A review of the cultivation potential of turbot (Scophthalmus maximus (L.)) in the Baltic Sea region: a promising candidate species for marine aquaculture in Russia

D Pyanov
Department of Aquaculture and Freshwater Fisheries, Atlantic Branch of the Russian Federal Research Institute of Fisheries and Oceanography (AtlantNIRO), Kaliningrad, Russia
E-mail: pyanov@atlantniro.ru

Abstract. Turbot (Scophthalmus maximus (L.)) is regarded as a good quality product that enjoys widespread acceptance and is therefore a popular choice for marine aquaculture in Europe. This study examines the potential and feasibility of the artificial cultivation of turbot in the Baltic Sea region. It takes into consideration both commercial farming and the implementation of artificial reproduction for stock enhancement programs. With regard to the latter, it discusses observed short-term trends towards reducing species catches in ICES 22-32 subdivisions as well as trends in hydrological process dynamics during the spawning process (especially low water salinity, which decreases the natural reproduction efficiency). The article also considers the potential of the research performed in 2018–2019 at the AtlantNIRO research facility on the development of cultivation techniques for turbot larvae. The findings demonstrate the possibility of cultivating turbot larvae under artificial conditions and are important for the development of marine aquaculture in the Kaliningrad region of Russia, since they can lead to the introduction of new fish species for farming in the future.

1. Introduction
Nowadays, fish come to the market from wild-capture fishing and aquafarming. The latter is becoming increasingly global, since more and more fish species are now being raised in ponds, floating net cages and recirculating aquaculture systems (RAS). It is meanwhile clear that such technology has a vast potential in reducing captures from the marine environment while maintaining supplies of fish to the market for human consumption. In some countries, the production volumes of artificially cultivated aquatic organisms (both in fresh and marine water) are already several times higher than the volume of wild catch, while marine aquaculture, the main activity of which is aimed at the cultivation of marine finfish species, molluscs and seaweed, can account for more than 50% of the country's seafood industry.

Currently, turbot (Scophthalmus maximus (L.)) is regarded as a valuable, nutritional marine water fish species in Europe. It is a coastal flatfish distributed in the marine or brackish waters of the North East Atlantic, the Baltic Sea and the Mediterranean Sea and has gained remarkable attention with respect to both fisheries and aquaculture [1]. However, the artificial cultivation of turbot is not yet widespread, and the production level of farmed turbot is relatively low compared to other fish species. The highest value was recorded in 2012, when production was 12,817 tons. It is now at about 10,000 tons, with most production being carried out in Spain and Portugal. According to the FAO, commercial turbot production in 2018 in the EU amounted to 8,396 tons [2]. The potential available from cultivating turbot under artificial conditions is due to its high palatability, good growth rate and attractive selling price.
In the countries of the Baltic Sea region, however, freshwater aquaculture production is better developed than marine aquaculture. The largest share of total aquaculture production is in the region represented by rainbow trout and common carp farming. Marine aquaculture species are only produced in small quantities, and only Finland, Denmark, and Sweden have suitable hydrological conditions for sea cage systems used to produce marine trout [3]. Even though turbot is native to the Baltic Sea, it is not commercially cultivated in the region, although there is a certain potential for this.

The aim of this study is to provide an overview of the potential and feasibility of artificial turbot cultivation in the Baltic Sea region, based on the example of the Kaliningrad, a coastal semi-exclave region of Russia. This article describes the features of the turbot population in the Baltic Sea that are essential for implementing artificial reproduction for stock enhancement programs and considers the results of applied research on the development of cultivation techniques for turbot larvae as performed at the Atlantic branch of the research facility of the Russian Federal Research Institute of Fisheries and Oceanography (AtlantNIRO) in 2018–2019.

2. Turbot in the Baltic Sea

The Baltic Sea has a very low salinity level, ranging from almost freshwater (3–8 ppt in the surface water) to 10 ppt in the deep basins. Adaptation to such environmental conditions has resulted in the turbot eggs in the Baltic Sea developing in a demersal manner, while all other populations of this species have pelagic eggs [4–8].

Turbot spawning occurs in the Baltic from May through early August in shallow waters on the sandy pebble seabed [9, 10]. The species winters away from the coast at depths that commonly reach up to 40 m.

Turbot attains sexual maturity at the age of 3–4 years. The species displays fractional spawning, which can result in up to 10 spawning events per female. A single female can carry between 70,000 and 5,000,000 eggs, depending on its body weight, length and age [10]. The fecundity of turbot in the Baltic Sea is slightly higher than in the North Sea, Atlantic and Black Sea Basins. The eggs have a diameter of 1.10±0.5 mm [11]. At the beginning of spawning, the individuals with lengths of between 27 and 45 cm have an absolute fecundity of 70,000 to 470,000 eggs, while a 35-centimeter-long turbot has a fecundity of about 1,200,000 eggs. The relative fecundity varies from 250,000 to 980,000 eggs per kg of female body weight [10].

The effectiveness of natural turbot spawning at a salinity level of below 6 ppt is low, and only a small number of newly hatched larvae survive [7, 12]. This is consistent with the data on turbot distribution in the Baltic Sea. Thus, the abundance of species is higher in the southern part of the sea and around the island of Gotland (ICES Subdivisions (SDs) 24-28), where the salinity level is about 7-8 ppt which is higher than that in the Northern Baltic (SDs 29-30), where it is about 5-6.5 ppt. The species is completely absent in the waters of the Gulf of Bothnia (SD 3), where the salinity level is 3 ppt [7]. The success of the natural reproduction of turbot is partly dependent on local upwelling events (mostly in SDs 25-28) and salty currents from the North Sea, which lead to a short-term increase in the salinity level of the water surface layer [7, 13, 14].

Turbot fishing is concentrated on the westerly parts of the Baltic Sea (SD 22–26) (Figure 1). Turbot catches have decreased steadily in commercial fisheries throughout the Baltic Sea since the mid-1990s, when the total landings in ICES subdivisions 22–32 were on average 800 to 1,200 t. In 2018, the total catch did not exceed 370 t, while the Russian catch (in SD 26) was only 7 t [15]. In Russian waters, turbot are usually caught in bottom-set gillnets with a mesh size of 110 to 240 mm, operated from small fishing vessels in April-May and September-November. Usually, this species represents about 0.1-0.4% of the overall fish catch [16].

Based on the above and taking into account any future increase in fish consumption as well as the impact of an unstable hydrological regime in the Baltic Sea, the realization of an artificial reproduction program for stock enhancement may be promising.

In conjunction with the implementation of the Strategy for the Development of the Fisheries Complex until 2030 developed by the Ministry of Agriculture of Russia, more investment will be made
not only into expanding existing aquatic bioresource production, but also in introducing the cultivation of new fish species in the Russian Federation. Thus, Baltic Sea turbot has recently attracted interest as a candidate for aquaculture in the Kaliningrad region, because the species is highly valued by local consumers, as it provides a supply of healthy food source and has a low body lipid content of about 2% of fish meat and a highly digestible protein content of 16%. It is usually sold frozen on the local market, as gutted whole fish, fillets with skin or skinned fillets.

The cultivation of turbot was introduced in the UK in the 1970s, and numerous individual aquaculture studies were performed in Europe in the last century. However, these were mainly focused on the Atlantic population. Until recently, there was only little regional interest in on turbot aquaculture originating from the Baltic Sea. Minor differences between species populations still exist, in particular, as mentioned above, due to variations in salinity. In Russia, interest in its cultivation began to develop in the mid-1990s at the facilities of the Atlantic Research Institute of Fisheries and Oceanography (now the Atlantic branch of the Russian Federal Research Institute of Fisheries and Oceanography) in Kaliningrad, which conducted experiments focusing on the incubation process and the early larval stages of cultivation. At the time, the results did not show much promise, due to the low survival rate of the turbot larvae. For this reason, in the period from 2018 to 2020, the aquaculture department of AtlantNIRO was tasked with developing proper cultivation protocols for turbot juveniles and adapting them to the conditions prevailing in the region.

3. The cultivation of turbot under artificial conditions at the AtlantNIRO aquaculture research facility

Normally, the aquaculture production cycle for turbot comprises the following stages: catch and transportation of wild turbot → broodstock management → artificial insemination and gamete management → egg incubation → larval culture up to 1 g → early fry culture up to 10 g → fry culture up to 100 g → cultivation up to a marketable size of 800-1000 g → establishment of broodstock. Once a full, captive broodstock has been established, there is no further need for catches of wild turbot.
The main aim of our project was to investigate the feasibility and features of the cultivation of Baltic turbot juveniles. Our efforts focused on providing robust documentation on the methods of the most crucial technical procedures leading to larval culture up to 1 g, as this is considered one of the most difficult stages of turbot cultivation. It concerns the period from the beginning of exogenous feeding until the complete metamorphosis of the larval body (turbot larvae are symmetrical after hatching), when mortality rates may be as high as 98%. Live feed is essential for turbot larvae, but after transformation to the juvenile stage, the fish body becomes asymmetrical, survival rates increase considerably, and the subsequent cultivation process is far more straightforward.

3.1. Material and methods
The research was carried out at the branch’s research facility located on the Curonian Spit (Lesnoy, Kaliningrad region) and focused on the first five stages of the turbot production cycle, as described above. We performed two trial runs in May-July 2018 and May-August 2019 respectively to familiarize ourselves with the methods used in the most crucial technical procedures.

3.1.1. Catch and transportation of wild turbot. In late May 2018, wild breeders were caught from the fishing vessel using bottom gillnets with a mesh size of 115–120 mm, exposed at a depth of 5–10 m along the coast of the Curonian Spit that separates the Curonian Lagoon from the Baltic Sea coast. Within 40-50 minutes, the captured turbot breeders were delivered to the facility and put in experimental RAS, while males and females were kept separate. The female breeders had a body length of no less than 35 cm and males no less than 20 cm. A total of 54 specimens were caught.

In June 2019, breeders were captured using a motorboat. The capture was carried out at night. The nets were set at 19:00 and withdrawal was at 7:00 the next day, corresponding to a net-soak time of 12 hours. The nets had a mesh size of 90–120 mm and were set at a depth of 4 m. A total of 52 specimens were captured.

In both cases, special plastic bags filled with fresh sea water were used for fish transportation.

3.1.2. Broodstock management. The wild breeders were kept at the small-scale RAS, which comprises four tanks, a circulation pump, a UV sterilization unit, a mechanical filtration unit and a water chiller. Males and females were kept in separate tanks filled with water of a natural salinity of 7 ppt. The initial water temperature of 12 °C in the RAS was close to the natural water temperature of the Baltic Sea that prevails in May and June. Sexual maturation of the breeders was achieved by temperature stimulation up to 14.0 °C. Individuals were separated as soon as they displayed any signs of maturation.

3.1.3. Artificial insemination and gamete management. Artificial fertilization was carried out using the semi-dry method, in which female eggs were collected by stripping and then putting them to soak in glass beakers, while male testes samples were homogenized and the resulting suspension with sperm introduced to the eggs through a gauze.

3.1.4. Egg incubation. The fertilized eggs were incubated in 20 l hatching jars using a water flow system with an increased salinity of 13 ppt in 2018 and 15 ppt in 2019. To ensure positive buoyancy of the eggs, the natural salinity of the seawater was adjusted using a synthetic salt. The water flow system (Figure 2) was specially designed for incubation trials involving turbot eggs, as follows. Seawater of the desired salinity passed from two fiberglass tanks (9) to the sump (4) from where it was pumped (2) to two chillers (1), the mechanical filtration unit (3), and the UV sterilization unit (5). Part of the water flowed back to the tanks (9) while another part flowed to the hatching jars (10). The water flow pressure to each jar was regulated by a small valve. The water from the jars passed through plastic drain tubes (6) into the raceway (7) from where it flowed through the drain hole and into the sewer (8).
Figure 2. Schematic diagram of the artificial incubator for turbot eggs. Water circulation; Aeration; 1 – Chiller; 2 – Circulation pump; 3 – Mechanical filtration unit; 4 – Sump; 5 – UV sterilization unit; 6 – Plastic drain tube; 7 – Raceway/stand for hatching jars; 8 – Water outlet to the sewer; 9 – Seawater tank; 10 – Hatching jar; 11 – Aeration tubes; 12 – To the air compressor.

In 2018, about 150,000 eggs were incubated. The incubation process was carried out at a water temperature of 12.5±0.8 °C; in 2019, a similar process was carried out with 200,000 eggs at 13.5±0.4 °C. In both cases, incubation took place at a room illumination of 30 lux.

3.1.5. Larval culture up to 1 g. The main differences between the two experiments are shown in Table 1. Newly hatched larvae were placed into tanks containing microalgae cultures (in 2018, these were *Nannochloropsis oculata* and *Dunaliella salina* in equal proportions, in 2019, only *Nannochloropsis oculata*) using the ‘green water’ technique. The enriched rotifers (*Brachionus plicatilis*) were introduced in a two-day post hatch (DPH) and maintained until 18 DPH in 2018 and until 20 DPH in 2019, when *Artemia salina* nauplii were offered at 12 and 8 DPH until 20 and 35 DPH in 2018 and 2019, respectively. Artemia metanauplii hatched from cysts were enriched with fat-soluble vitamins before being fed to the larvae and then offered in the period from 20 to 45 DPH and 30 to 45 DPH in 2018 and 2019 respectively. Starting from 30 to 40 DPH and until the end of experiment, turbot larvae were fed an artificial diet in parallel with the live feed. The nutrient composition of a commercial diet was 64% crude protein and 9% lipid. After feeding, the tanks were cleaned of any uneaten granules.

Over the course of the rearing processes, a number of differently shaped tanks were also employed. The water exchange rate in the tanks was 80% of total volume during the first 20 days of the cultivation process and 100% up to day 60.

In both trials, starting in the first days after incubation, the water temperature was increased gradually to 17.0 °C by 0.5 °C every day; it was maintained at this level until 15 DPH and then increased to 22.0 °C on the final day of the experiments. The light regime was 16 hours of light alternating with 8 hours of darkness.

3.2. Results and discussion

The sexual maturation of breeders by temperature stimulation in both years showed positive results, with about 30% of wild turbot females kept in the RAS after transportation maturing and producing eggs of good quality.
Table 1. Experimental conditions of turbot larvae cultivation in 2018–2019.

| Index                        | 2018                                      | 2019                                      |
|------------------------------|-------------------------------------------|-------------------------------------------|
| **Vessels containing larvae**|                                          |                                           |
| 0-20 DPH: cylindroconical tanks with a total water volume of 200 l | 0-20 DPH: cylindroconical tanks with a water volume of 200 l |
| 20-60 DPH: round, flat-bottomed fiberglass tanks with a water level of 20 cm and a total water volume of 200 l | 20-40 DPH: round, flat-bottomed fiberglass tanks with a water level of 20 cm and a total water volume of 200 l |
| 40-60 DPH: rectangular raceways with a water level of 20 cm and a total water volume of 300 l | 40-60 DPH: rectangular raceways with a water level of 20 cm and a total water volume of 300 l |
| **Stocking density**         | 2-20 DPH: 25,000 per m³                   | 2-20 DPH: 25,000 per m³                   |
|                              | 20-60 DPH: 20,000 per m³                  | 20-40 DPH: 20,000 per m³                  |
|                              | 40-60 DPH: 10,000 per m³                  |                                           |
| **Microalgae**               | 0-50 DPH: Nanochloropsis oculata           | 0-50 DPH: Nanochloropsis oculata           |
|                              | Dunaliella salina                         |                                           |
| **Feeding scheme**           | 2-18 DPH: Brachionus plicatilis            | 2-20 DPH: Brachionus plicatilis            |
| Live feeds                   | 12-20 DPH: Brachionus plicatilis           | 8-35 DPH: Artemia salina nauplii          |
|                              | Artemia salina nauplii                    | 30-45 DPH: Artemia salina                |
|                              | 20-45 DPH: Artemia salina metanauplii     | 40-60 DPH: Artemia salina metanauplii     |
| Artificial diet              | 40-60 DPH: 0.2-0.4 mm granules             | 30-40 DPH: 0.2-0.4 mm granules            |
|                              | 40-60 DPH: 0.4-0.6 mm granules             |                                           |

In the first trial, the incubation time of fertilized eggs at a water temperature of 12.5 °C was 164 h, with a hatching rate of 70%. In 2019, hatching occurred at 13.5 °C after 137 h at a rate of 77%. The hatching times for Black Sea turbot (*Scophthalmus maeoticus*), a species closely related to *Scophthalmus maximus*, were 162.7 h at 12 °C and 115.9 h at 14 °C [17] and are therefore similar to the results obtained in this study. In the case of turbot caught near the west coast of Spain, the incubation time was found to be 175 h at 13.0 °C [18]. This may be due to differences relating to the incubation process, salinity levels or illumination. In our case, incubation was carried out at a room illumination of 30 lux, compared with 60 lux in the trial performed for Atlantic turbot. Our data is similar to previous findings obtained by Karas and Klingsheim relating to North Sea turbot [19]. Our results are also comparable with the data of Kuhlmann et al. [4], where 50% of larvae hatched after 155 h at a temperature of 13°C.

Newly hatched larvae had a mean body length of 3.06±0.08 mm. Dissolved oxygen content in the larval rearing tanks was between 8.0–10.0 mg/l during both trials, while pH values were kept stable at a level of 8.5–8.8.

It was found that the main peaks of mortality in turbot larvae were observed from the moment of transition to exogenous feeding (when mouths of larvae are fully opened and they actively begin to consume live feed), as well as during the transition to feeding on nauplii and an artificial diet. Thus, in
2018, mortality peaks occurred at 7–10 DPH and 18 DPH. In the 2019 trial, the survival rate of larvae decreased to 20% at 30 DPH and to 10% at 30-60 DPH (Figure 3).

**Figure 3.** Survival rate and body length of turbot larvae.

These values are within normal ranges. The data obtained corresponds with the larva mortality findings for Atlantic turbot and Black Sea turbot [20, 21]. The concentration of microalgae in all experimental trials was maintained at a level of $7 \times 10^5$ cells/ml for 50 DPH. It is likely that the presence of certain types of microalgae in the water also affects the survival of the larvae. Thus, the survival rate of turbot larvae in 2019, when *Nanochloropsis oculata* was kept in water, was 48% up to 20 DPH. This species of microalgae is a good source of ω-3 fatty acids, in particular eicosapentaenoic acid (EPA), and is commonly used in marine aquaculture, especially for Black Sea turbot [20]. When larvae were reared in the presence of two microalgae cultures simultaneously, the survival rate of 20-day-old larvae was 25%. Obviously, the mortality rate may also be reduced if using other microalgae. Thus, the survival rate of Atlantic turbot larvae in the presence of *Tetraselmis suecica* and *Isochrysis galbana* microalgae in the rearing tanks can reach 63.5% at 15 DPH [22]. The lower survival rate in our case may also be due to the use of slightly different feeding schemes and a lower salinity level of 13-15 ppt. A higher survival rate can be attained with copepods as the initial feed, along with advanced hygiene and bacterial load control.

In both years, inflation of the swim bladder occurred at 5-6 DPH. Some larvae with an unfilled swim bladder decreased their feed consumption and lived for several more days. Resorption of the yolk sac ended at 9-11 DPH. At 14-17 DPH, the main elements of the fins were formed in the larvae, and the body height increased.

Throughout all the experiments, the concentration of rotifers and Artemia was maintained at a level of 1-3 ind./ml and 0.3-3 ind./ml, respectively. In 2018, the average concentration of rotifers in water was 2.9±2.4 ind./ml, while that of Artemia nauplii was 0.6±1.0 ind./ml. In 2019, the figures were 2.42±1.24 ind./ml and 0.51±0.60 ind./ml for rotifers and Artemia, respectively.

At 25 DPH, asymmetry of the eyes appeared in the larvae and they began a near-bottom lifestyle. At 30 DPH, some individuals began to lie on the bottom of the tanks. To speed up the completion of metamorphosis, the bottom of the fish tanks was covered in a thin layer of sand. During Artemia nauplii and metanauplii feeding, the larvae reacted poorly to the introduction of artificial feed. The feeds we used generally satisfied the total protein requirements for the species. Thus, for turbot weighing up to 10 g, it can be in the range of 50-69.8% [23, 24]. However, it is worth noting that the feeds used in our experiments sank quickly, and the rate of their immersion in water was only a few seconds. Some larvae captured food particles in the water column as they were settling. However, most of the granules quickly sank to the bottom. The larvae did not react to the settled granules and continued to consume Artemia in the tank. Within a few days of the first inclusion of artificial starter feeds in the diet, the larvae
developed a positive reaction to feed. According to the established features of the development of turbot at this and subsequent stages of cultivation, it is advisable to use floating or slowly sinking feed.

At 40 DPH, most of the larvae were concentrated at the bottom of the tanks. In some individuals, the right eye did not shift: such larvae were in constant motion and were unable to lie on the bottom of the fish tank.

At 45 DPH, when the presence of live feed in the tanks was minimized, the larvae were already actively consuming granules. The survival rate stabilized at an average level of 10-15%. On the same day, the completion of metamorphosis was noted in 80% of the individuals.

After passing through the metamorphosis stage, which is highly significant in the larval period, no further mortality was observed. The survival rate stabilized at an average level of 10-15%. The length and body weight of the larvae on day 60 were 35.10±4.90 mm and 0.52±0.22 g in 2019 and 33.00±4.69 mm and 0.48±0.07 g in 2018, respectively.

4. Conclusion

The results obtained demonstrate the possibility of turbot larvae cultivation under artificial conditions. In the experiments conducted in 2019, the survival rate at the end of the larval period was 10%, which can be considered a good result for rearing of this species. All the data acquired will be additionally processed by the institute branch’s aquaculture department and will subsequently form the basis of a handbook on methods of artificial cultivation of turbot, which local aquafarmers can adopt and use themselves within the region.

Considering the existing worldwide turbot farming techniques and our recent successful attempts at cultivating this species, it is fair to conclude that the potential of turbot aquaculture in the Kaliningrad region of the Baltic Sea region looks promising. However, the issues of possible restocking programs and large-scale farming will necessitate reliable marketing and thorough economic research [24].

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