1990). However not all fish will spawn every year. Extended daylength (LD24:0 and LD16:8) during the first winter results in increases overall growth and delays the age at first maturation (Imsland et al., 1997). Fewer males matured in the LDl6:8 (26%) and LD24:O (17%) groups than in the natural light (LDN) cycle (56%) group, whereas there were no differences between the experimental groups in the proportion of females that matured (range = 60-63%).

Depending on photoperiod to which hatchery reared breeders are exposed, a significant percentage of males will mature before their 36th month and a significant female percentage will lay eggs before their 48th month according to their photoperiod programation. However the earlier spawners are not numerous and do not produce a lot of gametes (Leclerq, personal communication).

Photoperiod induced maturation

Photoperiod is generally accepted as the most important factor synchronizing sexual maturation and reproduction in fish in temperate regions (Bromage et al., 2001) and numerous studies report the use of artificial photoperiods to modify, among other, fish maturation time (Imsland et al., 1997). Accelerated photoperiod are commonly used to advance fish maturation but, it is also possible to delay it with extended photoperiod regimes. Exposure to continuous light inhibits maturation in turbot, delays spawning of 4 weeks, individual spawning frequency is lowered (2.6 compared to 5.3 in the LDN group) and egg production reduced by 90% (545 g compared to 5645 g in the LDN group) (Imsland et al., 2003). Therefore these conditions are not advisable.

In turbot maturation occurs with increasing photo phase between 8.5 and 16.0 h of light per day and results in spawning when an optimal temperature of 14±1°C is present (Imsand et al., 2003). Light intensity preference for turbot broodstock is between 200 -500 lux. Under natural conditions of temperature and day length at Ifremer (Brest, France), running males are observed from November to the end of September. Milt production increases in February up to April/May before decreasing in summer. Females mature between February and May, are close to spawning by April whereas the first oviposition is recorded only in June. The spawning season ends at the beginning of August (Devauchelle et al., 1988) and sexual rest in females is recorded in late November/December. Table 1 shows optimal temperature and light conditions the turbot reproduction.

Photoperiod induced spawning

When turbot broodstock are first exposed to a constant photoperiod of 8L:16D, and subsequently to a sudden change to 16L:8D within 1 day, maturation begin within 15 days, most of the females ripen and spawn 2 months after the sudden shift in photoperiod, and all the males produce milt at the same period (Forés et al., 1990).

Based on these results, it is suggested to acclimatise the young broodstock to captivity at extended photoperiod of 16L:8D for fast growth until they reach sexual maturity. They are transferred to a natural photoperiod for gamete maturation prior to spawning for the first time (Imsland et al., 2003).

2.6.2. Effect of temperature

In most hatcheries water temperature is maintained at 11-13^{o}C throughout the year (Rodriguez, 2009). Under natural conditions, gonadal maturation continue to an advanced stage and is then suspended until temperatures are appropriate for final maturation and spawning. In turbot oocytes are fully mature in April, although spawning rarely occurs before June (Devauchelle et al., 1988). Temperature has a direct effect on ovulation in female turbot, but does not appear to influence gonad maturation when it remains below 16°C (Bromley et al., 1986; Devauchelle et al., 1988). McEvoy (1989) recommended to maintain husbandry temperature constant during the spawning period to reduce stress and to allow more accurate prediction of ovulation. Optimal temperature for spawning is 14±1°C and fertilization of the eggs does not occur above 16°C (Table 1).

Table 1. Optimal temperature and day length for turbot reproduction

	Duration	Temperature (^{o}C)	Day length (h)
Gametogenesis	160 days	9.4-10.5	11.40-12.40
Maturation	-	Increase 6 ->16	Increase 8.30 -> 16.20
Spawning	<70 days	13-15	15-16
Egg viability	<6 h, max 10 h	<16 (starting 1.5 month before spawning)	-

Source: Dechauvelle, N. Ponte en captivité et incubation de quatre poisons marins élevés en Europe. FAO Conferences.

Source: http://www.fao.org/docrep/field/007/af016f/AF016F08.htm

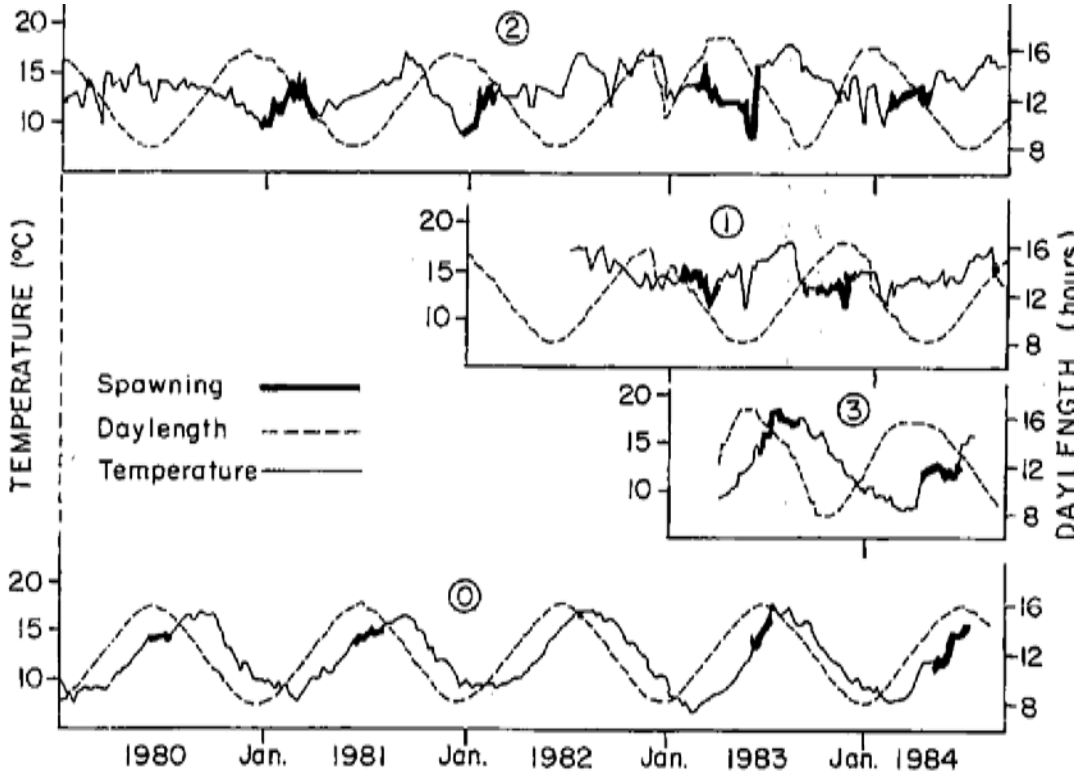


Figure 3. Occurrence of spawning in relation to daylength and temperature for spawners placed under natural (0) or shifted (1,2,3) conditions (Source: Devauchelle et al. 1988).

Note that in this figure 3 from Devauchelle et al. (1988), temperature increases (14 to 16°C) were not desired but came naturally at the work research facilities (Ifremer). 14°C is not an optimal spawning temperature as it often drives to overripe ovaries in females, 12-13°C is preferable (see 2.6.2. Effect of temperature).

2.7. Reproductive characteristics of turbot broodstock

Turbot does not display any external secondary sexual characteristics. They have a cyclical pattern of reproduction characterized by massive gonad development in female and morphological changes (volume and colour), particularly of the ovaries, immediately before the release of gametes. In males testis are difficult to locate.

2.7.1. Female broodstock

2.7.1.1. Ovulatory rythms

Females show successive alternating short and long ovulation periods (McEvoy, 1984). Under conditions of stable temperature and a steady LD18:6 photoperiod, captive hand-stripped turbot have a regular ovulatory rhythm, permitting the prediction of future ovulations. However, the interovulatory time period differs between females, so that the ovulations are not synchronized. The ovulatory periods generally fall within the range of 60-95 h. Howell & Scott (1989) suggested that the ovulatory periods is shortened with increasing temperature.

2.7.1.2. Fecundity and interval between spawning

Fecundity of female broodstock is very high in turbot and mature females can produce 5 to 10 million eggs (Danancher and Garcia-Vazquez, 2006). Absolute fecundity depends on female size and age and is around 250 000 eggs/kg (Rodriguez, 2009). The average production per kg of female over the spawning season is estimated to be 80 000-200 000 eggs (natural spawns) and 260 000-430 000 (stripping) (Devauchelle et al., 1988). Each female may spawn several times, up to 12 spawns per season (7 to 8 weeks) at a 3- to 6-days interval. Stripping should occur every 4 to 5 days. The average number of viable embryos produced by artificial reproduction depends on the delay observed between the ovulation time and the stripping.

2.7.1.3. Overripening of eggs

In turbot, ovulation occurs sequentially and eggs remain viable for a very short period. After ovulation the eggs are held for about 1 day within the lumen of the ovary, pending release at spawning. Ovulated turbot eggs over-ripen quickly when retained in the ovary lumen and this is probably a major cause of poor quality egg batches in captive broodstocks. Freshly-ovulated eggs show hatching rates of up to 97%, dropping to 0% after being retained in the ovary lumen for 1 day. Therefore, to ensure maximum egg viability, it is important to strip eggs from farmed turbot within 10 h (at a temperature between 10-12^{o}C) after ovulation to ensure a maximum viability of eggs (McEvov, 1984). The fertilization success starts already to decrease at 6 h after ovulation. Fauvel et al. (1992) recommended a daily examination of spawning broodstock females as a hatchery management practice to improve the viability and hatching rates and larval production per female but, in practice three visits a week are preferred. Eggs expelled shortly after ovulation by systematic stripping of females five times a week had high viability (72%) compared to eggs collected twice a week (46%). Handling frequency does not have any effect on female fecundity. Increase of stripping frequency raised hatching rates and larva production respectively from 11% to 35% and from 20000 to 65000 larvae/kg of spawning female (Fauvel et al., 1992).

2.7.2. Male broodstock

2.7.2.1. Milting period and sperm characteristics

The duration of the turbot reproduction period is longer in males than in females. The percentage of turbot with milt is maximum during the spawning season of the females, showing a synchronization reproduction. In captivity, the turbot spermiation period, lasts 6 months (Suquet et al., 1994) and some males can even still release sperm for up to 9 months (Devauchelle et al., 1988). In the wild, this period lasts only 4 months (Jones, 1974). The duration of the milting period may also be related to the rhythm of stripping: the shortest milting period is recorded in weekly stripped males and the longest one in monthly stripped animals (Suquet et al., 1992a). When compared to other fish species, turbot males are characterized by low values of maximum gonadosomatic index (0.6-0.8%) and are poor sperm producers in terms of volume (0.2-2.2 ml), concentration (0.7-11.0 x 10^9 spermatozoa/ml) and total sperm number released per stripping (0.2-12.0 x 10%pematozoa). The duration of cell movement is long (1-17 min). Increasing collection frequency of turbot males, from monthly to fortnightly and weekly stripping, results in the release of successive samples presenting decreasing semen volume and spermatozoa concentration (Suquet et al., 1994). Higher values were reported earlier by the same authors and ranged between 20-54.6 x 10^9 spermatozoa/ml, spermatocrit: 40.4% (Suquet et al., 1992b).

2.7.2.2. Hand stripping of males or milting

Male turbots are often selected by their smaller size compared to females, their chasing spawning behavior, the absence of eggs by catheterisation. Milt is obtained from running males by gentle pressure on the abdomen of the fish at the level of the testis.

2.7.2.3. Urine contamination of semen

Urine contamination of semen during stripping of turbot males is common (Dreanno et al., 1998). A 10% contamination of sperm by urine during 15 min is enough to decrease the percentage of motile sperm cell from 83.4 to 54.2% at 10-s post-activation reducing its fertilization capacity. In order to overcome this problem, two methods of sperm collection have been suggested by these authors:

- Emptying the urinary bladder using a catheter inserted in the ureter prior to sperm collection, allows elimination of the main part of urine.
- Collection of sperm in a syringe or tube partly filled with buffered ASL ($NaHCO_3$) to protect spermatozoa integrity.

2.7.3. Selection of breeding broodstock

In turbot there are no external indications that ovulation has occurred. Today in commercial hatcheries, females are not disturbed till they are inflated and then stripped. In the beginning of the turbot industry people were used to select broodstock for spawning based on the maturity stage of oocytes. During the months preceding spawning, ovarian samples were taken from females and checked for their maturity stage (see below). According to Wallace and Selman (1981) and West (1990), ovarian follicles are classified into five stages of maturity in turbot: I: early perinucleolar stage; II: late perinucleolar stage; III: Yolk vesicle stage; IV: vitellogenic stage and V: mature stage.

Procedure to check egg maturity stage:
To check the egg maturity stage in turbot, a two-step procedure is used:
-Insert a catheter tube with 3 mm internal diameter into the gonadal cavity of a female
-Take 2 ml of ovaries by applying suction whilst withdrawing the cacheter (Forés et al., 1990).
This procedure starts as soon as the abrupt change in photoperiod. Ovarian samples are initially taken 2 or 3 times each week. During the final stages of maturation, ovarian samples are taken on a daily basis. The abdominal pressure technique is used every 2-3 days to monitor the sequence of spawning once it had starten. Spawning takes place when egg diameters are approximately 1 mm. In each case, egg stages and numbers of buoyant and non-buoyant eggs is noted. In parallel, males are also sampled to see if they are running (see 3.3. Hand stripping of males).

2.7.4. Spawning behavior

In the wild, turbot spawn during the summer months. The spawning season occurs between April and August in Mediterranean populations and between May and August in Atlantic areas (Danancher and Garcia-Vazquez. 2006). In hatchery turbot do not or rarely spawn naturally. One particular type of behavior has been often observed by Bromley et al. (1986) during the spawning season. A female, closely pursued by a male, would swim up off the bottom and around the circumference of the tank at variable depth throughout the water column. Occasionally, the male moved in close above or below the female and the pair briefly swam in unison with synchronous undulations of the body musculature. After a few minutes the female returned to the bottom and the male settled near to or overlapping the female and was sometimes seen rippling his fin margins over the dorsal surface of the female. Even with such spawning behavior, no natural spawning occurs in tanks since aquaculture facilities lack of depth to generate sufficient pressure drops/increases by fast up/down swimming (Leclerq, personal communication).

3 Artificial reproduction

In turbot, egg production in captivity is mainly based on manual stripping and artificial fertilization. Artificial reproduction overcomes difficulties associated to unreliable natural spawning and eggs may be hand stripped every day or on alternate days and artificially fertilized. Turbot gametes are obtained by manual stripping and artificial fertilization (Leclerq, 1994).

The dry fertilization method is used on newly ovulated eggs: after stripping , eggs and sperm are mixed without seawater. Five to ten minutes later, clear seawater is poured over the cells. The eggs are then placed in the incubators. To avoid overripening (see section 2.4.1.3) egg stripping is performed within 10 h after ovulation (at 12-13oC). During egg manipulations, temperature control is necessary to ensure good quality eggs and it is important to avoid any temperature and salinity shock to the eggs. The mean value of the viability rate, the number of embryos issued from eggs stripped, is 33%. Depending on the quality of gametes and the fertilization success, viability rates can vary from 0 (failure) to 80% (success).

3.1. Hormonally induced synchronization of spawning

A hormonal approach is used in some turbot hatcheries to synchronise the spawning of many females at the same time during artificial reproduction [3 Diaz and Neira, 2005]. Significant synchronization and increased number of spawning females is obtained by using sustained-release pellets containing a gonadotropin-releasing hormone analogue (GnRH-a), D-Ala6-Pro9-Net]-luteinising hormone releasing hormone (Mugnier et al., 2000). Hormonal treatment with GnRH-a (25 µg/kg) is effective in inducing ovulation of 100% females compared to around 50% in non-treated broodstock and reduce by half the duration of the spawning period.

3.2. Hand stripping of females

Female turbot which are close to ovulating are checked daily during the spawning season. By pressing the abdomen at the level of the ovaries, hyaline eggs are expelled out of the ovaries and collected by stripping (Figure 4).

Figure 4. Stripping of turbot gamete.
Source: http://www.piscestt.com/FileLibrary%5C12%5CTurbot.pdf

3.3. Hand stripping of males

A gentle pressure is applied a few times, starting from the area just prior to the pelvic fins and ending near the urogenital pore. If the fish is fully mature, milt is usually extruded from the urogenital pore. If no milt is extruded, this fish is either immature or spent.

Summary protocol for spawning of turbot:
Eggs can be obtained from different groups of females the whole year around by modifying rearing temperatures and mostly day-night rhythms. However, individual females may not be spawning at each spawning season. Hormones treatments can also be used to manage advanced spawning in broodstock to synchronise maturation of females and to obtain egg production from different females all year round. If they are hormone treated female will shoot eggs more frequently during a shorter spawning season, which might be of help. However if the season is shorter, then the hatchery needs more groups of breeding fish in more tanks or should be well prepared to use a concentrated eggs spawning period. To induce spawning follow these steps:

- Prepare a breeding tank (constant water temperature:14°C, salinity between 32 and 34 ppt)
- Feed the fish to satiation every two days.
- Choose preferably broodstock that has been previously spawned in captivity and kept at a constant day length of 8 h of light.
- Select spawners showing spawning behaviour or spawning activity: swollen ovaries (females), running males.
- Take females weighing between 3.5 to 8 kg and males between 2 to 7 kg
- Put both sexes in a spawning tank containing at least 25 m3 of water, with a depth of 1 m at a density of less than 8-12 kg/m2 (1 kg/m3 and sex ratio of 1 or 2 males per female.
- Provide light using daylight fluorescent tubes giving a surface intensity of 200 lux.
- Once fish are adapted to spawning tanks change suddenly the photoperiod from 8 to 16 h of light. Maintain this light regime until spawning ends.
-Fish will start spawning after almost 2 months.

4 Hatchery period

4.1. Characteristics of turbot eggs at spawning

Turbot eggs (Figure 5) are very small (0.98 to 1.18 mm with an average 1.1 mm of diameter), smooth and spherical, non-adhesive, transparent and buoyant (Bromley et al., 1986). Eggs are pelagic and possess one single oil globule of 0.18-0.21 mm (Danancher and Garcia-Vazquez, 2006; Devauchelle et al., 1988). The optimal salinity ranged between 25-35 ppt and temperature between 15-17^{o}C for egg incubation.

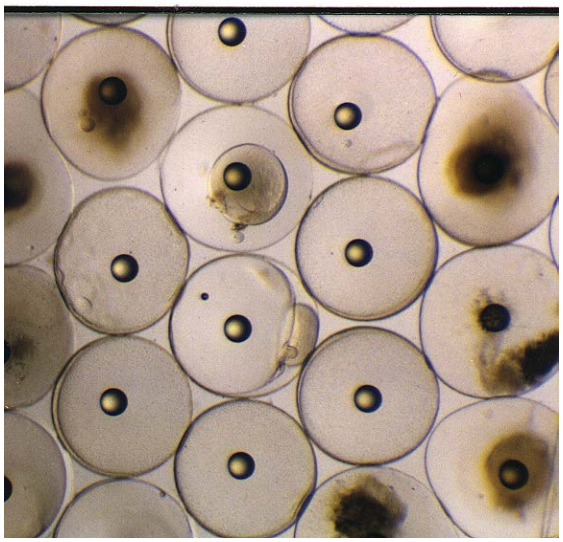


Figure 5. Turbot eggs. Spherical and translucent ova are viable; irregular shape and coalescent yolk are un-viable ova.
Source: http://www.ifremer.fr/drvraarn/images/poster_qualite_des_oeufs.pdf

4.1.1. Egg quality assessment

Unpredictable and variable egg quality is a limiting factor for the successful mass production of juveniles. Egg quality is defined as the egg's potential to produce viable fry and juvenile (Kjørsvik et al., 1990; Kjørsvik et al, 2003). Factors affecting fish egg quality are complex and include maternal age and condition factor, timing of the spawning cycle, overripening processes, genetic factors, and also intrinsic properties of the egg itself (Kjørsvik et al., 1990; Bromage, 1994; Brooks et al., 1997). Good quality pelagic eggs are usually more buoyant than poor quality eggs (McEvoy, 1984) and in some species such buoyant eggs have more hatching rate and normal development. Forés et al. (1990) have summarized in Table 2 the different factors for good quality turbot eggs. The quality of eggs can be determined at 2 different stages on readily spawned eggs and after fertilization.

Table 2. Quality criteria for turbot eggs

Egg stage	I	II	III	IV
Buoyancy	High	Middle		Low
Egg diameter		<1.1 mm	>1.1 mm	
% fertilization		>70%	<70%	
% symmetry		>50%	<50%	
First divisions size		Large	Small	

Stage I and II are the best egg quality to use.

Indicators of good quality eggs immediately after spawning (Figure 3) include:
- the percentage of buoyant eggs floating on the water surface. Choose egg batches with more than 50 ml of floating eggs in stages I and II which exhibit a high or medium degree of buoyancy and with diameter less than 1.1 mm (Forés et al., 1990).
- the egg transparency is a good early indicator of viability (Fernández-Palaicos et al., 1995).

4.1.2. Egg fertilization

In case of artificial reproduction stripped milt is either spread over the eggs before mixing with sea water (dry fertilization method) or is first diluted in seawater (wet fertilization). After 5 to 10 min, clear seawater is poured over the eggs. The supernatant eggs are placed in the incubator while sinking eggs are withdrawn. In the case of natural spawning, eggs are concentrated in automatic collector and collected once or twice a day (Chereguini et al., 1999). Eggs are classified as viable if they are buoyant, fertilized and appear to be developing normally (Bromley et al., 1986).

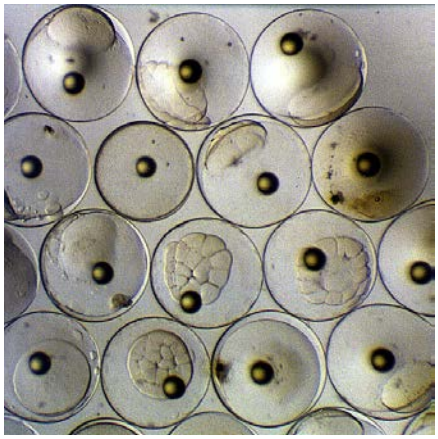


Figure 6. Fertilised turbot embryos.
Source: http://www.ifremer.fr/drvraarn/images/poster_qualite_des_oeufs.pdf

Calculation of fertilization and hatching rates:

Three and a half hour (3.5 h) after fertilization, a small sample of eggs is taken from the floating, surface layer of eggs. Two lots of 400 eggs are taken from each sample, the number of dividing eggs noted, and the percentage fertilisation calculated. The two sets of 400 eggs are then incubated until the eggs hatched. When hatching is completed (day 6-7), the number of larvae in each of the two tanks is counted and the mean percentage hatch calculated. During this process, dead eggs are counted daily and removed.

Indicators of good quality eggs after fertilization (Figure 6) include:

- -the fertilization rates and the ratio of normal cell cleavages (blastomere morphology) at the 8–32 cell stage calculated as the percentage of normal blastomeres (normal asymmetry) from fertilized eggs.
- - more than 70% fertilized, with more than 50% of the early divisions symmetrical, and with the first cell divisions of large size.
- -the hatching rates.
- -the skeletal malformations of yolk larvae.

4.1.3. Egg disinfection

Eggs can vehicle opportunistic pathogens present at the surface of the eggs and transmit disease from parent to offspring and between hatcheries. Egg disinfection improves both hatching and survival of eggs and reduce the risk of transfer of pathogenic bacteria within and between hatcheries (Salvesen and Vadstein, 1995). Agents used for surface disinfection of fish eggs include glutaraldehyde, iodophors, ultraviolet, ozone, hydrogen peroxide, sodium hypochlorite, antimicrobial peptides and antibiotics (Munor et al., 1995; Schachte, 1997; Birckbeck et al., 2006; Ben-Atia et al., 2007; Van der Eecken et al.,unpublished). Glutaraldehyde (400 ppm x 10 min) is commonly used for the disinfection of turbot eggs (Salvessen and Vadstein,1995; Salvesen et al., 1997). The use of antibiotics is however strictly controlled and minimized since resistance to antibiotic may result from abuse.

Procedure for egg disinfection *(Figure 7):*

Disinfection solutions are prepared with filtered (0.2 micrometer), UV treated or autoclaved seawater. Before treatment the eggs are collected on a nylon mesh and washed with sterile, filtered seawater to remove organic materials. Eggs are thereafter transferred to the disinfection solution maintained in a beaker (1:10 by volume). During disinfection, eggs are gently agitated to keep a constant concentration of the disinfectant around the eggs. After treatment, eggs are rinsed on the nylon mesh and washed with sterile, filtered seawater. Four hundred (400 mg/l) of glutaraldehyde applied for 10 minutes gave good results in the disinfection of turbot eggs (Salvesen and Vadstein, 1995; Salvesen et al., 1997). After disinfection, eggs are rinsed in sterile seawater at 25-35 ‰ salinity to remove excess disinfectant and are ready for incubation.

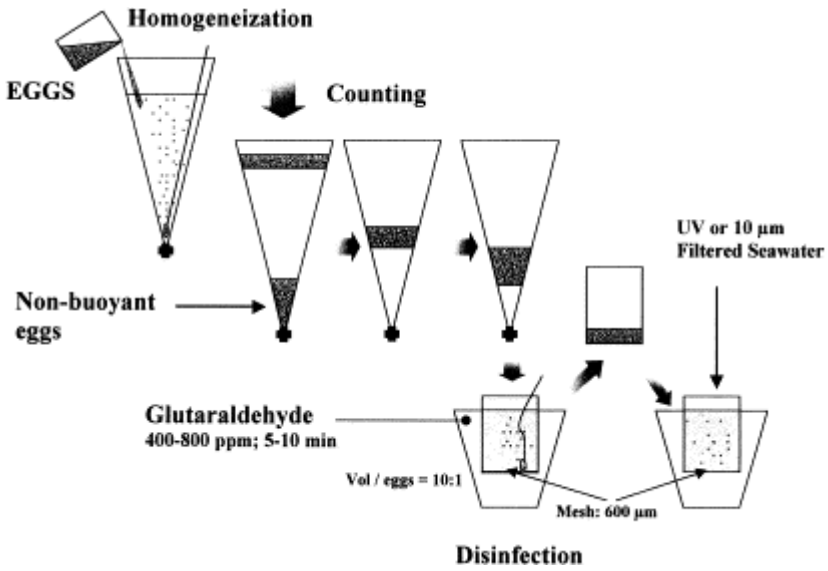


Figure 7. Procedure of surface egg disinfection for coldwater fish species (based on the work of Salvesen and Vadstein, 1995; Salvesen et al., 1997). Cited in Planas and Cunha (1999).

4.1.4. Incubation and hatching

Fertilised eggs are incubated per spawned batch at a density of 3000 eggs per liter (Declerq, personal communication). Munro et al. (1995) hatched fertilized eggs in 5-liter spherical flasks (40 eggs per flask = 8 eggs per liter) filled with full-strength (32 ‰ salinity) SW.

4.1.4.1. Incubation tanks

Regardless of the system used for larviculture, it is standard practice to rear the embryonic phase in a separate incubation facility. Round tanks with a conical bottom (Figure 8) and a volume of 80 -150 liters are commonly used (Rodriguez, 2009). They are supplied with flow-through filtered and UV treated seawater. These tanks are made of plastic or fiberglass and the inner surface is smooth to prevent damage to eggs and newly hatched larvae. The cylindro-conical shape gives a good water circulation and egg distribution, provided that a central mild aeration source is placed. Under these conditions water renewal every 2 to 3 hours is enough to keep the dissolved oxygen close to 100% saturation. Air and water flow is stopped every day to let dead eggs and hatching debris to sink to the bottom for the removal.

Figure 8. Egg incubation tanks used in Turkey

4.1.4.2. Incubation temperature

The optimum temperature for the incubation of eggs is between 13 and 15°C (Figure 9). At 15°C half of the eggs (50%) hatch within 4.5 days or 1590±90°C x hours (Devauchelle et al., 1988; Rodriguez, 2009), equivalent to 6–7 days at 13 °C (Iglesias et al., 1991). Leclerq (1994) stated a preferred stocking density of 3000 eggs/l and incubation temperature of 13 °C. The average hatching rate obtained is 78% from the viable morula egg stage.

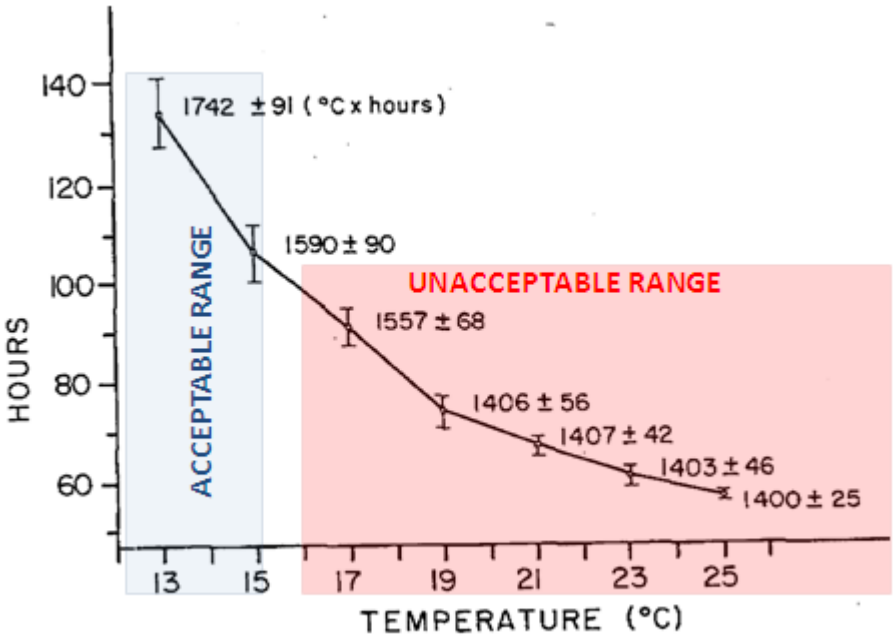


Figure 9. Relationship between temperature and the duration of turbot egg incubation

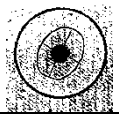
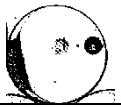
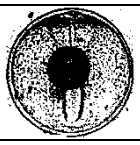
Source: Devauchelle et al., 1988.

In commercial hatcheries temperature during incubation of eggs should be closely monitored between 13 and 15°C max (12°C accepted but slows down the process and higher temperatures such as 16°C and above are not acceptable in commercial hatcheries (Declercq, personal communication).

4.1.5. Embryonic development of turbot

During incubation, fertilized eggs undergo different embryonic development (Table 3) before hatching (Devauchelle et al., 1988). Mechanical and thermal shocks must be minimized, especially at morula stage and just prior to hatching.

Table 3. Chronology of turbot embryogenesis at 15°C

Time	Stage	Description	Pictures
0h	Fertilisation		
1h45	1 cell	The blastomer appears at the animal pole, beginning of the 1st division	
2h30	2 cells		
3h	4 cells		
3h30	8 cells		
4h	16 cells		
5h	32 cells		
5h30	64 cells	Early morula	
15h	Morula	The cells begin to migrate to the equatorial zone	
18h			
18h	Beginning of gastrulation		
28h	Gastrula	Half-gastrula	
32h		The cephalo-caudal axis appeard	
38h		The head-tail axis appears	
42h		The somites are perceptible	
55h	Neurula	The embryo is developed on a half circumference	
68h		The embryo is pigmented black	
76h		The heart beats	
80h		The embryo occupies ¾ of the egg circumference	
87h	Pre-hatching	The embryo is pigmented red	
92h		The embryo is developed on the whole circumference	
100h		Embryo just prior to hatching	
106-112h	Hatching	50% hatching	

4.1.6. Larval and morphological development of turbot

Three distinct larval stages in turbot are described in an experimental research conducted at a temperature of 19°C.

4.1.6.1. Embryonic period

Just hatched larvae are about 3 mm long, are symmetric and the yolk vesicle is well developed. The digestive track is undifferentiated and closed anterior, and the eyes are not functional.

4.1.6.2. Vitellogenic period

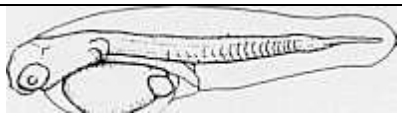
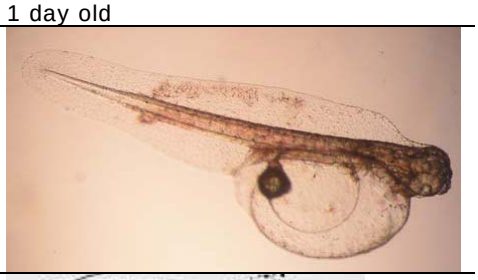
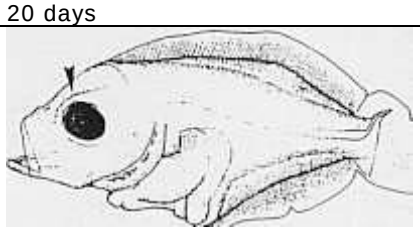
Between days 2 and 3, the mouth opens and exogenous feeding begins while yolk sac reserves and the oil globule are quickly mobilized and will disappear at days 5 and 7, respectively. During the vitellogenic period, important changes are observed: first differentiation of the digestive tract, heart organization (four cavities), swimmbladder inflation and pronephros differentiation.

4.1.6.3. Post-vitellogenic period

After day 7, the post vitellogenic period, the pneumatic canal degenerates (disappearing at day 9), the mesonephros differentiate and the digestive tract further develops. The formation of gastric glands (day 15) may be considered at the end of the physiological larval stage; Whereas from anatomical point of view, metamorphosis is just beginning at day 15 with the flattening of the body and the migration of the right eye. At day 30, turbot juveniles look like a small adult, but behaviorouly they are still pelagic; benthic behavior is definitely gained at day 40.

Morphological development of turbot larvae have been outlined by Jones (1972), Al-Maghazachi and Gibson (1984) and Gibson and Johnston (1995). They are presented in Table 4 and Figure 10 below.

Table 4. Morphological development of turbot larvae

Age (days)	Total length (mm)	Characteristics	Illustration
0	2.5	Newly hatch larvae have unpigmented eyes, unformed mouth and closed anus. Melanophores are distributed on the central notochord	 1 day old
5	4.0	The pectoral fin membrane appears.	
10	4.8	The pectoral fins are well developed. Melanophores are distributed on both the dorsal and ventral parts of the notochord	 11 days old
15	6.3	Differentiation of the anal fins from the common finfold begins and melanophores on the notochord are well developed except in the posterior region	
20	9.6	The dorsal, anal and caudal fins are all differentiated; the flexion of the notochordal end has advanced rapidly and the membrane of the ventral fins appears. Many melanophores are distributed in the posterior region of the notochord.	 20 days
25	14.1	All the fin rays are functionally differentiated. Many melanophores are distributed on the notochord except the anterior region of the head	 26 days
30	17.6	The migration of the eye from a symmetrical position to one side Begins	
70	43.3	The migration of right eye advances slowly compared to the relative growth of the other body parts. Fish is completely transformed at D-70.	

Larval development and staging has been extensively described by Al-Maghazachi and Gibson (1984) and are shown in Figure 10. Five major developmental stages can be recognized and are characterized as follows:

Stage 1: larvae symmetrical, yolk sac present.

Stage 2: larvae symmetrical, development of spines and air bladder.

Stage 3: appearance of fi rays, notochord straight.

Stage 4: asymmetry and eye migration, notochord posteriorly slanted dorsally.

Stage 5: completion of eye migration, spines and swim bladder resorbed.

There is no sharp distinction between successive stages; in general at least half the features characteristic of a particular stage must be developed before the onset of the next stage. For example, the right eye does not commence its migration until most of the fi rays have formed and the notochord within the caudal fin is inclined dorsally by 45^{o} or more.

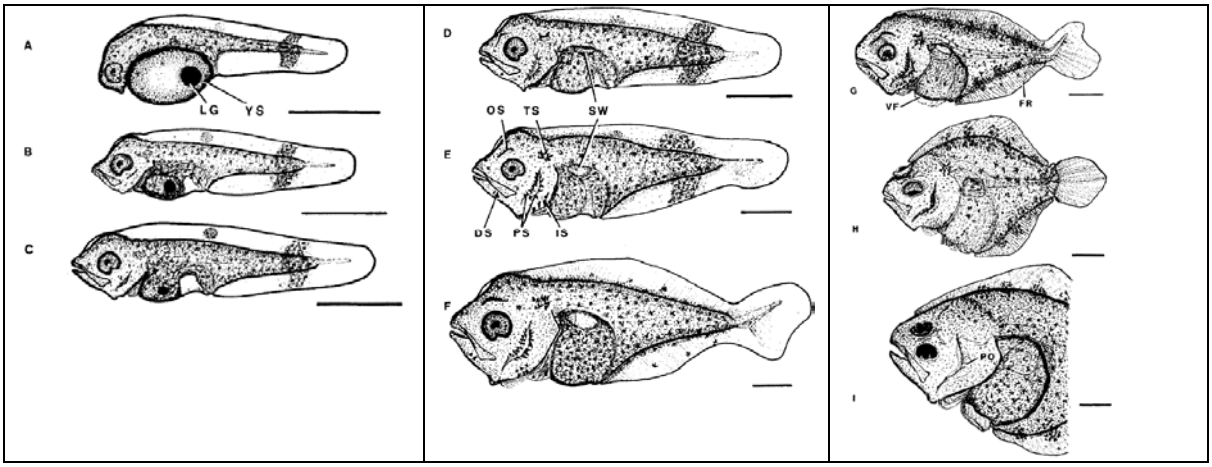


Figure 10. Stages and sub-stages in the larval development of the turbot, *Scophthalmus maximus:* A, Stage 1a, immediately after hatching, 2.8 mm long; B, Stage lc, 3.1 mm long; C, Stage 1d, 3.5 mm long; D, Stage 2b, 4.5 mm long; note the development of spines and the swim bladder; E, Stage 3b, 6.1 mm long; caudal fin rays are distinguishable; F, Stage 4a, 9.1 mm long; all fin rays are developed; note also the caudal portion of the notochord slanted upwards; G, Stage 4c. 14.2 mm long; the right orbital crest is now visible from the left side; H, Stage 5b, 20.2 mm long; the right eye is on top of the head and spines are fewer in number and smaller in size; I, Stage 5d, 38.4 mm long**;** the anterior body region of a fully metamorphosed juvenile; DS, dentary mandibular spines; FR, fin rays; IS, interopercular spines; LG, lipid globule; OS, orbital spines; PO, preoperculum; PS, preopercular spines; SW, swim bladder; TS, otocystic spines; VF, ventral fin; YS, yolk sac; scale bars, 1 mm (A-F), 2 mm (G-I). **Source**: Al-Maghazachi and Gibson (1984).

4.1.7. Metamorphosis in turbot

Metamorphosis starts at 15 DAH with the migration of the right eye and ends after 40-50 days when larvae are about 25 mm long (Danancher and Garcia-Vazquez, 2006*)*. During metamorphosis (Figure 11), the flatfish head undergoes striking anatomical changes. Starting from "usual" bilateral symmetry and "normal" swimming posture (top pictures) the right eye of the turbot migrates across the vertex (bottom pictures) to reach its final position on the left body side when the animal gets benthic, lying on the right body side.

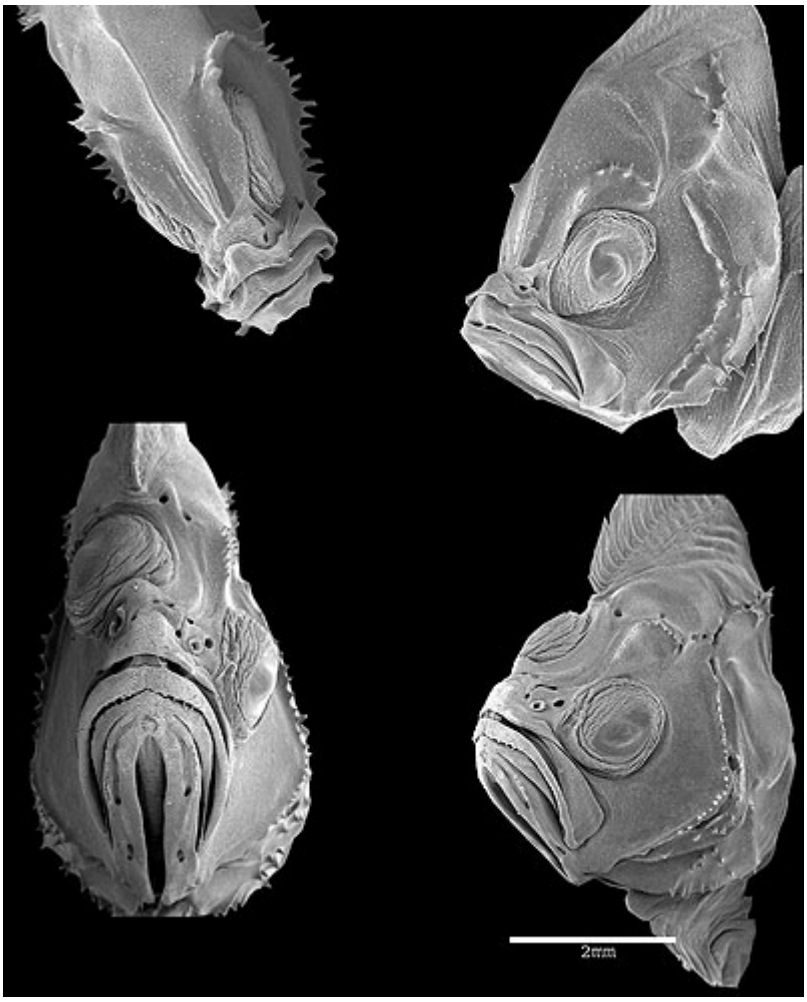


Figure 11. Metamorphosis of turbot larvae. Source: http://www.zsm.mwn.de/dir/biozentrum_rem.htm

4.1.8. Morphological malformations and pigmentation

Morphological abnormalities are observed commonly in hatchery-reared flatfishes and are assumed to develop during the period of asymmetric change and tissue differentiation associated with metamorphosis (Seikai *et al.*, 1987). Abnormalities tend to be related and include malpigmentation, failure of eye migration, and abnormal jaws and fins. These abnormalities have been attributed to environmental factors in hatcheries such as diet (fatty acids, vitamin), lighting, population density (Shelbourne, 1974; Seikai, 1985; Estévez and Kazanawa, 1995; Estévez et al., 1999). Malpigmentation is common in hatchery-reared flatfishes, decreasing the market value of whole fish (Bolker and Hill, 2000).

An evolution of the color is observed during ontogenesis. Being light brown at hatching, turbot larvae change to a dark brown color at first feeding and normally, between days 8 and 13, most larvae become orange-yellow. A small percentage of poor quality larvae stay dark. At early metamorphosis the color changes to white. But final pigmentation is not observed until days 20 and 25. At that age, normal and abnormal pigmentation can be clearly distinguished. Coloration abnormalities – partial or total lack of pigments- often occur at high rate in intensively grown turbot and other flat fish. Abnormal pigmented fish tend to be dark brown and white. In commercial hatcheries the problem of pigmentation is fully mastered.

4.2. Larval rearing

At hatching, the larvae are 2.4 to 3.4 mm long and well pigmented and weigh between 0.1 to 0.2 mg. They are transferred from the hatching units to larval rearing tanks in which the temperature is kept around 15 ^{o}C. Exogenous feeding starts 3 to 5 d post-hatch, depending on temperature. Larvae are fed live preys made of rotifers (*Brachiomus plicatilis*) and *Artemia* nauplii. The first month of feeding with live preys is the most critical.

4.2.1. Larval rearing facilities

4.2.1.1. Tanks

Newly hatched turbot larvae are stocked at densities of circa 30–40/l into rearing tanks, several cubic meter in volume, from 2 to 5 m^3 with the depth of 0.75 m. The depth of tanks can be up to 3-4 m in some hatcheries. Larval rearing tanks are circular, eventually with convex bottom. To maintain suitable larval rearing conditions, water is filtered by 5 µm filters and UV- sterilized.

4.2.1.2. Aeration

Aeration and circulation of the water are two of the most important issues in larval rearing. Turbot larvae do not support strong aeration in tanks; a gentle aeration without disturbances is preferred. Oxygen can be added using fine ceramic air-stones. Dhert et al. (1998) recommended avoiding the use of fine diffusers for aerating, to prevent larvae from ingesting small air bubbles. Water flow should be directed upward rather than downward to prevent direct physical effects of air and water flow on the larvae.

4.2.1.3. Temperature

During yolk resorption, water temperature is elevated from the initial egg incubation level, in preparation for start-feeding. Person-Le Ruyet (1989) reported a preferred rearing temperature of 18–19°C, while Riaza and Hall (1993) used a broader temperature range (16–21°C) in a semi-intensive rearing system. Water temperature is usually maintained at 18 to 21 °C. In commercial hatcheries, the temperature is mastered through the temperature of the room during the period when water renewal is very low, and then later through the temperature of the water flowing into the tanks.

4.2.1.4. Light

Light is one of crucial factors during feeding of larvae. Depending on the possibility to feed the larvae during the day light period, facilities are illuminated with 12L/12D, 24L or natural summer 16L/8D at the intensity of 200 lux using fluorescent lamps. Direct sunlight should be avoided in larval rearing facilities. If the green water (see 4.2.8.) rearing system is applied, continuous illumination (24 h) is provided.

4.2.1.5. Ammonia

Although turbot larvae are tolerant to ammonia, little is known about the toxic levels. With proper management, total ammonia never reaches 0.2 ppm in a good larval rearing facility (Leclerq, personal communication).

4.2.2. Transfer of larvae from hatching to rearing units

Just before transferring hatched larvae to rearing tank, aeration is stopped for a short time (10-15 minutes) in hatching tanks in order to allow undeveloped eggs and detritus to sink to the bottom of incubation tank and larvae or eggs to float at the water surface. Then, the undeveloped eggs and dirties are removed by siphoning or via the hose connected to the bottom of the incubator. Larvae are transferred gently to the rearing tanks by using beakers or 10-15 l pails. Larvae rearing tanks are stocked at a density between 15-30 larvae/l in intensive systems and at 5-10 larvae/l in semi-intensive tanks with open-circuit pumped seawater (Danancher and Garcia-Vazquez, 2006; Leclerq, personal communication). After transfer of larvae to the rearing tanks incubators are emptied, washed, disinfected and refilled for the incubation of the next egg batch. If no further incubations are foreseen, it is advised to breakdown and disinfect facilities used.

4.2.3. Live prey production

Production of marine fish larvae has been depending on live prey production of microalgae, rotifers, artemia and some enrichments to improve their nutritional quality as the fish larvae grow up. With recent developments algae paste and algae concentrates (ex. Chlorella sp. at 10-12 g/l) have proven to be far most effective hygienically and economically than the algae-yeast cultures and will soon kick-out algae cultures in fish hatcheries (Lee, 2003). They are commercially available (ex. Roquette, France). With Artemia carrying bacteria, viruses and other on-desired microorganisms, and sometimes pesticides and heavy metals, modern hatcheries and larval feed manufacturers have aimed to the reduction of dependence to Artemia by earlier weaning to micro-diets (See 4.3. Larval weaning).

Since the protocols for the production of live preys for marine larviculture has been extensively published in different handbooks, we refer to the most update version of one of these reports by Naser Agh & Patrick Sorgeloos (Eds). **Handbook of Protocols and Guidelines for Culture and Enrichment of Live Food for Use in Larviculture** (2005). We did not find useful to re-write protocol for live prey production since this handbook provides details of the production of rotifers and artemia as well as their enrichement and is available online http://www.urmia.ac.ir/artemia/Lists/List12/Attachments/4/203140_handbook%20final.pdf.

Like many other marine species, the successful rearing of turbot larvae depends on an adequate supply (Figure 12) of high quality live feeds, usually in the form of rotifers (fed on unicellular algae) and brine shrimp *(Artemia spp)* and their enrichments.

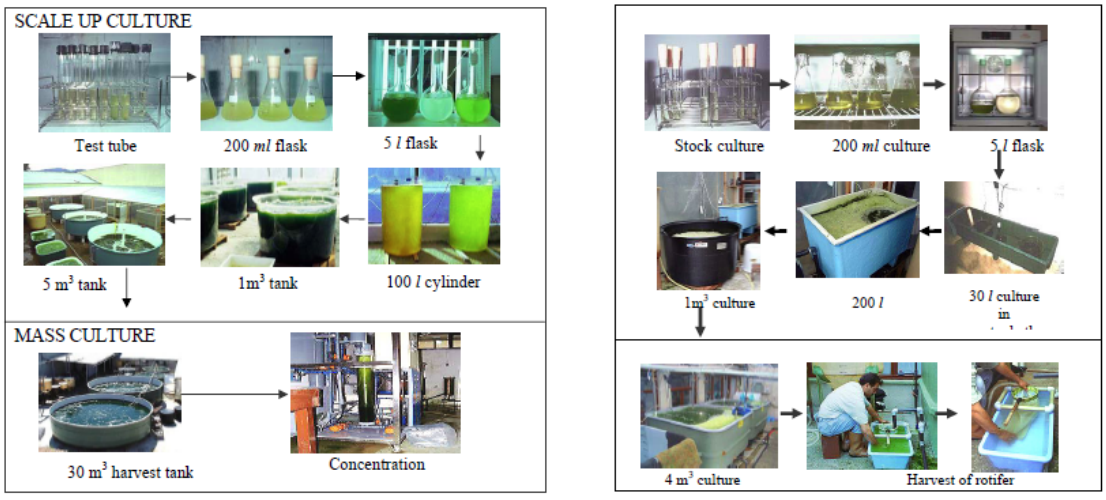


Figure 12. Example of an algae culture (left) and rotifer culture (right) flows at the Central Fisheries Research Institute in Turkey. Source: Çiftci, et al. (2002).

Notice that microalgae can also be mass-cultivated on a large scale for different purposes: aquaculture, biofuel and other markets in hanging plastic bags, photobioreactors, etc..

4.2.4. Copepod production

Numerous studies have demonstrated that copepods have a higher nutritional value than *Artemia*, as the nutritional profile of copepods appear to match better the nutritional requirements of marine fish larvae (Støttrup, 2000). They improve normal pigmentation, growth and survival. Furthermore, they can be administered under different forms, either as nauplii or copepodites at start feeding and as ongrown copepods until weaning, replacing Artemia. In a study of Kuhlmann et al. (1981), turbot larvae preferred copepod nauplii to rotifers. Different species of marine copepods have been used as live preys for marine fish species. The most commonly used is the calanoid copepod *Acartia tonsa* but other other species belonging to the harpacticoid groups are also used (Støttrup et al., 1986; FAO, 1996).
Copepods are produced in extensive, semi-intensive and in intensive systems. In extensive systems copepods are collected from the wild and concentrated within a specific size range using filters before being fed to the fish larvae. For first-feeding larvae, mesh sizes between 80 and 200 µm are used. Maximus A/S, a turbot hatchery in Denmark uses a copepod semi-intensive production system (Støttrup et al., 1998). Outdoor ponds (12 m diameter, 6 m high) are kept empty during the winter and filled with filtered saltwater before the introduction of the first feeding fish larvae. Agriculture fertilizers are added to enhance the production of phytoplankton and zooplankton. Planctonic copepods commonly found in these systems are calanoid genera such as *Acartia, Centropages* and *Temora* but carpacticoid copepods also proliferate. It is important to monitor the system regularly to consider the number of food organisms within the appropriate size range in relation to the size of the fish larvae (Støttrup, 2000). In extensive and semi-intensive systems, the use of wild harvested copepods or copepods allowed to bloom in the ponds present the risk to introduce parasites and diseases and is no longer in use (Shields, 2000). Intensive rearing systems of copepods are now possible and in some cases the provision of copepods for a short time during the larval stage is sufficient to ensure normal development.
Scaling-up small scale-production of copepods is still an issue but two species of copepods present a certain interest. They can be intensively produced within their optimal temperature range and density: the calanoid *Acartia tonsa* (16-18^{o}C, <100 adult/l) and the harpacticoid *Tisbe holothuriae* (16^{o}C, >13 000 adults/l). *A. tonsa* lead to low-volume output (±530 eggs/l). It is a free spawner and eggs can be removed from the adult culture by siphoning the tank bottom once or twice a day. The eggs are incubated for about 2 days with hatching percentages around 45%. In contrast the egg-sac-bearing harpacticoid *T. holothuriae* results in a more than 200-fold increase in output. Copepods with planktonic nauplii (*A. tonsa*) are preferred by fish larvae but require phytoplankton and low density while benthic copepods (*T. holothuriae*) do not require planktonic microalgae, can be cultivated at high density and can be fed on inert feeds for their production.

4.2.5. Live prey disinfection

With the upscaling and expansion of commercial larviculture, microbial control of marine hatcheries deserved a special attention because of an increased incidence of microbial diseases attributed to Vibrios and other potential fish pathogens. A big input of bacteria was brought to the fish larvae via the live food chain. Between 100 and 10 000 bacteria were counted per rotifer and per brine shrimp and increased with age in fish from 1000 to 100 000 bacteria per fish larvae (Sorgeloos et al., 1994). This necessitated the development of alternative measures (other than the use of antibiotics) to reduce the bacterial load and to selectively manipulate the microbial flora in live preys and in culture water.
Hygiene of the live prey production should be a concern from the initial strain maintenance down to the ultimate distribution to guarantee the larval feeding with “safe” preys. For example rotifers grown on algal paste are much cleaner than those produced with baker yeast. Rotifer culture requires hygienic care and the culture must be protected against the introduction of germs by disinfection of inlet water (UV or chlorine) and of tanks and maintenance material. In case hygienic conditions are deficient both microalgae and live preys (rotifer, artemia) have a high load of bacteria mainly made of *Vibrios,*

Aeromonas and *Pseudomonas*. In the past live preys have been disinfected using a combination of antibiotics but this is no longer advised to prevent the development of drug resistant bacterial strains. Exposure of rotifers to UV irradiation (38 mW/cm^2) was effective in reducing bacterial load by approximately 90% and to improve the surviva at D20 (Munro et al., 1999).
The way live preys are first cultivated determine how contaminated they'll be. The bacterial load of the prey is partially reduced by short starvation prior to enrichment to empty their guts and by rigorous washings with freshwater baths, especially after enrichment (Planas and Cunha, 1999). INVE Aquaculture has developed a 'Disinfecting Continuously' (DC) concept for *Artemia* enrichment to control microbial development during live food production. **DC DHA SELCO** incorporates optimal levels of DHA in the *Artemia* and also provides ow bacterial loads, esp. *Vibrio sp*. in enriched Artemia and reduced contamination with ciliates, fungi, etc. Ocean Nutrition has developed the Instant Baby Brine Shrimp, a revolutionary off the shelf product consisting of entirely sterile newborn nauplii in an aqueous solution.

4.2.6. Live prey enrichment

Several types of enrichments have been proposed and include enrichments of live preys with algae, oil emulsions, vitamins, proteins, probiotics and prebiotics (Dhert et al., 2001; Agh and Sorgeloos, 2005). Live preys can be used as carriers of nutrients and other protective compounds to the larvae using complete enrichments (Figure 13).

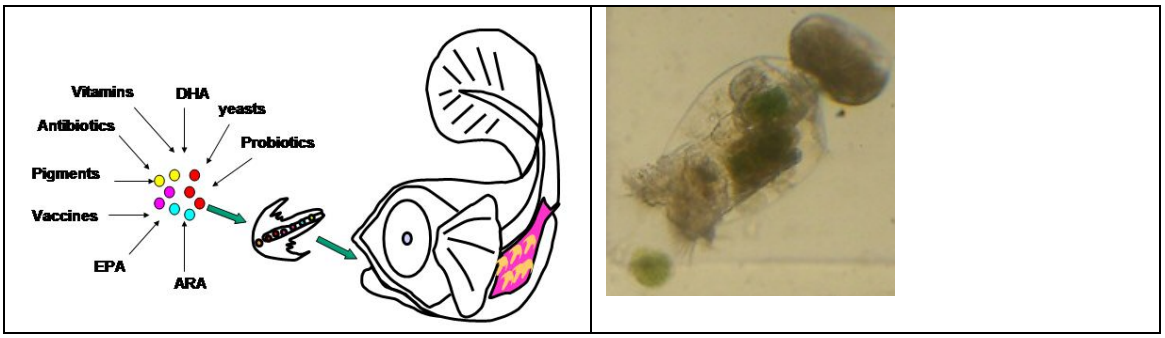


Figure 13. Different nutrients and bioactive compounds fed through live prey enrichment (left). Rotifer enriched with microalgae mixtures (right)

Rotifers (*Brachionus plicatilis*) followed by the branchiopod *Artemia* are the most common start feed for turbot larvae. However as the larvae grow, the nutritional quality of these prey is corrected by enrichment mixtures to increase the total content of nutrients: fatty acids, proteins, vitamin, and minerals in the larval foods in order to cover the nutritional requirements of turbot larvae.

Skretting has launched ORI-GO feeds that are efficient in live prey enrichment: **ORI-CULTURE**, a rotifer culture diet based on selected algae naturally consumed by rotifers; **ORI-GREEN** enrichment feed for rotifer and artemia is based on natural algae, proteins, phospho-lipids and marine oils, and rich in HUFAs. **ORI-GOLD** is a concentrated oil blend that converts quickly to a stable emulsion. The oil formulation is very stable, without the need for aggressive preservatives to prevent deterioration. It carries a blend of natural marine HUFAs (highly unsaturated fatty acids) and phospho-lipids with added protein micro-particles and algae derivatives to provide an excellent enrichment for *Artemia* while maintaining protein levels.

Finally, **ORI-PRO** is a high-performance blend of micro-encapsulated natural phytoproteins with marine HUFAs and phospho-lipids. Uniquely ORI-PRO raises both the protein and HUFA contents of the enriched *Artemia*. INVE (http://www.inve.com/) and Ocean nutrition (http://www.oceannutrition.eu) are commercialising different products for rotifer culture and enrichment of both rotifer and Artemia. Health products like Sanoguard® TOP F are also commercialized by INVE and incorporate immunostimulants and nutraceuticals to boost the health of larvae. A **Larviva Multigain** is also proposed by BIOMAR for the enrichment of both rotifers and Artemia for marine fish larvae (http://www.biomar.com). The product

contains single cell marine organisms, HUFA oil, vitamins, anti-oxidants, minerals and immunostimulants. A complete list of products and enrichment protocols are available as technical notes at the websites of the different companies (Table 5). Enrichment of preys can be made by a 24-h "second" culture with algae or fish oil emulsion.

Table 5. A range of products commercialized by INVE and Skretting (Ori-products) for the culture and enrichment of live preys.

	Rotifer	Artemia
Culture	Selco® S.parkle, Culture Selco® Plus, Selco® S.tream, Ori-Culture	*EG *Artemia* cysts, GSL ARTEMIA, High5, AF, AF480, MC450, OF SEPart,
Enrichment	Selco® S.presso, DHA Protein Selco® Ori-green,	Easy Selco's (Easy Selco, Easy DHA Selco, Easy Super Selco); Hygiene Selco (DC DHA Selco), Species specific Selcos Ori-green, Ori-Gold, Ori-Pro

* Artemia from different sources, size and quality available for the culture

4.2.7. Live prey feeding

Newly hatched larvae rely on endogenous reserves for the following 2–3 days (Person-Le Ruyet, 1989). Following absorption of the yolk sac the larvae are fed live feed, rotifers followed by *Artemia*. Enriched Artemia nauplii are used before weaning to dry feeds. When the mouth opens, often at day 3 depending on the temperature, enriched rotifers (*B. plicatilis*) are given and introduced slowly into the rearing water by using beakers. The density of rotifers in the rearing tank depends on larval stage, and is maintained at 2 to 5 rotifers/ml. Rotifers are fed several times per day and the feeding frequency is increased as the larvae grow. The density of rotifers in the tank is examined at 10:00 & 14:00 h and additional rotifers are provided when the density of rotifer is lower than the required density to be maintained. On D-8 to D-12, newly hatched nauplii of *Artemia* are given to the larvae and gradually replace rotifers. Depending on the mouth size, from D-13 or earlier whenever possible, both newly hatched and enriched *Artemia* are given. The density of *Artemia* fed to larvae increases from 0.2 to 0.4 ind./ml and is adjusted based on consumption by the larvae. In general, *Artemia* are consumed by the larvae within two hours. Since micro-diets for turbot appeared on the market, artemia is at risk to disappear from hatcheries since early weaning from live feed is always preferred and is possible for turbot larvae from D-18 on or earlier. Anyhow the weaning should be completed by day 24-25 at the latest. Although it is difficult to estimate the daily quantity of prey needed per larvae, it is necessary to avoid both underfeeding of the larvae and the decrease of the nutritive value of uneaten prey. In any case, 90% of the prey distributed must be ingested within 24 h.

4.2.8. Green water culture system

In some hatcheries, axenic algae are added to the rearing tanks to create a ''green-water'' system before introduction of larvae in rearing tanks. Algae maintain the nutritional quality of the rotifers in the larval rearing water, reduce the levels of dissolved ammonia in the culture system and thus, indirectly, improve the growth and survival of larvae (Reitan et al., 1993; Munro et al., 1995; Øie et al., 1997). Micro-algae are believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control. Microalgae such as *Nannochloropis oculata, Tetraselmis suicica, Isochrisis galbana* are the most common

algae used in the culture of rotifers (Dhert et al., 2001; Agh and Sorgeloos, 2005). Some other micro-algae including *Dunaliella tertiolecta, Pavlova lutheri, Chlorella sp. and Stichococcus sp.* have also been used as food for rotifer cultures. Apart from maintaining the rotifer high nutritional value, their other positive effects in the intensive rearing environment are thought to be a certain bacteriostatic capacity and a shading effect that reduces the larval aggressive behaviour. Larval culture in "clear" water is also feasible, but it gives lower average results in terms of survival and size homogeneity.

4.2.9. Microbially matured water

Establishment of a beneficial, protective microflora of marine larvae can be obtained by use of microbial matured water and probiotics (Skjermo and Vadstein, 1999). Microbial maturation of the rearing water before use in the larval tanks, can be obtained by running the water through a maturation unit that selects for non-opportunistic microflora which inhibits the proliferation of opportunistic pathogenic bacteria in the water and the larvae. Different filtration steps (sandfilter, membrane pore of 0.2µm) reduce the bacteria in the water by >95%. The recolonization and selection of bacteria in microbially matured water is performed by a heavily oxygenated biofilter and controlled water temperature. When microbially matured water is used in the earliest developmental stages of marine fish larvae, enhanced growth and survival can be obtained.

Also, the introduction of probiotic bacteria may promote the defence of the gut flora against pathogenic bacteria. Probiotic bacteria can be added directly to the water or administered to the larvae via live food, such as rotifers and *Artemia*. Turbot larvae maintained in matured water showed faster growth than larvae maintained in membrane filtered water, and reached 51% higher weights during the experimental period (Salvesen et al., 1999; Skjermo et al., 1997). Microbiological studies of turbot larvae reared in membrane filtered water have shown that the onset of the first feeding may induce a 10,000-fold increase in the number of intestinal bacteria in the turbot larvae from day 1 to 5 after hatching while larvae maintained in tanks with microbial matured water added microalgae the increase in bacterial density was only 10-fold during the same period. Improved larval feeding rates and growth were obtained by the use of matured water. BactoSafe, a probiotic product, is commercialized by Bern Aqua (http://www.bernaqua.com/fish.html) as water conditioner and larval feeds. It is a concentrated complex of live bacteria (*Bacillus subtilis, B. licheniformis, B. cereus, Pediococcus acidilactici*).icOther probiotic bacteria for turbot larvae include *Vibrio pelagius* (Ringø and Vadstein, 1998), *B. toyoi* and *Bacillus* sp. spores via rotifers (Gatesoupe, 1989; 1991); lactic bacteria via rotifers (Gatesoupe, 1990); *Lactobacillus plantarum* and *L. helveticus* via rotifers (Gatesoupe, 1991). *L. bulgaricus* and *Streptococcus lactis* via *Artemia* (Garcia-de-la-Banda et al., 1992). Since selected strains of probiotics might be easily out-competed for growth substrates in the gut, Munro et al. (1999) suggested that a diverse probiotic flora may be more effective than using a single isolate.

4.3. Larval weaning

Marine and some freshwater fish larvae have been fed in the past with Artemia nauplii followed enriched metanauplii before weaning to dry feeds. In order to reduce production costs linked to Artemia and the recurrent risk of transfer of harmful bacteria to the larvae, fish feed companies are offering since recently alternatives to Artemia in the form of micro-diets. Skretting has put on the market the Gemma-Micro diet which is co-fed with rotifers and is thought to replace Artemia. In good conditions, 50% of larvae establish artificial food intake in less than 2 days and the rest start eating later on.

4.3.1. Weaning tanks

Weaning can be started directly in larval tanks 1 week before changing of facilities. Weaning tanks are either circular with a conical or a hemi-spherical bottom or, preferably flat, circular or square. Fiber glass tanks of 2x2x0.5 m are most common. Weaning is done at a photoperiod of 18h L / 6h D and light intensity of 2-4 watts/m2.

With developments made during the last 25 years with inert feed, water management of larval rearing tanks, automation of feed distribution, better enrichments, the scale of time of feeding scheme in Figure 14. has been shortened and further progress in micro-diet formulation is expected to kick out completely the artemia in larval feeding (Declerq, personal communication).

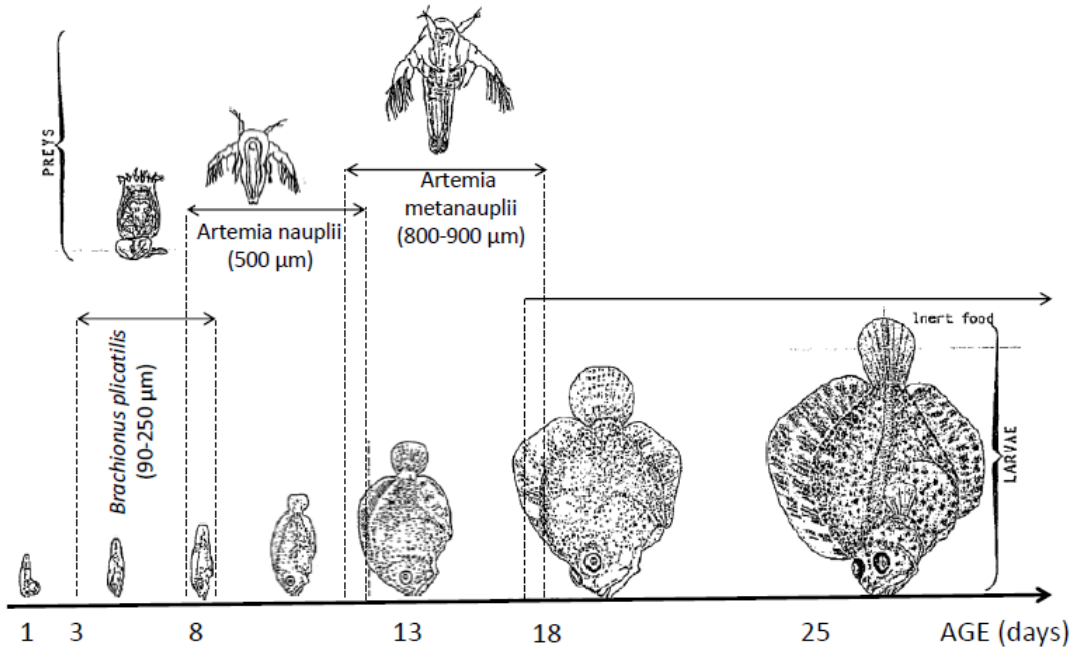


Figure 14. Larval development and feeding scheme during the first. Adapted from Pearson-Le Ruyet, J. Aquaculture, Technique et Documentation, Vol. 2 Lavoisier, Paris , 1986. With recent progress, rotifer are fed from D-3 to D-8, Artemia nauplii from D-8 to D-12, enriched artemia metanauplii from D-13 to D-18 and inert food (micro-diet) from D-18 on and complete weaning at D24-25 at the latest.

4.3.2. Weaning feeds

At the end of the live prey-feeding phase, turbot larvae are about 25 days post-hatch old and weigh between 100 and 120 mg. At this age they are still pelagic but ready for weaning onto microparticulate diet. When rotifers or *Artemia* are available in the rearing water, larvae prefer the live food than microdiet. As a result, weaning them to artificial diet becomes difficult. With progressive weaning, the artemia ration is delayed from morning to evening, from days 3 to 5 days post weaning and the compound feed is offered continuously. It is therefore recommended not to overfeed them with live food. Weaning may be direct if an automatic feeder is used. Starter weaning diets contain crude protein and fat levels ranging from 50 to 60% and from10 to 15% of dry matter respectively. They must be attractive and palatable, i.e., containing food stimulants such as inosine (0.6-1% of the dry matter) and having a soft texture. This is achieved by expanded rehydratable, water stable pellets.

On the website of Skretting **GEMMA Wean** is described as an end co-feed and weaning diet aimed for *Artemia* optimisation during larval rearing phases and **GEMMA Diamond** as one marine post-weaning diet designed to give juveniles the best start by assuring fast and efficient growth and low feed conversion ration under pre-growing conditions. In practice, other weaning diets have shown better results. These are from Biomar (Weanex), Bernaqua (Caviar) and Ewos (Aglonorse) but the latter is still too expensive.

4.4. Larval survival

Hygiene, bacterial load and bacterial types under development in the larval rearing tank and live preys facilities are said to be the key factors for getting better survival. Survival taken as an average over a whole production season is rather low during the turbot hatchery phase (15% versus 25%) and the variability in hatchery success is also high among batches, ranging from close to 0% up to 50% survival (Lavens et al., 1999; Leclerq, 1994). The most critical phase in terms of survival is the diet transition phase, between weeks one and two, when catastrophic mortality can occur (Person-Le Ruyet, 1989). Mortality peaks during larviculture occur at three culture periods:
-at the beginning and is attributed to the spawn quality or to stress during transfer of larvae into rearing tanks;
-between 6 and 8 days and correspond to the time unfed larvae die. This mortality is attributed to the unpalatibility of unsuitability of prey and/or the bacterial problems.
-between days 10 and 15, sometimes leading to the complete loss of larvae.

A survival rate of 20 to 30% during the first month is acceptable but difficult to obtain routinely. In 2002, Person-Le Ruyet indicated that despite improvements, the average survival from hatching to 5 g juveniles was about 20% (range 10-40%) (Person-Le Ruyet, 2002).

5 Ongrowing period

5.1. Tanks

During the nursery phase or pre-growing stage, weaned turbot larvae are grown further to a size suitable for transfer to farms. At the end of the hatchery-nursery phase, juvenile turbot weighing about 10 g (4-5 months post-hatching) are ready for another 4-5 months on-growing phase in indoor facilities. Turbot hatcheries and some farms keep juveniles in small tanks (10-20 m^2, 0.25-0.50 m useful water depth) under controlled environmental conditions in a closed recirculation system in which the water can be heated or cooled as required. Tanks are usually square with rounded corners, with flat bottom and a height of 0.5-0.7 m.

5.2. Culture environment

Optimal temperature for the growth of turbot juveniles is more likely 21°C although optimal temperature for a best FCR is closer to 18-19°C. Optimal temperature for survival is probably as low as 16-18°C. The optimum salinity is around 18-20 ‰ but turbot can adapt to salinity between 10 ‰ and 35 ‰ under optimal temperatures. Optimal light intensity is around 200 lux at the water surface and different light regimes do not seem to affect the feed intake and growth. Under certain rearing conditions, long photoperiods may be used to stimulate feeding activity (Person-Le Ruyet et al., 2002). Oxygen is one of the major limiting factors. The minimum oxygen required for maximum growth is 6 mg/l. Feeding stops at 3mg/l O_2 and the lethal concentration is 0.75-1.3 mg/l O_2. Concentrations below 2-3 mg/l total ammonia are considered as safe (Person-Le Ruyet, 2002).

5.3. Stocking density

Turbot juveniles tolerate overcrowding, thus stocking density can reach 2500 individuals/m^2 (Table 6). Starting densities are low density (10 kg/m^2) at the start increasing to 30 kg/m^2 at the end of the phase. The first grading is done when fish are about 50-60 g, which marks the end of the on-growing phase (Person-Le Ruyet, 2002).

Table 6. Stocking densities from weaning up to 1 year at IFREMER.

Wet fish weight (g)	Age (months)	Number/m2
0.1	1	2500
2	3	1000
10	5	500
35	7	250
75	9	150
125	11	100

5.4. Feeding and feeds

Fish are fed dry pellets of small size and pellet size and feeding rate (Table 7) must be adjusted to fish size to maximize growth. Buoyant non-floating pellets are preferred and should be ingested during their slow descent to the bottom.

Table 7. Indicative feeding table of turbot juveniles to adults.
Source: http://www.biomar.com

Size range (g)	Size pellets (mm)	Feeding (kg feed/day/100 kg fish)		
		Temperature (°C)		
		16	18	20
20-50	3.0	1.41	1.69	1.80
50-100	4.5	1.00	1.20	1.30
100-300	6.5	0.65	0.77	0.80
300-600	9.0	0.45	0.54	0.50
600-1000	12.0	0.35	0.42	0.40
1000-1500	15.0	0.29	0.34	0.30
1500-2000	15.0	0.25	0.29	0.20

5.5. Growth and survival

Turbot growth during the on-growing phase depends on temperature, feeding conditions, and on juvenile quality in terms of capacity to adapt to environmental conditions on the site. The growth potential of turbot during the first year is high: 200 g at 9 months and 350 g or more at 12 months. The high efficiency of commercial dry pellets allow an apparent FCR of about 0.8.

The survival is in the range of 75-85%, the better the water quality, the higher the survival (Person-Le Ruyet, 2002). To prevent diseases during this phase, turbot are most oftern vaccinated against vibriosis and furunculosis. In some sites they should be vaccinated against bacterial diseases caused by *Flexibacter* and *Streptococcus*. Turbot are also highly susceptible to parasite infestation, mainly by the ciliates *Trichodina* and *Uronema*

6 Grow-out period

6.1. Rearing facilities

Turbot are reared in land-based circular or square with cut angles concrete tanks with open-circuit pumped seawater or in re-circulation systems. The tank volume ranges from 20 to 100 m^3 with a depth of 0.4-1.2 m (shallow raceways exhibit depth of 0.4-0.5m). Land based rearing tanks are made of concrete, less commonly metallic structure and sometimes polyesther (shallow raceways). Tanks are covered individually or housed under light building like greenhouses or wharehouse like constructions in order to limit fouling and to provide reduced lighting (it as well protects workers!). To reduce heating costs to a minimum, turbot farms tend to reuse water after specific treatment and limit fresh seawater supply to 5-10% of water volume per day.

6.2. Stocking density

Turbot can be grown out at high stocking densities in onshore tanks and raceways. Turbot can tolerate densities equivalent to 50-75 kg/m2. The normal operational stocking range varies from 25 to 50 kg/m^2 in tanks. Note that the density as an average for a farm growing fish from 10 g to 2 kg is in the order of 42-50 Kg/m^2 (Pearson-Le Ruyet, 2002). In most intensive rearing systems, stocking densities are about 30-35 kg/m^2 of 300 g fish, 45 kg/m^2 of 750 g fish, and up to 60-80 kg/m^2 of larger fish (Person-Le Ruyet, 2002). To increase the resting surface available for the fish, tanks can be fitted with 1-2 rigid netted carpets about 30 cm off tank bottom. During grow-out, it is advisable to maintain fish sizes homogeneous through regular grading, at least twice during the grow-out. Survival is near maximum during grow-out.

6.3. Feeds

Commercial turbot feeds contain around 55 % proteins and 14% lipids and are commercialised by Biomar, Skretting and other feed companies. Larger fish have lower feed requirements than juveniles and are fed by hand once or twice a day. With acceptable feed quality available from the industrial suppliers, commercial FCR is in the surroundings of 1.1:1 from 10 g to 2 Kg. It evolves of course, as 10 g fish are capable of 0,8 when 1,5 kg+ are more likely in the 1,3-1,8 range.

7 Turbot diseases

Increased development of turbot culture has been accompanied by an increase in disease problems involving bacterial, viral and parasitic infections (Toranzo et al., 1990, Fernandez-Puentes et al. 1991, Beatriz et al., 1993, Castro et al., 2006)

7.1. Viral diseases

-Herpes virus is associated with excessive growth of epidermal cells in skin and gills.
-Infectious pancreatic virus causes muscular hemorrhages and a severe necrosis of the hematopoietic tissue. IPNV produced mortality only in small fish (2 g), although the larger fish (30 g) harbored the virus for at least 35 d.
-Viral Hemorrhagic Septicaemia (HVS) cause sudden death.

7.2. Bacterial diseases

-Vibriosis is responsible for the mortality of young turbot and is caused by a few pathogenic strains of *Vibrio anguillarum*
-Yersiniosis (*Yersinia ruckeri)*
-Furunculosis (*Aeromonas salmonicida*)
-Bacteriosis caused *Photobacterium leiognathi.*
-Flexibacteriosis (*Flexibacter maritimus*)
-Winter disease (*Pseudomonas anguilliseptica)*
-*Edwarsielle tarda*
-Mycobacteriosis (*Mycobacterium marinum*).

Multiple vaccines are available for Furunculosis, Flexibacter and Vibrio in Spain and France. *Vibrio* spp. and *Pseudomonas* spp. were the most prevalent bacteria recovered from diseased turbot in farms in Galicia (Spain). Haemorrhages in palate and jaws, tail and fins, and ulcerative lesions were the most frequent external clinical sings of diseased fish (Toranzo et al., 1993).

7.3. Parasites

-Ciliates (*Cryptocaryon* and *Trichodina*) feed on the surface of the gills and skin.
-*Haemogregarina sachai* causes a myeloid necrosis.
-Trematode *Cryptocotyle lingua* causes black spot disease.
-Myxosporean (*Enteromyxum scophthalmi*)
-*Uronema marinum* is by far the one giving more difficulties to farmers. It is an internal parasite which is not easy to cure when installed. There are intents for vaccines and other preventive measures but it remains a big threat for turbot farmers (Leclerq, personal communication).

Some turbot diseases have been summarized in Table 8. Source FAO:
ftp://ftp.fao.org/fi/document/aquaculture/CulturedSpecies/file/en/en_turbot.htm

Table 8. Diseases, causative agents and control measures in turbot.

DISEASE	AGENT	TYPE	SYNDROME	MEASURES
Amoebic Gill Disease (AGD)	*Neoparamoeba pemaquidensis*	Ectoparasite	Gill parasitism; respiratory impairment	Freshwater bath
Trichodiniasis	*Trichodina* spp.	Ectoparasite	Flashing; skin darkening; lethargy; breathing difficulties; rubbing gill cover & body against rearing surfaces	Disinfectant bath
Scuticociliatosis	*Philasteridis dicentrarchi*	Ecto, Endoparasite	Cutaneous ulcers; darkened skin; swimming behaviour alterations; bulging eyes; abdominal distension	Density reduction
Microsporidiosis	*Tetramicra brevifilum*	Endoparasite	–	Density reduction
Myxosporidiosis	*Enteromysum scophthalmi*	Endoparasite	Numerous white cysts on the skin and gills	Density reduction; complete disinfection of facilities
Flexibacteriosis	*Tenacibaculum maritimun*	Bacterium	Grey patches in dorsal fin area at first; lesions in head and mouth; sometimes gill rot	Vaccine; antibiotics
Furunculosis	*Aeromonas salmonicida*	Bacterium	Boil-like skin lesions	Antibiotics; farm-made vaccines
Streptococcosis	*Streptococcus parauberis*	Bacterium	Haemorrhaging of fins, skin, and serosal surfaces; ulcers	Vaccine
Vibriosis	*Vibrio anguillarum*	Bacterium	Darkened skin; lethargy; frayed fins; skin ulcers; exophthalmia	Vaccine; antibiotics

8 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 57846-2009-AQ-NLD-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

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Justification

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Date: December 2011

Appendix A. Voortplantingsprotocol voor tong (Solea solea) in een notendop

1 .Broodstock

1.1 Systeem

De bakken voor de houderij van tong ouderdieren moeten een diameter hebben van ± 3m en een diepte van ± 1 m (Baynes et al., 1994; Kamstra and van der Heul,2000). Door Imares wordt er gebruik gemaakt van verschillende typen zout water om succesvol tong voort te laten planten. Zowel met natuurlijk zeewater (persoonlijke communicatie van der Heul) als met zout grondwater (persoonlijke communicatie Blom) worden goede resultaten behaald. Een eenvoudig systeem bestaat uit een grote bak met de vissen en een kleine pompbak. In de pompbak staat een bolletjesfilter en het water wordt vanaf de pompbak door middel van een zandfilter naar de vissen gepompt.

1.2 Houderij

Het hoofddoel van het houden van ouderdieren is het produceren van eieren in een voldoende kwantiteit en kwaliteit. Om dit te bereiken zijn de volgende zaken van belang: waterkwaliteit, voeding, leefomstandigheden, lichtregime en temperatuur. De waterkwaliteit, voeding en leefomstandigheden zijn bepalend voor het welzijn van de vissen en het lichtregime en de temperatuur worden met name gebruikt om de vissen door een “natuurlijke cyclus” te laten gaan om de aanmaak van eieren en sperma te stimuleren.

1.3 Waterkwaliteit

Als er gebruik gemaakt wordt van een doorstroom systeem is de waterkwaliteit niet of nauwelijks te beïnvloeden. Groot nadeel is dat de omgeving uiteindelijk bepaald wanneer de vissen eieren gaan produceren en er een kans is op infectie door ziekteverwekkers met name in de zomermaanden. Recirculatie heeft als groot voordeel dat de temperatuur beïnvloed kan worden en de hatcherymanager kan besluiten wanneer er eieren worden geproduceerd. Door de relatief lage dichtheid van vissen 1-3 kg/ m^2 (Blonk et al., 2005; Baynes et al 1994; Hong et al 2006; Kamstra en Heul 2000) volstaat een relatief eenvoudig zuiveringssysteem bestaande uit een zandfilter en een bolletjesfilter (Kamstra en Heul 2000). De waterkwaliteit in de tank wordt bepaald door het tankhydrolica en het voerregime. Bij een onvoldoende debiet in combinatie met overvoeren kan de waterkwaliteit negatief beïnvloed worden wat uiteindelijk stress veroorzaakt bij de vissen en van invloed kan zijn op de ei-productie. Ammonium en nitriet moeten rond de nul zijn en de nitraatwaarden moeten de 60 mg/l niet overschrijden (persoonlijke communicatie Blom).

1.4 Voeding

De voeding van de ouderdieren is van directe invloed op de ei-kwaliteit, het voeren van enkel droogvoer geeft slechte resultaten (Baynes et al 1993). Op de markt bestaan speciale droogvoeders voor ouderdieren, deze korrels zijn voor tong relatief groot en hard en worden door de vissen slecht gegeten.
In het jaar zijn er twee periodes, een winterperiode en een zomer periode. Gedurende de zomerperiode worden de vissen gevoerd met een moistpellet, bestaande uit vismeel, mosselvlees en visolie (0,6%/dag) en in de wintermaanden met zagers (0,3%/dag) (Kamstra en van der Heul, 2000). In de zomermaanden moet de temperatuur rond de 18 graden zijn en sterken de vissen aan na het paaiseizoen. De waterkwaliteit in recirculatie systemen zal sterk verminderen als niet alle moistpellets worden opgegeten en het is te adviseren in meerdere porties te voeren.
Als de temperatuur beneden de 11 graden zakt wordt er omgeschakeld naar levende zagers. Dit is een natuurlijke voedselbron van de tong en biedt de garantie dat de voedingsstoffen in voldoende mate aanwezig zijn om de vrouwtjes te laten matureren. Beneden de 11 graden worden de tongen een stuk minder actief en eten nog nauwelijks. Groot voordeel van zagers is dat eventueel overgebleven zagers de tank niet vervuilen en in een later stadium kunnen worden opgegeten.

1.5 Leefomstandigheden

In het wild verblijft tong gedurende een groot gedeelte van de dag in het zand. Gedurende de nacht verlaat hij die omgeving en gaat actief op zoek naar eten. Het houden van tong in een bassin zonder zand zorgt voor een verhoogde stress bij de vissen en resulteert in slechte prestaties tijdens de paaiperiode. Het toevoegen van zand geeft wel de gewenste resultaten (Kamstra en Heul, 2000). De korrelmaat van het zand moet niet te klein worden gekozen omdat er anders een zuurstofloze laag kan ontstaan waarin H_2S wordt gevormd, dat toxisch is voor vissen.
Tong is een lichtschuwe vis en is met name gedurende de nacht actief. De verlichting boven de bak mag niet meer zijn dan 100 lux aan het oppervlak van de waterkolom (Kamstra en van der Heul, 2000). Temperatuurfluctuaties moeten zoveel mogelijk vermeden worden en bij het afkoelen of opwarmen wordt niet meer dan 1 graad per dag aangehouden (persoonlijke communicatie Blom).
Ondanks het optimaal houden van de omstandigheden kunnen er problemen optreden met de gezondheid van de vissen. Dit zal zich in eerste instantie uiten in verminderd eetgedrag. Broodstockvissen zijn kostbaar en indien nodig moet er snel gereageerd worden met een formaline of antibiotica behandeling.

1.6 Lichtregime en temperatuur

De daglengte en de temperatuur zijn de factoren die de aanmaak van eieren en sperma stimuleren. De daglengte in Nederland is op internet eenvoudig te vinden http://bernardwx.blogspot.com/2010/12/de-zonnewende.html. Door middel van een tijdklok die iedere week aangepast word, kan een vergelijkbare situatie met de natuurlijke omstandigheden gecreëerd worden. Met de daglengte moet ook de temperatuur aangepast worden naar zomer en winter. Voor een goede simulatie van de winterperiode dient de temperatuur gedurende enige tijd 7°C geweest zijn (Hong et al., 2006).

2 Ei-productie

De ei-productie kan na de winterperiode gestart worden door de temperatuur in de broodstocktank geleidelijk te verhogen van 7°C naar boven de 11°C (persoonlijke communicatie Blom). Gedurende de nacht paaien de tongen op natuurlijke wijze. Bij een saliniteit van 34 g/l zullen de eieren drijven en kunnen worden opgevangen. De temperatuur stuurt uiteindelijk de voortplanting en wanneer er voldoende eieren geproduceerd zijn kan de temperatuur weer verlaagt worden totdat er weer ruimte beschikbaar is voor een nieuwe ronde.

2.1 Incubatie

De gedurende de nacht geproduceerde eieren kunnen met een overflow collector (doorstroom) of een ei-collector in de pompbak (recirculatie) opgevangen worden. De eieren blijven in de collector door een 400 µm nylon net. Tongeieren zijn relatief ongevoelig voor mechanische beschadigingen, debieten moeten echter niet te hoog gekozen worden.
De geproduceerde eieren kunnen voorzichtig met een fijn 100 µm net verzameld worden in een getarreerde emmer. Een gram eieren bevat ongeveer 600 stuks (Kamstra and van der Heul, 2000) en met het totaal gewicht kan het aantal geproduceerde eieren van de batch geschat worden.
De batch eieren wordt vervolgens uitgezet in een incubatie systeem, bestaande uit conische trechters (±100-250 l) aangesloten op een broodstocktank of een los systeem met zandfilter, koeling en een pompbak. Het zeewater in de trechters moet 34 g/l zijn en de temperatuur ongeveer gelijk aan de temperatuur waarbij de eieren geproduceerd zijn. Beluchting onderin de trechter zorgt voor een voorzichtige beweging van de eieren. Het water in de trechter wordt meerdere keren per uur ververst. Tot slot houdt een filter van 400 µm de eieren in de trechter. Het bevruchtingspercentage van de batches met eieren kan sterk fluctueren. Niet bevruchte eieren zijn een voedingsbron voor bacteriën en dienen zo snel mogelijk verwijdert te worden.
Het verwijderen van niet bevruchte eieren gebeurt iedere dag door de doorstroming en beluchting even stil te zetten. Niet bevruchte eieren zakken naar de bodem en kunnen door het openzetten van de kraan aan de onderzijde van de trechter verzameld worden in een bekerglas. De dode eieren kunnen vervolgens gewogen worden. Het aantal van de overgebleven eieren kan bepaald worden nadat de gewichten van de dode eieren afgetrokken wordt van het begin gewicht.
De bevruchte eieren hatchen na ongeveer 70 daggraden (persoonlijke communicatie blom). Op het moment van hatchen komen er lege ei schillen vrij en deze moeten zo spoedig mogelijk afgevoerd worden omdat dit een potentiële voedingsbodem is voor bacteriën. Na het hatchen kunnen de larven getransporteerd worden naar de larventanks.

3 Larvale opkweek

3.1 Systeem

Voor de opkweek van larven kan gekozen worden voor zowel een doorstroom systeem als een recirculatie systeem. Hier zal verder alleen in gegaan worden op het ontwerp van de larvenbakken. Een volume van tussen de 100 en 400 liter geniet de voorkeur en de bakken kunnen zowel rond als rechthoekig zijn. Voor elke ronde met larven moet het systeem volledig ontsmet zijn.
Per liter worden er 50- 200 larven (Kamstra and van der Heul, 2000; Palazzi et al, 2006; Canavate and fernandez-Diaz, 1999) uitgezet. De temperatuur van het systeem moet tussen de 18 en 22 graden en een saliniteit van 34 (±1) g/l gehouden worden (Kamstra and van der Heul, 2000; Palazzi et al, 2006; Canavate and fernandez-Diaz, 1999). In het larvensysteem ontwikkelen de pelagische larven (bij aanvang) zich tot een benthische platvis die na een periode van levend voer volledig wordt overgeschakeld naar het eten van droogvoer.

In de larvenbak wordt centraal een standpijp geplaatst met daarom heen een filterkorf die aan de onderkant belucht wordt door een ronde beluchtingsslang. De beluchting voorkomt verstopping van het filter. Tijdens de levend voer fase worden de filters twee maal vervangen. Gedurende de dag een filter met gaas van 200 µm en gedurende de nacht een 500 µm tot 1000 µm filter. Het 200 µm filter houdt overdag de artemia in de tank en het levende voer blijft zo beschikbaar voor de larven. Aan het einde van de dag worden de grove filters geplaatst en de pomp enige uren stil gezet. Na enkele uren gaat de pomp d.m.v. een tijdklok weer aan en spoelen de overgebleven artemia uit de tank. Tijdens de droogvoer fase wordt enkel een grover filter gebruikt die wel 1x per dag schoongemaakt moet worden.

3.2 Onverrijkte artemia (5-10 dagen na hatchen)

Wanneer de larven uit het ei komen zijn de larven nog niet volledig ontwikkeld en functioneren de ogen de de mond nog niet optimaal. Daarnaast vertonen de larven nog geen actief zwemgedrag. Gedurende de eerste 3 tot 4 dagen na het hatchen zijn de larven nog volledig afhankelijk van hun dooierzak. Daarna kan er gestart worden met het voeren van onverrijkte artemia.
De artemia cysten moeten bij een temperatuur van 28 °C en een sterke belichting zo'n 24 uur worden uitgebroed. Na de 24 uur kunnen de relatief kleine artemia's gevoerd worden in twee porties aan de larven. Belangrijk is dat artemia van de tweede voedering koel worden gezet (< 7°C) om hun voedingswaarde zoveel mogelijk te behouden. De dichtheid van de artemia dient zo'n 5-10/ml te zijn.
In deze fase zijn de larven nog relatief klein en moet de doorstroming en beluchting relatief laag zijn om mechanische beschadiging aan de larven te voorkomen.

3.3 Verrijkte artemia (10-22 dagen na hatch)

Na tien dagen zijn de larven dusdanig groot geworden dat er gevoerd kan gaan worden met verrijkte artemia. Na het uitkomen worden de larven nogmaals een dag bij 28 graden gehouden en verrijkt. Op de markt zijn diversen verrijkingen beschikbaar die goede resultaten geven.
De debieten in de bak kunnen ook iets verhoogd worden en in deze periode zullen de larven meer artemia gaan eten en is het zaak om de dichtheid goed in de gaten te houden. Dit kan met behulp van een 1ml pipet. Eventueel kan besloten worden om tussentijds een extra dosis voer te geven om de dichtheid op peil te houden.
Tijdens deze fase wordt ook voorzichtig begonnen met het voeren van kleine porties droogvoer, het gebruik van aglonorse wordt aangeraden (Kamstra en van der Heul, 2000). Tweemaal per dag kan een kleine dosis gegeven worden, voor de voederbeurten met artemia.

3.4 Weaning

Aan het einde van de periode van het voeren van de verrijkte artemia zijn de larven verandert in benthische platvissen en vertoeven dan voornamelijk op de bodem. Op dag 23 wordt er volledig overgeschakeld op droogvoer. In tegenstelling tot artemia beïnvloedt niet opgegeten droogvoer de waterkwaliteit sterk. Overvoeren moet ten allertijden voorkomen worden, desondanks zal de vervuiling toenemen en moeten de tankwanden en bodem geregeld schoon gemaakt worden om te voorkomen dat er een biofilm ontstaat. Als de platvissen goed op het droogvoer zitten kunnen zij verplaatst worden naar een pootvissysteem.