

# Stocking density effect on survival and growth of early life stages of maraena whitefish, *Coregonus maraena* (Actinopterygii: Salmoniformes: Salmonidae)

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<http://zoobank.org/00057267-4821-40E4-928B-6D2EDC396A70>

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**Academic editor:** Predrag Simonović ♦ **Received** 8 February 2021 ♦ **Accepted** 20 March 2021 ♦ **Published** 14 June 2021

**Citation:** Stejskal V, Matousek J, Sebesta R, Nowosad J, Sikora M, Kucharczyk D (2021) Stocking density effect on survival and growth of early life stages of maraena whitefish, *Coregonus maraena* (Actinopterygii: Salmoniformes: Salmonidae). Acta Ichthyologica et Piscatoria 51(2): 139–144. <https://doi.org/10.3897/aiiep.52.64119>

## Abstract

The maraena whitefish, *Coregonus maraena* (Bloch, 1779), is often considered a suitable candidate for intensive aquaculture diversification in the EU. However, only a few such farms in Europe are in operation. Rearing this species in recirculating aquaculture systems is a recent innovation, and optimisation is necessary to standardise aspects of larviculture. This 30-day study investigated the effect of stocking densities of 25/L, 50/L, 100/L, and 200/L on the survival and growth of maraena whitefish larvae in a recirculating aquaculture system. The four groups of larvae (initial weight =  $7.4 \pm 0.1$  mg; initial total length =  $13.0 \pm 0.1$  mm) in three repetitions were reared in a recirculating system. Larvae were fed fresh live brine shrimp metanauplii every 3 h at a rate converted to larval stocking density. After the experiment, 10 larvae from each tank (30 of each density group) were weighed on a digital microbalance (ABJ 220-4M KERN, Germany, readout = 0.1 mg) and measured manually on images taken with Leica MZ16 A stereomicroscope and a digital colour camera with 5-megapixel resolution for Leica DFC420 Image Analysis. No significant differences in final body weight, total length, size heterogeneity, condition factor, or survival were found among treatments ( $P > 0.05$ ). The highest non-significant survival rate and growth parameters were observed in larvae reared at 25/L. On the contrary, it is possible to rear maraena whitefish larvae at high stocking density without any subsequent negative consequences for growth and survival. As no significant differences in any evaluated parameter were observed between groups of larvae at the highest and lowest stocking density, we conclude that it is possible to rear maraena whitefish larvae at high stocking density (and 200/L) without any subsequent negative consequences for growth and survival.

## Keywords

coregonids, fry, growth metrics, larviculture, recirculation systems

## Introduction

The maraena whitefish, *Coregonus maraena* (Bloch, 1779), is a promising species for inland freshwater aquaculture throughout east-central Europe (Mukhachev and Gunin

1999), and northern Europe, especially Finland (Jobling et al. 2010) and Norway (Siikavuopio et al. 2011). Several decades ago, due to predation by the great cormorant *Phalacrocorax carbo*, the population dramatically declined (Suter 1997). Eutrophication has also contributed to the decrease

(Thomas and Eckmann 2007). At present, it is important that re-establishment of whitefish natural production be accompanied by the culture in intensive aqua systems. The recirculating aquaculture system is an important model in worldwide aquaculture, given its cost-effectiveness, low environmental impact, ease of regulating water quality, and final product quality control features (d'Orbecastel et al. 2009). The establishment of coregonid production in recirculating systems requires that optimal larviculture conditions, including stocking density as a crucial factor in the productivity of fish culture systems, be identified.

Excessively high density can produce a stress response, particularly increased plasma cortisol level (Li et al. 2012), impede thyroid hormone production (Herrera et al. 2016), and affect growth (Żarski et al. 2008) and survival (Molnár et al. 2004; Szkudlarek and Zakes 2007). High density can lead to fin erosion, gill damage, fish welfare impairment (Ellis et al. 2002), and promote cannibalism (Liao and Chang 2002). It can decrease food utilization (Sharma and Chakrabarti 1998) and alter metabolic rate (Tolussi et al. 2010) with respect to lipids (Mommensen et al. 1999), carbohydrates (Sangiao-Alvarellos et al. 2005), and proteins (Costas et al. 2008). Finally, high fish density can impair water quality (Montero et al. 1999), reducing oxygen levels and increasing ammonia concentration accompanied with parasitological incidence, moderate hyperplasia and absence of congestion (Azevedo et al. 2006) in commercial production systems. These negative aspects such as cannibalism or aggressive behaviour lead to mass mortality of larvae and cause economic loss (Smith and Reay 1991; Ruzzante 1994) as well as disrupt production stability (Rowland et al. 2006). On the other hand, low stocking density is associated with high production costs (Luz and Santos 2008). Stocking density has been shown to be a limiting factor in fish growth during early development (Webb et al. 2007), while its impact is mitigated in adult fish (Duarte et al. 2004).

In Europe, the initial rearing of coregonid larvae in intensive indoor tanks is usually practised with stocking densities ranging from 5 to 100 larvae/L (Dabrowski and Poczyczynski 1988; Beltran and Champigneulle 1992; Esmaeilzadeh-Leithner and Wanzenböck 2018). Moreover, larvae rearing of lake whitefish, *Coregonus clupeaformis* (Mitchill, 1818), as similar commercially important species from North America is usually done with low density (30 larvae per L) (Zitzow and Millard 1988, Harris and Hulsman 1991).

The optimal stocking density needs to be determined for each fish species and developmental/reproductive stage to facilitate survival and growth and enable efficient management to maximise production and profitability, as well as to provide proper conditions for fish. Information on stocking density effects on maraena whitefish larvae growth performance and survival is scarce. The goal of the presently reported study was to determine whether stocking density affects the survival and growth of maraena whitefish larvae reared in a recirculating aquaculture system (RAS).

## Materials and methods

### Eggs and larvae

Maraena whitefish were obtained from in the Szczecin Lagoon (the River Odra estuary), north-western Poland. The broodstock comprised 120 fish at a 1:1 sex ratio. Gametes of three-year-old 60 females (mean weight,  $800.4 \pm 80.1$  g, mean  $\pm$  SEM; mean total length,  $30.2 \pm 1.1$  cm) and three-year-old 60 males ( $650.5 \pm 49.7$  g, mean  $\pm$  SEM; mean total length,  $26.4 \pm 0.9$  cm) were stripped manually (no hormone stimulation) by commercial fishermen in December 2016 shortly after fish capture and transported to local hatcheries for fertilization and incubation. Eggs (100 mg) were fertilized with 0.5 mL of milt mixed with 50 mL of hatchery water and incubated at the ambient water temperature of the river (2–3°C) with initial water inflow 3 L/min, oxygen saturation to 90%, and pH near 7.0. In February 2017, the eggs were taken to the Department of Lake and River Fisheries (Olsztyn, Poland) where they were distributed among five 8-L Zug jars ( $n = \sim 150\,000$  eggs/jar) in a recirculating system and incubated at 3.0–3.5°C with water inflow 3 L/min, oxygen saturation to 90%, and pH near 7.0. In total,  $\sim 750\,000$  eggs were incubated. After 60 days, eggs were transferred to the second set of 8-L Zug jars and incubated at 8–9°C to accelerate development and hatching. After 5 days, the temperature was increased to 10°C for mass hatching. Hatching success was estimated at 90%, and about 675 000 larvae were available for the experiment. Hatched larvae swam across to a tank (total volume 1 m<sup>3</sup>) underlain with 0.2 mm mesh. After 24 h, larvae were transferred to tanks in the RAS.

### Experimental system

Four groups of larvae in three replicates were transferred to the experimental aqua system consisting of twelve 2 L aquaria, 96 × 154 × 200 mm. The recirculating system (2300-L total water volume) included a series of filtration sections (total biofilter volume 1500-L), a settling tank (500-L water volume). Thirty fish were weighed and measured to obtain the initial values for weight and length. Maraena whitefish larvae (initial weight,  $7.4 \pm 0.1$  mg, mean  $\pm$  SEM; initial total length,  $13.0 \pm 0.1$  mm) were placed into each aquarium at stocking density of 25/L (S25), 50/L (S50), 100/L (S100), and 200/L (S200). A biomass by litre (g/L) was 0.185 (S25), 0.370 (S50), 0.740 (S100), 1.480 (S200). A total of 2250 larvae were used in the experiment.

### Rearing conditions

The oxygen level, water temperature, and pH were checked daily at 0800 and 1600 h. The pH range was monitored using an OxyGuard H04PP Handy pH meter (OxyGuard International, Denmark). The initial tempera-

ture without supplemental heat was 10°C. Water temperature ~19°C was regulated by a HAILEA HC-1000A cooler (China). The temperature was gradually elevated from 10°C to 19°C (3°C/day). Oxygenation was maintained using two SICCE Syncra 5.0 pumps (5000 L/h) (Italy). Ammonia, nitrate, and nitrite concentrations were analysed using HACH, LCK 304, LCK 339, LCK 341 (Germany) with a HACH DR5000 spectrophotometer (Germany). Disinfection used a 30 W UV MCT Transformatoren GmbH steriliser (Germany). NaCl was added at 1 g/L weekly to maintain a 16:1 chloride:nitrogen ratio. A constant inflow of 0.4 L/min was ensured. Dead larvae were removed and counted during daily cleaning. The level of organic matter remained low. A low CO<sub>2</sub> level was maintained via aeration and keeping alkalinity stable. During the 30-day trial, basic physico-chemical parameters were following: temperature = 19.1 ± 0.0°C, pH = 8.7 ± 0.0, O<sub>2</sub> saturation = 85.8 ± 0.9%, O<sub>2</sub> concentration = 7.9 ± 0.1 mg/L, NH<sub>4</sub><sup>+</sup> = 0.1 ± 0.0 mg/L, NO<sub>2</sub> = 0.8 ± 0.1 mg/L, NO<sub>3</sub> = 21.2 ± 5.4 mg/L.

## Feeding

Larvae were fed fresh live metanauplii of brine shrimp, *Artemia salina* (Ocean nutrition, HE > 230 000 NPG, Belgium) (20–24 h old, 0.4–0.5 mm) four times daily at 3 h intervals during the light phase (0830 to 1730 h). The feeding level was fixed to the range of 500–700 *Artemia* sp. metanauplii per fish per day at a rate converted to larval stocking density (Table 1). The daily ratio was based on a previous experiment (unpublished data). Furthermore, this ration was in slight excess as some uneaten metanauplii were observed in tanks at the end of the day. The feeding level was adapted according to losses of larvae during the experiment and fish body weight (Fiogbé and Kestemont 2003) using the formula

$$R_{\text{opt}} = 4.89W^{-0.27}$$

where  $R_{\text{opt}}$  = optimal daily feeding level,  $W$  = body weight [g].

## Sampling and measurements

After the experiment, 10 larvae from each tank (30 of each density group) were weighed on a digital

**Table 1.** Concentration of brine shrimp (*Artemia salina*) fed to larvae of maraena whitefish, *Coregonus maraena* (Bloch, 1779) in a 30-day trial.

Group	Whitefish stocking density		Artemia feeding dose	
	[larvae/L]	[larvae/2L]	[mL/L]	[mL/2L]
S25	25	50	2.5	5
S50	50	100	5.0	10
S100	100	200	10.0	20
S200	200	400	20.0	40

Values for 2 L relate to the volume of 2-L aquarium.

microbalance (ABJ 220-4M KERN, Germany, readout = 0.1 mg) and measured manually from images taken with Leica MZ16 A stereomicroscope and a digital colour camera with 5-megapixel resolution for Leica DFC420 Image Analysis.

A sample size of ten larvae per tank, 30 larvae per treatment, was used as in a number of studies (Kaiser et al. 2003, Mahmood et al. 2004, Fletcher et al. 2007, Celada et al. 2008, Nowosad et al. 2013, Palińska-Żarska et al. 2014, Laczynska et al. 2016).

The survival rate (SR), size heterogeneity (SH), and condition factor ( $K$ ) and specific growth rate (SGR) were assessed as follows:

$$\text{SR (\%)} = 100 \times (N_f/N_i)$$

in which  $N_i$  and  $N_f$  = initial and final number of larvae, respectively;

$$\text{SH (\%)} = 100 \times (\text{SD}/W_m)$$

in which SH = size heterogeneity; SD = mean standard deviation of weight of 10 randomly selected larvae/tank;  $W_m$  = mean weight [mg] of 10 larvae/tank.

$$K = 100\,000 \times W \times (\text{TL}^3)^{-1}$$

in which  $W$  = mean weight [g] of 10 larvae/tank; TL = mean total length [mm] of 10 larvae/tank

$$\text{SGR (\%)} = 100 \times [(\ln W_t - \ln W_0)/d]$$

in which  $W_t$  and  $W_0$  are final and initial weight of larvae, respectively [g];  $d$  = duration of the experiment [days].

## Statistical analysis

Statistical analyses were performed using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). Data are presented as mean ± SEM. The effects of stocking density on  $W$ , TL, SR,  $K$ , SH, and SGR were analysed by one-way ANOVA with stocking density as a fixed variable. Differences were considered significant at  $P < 0.05$ . Prior to ANOVA, SR,  $K$ , SH, and SGR were arcsine-transformed. All data were tested for homogeneity of variance using the Cochran, Hartley, and Bartlett test, and for normality with the Shapiro–Wilk normality test. The parametric Tukey test was used for assessing differences among groups in  $W$ , TL, SR, SH,  $K$ , and SGR (Table 2).

## Results

At the conclusion of the trial, no significant ( $P > 0.05$ ) differences among treatments were observed in SR,  $W$ , TL, SH,  $K$ , or SGR (Table 2). The highest SR (92.7% ± 2.4%),

**Table 2.** One-way ANOVA results for the factor stocking density on total length (TL), body weight (*W*), size heterogeneity (SH), condition factor (*K*), survival rate (SR), and specific growth rate (SGR) of larvae of maraena whitefish, *Coregonus maraena* (Bloch, 1779).

Parameter	Source of variation	SS	DF	F	MS	P
TL	SD	0.9	3.0	0.3	2.3	0.2
<i>W</i>	SD	466.3	3.0	155.4	2.7	0.1
SH	SD	22.7	3.0	7.6	0.2	0.9
<i>K</i>	SD	0.0	3.0	0.0	2.2	0.2
SR	SD	3.6	3.0	1.2	0.2	0.9
SGR	SD	0.0001	3.0	2.1	0.00005	0.2

SD = stocking density; SS = sum of square; DF = degrees of freedom; *F* = distribution fitting; MS = mean square; *P* = probability.

**Table 3.** Effect of stocking density on growth and survival of larvae of maraena whitefish, *Coregonus maraena* (Bloch, 1779), in a 30-day growing trial.

Group	SR [%]	TL [mm]	<i>W</i> [mg]	SH [%]	<i>K</i>	SGR [%]
S25	92.7 ± 2.4	30.7 ± 0.3	147.9 ± 5.8	22.5 ± 4.3	0.51 ± 0.01	0.50 ± 0.003
S50	91.3 ± 1.5	30.4 ± 0.2	135.7 ± 1.6	20.3 ± 3.6	0.48 ± 0.01	0.49 ± 0.001
S100	91.33 ± 1.1	30.4 ± 0.1	135.1 ± 3.5	21.1 ± 4.9	0.48 ± 0.00	0.49 ± 0.003
S200	91.8 ± 1.0	30.0 ± 0.2	131.3 ± 5.2	18.7 ± 2.3	0.49 ± 0.01	0.49 ± 0.004

Groups represent stocking densities of 25, 50, 100, and 200 larvae/L, respectively; SR = survival rate, TL = total length, *W* = body weight, SH = size heterogeneity, *K* = condition factor, SGR = specific growth rate.

*W* (147.9 ± 6.3 mg), TL (30.7 ± 0.4 mm), SH (22.5% ± 1.1%), *K* (0.51 ± 0.01), and SGR (0.50 ± 0.003%) was observed at S25 (Table 3).

## Discussion

The fact that growth–weight parameters did not differ significantly means that maraena whitefish growth was not influenced by stocking density at the tested levels. Slightly lower (non-significant) growth was found with increasing stocking density. It is important to sustain uniformity of fish size in aquaculture (Biswas et al. 2010). The effect of stocking density on larva size heterogeneity may be species-dependant. For instance, the relation of stocking density to size heterogeneity has been reported to be positive in red tilapia *Oreochromis niloticus* (Linnaeus, 1758) × *Oreochromis mossambicus* (Peters, 1852), when stocking density was 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 fry per litre (Huang and Chiu 1997), but negative in Arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), with stocking density 10, 20, 28, 40, 60, 80, and 100 fry per litre (Wallace et al. 1988). We found size variation with respect to stocking density at the levels tested to be negligible with the only non-significant more uniform size in the S200 group and the least uniform in the S25 group. North et al. (2006) observed the same trend, with the highest size heterogeneity observed in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), reared in low stocking density and vice versa.

Stocking density can influence mortality rate, with survival often negatively correlated with stocking densi-

ty as shown for silver perch, *Bidyranus bidyanus* (Mitchell, 1838) (see Rowland et al. 2006). Fish species can be classified as density-independent or density-dependent. Tilapia larvae (Huang and Chiu 1997) were reported to be density-dependent. Survival was high and not significantly affected by stocking density in the presently reported study, thus maraena whitefish seem to be density-independent, and stocking density is not likely a limiting factor in their survival in intensive rearing. High survival in all groups indicates that high-density aquaculture may be suitable for the production of this species. This phenomenon was also seen in Kupren et al. (2011) for asp, *Leuciscus aspius* (Linnaeus, 1758); ide, *Leuciscus idus* (Linnaeus, 1758); and chub, *Squalius cephalus* (Linnaeus, 1758).

Stocking density has been reported to be an important factor in fish growth (Saoud et al. 2008) and is of particular concern in the welfare of intensively farmed fish (Ashley 2007, Woche et al. 2011). Mortality (Ellis et al. 2012), as well as susceptibility to pathogen infections and fin damage (Turnbull et al. 1998, Jones et al. 2011), in farmed fish, are generally considered important indicators of welfare. Ashley (2007) suggests that unsuitable stocking density can result in damage or death of fish. Negative effects of high stocking density on fish growth and survival can be attributed to impaired water quality associated with accumulation of fish metabolites and carbon dioxide, with accompanying decline in pH level (Hosfeld et al. 2009). As no technical problems or disease occurred during the course of our study, we can conclude that water quality and stocking density effects were accurately evaluated. The high survival rate at all density levels and lack of observable damage to fins are evidence of appropriate rearing conditions with respect to fish welfare.

## Conclusions

No significant differences in any evaluated parameter were observed between groups of larvae at the highest and lowest stocking density. It is possible to rear maraena whitefish larvae at high stocking density with no subsequent negative consequences for growth and survival. This study examined fry and early-stage larvae, but a further study, focusing on juvenile and adult maraena whitefish, is warranted. The effects of stocking density on stress hormone response, body composition, and haematological and biochemical parameters of maraena whitefish should be studied.

## Acknowledgements

The study was financially supported by the Ministry of Agriculture of the Czech Republic and NAZV project (QK1810296).

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