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Selected aspects of intensively cultured European whitefish (*Coregonus maraena*, Bloch) and peled (*Coregonus peled*, Gmelin)

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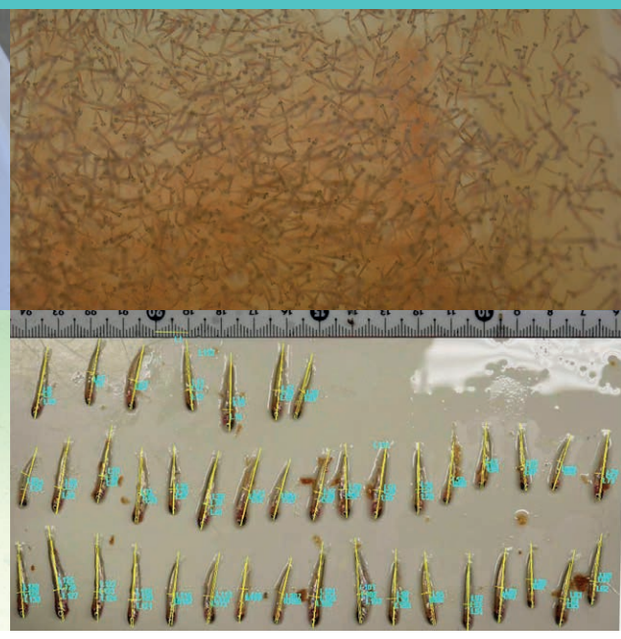

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. COREGONIDS

Whitefish (*Coregonus* sp.) have a northern circumpolar distribution with occurrence in North America (Canada), Asia (Chukchi peninsula) and Europe (British Isles) (Bodaly et al., 1991). *Coregonidae* subfamily belongs to the *Salmonidae* family. The *Coregoninae* subfamily is actually divided into genus *Coregonus, Prosopium, Stenodus* and *Leucichthys* including marine, anadromous and freshwater species. *Coregonus* evolved in the lake and stream area of northwest Eurasia, *Stenodus* and *Prosopium* originated in the rivers of Siberia and northwest America respectively, and *Leucichthys* became differentiated in the lake-studded area of northeast America (Smith, 1957). Some species are extinct following the formation of hybrids and habitat modification. The genetic integrity of several wild populations and species is threatened. This leads to uncertainty and confusion in the classification and correct taxonomy of this fish genus. There exist a lot of speculations about number of coregonid species and subspecies which differ in size, shape, number of gill rakers, scales etc. In several lakes, several populations (up to 11 in Lake Onega) are morphologically and genetically distinct. They live in sympatry, and they have different habitat, ecology, preffered prey and spawning season requirements. These populations negate the idea that only single species could be involed within one lake (Kottelat and Freyhof, 2007). Beveridge (1989) mentioned 20 - 30 species belonging to the genus *Coregonus*. Hanel and Novák (2007), state 67 species of genus *Coregonus* and 6 species of genus *Prosopium*. Until 1950, 92 subspecies of whitefish had been described in Europe, and 300 local forms had been described all over the world (Brylińska, 2000).

1.2. COREGONIDS IN THE CZECH REPUBLIC

Coregonids farming has a long history in the Czech Republic. In 1882, Maraena whitefish (*Coregonus maraena*) were introduced to the Czech Republic, whereas peled (*Coregonus peled*) were introduced from Siberia in 1970. Both species are suitable for polyculture with common carp (*Cyprinus carpio*) in deep ponds with cool water conditions (Hochman, 1987). In the past, the production was stable and high, but due to the global threats including predation by the great cormorant (*Phalacrocorax carbo*) (Suter, 1997), overfishing (Jackson et al., 2001), hybridization (Luczynski et al., 1992), eutrophication (Thomas and Eckmann, 2007), impairments of natural spawning sites (Winfield et al., 2004), pollution and environmental changes (Walther et al., 2002) the yield has rapidly declined. In 1997, market-size whitefish production ranged approximately 140 tons (Annual report 2002, Czech Ministry of Agriculture). The annual production decreased dramatically to 24 tons in 2007 (Annual report 2011, Czech Ministry of Agriculture) and to 4 tons in 2016 (Annual report 2017, Czech Ministry of Agriculture). The demand for coregonids is still high but current whitefish production do not sufficiently support internal fish market (general knowledge).

Production of whitefish in intensive culture can mitigate the decline in wild populations from lakes and ponds. Furthermore, artificial production provides juvenile fish for re-stocking lakes, as well as for market demands (Heikinheimo-Schmid, 1992). Nowadays, whitefish are candidates for inland freshwater aquaculture, particularly in local markets in central and east Europe (Turkowski, 1999) and they show potential for rearing in RAS systems (Siikavuopio et al., 2012). They are well known for high palatability of their flesh and high content of polyunsaturated fatty acids (Orban et al., 2006). On the other hand, rearing of peled in RAS is a recent innovation, and optimization is necessary to standardize aspects of culture, including weaning of larvae from live feed to artificial diet (Stejskal et al., 2017).

1.2.1. Peled (*Coregonus peled*)

Peled has been considered valuable commercial species of coldwater aquaculture in Russia. This species has been introduced both within and far outside its natural range, for instance Estonia, Lithuania, Latvia, Belarus, Finland, Poland, Germany, Czech Republic, France and Japan. Introduced fish were stocked in lakes or produced in ponds and cage systems (Gordeeva, 2008). The intensive fish farms have been gradually developed in countries with a high potential for whitefish rearing, such as Finland, Sweden, Poland, Germany and Italy (Jobling et al., 2010).

Peled is characterized by fast growth, as well as early maturation at 2 years and higher temperature tolerance compare to maraena whitefish. Spontaneous mass ovulation may occur due to a sharp drop of winter temperature below 2 °C (Hochman, 1987). Presuming an optimal oxygen conditions, the maximum tolerable water temperature for normal activity of peled is 28 °C (Hochman and Klaus, 1976). The optimal recommended temperature for rearing of peled is 22 °C (Szczerbowski et al., 1974). Matousek et al. (2017) recommended an optimal temperature range 16-22 °C for peled juveniles. It is naturally fed particularly on copepods, cladocerans and rotifers (Furgala-Selezniow et al., 2005). Combined feeding of live prey, especially *Artemia* sp, together with commercially formulated feed, known as co-feeding technique, is a strategy used to enhance larval performance compare to feeding either type of feed alone (Canavate and Diaz, 1999). In some species co-feeding techniques stimulated growth and supported higher survival (Wilcox et al., 2006).

1.2.2. Maraena whitefish (*Coregonus maraena*)

Maraena whitefish is an anadromous freshwater whitefish with its former distribution in the Southern Baltic Sea and its neighboring rivers. In the Baltic Sea, two main reproductive forms of whitefish are described. A migratory form spawning in coastal rivers and creeks (therefore known to as river spawning ecotype/form), and a more resident form spawning in shallow bays of the Baltic Sea (hereafter referred as sea spawning ecotype/form) (Lehtonen, 1981). Both forms distribution is in coastal waters from the very north to the more southern parts of the Baltic Sea (Swedish Board of Fisheries, 2010).

The origin of maraena whitefish is Masurian lakes in Poland. Attempts at classifying the whitefish in Poland resulted in differentiating one species further divided into four subspecies (Brylińska, 2000) according to different lakes Miedwie Lake, Gorzynskie Lake, Wigry Lake and Łebsko Lake (Heese, 1990). Miedwie Lake is considered to be the original habitat of this whitefish form (*Coregonus lavaretus maraena*) (Trzebiatowski et al., 1988). The Miedwie whitefish is characterized by a really high growth rate. For this reason, it has been used for stocking of most European lakes. For instance, it has been introduced into the Czech Republic, Austria (Jagsch, 1992) Slovakia (Mužik et al., 2003) and even in distant Japan (Toshikazu and Tetsuro, 2004) and Vietnam (Hai et al., 2014). According to a suggestion of Kottelat and Freyhof (2007), *C. lavaretus* should be applied only to French and Swiss whitefish populations. Østbye et al. (2006) state that the occurrence of ecologically differentiated forms (ecotypes) reflects ecological divergence since the last glaciations. On the west coast of Sweden, where the conditions are more marine, the occurrence is, however, restricted to areas with close connection to freshwater (Swedish Board of Fisheries, 2010).

This species requires high quality clean water conditions. On account of excessive fishing, eutrophication, deterioration of spawning sites, and habitat fragmentation have brought the population of *C. maraena* to the edge of extinction (Olsson et al., 2012). For these reasons, intensive restocking activities endeavored to stabilise the production and to establish the

aquaculture of maraena whitefish (Jennerich and Schulz, 2009). Farming of *C. maraena* was successfully launched in Germany (Arndt and Jansen, 2008), Finland (Kause et al., 2011), Sweden (Säisä et al., 2008) and Poland (Heese, 1990). Nevertheless, maraena whitefish is categorised as vulnerable species with a decreasing population trend (Freyhof and Brooks, 2011). During the late 1980s, the annual commercial landing reached 1000 t in the Bothnian Bay alone. Since 2000, catches have decreased from 276 to 139 t (Swedish Agency for Marine and Water Management, 2013). A similar pattern is observed on the Finnish side of the Baltic, and the stocks are here nowadays mainly sustained by stocking of hatchery-reared fish (Säisä et al., 2008). The rearing of *C. maraena* is connected to some problems such as slow growth rates or susceptibility towards husbandry stress and pathogens (Altmann et al., 2015). Nowadays, In the Czech Republic, *C. maraena* production is connected to instable temporary populations based on released material obtained from fish farms and ponds (Lusk et al., 2009).

1.3. CULTURE OF FISH IN RECIRCULATED AQUACULTURE SYSTEMS

Limited fresh water availability, and pollution are regarded as the main obstacles for further expansion of conventional cage-based and flow-through aquaculture systems. Hence, existing aquaculture producers from European countries – United Kingdom, Ireland, Italy (Eurostat, 2010) and Norway (Eurostat, 2011) have promoted Recirculating Aquaculture Systems (RAS) as one of the possible solutions and opportunities aquaculture development. The rearing in RAS satisfies demand for a lot of freshwater fish species, particularly predatory fish, thermophilic fish, sea fish, sturgeons and another water organisms like crustaceans, molluscs, and algae (Martins et al., 2010).

The RAS represents a combination of biological filtration (ammonia nitrification by biofilter, disinfection by UV), mechanical filtration (solid removal, decantation), gas control (oxygen supply, CO_2 degassing), systems for control of physiochemical parameters and culture tanks of appropriate design. In recirculating systems under aerobic conditions, the biological filter oxidises ammonia into nitrites and nitrates. Control of the physicochemical parameters is one of the advantages of the recirculating systems (Heinen et al., 1996). An inappropriate combination of water quality factors such as dissolved O_2 (Foss et al., 2003), salinity (Alabaster et al., 1979), nitrite (Lemarié et al., 2004), CO_2 (Randall and Wright, 1989), and ammonia may cause fish health problems.

1.4. ASPECTS INFLUENCING EARLY REARING OF FISH IN RAS

For a long time, the influence of environmental factors on fish has been studied with regards to their impacts on reproduction, growth, feed conversion and overall quality of reproduced fish. Fish, as poikilothermic organisms, are strongly dependant on temperature. Another crucial factors which are involved in the control of physiological functions include light conditions, oxygen availability, pH value, the presence of toxicants, such as ammonia, nitrite, nitrate, carbon dioxide, etc. There are also other rearing aspects which play a major role on survival, grow and complex fish development, for instance stocking density, quality, composition and techniques of feeding, as well as the occurrence of body deformities.

1.4.1. Effect of light

Light is a complex of external and ecological factors, including colour spectrum, intensity and photoperiod and can strongly influence fish performance. It is much easier to control light

regime under the controlled conditions and investigate its effects on fish reproduction and larvae growth and development. Most of fish species needs a minimal illuminance for normal development and growth. This is presumably in relation to the ability to localize, catch and ingest prey. Light is also essential for body pigmentation, what is an important phenomenon involved in early development and growth. A relationship between survival and growth can be established, and often optimal light for growth is not the same as for survival. A compromise has to be found (Boeuf and Lebail, 1999).

Generally, upper light intensity levels are required for growth optimisation of larvae, but too intensive light can be stressful or lethal for them. On the contrary, some species can develop and grow at very low intensities as is the case for some pelagic marine species larvae, fish living in estuarines with turbid water (Blaxter, 1980). Rarely, in the total absence of light (Batty et al., 1986). Both, diurnal or seasonal biorhythms are related to the periodicity of light. Fish, exhibit a 24-h cycle in their activities which may often a signal of photokinesis (Clarke, 1965). Fish are either more active in light, less active in darkness, or vice versa. Several factors affect fish light sensitivity and their behaviour such as concomitant diurnal changes in other factors, for instance temperature or oxygen availability (Beouf and Lebail, 1999). Fish light receptivity also changes with the developmental stage. The number of cones in the retina increases during ontogenesis and early development (Blaxter, 1975).

1.4.2. Effect of temperature

Water temperature is one of the most important factors in intensive rearing and has an influence on fish because of its crucial role in controlling metabolism, survival, growth, feed conversion and the economics of rearing (Jobling, 1997; Bernier, 2010). The thermal niche and the ability to tolerate thermal stress vary between fish species and certain developmental periods of a species (Reist et al., 2006a). Thermal limits are narrower for embryos and larvae compare to juveniles and adults (Kupren et al., 2010). Increasing egg incubation temperature leads to higher prevalence of abnormalities, in relation to heart, vertebrae (Ørnsrud et al., 2004) and jaw (Bolla and Holmefjord, 1988) which affects vertebral morphology (Ytteborg et al., 2010a) and meristic counts (i.e. vertebral number, Taning, 1952).

Fish can be classified as stenothermal which tolerate narrow temperature range or eurythermal tolerating wide thermal range (Wieser, 1991). Next division contains coldwater, warmwater and mesothermal fish species (Kamler, 2002). Among temperate fish, there is a common assumption that low-temperature tolerance is near 0 °C based on the freezing point of blood plasma (-0.5 to -1.0 °C). For fishes with lower lethal temperatures above the freezing point, the physiological source of mortality is caused by inability to maintain homeostasis at the cellular and organismal level. At the cellular level, incapability to maintain ionic gradients affects the central nervous system and physiological functions, by reducing the effectiveness of synapse transmission (Cossins and Bowler, 1987).

1.4.3. Effect of stocking density

Optimal stocking density varies according fish species but also depends on the rearing method and fish live cycle (Papoutsoglou et al., 1998; Kwiatkowski et al., 2008). In larviculture, stocking density has a strong impact on freshwater and marine fish performance, especially survival and growth (Hitzfelder et al., 2006). Identifying the optimum stocking density for a species is a critical factor for both enable efficient management to maximize production and profitability and also for optimum husbandry practices (Rowland et al., 2006). On the other hand, determination of the optimum stocking density and its impact on fish fry development

is quite difficult with regards to the other factors as feeding regimes, rearing temperatures and different photoperiod which simultaneously affect the fish performance (Wolnicki, 2005).

At some fish, high density can cause mortality induced by the disruption of feeding behaviour and the occurrence of cannibalism (Liao and Chang, 2002). Cannibalism and aggressive behaviour affects mass mortality of larvae followed by economic loss (Ruzzante, 1994). Furthermore, larvae which are damaged and injured by cannibalism, basically in the fin area (necrosis, splits, rot) are sensitive to infection with bacterial diseases (Iwamoto and Fujimoto, 1997). Elevated stocking density also impairs water quality, especially reduces dissolved oxygen levels and increases ammonia concentration (Azevedo et al., 2006), worsens fish welfare (Conte, 2004) and has impact on the food intake impairment (Lemarié et al., 2004), as well as physiological functions accompanied by alteration in metabolic rate (Tolussi et al., 2010). On the other hand, low stocking densities are associated with high production costs, thus effectiveness of rearing declines (Luz and Santos, 2008).

1.4.4. Effect of weaning time and co-feeding technique

Food type preference, prey detection, capture and consumption depend on larval age and ontogeny what is in connection with sensory and visual development (Pankhurst, 2008), weight (Olsen et al., 2000), length (Mayer and Wahl, 1997), locomotive ability (Blaxter, 1986) and mouth gape (Fernandez et al., 1994). Live food in aquaculture is currently limited to few species, most often the brine shrimp (*Artemia salina*), and rotifers especially *Brachionus* sp. (Lavens and Sorgeloos, 1996). Live food organisms stimulate larval-feeding activity by their movement and excretion of metabolic wastes and chemicals, such as amino acids, peptides and ammonium salts which act as visual stimuli and attractants (Kolkovski et al., 1997a,b). Cultivation methods of live organisms such as *Brachionus* rotifers and *Artemia* brine shrimp are simple and well known, on the other hand, their usage is usually unreliable and costly. *Artemia* nauplii is responsible for approximately 40% of the total amount of live food in aquaculture (Lavens and Sorgeloos, 2000). *Artemia*, represented 40% of the feed cost, and 80% of the live prey feeding cost (Le Ruyet et al., 1993). Furthermore, high amounth of pathogenic bacteria associated with live food organisms may influence fish health (Olafsen, 2001).

The requirement of hatcheries for live food organisms is accelerating and the search for non-*Artemia* alternatives is also increasing (Sorgeloos et al., 2001). Recently, nematodes were suggested as cost-effective alternatives to fish and crustacean larvae (Weber and Traunsburger, 2014). Nematodes are successful when used in combination with commercial food as co-feeding regimes (Kahan, 1980). Combined feeding of live prey, in particular *Artemia* sp. plus artificially formulated nutrition, known as co-feeding, is a strategy used for enhancement of larval performance compare to either type of feed alone (Ronyai and Feledi, 2013).

Early larval weaning onto artificial feed is really important for successful culture of fish because live prey is an expensive, time consuming and sometimes unreliable procedure. For instance, cultured zooplankton, such as rotifers and *Artemia*, require enrichment with commercial products or microalgae prior to larval feeding as they lack essential nutrients required for optimal growth and survival (Aragão et al., 2004). The value of polyunsaturated fatty acids enrichment strategies such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been displayed to elevate growth and survival of many fish larvae during the production process (Noori et al., 2011a). A manufactured microparticulate diet optimal for the total live food replacement at the beginning of exogenous feeding has not yet been developed for most larval finfish species. Bad performance of manufactured diets to first-feeding larvae may be affected by: 1. low ingestion rates caused by the low palatability or low residence time

in the water; 2. low digestibility of the diet due to inadequate digestive enzyme activity; 3. poor nutritional composition of the diet (Baskerville-Bridges and Kling, 2000).

1.4.5. Effect of oxygen level

Apart from temperature and feed, oxygen is one of the most important aspect influencing fish development and welfare. Fluctuations of dissolved oxygen are typical natural incidence in most aqua ecosystems (Pihl et al., 1992). It is generally known that compare to juveniles and adult fish, the eggs and larvae are more sensitive to the disruption of dissolved oxygen. Too high temperatures increase the respiratory oxygen demand of fish (Harris et al., 2006) and reduce oxygen solubility (Carpenter, 1966), but increases food intake, growth and so fish biomass (Cecchini and Caputo, 2003). Technology for regulating water oxygen content has a big potential for water quality improvement in aquaculture, especially in RAS systems (Dwyer et al., 1991) to increase biomass and production (Olsvik et al., 2006). Exposure of some fish species to both hyperoxia and hypoxia may be detrimental to water organisms, resulting in suboptimal growth followed by lower biomass production (Wedemeyer, 1997),

Hypoxia, defined as an insufficient level of dissolved oxygen in water, causes physiological stress in water organisms leading to potential death in most fish species (Diaz and Breitburg, 2009). Fish species which do not have the capability to tolerate reduced oxygen level could be escape from its original area as a result of hypoxia (Doudoroff and Shumway, 1970). Dissolved oxygen concentrations below 1 $mg \cdot L^{-1}$ are considered fatal to most fish species (Fry, 1971) although some species display individual tolerance (Vaquer-Sunyer and Duarte, 2008). Low dissolved oxygen concentrations between 2 and 4 $mg \cdot L^{-1}$ may affect food consumption, growth, reproduction, distribution and behaviour of fish (Breitburg et al., 1997; Robb and Abrahams, 2003). The concentration 6 $mg \cdot L^{-1}$ may cause chronic stress affecting growth (Neill and Bryan, 1991). Most fish react to hypoxia by increasing their ability to maintain oxygen delivery for satisfaction of respiratory needs (Wu, 2002). In teleosts, this is usually managed by activities ensuring an increase in the rate of water flow over the gills and the diffusional gills capacity enhancement (Randall, 1970) as apparent at the European flounder (*Platichthys flesus*) (Soldatov, 1996) and the eel (*Anguilla anguilla*) (Wood and Johansen, 1972). Other hypoxia response mechanisms involved is a reduction in locomotor activities ensuring the depression of overall energy metabolism as demonstrated by the common carp (*Cyprinus carpio*) (Zhou et al., 2000), common sole (*Solea solea*) (Dalla Via et al., 1994) and Atlantic cod (*Gadus morhua*) (Schurmann and Steffensen, 1994).

Hyperoxia, an excessive level of dissolved oxygen in water may stimulate growth (Hosfeld et al., 2008), boost ammonia tolerance (Fridovich, 1977) or increase fish feed intake and growth (Hosfeld et al., 2008). On the other hand, consistent oxygen saturation of 140%–150% may cause oxidative stress leading to increased susceptibility to diseases and affecting increased mortality (Fridell et al., 2007). Tolerance level for environmental hyperoxia exists for all species depending on life stage, environmental and physiological conditions (Foss et al., 2003).

1.4.6. Effect of rearing environments on body deformities

Deformities of fish are a serious problem in aquaculture. It is followed by negative consequences for the marketing, production costs, product value, biological performance of the fish and welfare. Skeletal deformities have been reported in almost every reared fish species, with divergent incidence and severity (Koumoundouros et al., 1997a,b; Planas and Cunha, 1999). Skeletal deformities occurrence is frequent especially during ontogeny up to metamorphosis, mainly affected by unsuitable abiotic conditions (Wang and Tsai, 2000; Takle

et al., 2005), nutritional imbalances (Cahu et al., 2003), as well as diseases and genetic factors (Gjerde et al., 2005). The most common skeletal deformities are vertebral body deformities with cranio-caudal compressions (Witten et al., 2005), ankylosis - fusion of adjacent vertebrae (Witten et al., 2006) or dislocations (Fjelldal et al., 2007a). Deformities occurring in freshwater fish are likely to develop in the trunk region of the vertebral column (Sullivan et al. 2007), while those that occur in marine species are identified in the tail region (Fjelldal et al. 2007a).

Opercular abnormalities are commonly associated with severe foldings and twists of the operculum and suboperculum. This affect both the morphology (Koumoundouros et al., 1997b) and the biological performance growth, survival of a variety of cultured fish (Hilomen-García, 1997). Fin deformities are characterised by rays curvature which is mostly affected by fish fighting in intensive aquaculture (especially salmonids and percids) (Policar et al., 2016; Stejskal et al., 2011).

1.5. OBJECTIVES OF THE THESIS

The overall aim of this thesis was to investigate effect of selected aspects influencing performance of European whitefish and peled in intensive culture. All the experiments were carried out using recirculation aquaculture system (RAS).

The specific objectives were to:

1) Investigate the effect of light intensity and tank wall color on survival and growth of peled (Chapter II.).
2) Examine the effect of temperature on growth and survival of European whitefish larvae under controlled conditions (Chapter III.).
3) Asses the effect of stocking density on growth and survival of European whitefish larvae under controlled conditions (Chapter IV.).
4) Evaluate the effect of different feeding strategy on growth performance, survival, intestine development and liver status of European whitefish larvae under controlled conditions (Chapter V.).
5) Uncover effect of timing and co-feeding duration on success of weaning of peled larvae (Chapter VI.).
6) Study the effect of water oxygen saturation on growth and haematological profile of juvenile peled (Chapter VII.).
7) Reveal prevalence of deformities in intensively reared peled and comparative morphometric with pond reared fish (Chapter VIII.).

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CHAPTER 2

THE EFFECT OF LIGHT INTENSITY AND TANK WALL COLOUR ON SURVIVAL AND GROWTH OF PELED *COREGONUS PELED* GMELIN 1788 LARVAE

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RESEARCH PAPER

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The Effect of Light Intensity and Tank Wall Colour on Survival and Growth of Peled *Coregonus peled* Gmelin 1788 Larvae

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Abstract

A 33-day experiment was carried out to investigate the effect of light at 80, 380, and 3800 lux and tank wall colour (black, grey, white, red, green, blue, clear) separately and in combination on growth, survival, yield, and size heterogeneity of peled *Coregonus peled* larvae. 7 groups of larvae in 3 repetitions were transferred to the experimental system. Each group comprised 300 larvae. Larvae were fed fresh live brine shrimp *Artemia salina* and artificial dry food LARVIVA ProWean. Significantly higher ($P < 0.05$) survival was observed in black and white tanks in comparison with grey tanks. Significantly lower ($P < 0.05$) size heterogeneity was observed in red and grey tanks compared to clear, black, green, and blue tanks, and in RD-H compared to WH-H, WH-M, WH-L, CL-H, CL-M, CL-L, BK-H, BK-M, BK-L, GN-H, GN-M, GN-L, BE-H, BE-M, BE-L, GY-H, and RD-M compared to WH-M, WH-L, CL-H, CL-M, CL-L, BK-H, BK-M, BK-L, GN-H, GN-M, GN-L, BE-H, BE-M, BE-L, and RD-L, GY-L, GY-M compared to WH-L, CL-H, CL-M, CL-L, BK-H, BK-M, BK-L, GN-H, GN-M, GN-L, BE-H, BE-M, BE-L. Based on our results, peled larvae are independent of light intensity. Rearing of peled in black tanks can be recommended for the highest survival.

Introduction

Peled *Coregonus peled* (Gmelin 1788) is a promising species for freshwater culture, especially in central and Eastern Europe (Mukhachev & Gunin, 1999). Recently, due to the predation, especially by cormorants *Phalacrocorax carbo* (L., 1758) its production has rapidly declined (Suter, 1997). Establishing stable whitefish production using recirculating aquaculture systems (RAS) requires determination of optimal larviculture conditions. Optimal light intensity (LI), tank wall colour (TWC), photoperiod, and light spectrum are critical factors in fish development (Boeuf & Le bail, 1999) from

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
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eggs (Prokesova, Stejskal, Matousek, Kouril, & Baras, 2017) to sexually mature fish (Suquet, Omnes, Normant, & Fauvel, 1992).

Many fish species have a minimum threshold of LI (Table 1) as well as optimal TWC (Table 2) for normal development and growth of larvae. Low LI is reported to affect detection and capture of prey (Buchet et al., 1995; Link & Edsall, 1996), searching activity (Ounais-Guschemann, 1989), and swimming speed in European whitefish *Coregonus lavaretus* L. and vendace *Coregonus albula* L. (Gjelland, Bøhn, Knudsen, & Amundsen, 2004) as well as disturbance of diurnal activity in European whitefish (Müller, 1978a) and photo-kinetic responses in lake whitefish *Coregonus clupeaformis* (Mitchill 1818) (Scherer & Harrison, 1988). Conversely, excessively high LI can cause severe retinal damage, as reported in European sea bass *Dicentrarchus labrax* (L., 1758), Atlantic cod *Gadus morhua* (L., 1758) and Atlantic salmon *Salmo salar* (L., 1758) (Vera & Migaud, 2009) and increased aggressiveness in African catfish *Clarias gariepinus* (Burchell 1822) (Britz & Pienaar, 1992). Different TWC induce a variety of responses in relation to growth (Luchiari & Pirhonen, 2008), survival (Raghavan et al., 2013), aggression (Hoglund, Balm, & Winberg, 2002), stress response (Rotllant et al., 2003; Papoutsoglou, 2005), behaviour and feed acceptance (Strand, Alanara, Staffan, & Magnhagen, 2007). The LI and TWC can influence fish survival (Brannas, Alanara, & Magnhagen, 2001) and inflation of larva swim bladder (Martin-Robichaud & Peterson, 1998), and optimal TWC is associated with lower incidence of mouth deformities (Cobcroft, Shu-Chien, Kuah, Jaya-Ram, & Battaglene, 2012). Some levels of LI and TWC has been demonstrated to induce stress in fish (Rotllant et al., 2003).

Table 1, Table 2

Larviculture is considered to be the most critical period in fish rearing with light being regarded as a crucial abiotic factor that can limit its quality. Furthermore, the optimal light conditions need to be determined for each fish species and developmental/reproductive stage to facilitate survival and growth and enable efficient management to maximize production and profitability, as well as to provide proper conditions for fish. Previous light studies in coregonids were conducted on European whitefish, vendace, and lake whitefish. However, there is no knowledge on the effect of light in early rearing of peled larvae in intensive culture. This study aimed to assess effects of tank wall colour, light intensity, and their combination on survival and growth of peled larvae.

Materials and Methods

Larvae

Peled larvae were obtained immediately after hatching from Kinský Žďár, a.s (49°58'N; 15º93'E) and transported to storage tanks at the wet laboratory of the Institute of Aquaculture and Protection of Waters (48°97'N; 14°45'N). After absorption of the yolk-sac, 300 larvae (2.12 ± 0.45 mg) were stocked into each of 63 tanks. A total of 18 900 of larvae were used.

Experimental system and design

The experiment was conducted for 33 days in a small recirculating aquaculture system (RAS) comprising two storage tanks (400 L, 480 × 555 × 1500 mm) and 63 rearing tanks (6.8 L, 120 × 190 × 300 mm) with flat bottoms and overflow with mesh size of 0.31 mm. Tanks were divided into a rearing section with water inflow and an aeration section using an air stone, separated to avoid contact of larvae with air bubbles. Water flow was at 3 L $m^{-1}h^{-1}$. Tank wall colours were black (BK), grey (GY), white (WH), red (RD), green (GN), blue (BE), and clear (CL) in combination with three light intensities: 80 lux (low = L), 380 lux (medium = M), and 3800 lux (high = H) provided by LED bulbs (Aquatlantis Easy LED Universal, Portugal). The combinations (experimental groups) BK-L, GY-L, WH-L, RD-L, GN-L, BE-L, CL-L, BK-M,

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

GY-M, WH-M, RD-M, GN-M, BE-M, CL-M, BK-H, GY-H, WH-H, RD-H, GN-H, BE-H, and CL-H were tested. The tank bottom and the walls were covered with opaque plastic film. CL, WH and GY were considered light colours and GN, BE, BK and RD dark colours. The lights were positioned approximately 40 cm above tanks. Each experimental group was tested in triplicate. A consistent photoperiod 12D:12L was maintained using automatic timers (Ever Flourish EMT 445-F, Germany).

Culture Conditions

The oxygen level and pH were checked daily at 8.00 and 16.00. The pH range was monitored using a HACH HQ 40 multimeter (Germany) and maintained near neutral 6.8-7.2. Water temperature was kept at 13–15 °C using a HAILEA HC-1000A cooler (China). Oxygenation was near 100% saturation in the rearing aquaria and 80% at the tank outlets using SECOH and AIRMAC pumps (Japan, Taiwan). Ammonia, nitrate, and nitrite concentrations were analysed using HACH, LCK 304, LCK 339, LCK 341 (Germany) with HACH DR2800 spectrophotometer (Germany). NaCl was added at 1 g L^{-1} weekly to maintain a 16:1 chloride:nitrogen ratio. The aquaria were cleaned and dead larvae removed and counted daily.

Feeding

Brine shrimp *Artemia salina* L. metanauplii, 20–24 h old, 0.4-0.5 mm, 240 000 nauplii g^{-1} (Ocean Nutrition Europe, Belgium) were incubated following supplier's instructions. Fresh metanauplii were fed from 4 to 25 days post hatching (dph) at 500–700 $larvae^{-1}$ day^{-1}. Larvae were fed 7 times daily at 2 h intervals during the light phase (7.00 to 19.00).

Artificial dry food was fed from day 26-37 dph without co-feeding, since peled larvae easily adapt to artificial food (Stejskal et al., 2017). LARVIVA ProWean (BioMar, France) of particle size (80-200 µm) was provided six times per day at 2 h intervals. Composition of commercial feed was crude protein 58%, crude lipids 12%, crude ash 11.1%, crude cellulose 0.5%, vitamin C 1000 mg kg^{-1}, vitamin E 800 mg kg^{-1}, vitamin A 2.6 mg kg^{-1}, vitamin D3 0.044 mg kg^{-1}, phosphorus 1.64%, and n-3 HUFA 2.50% (manufacturer's data).

Sampling and Measurements

Dead larvae were removed from each tank during daily cleaning, counted, and preserved in 4% formalin. At completion of the experiment (37 dph), effect of LI, TWC, and their combination, on survival rate (SR), larval yield (LY), and size heterogeneity (SH), final weight (FW), total length (TL), standard length (SL), body height (BH) was assessed as follows:

$SR\ (\%) = 100 \times Nf\ (Ni - Ns)^{-1}$

in which Ni and Nf = initial and final number of larvae, respectively, Ns = number of dead larvae and sampled larvae per tank (day 7, 14, 21, 28)

LY was calculated using following formula:

$$LY\ (g) = \left(\left(\frac{initial\ number\ of\ larvae}{100}\right) \cdot survival\right) \cdot weight$$

with survival and weight = % surviving and mean weight (g) of larvae

SH was defined as coefficient of variance of weight, calculated as follows:

$CV\ (\%) = 100 \times (SD / W_m)$

in which CV = coefficient of variance; SD = mean standard deviation of weight of 30 randomly selected larvae per tank; W_m = mean weight (mg) of 30 larvae per tank.

At the conclusion of the experiment, 30 larvae from each tank were preserved in 4% buffered formalin and weighed on a digital microbalance (Mettler Tolledo, Excellence Plus, Switzerland, d = 0.0001 g). Larvae were digitally photographed, and TL, SL, BH were measured using image analysis in MicroImage 4.0 (Olympus, Japan).

Statistical Analysis

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Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

The data are presented as mean ± SEM. Statistical analyses were performed using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). The effects of tank conditions on body weight, total length, standard length, total height, survival rate, larval yield, and size heterogeneity were analysed by two-way ANOVA with TWC and LI as fixed variables. The level of significance used for all tests was $\alpha = 0.05$ (Zar, 1999). Prior to ANOVA, survival percentages were arcsin-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.

Results

Survival rate

A significantly ($P < 0.05$) higher survival rate was observed in black (69.0 ± 3.43%) and white (66.4 ± 1.64%) compared to grey (49.9 ± 1.96%) tanks (Figure 1a). No significant differences were observed in grey compared to red, clear, green, blue or white compared to black. Light intensity and combined effect of LI*TWC showed no significant effect on survival rate (Figure 1b and Figure 1c) (Table 4).

Figure 1a, Figure 1b and Figure 1c, Table 4

Growth

Light intensity, TWC, and their combination were not associated with significant differences $P < 0.05$) in body weight, total length, standard length, body height, or larval yield (Table 3 and Table 4). Tank wall colour was associated with significantly higher size heterogeneity in black (52.4 ± 1.77%), green (50.9 ± 3.27%), clear (46.6 ± 1.16%), blue (46.5 ± 1.51%), white (38.5 ± 1.66%) compared to red (20.5 ± 4.55%), and black, green, clear, blue compare to grey (27.3 ± 4.55%) and red (Figure 2a). No differences ($P < 0.05$) were observed in SH in the LI groups (Figure 2b). Significantly lower SH ($P < 0.05$) was found in RD-L, RD-M, RD-H, GY-L, GY-M compared to WH-L, CL-H, CL-M, CL-L, BK-H, BK-M, BK-L, GN-H, GN-M, GN-L, BE-H, BE-M, and BE-L and in RD-H compared to WH-L, WH-M, WH-H, CL-H, CL-M, CL-L, BK-H, BK-M, BK-L, GN-H, GN-M, GN-L, BE-H, BE-M, BE-L, and GY-H (Figure 2c).

Table 3 and Table 4, Figure 2

Discussion

Peled *Coregonus peled* is a recent addition to intensive aquaculture. Ensuring optimal breeding conditions is a basic precondition for successful larva rearing. The present study provided evidence that peled larvae can be cultured at a wide range of light intensities without significant effects on growth, survival rate, or larval yield. Whitefish larvae reared at 380 lux showed slightly higher survival rate compared to larvae reared at 80 lux and at 3800 lux, suggesting that LI in the intermediate range may be suitable for rearing peled larvae, although this is only speculation. Determining the proper light regime for peled culture systems is important from an economic standpoint. This study was unique, and we cannot extrapolate the effects of LI on growth, survival rate, and size heterogeneity to other coregonids. A wide range of intensity has been used in other research: Stejskal et al. (2017) used 200-400 lux in peled; Scherer and Harrison (1988) employed 1800 lux for whitefish *Coregonus clupeaformis*; Beier (2016) used 1 and 10 lux for vendace *Coregonus albula*; and Link and Edsall (1996) used 2, 5, 10, 40, 100, 400, 1000, and 1500 lux for lake herring *Coregonus artedi* (Lesueur 1818). Our results are similar to findings obtained by Kestemont et al. (2003), who stated that LI did not significantly affect survival rate and size heterogeneity of Eurasian perch *Perca fluviatilis* (L. 1758) larvae. Perch reared under 400 lux showed slightly higher survival rate than those reared under 5 lux. Hinshaw (1986) reported that yellow perch *Perca flavescens* (Mitchill 1814) larvae reared under 250 lux

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

exhibited slightly higher survival rate compared to those reared at 75 lux. An intermediate level of LI seems to be optimal for peled and other visual feeders (Kestemont et al., 2003), whereas Hecht and Appelbaum (1987) stated that a low level of LI appears to be optimal for nocturnal feeders, which rely little on vision.

Tank wall colour had no significant effect on peled total length, standard length, body height, final weight, or larval yield. A significantly higher survival rate was observed in white and black tanks compared to grey. Size heterogeneity in red and grey tanks was significantly lower than in clear, black, green, and blue variations. Information regarding TWC and its effects on growth, survival rate, and size heterogeneity of other coregonids is scarce, and published reports do not provide detailed description of TWC in their experimental designs. Results similar to those of the present study regarding growth and size heterogeneity have been reported: Monk, Puvanendran, and Brown (2008) observed no significant difference in growth rate of Atlantic cod *Gadus morhua* larvae in tanks with black walls and dark bottoms compared to tanks with black walls and light bottoms. Jentoft, Øxnevad, Aastveit, and Andersen (2006) reported values of size heterogeneity for Eurasian perch *Perca fluviatilis* larvae reared in black tanks (51.1 ± 23.8%) similar to our findings (52.4 ± 27.3%) for the same colours.

We found no significant LI/TWC combined effects on growth parameters or on survival. Significant differences were observed only in size heterogeneity. Our results are similar to those of Downing and Litvak (2000) who reported no significant differences in survival rate of haddock *Melanogrammus aeglefinus* (L., 1758) larvae with LI 100 and 1500 lux combined with black or white TWC.

Fish growth rate and survival are affected by biochemical and neuro-hormonal processes with complex interactions (Papoutsoglou, 1998). Tank wall colour and LI may correlate with stress levels, especially plasma cortisol response (Rotllant et al., 2003). Stress can increase catabolic processes of cultured fish and may reduce growth (Strand, Alanara, Staffan, & Magnhagen, 2007; El Sayed & El Ghobashy, 2011) and survival rate (Okada et al., 2015). Papoutsoglou, Mylonakis, Miliou, Karakatsouli, and Chadio (2000) reported that fish reared in black tanks had significantly higher plasma cortisol levels than those reared in white tanks, and specific growth rate and final weight was significantly higher and feed conversion ratio significantly lower in white tanks. This was also observed by Eslamloo, Akhavan, Eslamifar, and Henry (2015), Rahnama, Heydarnejad, and Parto (2015), Wang et al. (2016), and Wang et al. (2017). On the contrary, Downing and Litvak (2000) and Martin-Robichaud and Peterson (1998) found larvae reared in dark coloured tanks to show lower stress levels, higher food intake, and less body damage.

Conclusion

Our results showed peled total length, standard length, body height, final weight, and larval yield to be independent of TWC, LI, or their combination. Survival rate in black and white tanks was higher than in grey tanks, and larvae in red and grey tanks showed significantly lower size heterogeneity compare to clear, black, green, and blue tanks. Based on our results, intermediate LI combined with black TWC can be recommended. The combined effect of LI and TWC needs to be further studied considering additional biotic and abiotic factors such as prey perception, the mirror effect (Hinshaw, 1986), retinal development (Guma'a, 1982), and walling behaviour (Cobcroft & Battaglene, 2009). Further studies investigating chronic effects of background colour on peled growth, survival, stress, and immune reactions are recommended.

Acknowledgements

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Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
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Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
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Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
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Table 1. Minimal light intensity thresholds for normal development and growth of different fish larvae

LI (lux)	Species	Source
< 1	*Clupea harengus* (L., 1758)	(Blaxter, 1975)
1	*Morone saxatilis* (Walbaum 1792)	(Chesney, 1989)
1-10	*Hippoglossus hippoglossus* (L., 1758)	(Hole & Pittman, 1995)
50	*Salvelinus alpinus* (L., 1758)	(Wallace, Kolbeinshavn, & Aassjor, 1988)
50-150	*Sparus aurata* (L., 1758)	(Ounais-Guschemann, 1989)
150	*Clarias gariepinus* (Burchell 1822)	(Almazan-Rueda, Schrama, & Verreth, 2004)
200-600	*Salmo salar* (L., 1758)	(Mortensen & Damsgard, 1993)
350	*Paralichthys lethostigma* (Jordan & Gilbert, 1884)	(Daniels, Berlinsky, Hodson, & Sullivan, 1996)
600	*Dicentrarchus labrax* (L., 1758)	(Barahona-Fernandes, 1979)
1000	*Siganus guttatus* (Bloch 1787)	(Duray & Kohno, 1988)

*LI = light intensity

J

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx

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Table 2. Optimal tank wall colour for development and growth of different fish larvae

TWC	Species	Source
YE	*Oreochromil niloticus* (L., 1758)	(Luchiari, Duarte, Freire, & Nissinen, 2007)
BE	*Oncorhynchus mykiss* (Walbaum 1792)	(Ustundag & Rad, 2015)
RD	*Danio rerio* (Hamilton 1822)	(Spence & Smith, 2008)
BE	*Lates calcifer* (Bloch 1790)	(Ullmann et al., 2011)
WH	*Carrasius auratus* (L., 1758)	(Eslamloo, Akhavan, Eslamifar, & Henry, 2015)
WH	*Epinephelus coioides* (Hamilton 1822)	(Zhang et al., 2015)
DBE	*Pelteobagrus fulvidraco* (Richardson 1846)	(Rahnama, Heydarnejad, & Parto, 2013)
BK	*Pelteobagrus fulvidraco* (Richardson 1846)	(Rahnama, Heydarnejad, & Parto, 2013)
BE	*Scophthalmus maximus* (L., 1758)	(Li et al., 2016)

*TWC = tank wall colour – YE = yellow, BE = beige, RD = red, BE = blue, WH = white, DBE = dark blue, BK = black, GN = green, BE = blue

K

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

Table 3. Effects of light intensity, tank wall colour and interaction of light intensity and tank wall colour on growth of *Coregonus peled* (Gmelin 1788) larvae in a 33 day growing trial.

TWC	LI	Group	BW (mg)	TL (mm)	SL (mm)	BH (mm)	LY (g)
RD	H (3800 lux)	RD-H	53.5 ± 1.60	22.2 ± 0.08	19.6 ± 0.06	3.2 ± 0.03	8.40 ± 0.31
	M (380 lux)	RD-M	58.0 ± 1.61	22.5 ± 0.08	19.5 ± 0.07	3.3 ± 0.03	10.1 ± 0.31
	L (80 lux)	RD-L	64.6 ± 1.64	22.5 ± 0.08	19.5 ± 0.06	3.2 ± 0.03	11.0 ± 0.32
WH	H (3800 lux)	RD-H	64.0 ± 1.69	22.1 ± 0.08	19.0 ± 0.06	3.3 ± 0.03	10.4 ± 0.33
	M (380 lux)	RD-M	69.6 ± 1.74	22.7 ± 0.08	19.5 ± 0.06	3.4 ± 0.03	12.1 ± 0.34
	L (80 lux)	RD-L	65.5 ± 1.78	22.5 ± 0.07	19.4 ± 0.06	3.3 ± 0.03	11.1 ± 0.35
CL	H (3800 lux)	RD-H	60.8 ± 1.71	23.1 ± 0.06	19.9 ± 0.04	3.6 ± 0.03	10.2 ± 0.33
	M (380 lux)	RD-M	58.3 ± 1.52	22.7 ± 0.03	19.6 ± 0.03	3.3 ± 0.02	9.50 ± 0.30
	L (80 lux)	RD-L	60.8 ± 1.56	22.3 ± 0.03	19.4 ± 0.03	3.3 ± 0.02	10.4 ± 0.32
BK	H (3800 lux)	RD-H	62.6 ± 1.55	22.1 ± 0.03	19.1 ± 0.03	3.2 ± 0.02	9.80 ± 0.31
	M (380 lux)	RD-M	70.3 ± 1.61	22.5 ± 0.03	19.4 ± 0.03	3.3 ± 0.03	10.5 ± 0.32
	L (80 lux)	RD-L	74.2 ± 1.57	22.6 ± 0.03	19.7 ± 0.03	3.2 ± 0.02	13.2 ± 0.34
GN	H (3800 lux)	RD-H	55.5 ± 1.66	23.1 ± 0.03	19.7 ± 0.03	3.5 ± 0.02	9.90 ± 0.36
	M (380 lux)	RD-M	59.7 ± 1.76	22.7 ± 0.03	19.4 ± 0.03	3.4 ± 0.02	10.2 ± 0.38
	L (80 lux)	RD-L	53.3 ± 1.75	22.7 ± 0.03	19.7 ± 0.03	3.1 ± 0.02	7.80 ± 0.38
BE	H (3800 lux)	RD-H	48.9 ± 1.90	21.6 ± 0.03	18.6 ± 0.03	3.2 ± 0.02	7.40 ± 0.41
	M (380 lux)	RD-M	63.8 ± 2.07	22.6 ± 0.03	19.5 ± 0.03	3.3 ± 0.02	10.2 ± 0.45
	L (80 lux)	RD-L	65.2 ± 2.39	22.5 ± 0.02	19.7 ± 0.01	3.1 ± 0.01	10.0 ± 0.51
GY	H (3800 lux)	RD-H	46.2 ± 1.69	21.6 ± 0.02	19.0 ± 0.01	3.0 ± 0.01	7.60 ± 0.24
	M (380 lux)	RD-M	52.9 ± 2.24	22.3 ± 0.01	19.4 ± 0.02	3.3 ± 0.01	8.40 ± 0.20
	L (80 lux)	RD-L	50.6 ± 1.60	22.5 ± 0.08	19.6 ± 0.06	3.1 ± 0.03	8.80 ± 0.31

Data are presented as mean ± S.E.M.

*BW= final body weight; TL= total length; SL, standard length; BH= body height; LY= larval yield

*LI = light intensity – H = H (3800 lux), M = M (380 lux), L = L (80 lux)

*TWC = tank wall colour – RD = red, white = WH, CL = clear, BK = black, GN = green, BE = blue, GY = grey

L

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

Table 4. Two-way ANOVA results for the factors light intensity and tank wall colour and their interaction on final SR, BW, TL, SL, BH, LY, SH of *Coregonus peled* (Gmelin 1788) larvae.

Parameters	Source of variation	SS	DF	F	MS	*P*
SR	LI	500.10	6	250.10	2.37	0.11
	TWC	2170.50	6	361.70	3.43	0.01
	LI × TWC	573.80	12	47.80	0.45	0.93
BW	LI	0.00	2	0.00	0.11	0.90
	TWC	0.11	6	0.02	0.97	0.46
	LI × TWC	0.16	12	0.01	0.75	0.70
TL	LI	1.07	2	0.54	1.36	0.27
	TWC	2.94	6	0.49	1.24	0.30
	LI × TWC	4.97	12	0.38	0.97	0.49
SL	LI	0.98	2	0.49	1.84	0.17
	TWC	1.06	6	0.18	0.66	0.68
	LI × TWC	3.15	12	0.26	0.98	0.48
BH	LI	0.03	2	0.01	2.51	0.09
	TWC	0.04	6	0.01	1.01	0.43
	LI × TWC	0.04	12	0.00	0.51	0.90
LY	LI	21.64	2	1.85	1.85	0.17
	TWC	63.61	6	1.81	1.81	0.12
	LI × TWC	40.20	12	0.57	0.57	0.85
SH	LI	28.50	2	14.30	0.33	0.72
	TWC	8084.50	6	1347.40	31.32	0.00
	LI × TWC	1080.60	12	90.00	2.09	0.04

*SR = survival rate; BW = body weight; TL = total length; SL = standard length; BH = body height; LY = larval yield; SH = size heterogeneity.

*SS = sum of squares; DF = degrees of freedom; F = distribution fitting; MS = mean squares, *P* = probability

*LI = light intensity; TWC = tank wall colour; LI × TWC = interaction of light intensity and tank wall colour

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

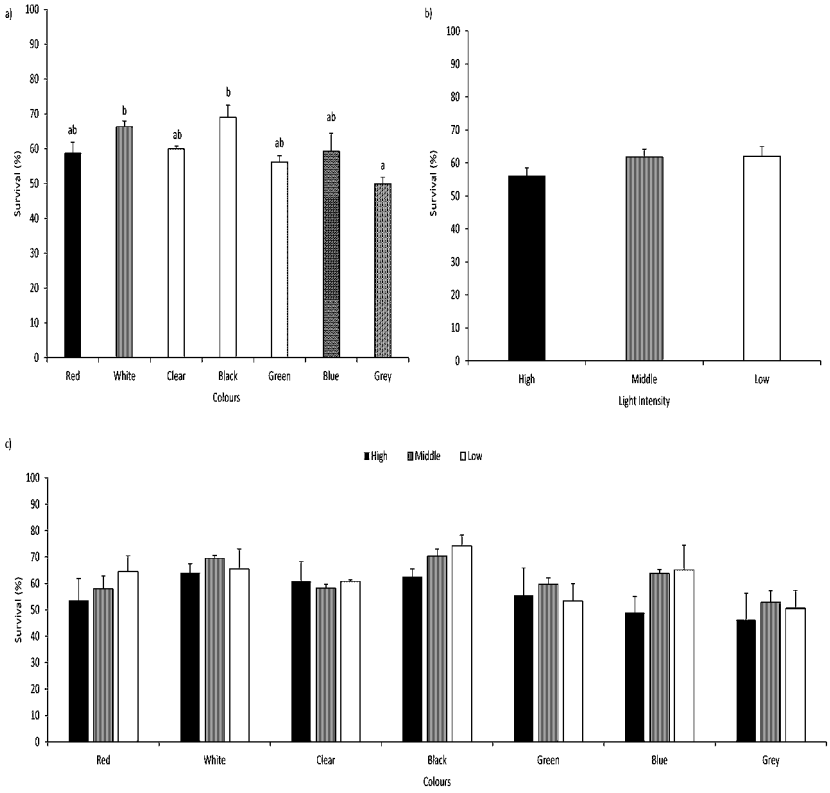

Figure 1. Mean survival rate (%) at tested tank wall colours (red, white, clear, black, green, blue, grey) (a) light intensities (high - 3800 lux, middle - 380 lux, low – 80 lux) (b) and light intensity*tank wall colours (c). The same letters indicate no significant differences ($P > 0.05$) among tank wall colours. Bars show the mean and whiskers indicate S.E.M. (n = 63).

Turk. J. Fish.& Aquat. Sci. 19, xxx-xxx
http://doi.org/10.4194/1303-2712-v19_7_10

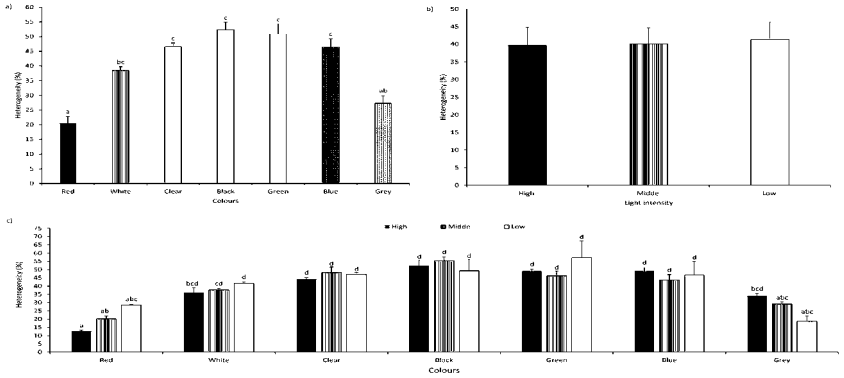

Figure 2. Size heterogeneity (%) at tested tank wall colours (red, white, clear, black, green, blue, grey) (a) light intensities (high - 3800 lux, middle - 380 lux, low – 80 lux) (b) and light intensity*tank wall colours (c). The same letters indicate no significant differences ($P > 0.05$) among tank wall colours. Bars show the mean and whiskers indicate S.E.M. (n = 63).

CHAPTER 3

EFFECT OF TEMPERATURE ON GROWTH AND SURVIVAL OF MARAENA WHITEFISH *COREGONUS MARAENA* (BLOCH 1779) LARVAE IN CONTROLLED CONDITIONS

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Effect of temperature on growth and survival of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae in controlled conditions

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Abstract

This 28-day study investigated the effect of three rearing temperatures, 11, 15 and 19°C, on survival and growth of maraena whitefish fry in a recirculating aquaculture system. Three groups of larvae in three repetitions were reared in recirculating system. Each group comprised 200 larvae. Feeding level was fixed at 500–700 *Artemia* sp. metanauplii per fish per day. Larvae were fed fresh live brine shrimp at 10 ml/tank every 3 hr. Significantly higher body weight (p = 0.00), total length (p = 0.00), larval yield (p = 0.00) and condition factor (p = 0.00) were obtained at 19°C compared to 15 and 11°C, as well as at 15°C compared to 11°C. Significantly higher survival (p = 0.00) was observed in larvae reared at 11 and 15°C compare to 19°C and no significant differences were observed between 11°C compared to 15°C. No significant differences in size heterogeneity among treatments were found (p = 0.46). In larviculture, the optimal assessed temperature for growth of maraena whitefish was 19°C, with highest survival observed at 11°C, at the end of this 28 days trial. The findings in this study apply to the particular study location and may not be applicable more broadly.

KEYWORDS

brine shrimp, fry, growth, larviculture, mortality, temperature

1 | INTRODUCTION

The maraena whitefish *Coregonus maraena* (Bloch 1779) is a promising species for inland freshwater aquaculture throughout East and Central Europe (Mukhachev & Gunin, 1999) as well as in northern Europe, especially Finland (Jobling et al., 2010) and Norway (Siikavuopio, Knudsen, Amundsen, Sæther & James, 2010). Recently, due to predation by the great cormorant *Phalacrocorax carbo* L. production in traditional pond-based aquaculture has declined dramatically (Suter, 1997). Commercial overfishing has also contributed to the overall decrease (Thomas & Eckmann, 2007). In Finland, aquaculture production of maraena whitefish is low, but has increased from about 50 tonnes to ~1,000 since 2005–2010 and is predicted to soon reach 4,000 tonnes in the near future (Jobling et al., 2010). In nature, it spawns during November and December and incubation period is 280–300 days. Hatching of larvae usually appears at the end of March (Kottelat & Freyhof, 2007). Currently, it is very useful that coregonid production be established throughout intensive culture in closed systems. In recent years, recirculating aquaculture systems (RAS) provide safe and controlled conditions for fish rearing. The optimal larviculture conditions should be identified, including appropriate oxygen concentration, pH, feeding, illumination, salinity and temperature.

Temperature exerts a primary influence on the growth of fish of any species. Inappropriate temperature can affect fish survival, growth, feeding, maturation (Jobling, 1994), reproduction success (Nowosad, Targońska, Chwaluczyk, Kaszubowski & Kucharczyk,

2014), embryonic (Eckmann & Pusch, 1989) and gonad (Kamler, 1992) development, timing of hatching (Keinänen, Tigerstedt, Kålax & Vuorinen, 2003), feed conversion, economics of rearing (Bernier, 2010; Jobling, 1997) and prevalence of deformities (Stejskal et al., 2018). Temperature tolerance is species-dependent (Jobling, 1994; Reist et al., 2006). Data with respect to optimal temperature for maraena whitefish, especially in intensive culture, are lacking.

Previous temperature studies in maraena whitefish were conducted on juveniles (6.5–444 g). However, there is a lack of knowledge on the effect of water temperature in early rearing of larvae in intensive culture. Larviculture is considered to be the most critical period in fish rearing with temperature being regarded as a crucial factor that can limit its quality. This study aimed to test effects of water temperature on survival and growth of maraena whitefish larvae reared in a recirculating aquaculture system.

2 | MATERIALS AND METHODS

2.1 | Eggs and larvae

Maraena whitefish were obtained from lagoons in Szczecin in the River Odra, north-western Poland. The broodstock comprised 120 fish at a 1:1 sex ratio. Gametes of 35 females and 35 males 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 = ~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, ~750,000 eggs were incubated. After 60 days, eggs were transferred to a second set of 8-L Zug jars and incubated at 8–9°C to accelerate development and hatching. After 5 days, 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 fry swam across to a tank with water temperature 12°C (total volume 1 m^3) underlain with 0.2 mm mesh. During 24 hr, temperature was elevated to 15°C. Larvae were transferred to tanks in the RAS.

2.2 | Experimental system, feeding and rearing conditions

Three groups of larvae in three repetitions were transferred to the experimental system consisting of nine 2-L aquaria, 96 × 154 × 200 mm. Each group comprised 200 larvae (mean initial weight, 7.4 ± 0.1 mg; mean initial total length, 13.0 ± 0.1 mm). Initial stocking 100 larvae/L were also used in study Champigneulle (1988) with no crowding stress effect. The nine aquaria were separated into two RAS units: one comprised three aquaria reared at 19.0 ± 0.02°C, and the second further subdivided to include three aquaria reared at 11.0 ± 0.02°C and three at 15.0 ± 0.03°C. Aquaria were placed in three larger tanks (in water bath with a capacity of 128 L) in order to regulation of water temperature in each larger tank. Subdivided unit with temperature maintained at 11 and 15°C was automatically regulated by electric heaters (2 × 500 W) ViaAqua Titanium Aquarium TH-500 (Germany) and thermoregulators Dixell S.p.A., diHell XT (Italy). Disinfection was provided using 30 W UV sterilizer MCT Transformatoren GmbH (Germany).

Larvae were fed fresh live brine shrimp *Artemia salina* L. metanauplii (Ocean nutrition, HE >230,000 NPG, Europe, Belgium), 20–24 hr old, 0.4–0.5 mm. Larvae were fed four times daily at 3 hr intervals during the light phase (8.30–17.30). Feeding level was fixed at 500–700 *Artemia* sp. metanauplii per fish per day. Feeding dose 10 ml of *Artemia* homogenous suspense per tank was provided in every feeding period. During the experiment, feeding dose was lowered correspondingly with larvae mortality and actual total number of surviving larvae in the individual tanks.

The oxygen level and pH were checked daily at 8.00 and 16.00. The pH range was monitored using an OxyGuard H04PP Handy pH meter (OxyGuard International, Denmark). Water temperature was kept automatically at 11, 15 and 19°C using a HAILEA HC-1000A cooler (China). Oxygenation was maintained using two pumps: SICCE Syncra 5.0 (5,000 L/hr; Italy). Ammonia, nitrate and nitrite concentrations were analysed using HACH, LCK 304, LCK 339, LCK 341 (Germany) with HACH DR5000 spectrophotometer (Germany; Table 1). NaCl was added at 1 g/L weekly to maintain a safe 16:1 chloride:nitrogen ratio. A constant inflow of 0.4 L/min was ensured. Dead larvae were removed and counted during daily cleaning, but not conserved in formalin. The level of organic matter remained low. A low CO_2 level was maintained via aeration and keeping alkalinity stable. The experiment lasted 28 days.

2.3 | Sampling and measurements

At the end of the experiment, after 28 days (30 dph), the survival rate (SR), size heterogeneity (SH), condition factor (K) and larvae yield (LY) was assessed as follows:

TABLE 1 Physicochemical parameters of water in two separated systems presented as mean ± standard error of the mean (*SEM*) from the whole experiment in maraena whitefish *Coregonus maraena* (Bloch, 1779) larvae

Parameter	Unit	*T*11	*T*15	*T*19
pH	-	8.8 ± 0.02	8.8 ± 0.02	8.8 ± 0.01
O_2	%	87.8 ± 0.55	87.8 ± 0.55	87.8 ± 0.55
O_2	mg/L	7.9 ± 0.05	7.9 ± 0.05	7.9 ± 0.05
NH_4^+	mg/L	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01
NO_2^-	mg/L	0.25 ± 0.11	0.25 ± 0.11	0.4 ± 0.11
NO_3^-	mg/L	9.4 ± 0.86	9.4 ± 0.86	22.3 ± 4.90
T	°C	11.0 ± 0.02	15.0 ± 0.03	19.0 ± 0.02

Note. *T* = temperature 11, 15, 19°C.

$$SR(\%) = 100 \times N_f(N_i - N_s)^{-1}$$

in which N_i and N_f = initial and final number of larvae, respectively, N_s = number of sampled larvae per tank (day 7, 14, 21)

$$SH(\%) = 100\,(SD/W_m)$$

in which SH = size heterogeneity; *SD* = mean standard deviation of weight of 10 randomly selected larvae per tank; W_m = mean weight (mg) of 10 larvae per tank.

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

in which W = mean weight (g) of 10 larvae per tank; TL = mean total length (mm) of 10 larvae per tank

$$LY\,(g/group) = \left(\left(\frac{\text{initial number of larvae}}{100}\right)\text{survival}\right)\text{weight}$$

with survival and weight = % surviving and mean weight (g) of larvae respectively.

Ten larvae from each tank (30 of each temperature group) was taken for W and TL measurements, as was also described by Laczynska et al. (2016), Celada, Aguilera, Carral, Saez-Royuela and Melendre (2008), Fletcher et al. (2007), Kaiser, Endemann and Paulet (2003), Mahmood, Ali and Anwar-Ul-Haque (2004), Nowosad et al. (2013) and Palińska-Żarska et al. (2014). Larvae were anaesthetized (2% Etomidate- 0.4 ml/L; IRS, Poland), weighed on a digital microbalance (ABJ 220-4M KERN, Germany), and measured manually from images taken with Leica MZ16 A stereomicroscope and a digital colour camera with 5 Mpixel resolution for Leica DFC420 Image Analysis. Total length (TL ± 0.01 mm) and body weight (BW ± 0.1 mg) were measured on days 0, 7, 14, 21 and 28.

2.4 | Statistical analysis

The data are presented as mean ± *SEM*. Statistical analyses were performed using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). The effects of temperature on BW, TL, LY, SR, *K* and SH were analysed by one-way ANOVA with temperature as fixed variable. Differences were considered significant at $p < 0.05$. Prior to ANOVA, SR, *K* and SH were arcsin-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 (Table 2).

3 | RESULTS

3.1 | Survival rate

Mortality was similar in all temperature groups during the first 7 days of the experiment. In time horizon 7–21 day, significantly ($p = 0.00$) lower SR was observed in 11°C compared to 15 and 19°C (Figures 1 and 2). After 28 days, the reversed situation was observed, when SR at 19°C was significantly ($p = 0.00$) lower (72.3 ± 0.60%) compared to 11°C (78.2 ± 0.67%) and 15°C (77.5 ± 0.76%; Figures 1 and 2; Table 2). Cumulative survival in the time horizon of 28 days is indicated in Figure 1. Survival in 7-day increments is shown in Figure 2.

TABLE 2 One-way ANOVA results for the factor temperature on total body length, final body weight, size heterogeneity, condition factor, larval yield, survival rate of maraena whitefish *Coregonus maraena* (Bloch, 1779) larvae

Parameters	Source of variation	SS	*df*	*F*	MS	*p*
TBL	*T*	94.9	2.0	47.4	183.3	0.00
FBW	*T*	13610.7	2.0	6805.3	112.8	0.00
SH	*T*	26.6	2.0	13.3	0.9	0.46
K	*T*	0.0	2.0	0.0	104.0	0.00
LY	*T*	269.6	2.0	134.8	74.3	0.00
SR	*T*	61.2	2.0	30.6	22.0	0.00

Notes. Parameter abbreviations: FBW: final body weight; FSH: size heterogeneity; K: condition factor; LY: larval yield; SR, survival rate; TBL: total body length.
Statistical abbreviations: *df*: degrees of freedom; *F*: distribution fitting; MS: mean square; *p*: probability; SS: sum of square.
Factor parameter: *T*: temperature.

3.2 | Growth, size heterogeneity, yield and condition factor

Significantly ($p = 0.00$) higher BW was observed in 19°C (139.7 ± 3.8 mg) compared to that in larvae at 15°C (103.1 ± 6.31 mg), and at 15°C in comparison with 11°C (87.9 ± 2.9 mg; Table 2). Body weight in 7-day increments is shown in Figure 3. The highest final TL was observed at 19°C (30.7 ± 0.2 mm) and the lowest at 11°C (26.8 ± 0.2 mm), with a significant difference at 19°C compared to 15°C, and at 15°C compared to 11°C ($p = 0.00$; Table 2). Total length measured at 7-day increments is shown in Figure 4. The highest LY was found at 19°C (25.2 ± 1.1 g/tank) and the lowest at 11°C (15.9 ± 0.3 g/tank). Larval yield was significantly higher at 19°C compared to 15°C, and at 15°C in comparison with 11°C (Figure 5). Significantly higher ($p = 0.05$) *K* was observed at 19°C (0.5 ± 0.0), compared to 15°C and 11°C (0.4 ± 0.0; Figure 6; Table 2). No significant ($p = 0.46$) differences were observed in SH at the conclusion of the trial. Highest SH was observed at 15°C (17.9 ± 1.3%), intermediate SH at 11°C (17.6 ± 3.02%), and lowest at 19°C (13.8 ± 2.9%; Table 2).

4 | DISCUSSION

Recommendations regarding optimal coregonid rearing temperatures include thresholds for minimum and maximum temperature. Temperature range 12–18°C is considered supportive for the maximal growth of whitefish in culture (Jobling et al., 2010). According to Koskela and Eskelinen (1992), optimal temperature for whitefish larva growth is 19.3–20.6°C. Kucharczyk, Czerkies and Leskelä

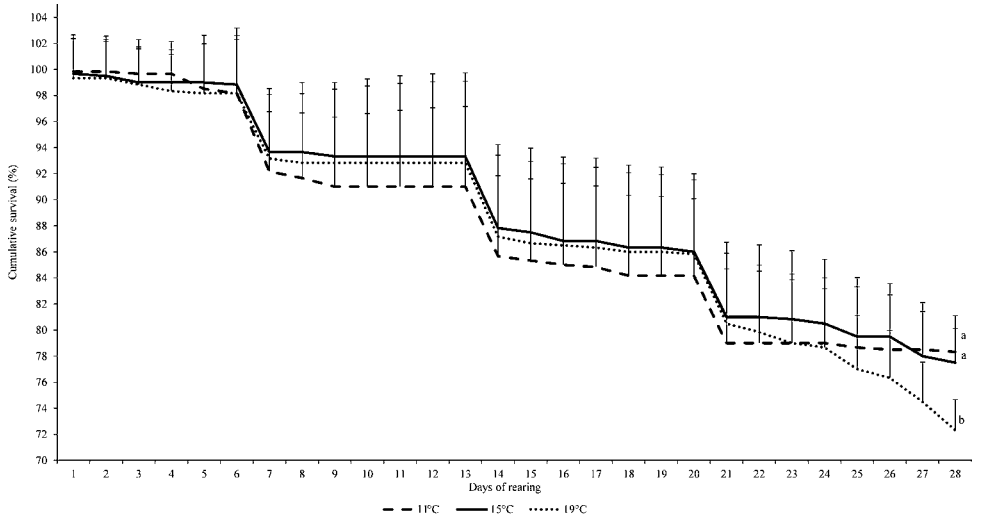

FIGURE 1 Cumulative survival of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae reared 28 days at three temperatures. Line is mean and whiskers indicate *SEM* of three replicates (n = 3). A sudden decline in day 7, 14, 21 includes sampling to growth-weight measurement

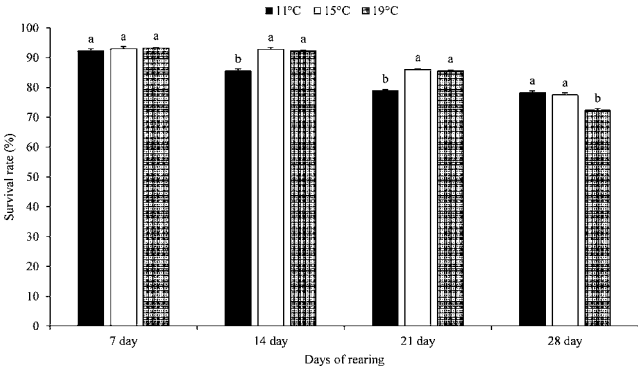

FIGURE 2 Mean survival rate (%) in maraena whitefish *Coregonus maraena* (Bloch 1779) measured at 7-day intervals during 28 days of exposure. Different letters indicate significant differences ($p < 0.05$) among temperatures. Bars represent means and whiskers indicate *SEM* of three replicates (n = 3)

(1994) state that thermal preference can vary with respect to origin from different populations. Mortality is often related to temperature, life stage, nutrition and antagonistic behaviour (Hurst, 2007). The study presented here also indicated that temperature had a strong impact on the rearing of whitefish fry in a recirculating system, in particular, connected to survival and growth. Whitefish larvae are known for being extremely sensitive to handling and usually die after manipulation.

It seems that slightly higher temperature (19°C) is beneficial for maraena whitefish survival in comparison with mildly lower temperature (11°C) or comparable to recommended thermal optimum (15°C) during the first 21 days of rearing. Taken into the consideration the highest survival in 11°C (day 28), relatively stable survival in 15°C (day 28) and sudden decline in 19°C (day 22–28; Figures 1 and 2), it appears that after 21 days, the temperature should be reduced. Similar mortality trend was also observed by Siikavuopio, Knudsen, Amundsen, Sæther and James (2013), but they did not see the accelerated growth displayed in our study. Bergot, Charlon and Durante (1986) reported that water temperature of 14°C promoted a survival rate >95% in European whitefish larvae during 54 days of rearing. Conversely, Szczepkowski, Szczepkowska and Krzywosz (2006) exposed European whitefish to 22°C water with survival rates of 95% and the highest growth at temperatures measured compared to those reared at 20 and 24°C. This study used whitefish population

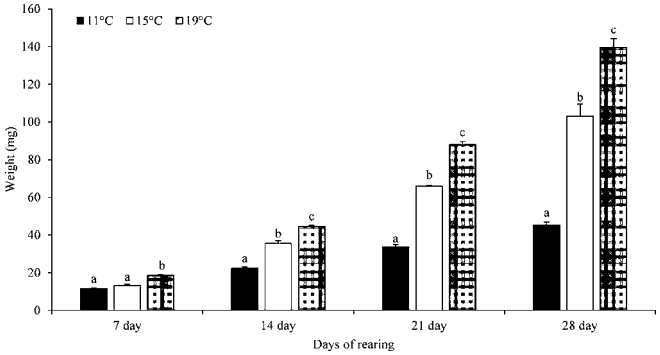

FIGURE 3 Mean body weight (mg) in maraena whitefish *Coregonus maraena* (Bloch 1779) measured at 7-day intervals during the 28 days of exposure. Different letters indicate significant differences ($p < 0.05$). Bars represent means and whiskers indicate *SEM* of three replicates ($n = 3$)

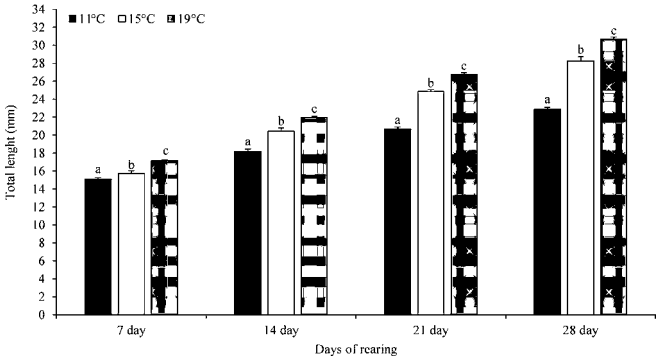

FIGURE 4 Mean length (mm) in maraena whitefish *Coregonus maraena* (Bloch 1779) measured at 7-day intervals during 28 days of exposure. Different letters indicate significant differences ($p < 0.05$) among temperatures. Bars represent means and whiskers indicate *SEM* of three replicates ($n = 3$)

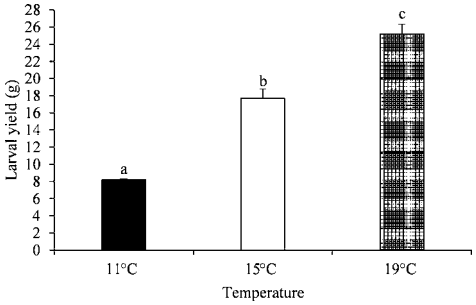

FIGURE 5 Mean larval yield (g/tank) in maraena whitefish *Coregonus maraena* (Bloch 1779) among tested temperatures. Different letters indicate significant differences ($p < 0.05$). Bars represent means and whiskers indicate *SEM* of three replicates ($n = 3$)

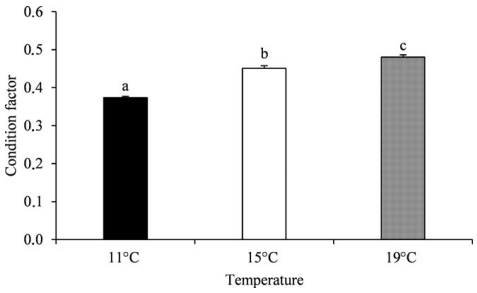

FIGURE 6 Mean values of condition factor in maraena whitefish *Coregonus maraena* (Bloch 1779) among temperatures. Different letters indicate significant differences ($p < 0.05$) among temperatures. Bars represent means and whiskers indicate *SEM* of three replicates ($n = 3$)

from lakes and so it seems that various intraspecific tolerance to the upper temperature limit is displayed, as was described in study Kucharczyk et al. (1994).

A temperature of 19°C exceeded the temperature range (12–18°C) previously reported to support maximum growth rates of cultured whitefish (Jobling et al., 2010). After 28 days, 19°C considered

as a suboptimal water temperature for survival did not result in growth reduction. Consistently to present study, the equal mortality trend which corresponds to decreasing survival with higher water temperature, as well as, increased growth coinciding with increasing temperature was observed in following studies. Matoušek, Stejskal, Prokešová and Kouřil (2017), with optimal growth of peled *Coregonus peled* Gmelin juveniles at 19–22°C, although peled is known for higher temperature tolerance. Similar findings were reported by Edsall (1999) who suggested 18.5°C as optimal for North American lake whitefish *Coregonus clupeaformis* Mitchill juveniles growth, but individuals at 10.1°C and 15°C displayed the highest survival. The next supporting study, Luczynski (1991) exposed vendace *Coregonus albula* L. larvae to 5, 7, 10, 12, 15, 17, 20 and 22°C. The temperature range 15–20°C was found to be the most suitable for sustained production of vendace larvae. On the other hand, mortality increased over the range 15–20°C, although still accompanied by an increase in net biomass gain.

Our results were similar to those of Siikavuopio et al. (2013) who reported significantly higher *K* in maraena whitefish reared at 18°C compared to those reared at 15 and 21°C. Matoušek et al. (2017) observed higher *K* values at 19, 22 and 25°C compared to 13 and 16°C. We found no significant effect of temperature on SH, and all temperature groups showed values below 20% at the conclusion of the trial. Siikavuopio, Knudsen, Amundsen and Sæther (2012) found out similar low coefficient of variation in SH below 20% at maraena whitefish.

No higher mortality or other impacts on larvae fitness and development was observed at the beginning of experiment. It seems that sudden temperature increment (4°C) or decline (4°C) is acceptable for performance of maraena whitefish larvae. Neither slightly higher concentration of nitrites 0.4 ± 0.11 mg/L nor nitrates 22.3 ± 4.90 affect the growth and survival of maraena whitefish (Table 1), because 16:1 chloride:nitrogen ratio was maintained during the whole experiment.

5 | CONCLUSIONS

We conclude that the optimal assessed temperature for growth of maraena whitefish was 19°C, with highest survival observed at 11°C. Further studies are required to investigate the optimal temperatures for survival and growth at different life stages and sizes of maraena whitefish.

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CHAPTER 4

EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL OF MARAENA WHITEFISH *COREGONUS MARAENA* (BLOCH 1779) LARVAE IN CONTROLLED CONDITIONS

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EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL OF MARAENA WHITEFISH *COREGONUS MARAENA* (BLOCH 1779) LARVAE IN CONTROLLED CONDITIONS

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ABSTRACT

This 30-day study investigated the effect of stocking densities of 25 L^{-1}, 50 L^{-1},100 L^{-1}, and 200 L^{-1} on survival and growth of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae in a recirculating aquaculture system. The four groups of larvae (total n = 2250) (initial weight = 7.4 ± 0.1 mg; initial total length = 13.0 ± 0.1 mm) in three repetitions were reared in a recirculating system. Larvae were fed fresh live brine shrimp metanauplii every 3 hours at a rate correlated with stocking density. At the completion of the experiment, 10 larvae from each tank (30 of each temperature group) were weighed and measured. No significant differences in final body weight, total length, size heterogeneity, condition factor, or survival was found among treatments ($p > .05$). The highest non-significant survival rate (92.7 ± 2.4 %), body weight (147.9 ± 6.3 mg), total length (30.7 ± 0.4 mm), size heterogeneity (22.5 ± 1.1 %), and condition factor (0.51 ± 0.01) were observed in larvae reared at 25 L^{-1}. For the best growth and survival, rearing of maraena whitefish 25 larvae L^{-1} could be recommended. Stocking density 200 larvae L^{-1} is advised to maintain the lowest size heterogeneity.

Keywords: *Stocking density, larviculture, fry, mortality, coregonids*

1. INTRODUCTION

The maraena whitefish *Coregonus maraena* (Bloch 1779) is a species promising for inland freshwater aquaculture throughout East, Central Europe (Mukhachev & Gunin, 1999), and northern Europe, especially Finland (Jobling et al., 2010) and Norway (Siikavuopio, Knudsen, Amundsen, Sæther & James, 2011). Several decades ago, due to predation by the great cormorant *Phalacrocorax carbo* (L.), the population dramatically declined (Suter, 1997). Eutrophication has contributed to the decrease (Thomas & Eckmann, 2007). At present, it is important that re-establishment of whitefish natural production be accompanied by the culture in intensive aquasystems. 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'Orbcastel, Person-Le Ruyet, Le Bayon & Blancheton, 2009). Establishment of coregonid production in recirculating systems requires that optimal larviculture conditions, including stocking density to be identified.

Stocking density is a crucial factor in productivity of fish culture systems. Excessively high density can produce a stress response, particularly increased plasma cortisol level (Barton,

Rahn, Feist, Bolling & Schreck, 1998), impede thyroid hormone production (Herrera, Rodiles, Sanchez, Lopez, & de La Roca, 2016), and affect growth (Zarski et al., 2008) and survival (Molnar et al., 2004; Szkudlarek & Zakes, 2007). High density can lead to fin erosion, gill damage, fish welfare impairment (Ellis et al., 2002), and promote cannibalism (Liao & Chang, 2002). It can decrease food utilization (Sharma & Chakrabarti, 1998) and alter metabolic rate (Tolussi, Hilsdorf, Caneppele & Moreira, 2010) with respect to lipids (Mommsen, Vijayan & Moon, 1999), carbohydrates (Sangiao-Alvarellos et al., 2005), proteins (Costas, Aragão, Mancera, Dinis & Conceição, 2008), and enzymes (Wendelaar Bonga, 1997). Finally, high fish density can impair water quality (Montero, Izquierdo, Tort, Robaina, & Vergara, 1999), reducing oxygen levels and increasing ammonia concentration (Azevedo, Martins, Bozzo & Moraes, 2006) in commercial production systems. These negative aspects lead to economic loss (Ruzzante, 1994) as well as disrupt production stability (Rowland, Mifsud, Nixon & Boyd, 2006). On the other hand, low stocking density is associated with high production costs (Luz & Santos, 2008). Stocking density has been shown to be a limiting factor in fish growth during early development (Webb, Hitzfelder, Faulk & Holt, 2007), while its impact is mitigated in adult fish (Duarte et al., 2004).

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 maximize 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 present study was to determine whether stocking density affects survival and growth of maraena whitefish larvae reared in a recirculating aquaculture system (RAS).

2. MATERIALS AND METHODS

2.1. Eggs and larvae

Maraena whitefish were obtained from lagoons in Szczecin in the River Odra, north-western Poland. The broodstock comprised 120 fish at a 1:1 sex ratio. Gametes of three years old 35 females (average weight, 800.4 ± 80.1 g, mean±SEM; average total length, 30.2 ± 1.1 cm) and three years old 35 males (650.5 ± 49.7 g, mean±SEM; average total length, 26.4 ± 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 fertilised 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^{-1}, 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 = ~150 000 eggs/jar) in a recirculating system and incubated at 3.0–3.5°C with water inflow 3 L min^{-1}, oxygen saturation to 90%, and pH near 7.0. In total, ~750 000 eggs were incubated. After 60 days, eggs were transferred to a second set of 8-L Zug jars and incubated at 8–9°C to accelerate development and hatching. After 5 days, 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 fry swam across to a tank (total volume 1 m^3) underlain with 0.2 mm mesh. After 24 h, fry were transferred to tanks in the RAS.

2.2. Experimental system and feeding/rearing conditions

Four groups of larvae in three repetitions were transferred to the experimental aquasystem consisting of twelve 2 L aquaria, 96 × 154 × 200 mm. The recirculating system (2300-L total water volume) included series of filtration sections (total biofilter volume 1500-L), a settling tank (500-L water volume). 30 fish were weighted and measured to obtain the initial values for weight and length. Maraena whitefish larvae (initial weight, 7.4 ± 0.1 mg, mean±SEM; initial total length, 13.0 ± 0.1 mm) were placed into each aquarium at stocking density of 25 L^{-1} (S25), 50 L^{-1} (S50), 100 L^{-1} (S100), and 200 L^{-1} (S200). A total of 2250 larvae were used in the experiment.

Larvae were fed fresh live brine shrimp *Artemia salina* (L.) metanauplii (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 (8.30 to 17.30). *Artemia* was fed as a homogenous suspension of 500–700 metanauplii mL^{-1} at a rate converted to larval stocking (Table 1). The daily ratio was based on previous experiment (unpublished data). Furthermore, this ratio was in slight excess as some of uneaten metanauplii were observed in tanks at the end of day. The feeding level was adapted according to fish body weight and losses of larvae during the experiment.

The oxygen level, water temperature, and pH were checked daily at 8.00 and 16.00. The pH range was monitored using an OxyGuard H04PP Handy pH meter (OxyGuard International, Denmark). The initial temperature without supplemental heat was 10°C. Water temperature ~19°C was regulated by a HAILEA HC-1000A cooler (China). Oxygenation was maintained using two SICCE Syncra 5.0 pumps (5000 L h^{-1}) (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^{-1} weekly to maintain a 16:1 chloride:nitrogen ratio. A constant inflow of 0.4 L min^{-1} was ensured. Dead larvae were removed and counted during daily cleaning. The level of organic matter remained low. A low CO_2 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_2 saturation = 85.8 ± 0.9%, O_2 concentration = 7.9 ± 0.1 mg L^{-1}, NH_4^+ = 0.1 ± 0.0 mg L^{-1}, NO_2 = 0.8 ± 0.1 mg L^{-1}, NO_3 = 21.2 ± 5.4 mg L^{-1}.

2.3. Sampling and measurements

At the completion of the experiment, 10 larvae from each tank (30 of each temperature group) were weighed (d = 0.1 mg) on a digital microbalance (ABJ 220-4M KERN, Germany, d = 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 $tank^{-1}$, 30 larvae $treatment^{-1}$, was used by Kaiser, Endemann & Paulet (2003); Mahmood, Ali & Anwar-ul-Haque (2004); Fletcher et al. (2007); Celada, Aguilera, Carral, Saez-Royuela & Melendre (2008); Nowosad et al. (2013); Palinska-Zarska et al. (2014); and Laczynska et al. (2016).

The survival rate (SR), size heterogeneity (SH), and condition factor (K) was assessed as follows:

$SR\ (\%) = 100 \times (Nf \times Ni^{-1})$

in which Ni and Nf = initial and final number of larvae, respectively;

$SH\ (\%) = 100 \times (SD \times W_m^{-1})$

in which SH = size heterogeneity; SD = mean standard deviation of weight of 10 randomly selected larvae $tank^{-1}$; W_m = mean weight (mg) of 10 larvae $tank^{-1}$.

$K = 100\ 000 \times W \times (TL^3)^{-1}$
in which W = mean weight (g) of 10 larvae tank^{-1}; TL = mean total length (mm) of 10 larvae tank^{-1}

2.4. Statistical analysis

Statistical analyses were performed using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). Data are presented as mean ± SEM. The effects of temperature on body weight (BW), TL, SR, K, and SH were analysed by one-way ANOVA with temperature as fixed variable. Differences were considered significant at $p < .05$. Prior to ANOVA, SR, K, and SH were arcsin-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 BW, TL, SR, SH and K. (Table 2)

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

Stocking density	*Artemia* feeding dose (mL)
*S25 (**50)	2.5 (**5)
*S50 (**100)	5.0 (**10)
*S100 (**200)	10.0 (**20)
*S200 (**400)	20.0 (**40)

*(Sn) values indicate number of larvae L^{-1} in each group

**conversion of stocking density and *Artemia* feeding dose to 2L aquarium volume

Table 2. *One-way ANOVA results for the factor stocking density on total length, body weight, size heterogeneity, condition factor, survival rate of maraena whitefish (Coregonus maraena Bloch, 1779) larvae.*

Parameters	Source of variation	SS	DF	F	MS	*p*
TL	SD	0.9	3.0	0.3	2.3	0.2
BW	SD	466.3	3.0	155.4	2.7	0.1
SH	SD	22.7	3.0	7.6	0.2	0.9
K	SD	0.0	3.0	0.0	2.2	0.2
SR	SD	3.6	3.0	1.2	0.2	0.9

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 maraena whitefish (Coregonus maraena Bloch, 1779) larvae in a 30-day growing trial.*

Group	SR (%)	TL (mm)	BW (mg)	SH (%)	K
S25	92.7 ± 2.4	30.7 ± 0.3	147.9 ± 5.8	22.5 ± 4.3	0.51 ± 0.01
S50	91.3 ± 1.5	30.4 ± 0.2	135.7 ± 1.6	20.3 ± 3.6	0.48 ± 0.01
S100	91.33 ± 1.1	30.4 ± 0.1	135.1 ± 3.5	21.1 ± 4.9	0.48 ± 0.00
S200	91.8 ± 1.0	30.0 ± 02	131.3 ± 5.2	18.7 ± 2.3	0.49 ± 0.01

S(n) = stocking density: larvae L-1; SR = survival rate; TL = total length; BW = body weight; SH = size heterogeneity; K = condition factor

3. RESULTS

At the conclusion of the trial, no significant ($p > .05$) differences among treatments were observed in SR, BW, TL, SH, or K (Table 2). The highest SR (92.7 ± 2.4 %), BW (147.9 ± 6.3 mg), TL (30.7 ± 0.4 mm), SH (22.5 ± 1.1 %), *and K* (0.51 ± 0.01) was observed at S25 (Table 3).

4. DISCUSSION

Growth-weight parameters did not differ significantly; hence 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, Thirunavukkarasu, Sundaray & Kailasam, 2010). The effect of stocking density on larva size heterogeneity may be species-dependant. For instance, the relationship of stocking density to size heterogeneity has been reported to be positive in red tilapia *Oreochromis niloticus* (L.) × *Oreochromis mossambica* (Peters) fry (Huang & Chiu, 1997), but negative in Arctic charr *Salvelinus alpinus* (L.) fry (Wallace, Kolbeinshavn & Reinsnes, 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 highest size heterogeneity observed in fish reared in low stocking density and vice versa.

Stocking density can influence mortality rate, with survival often negatively correlated with stocking density (Rowland, Mifsud, Nixon & Boyd, 2006). Fish species can be classified as density-independent or density-dependent. Tilapia larvae (Huang & Chiu, 1997) were reported to be density-dependent. Survival was high and not significantly affected by stocking density in the present study, thus maraena whitefish seem to be density-independent, and stocking density is not likely a limiting factor in their survival in intensive rearing.

Stocking density has been reported to be an important factor in fish growth (Saoud, Ghanawi & Lebbos, 2008) and is of particular concern in the welfare of intensively farmed fish (Ashley, 2007; Wocher, Harsányi & Schwarz 2011). Mortality (Ellis, Berrill, Lines, Turnbull & Knowles, 2012), as well as susceptibility to pathogen infections and fin damage (Turnbull, Adams, Richards & Robertson, 1998; Jones, Noble, Damsgård & Pearce, 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 (Ruyeta, Bayon & Gros, 2007; Hosfeld, Hammer, Handeland, Fivelstad & Stefansson, 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.

5. CONCLUSIONS

Studies suggest that fish grown at low stocking densities perform better than at higher densities. During a 30-day trial, slightly better maraena whitefish larvae survival, growth, and condition factor was observed at 25 larvae L^{-1} compared to larvae reared at 50, 100, and 200 larvae L^{-1}. However, no significant differences in any evaluated parameter were observed between groups of larvae at the highest and lowest stocking density. This study examined fry and early stage larvae; further study focusing on juvenile and adult maraena whitefish is

warranted. Effects of stocking density on stress hormone response, body composition, and haematological and biochemical parameters of maraena whitefish juveniles should be studied.

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CHAPTER 5

EFFECT OF FEEDING STRATEGY ON SURVIVAL, GROWTH, INTESTINE DEVELOPMENT, AND LIVER OF MARAENA WHITEFISH *COREGONUS MARAENA* (BLOCH 1779) LARVAE CULTURED UNDER RAS CONDITIONS

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EFFECT OF FEEDING STRATEGY ON SURVIVAL, GROWTH, INTESTINE DEVELOPMENT, AND LIVER OF MARAENA WHITEFISH *COREGONUS MARAENA* (BLOCH 1779) LARVAE CULTURED UNDER RAS CONDITIONS

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ABSTRACT

Study compared efficacy of larva feeding strategies. Seven groups of 250 maraena whitefish *Coregonus maraena* larvae in three repetitions were reared in a recirculating system. Effects of live (LF) (brine shrimp metanauplii) and artificial feed (AF) (dry commercial diet) and first weaning from live feed to a commercial dry diet at 5 (FW5), 10 (FW10), 15 (FW15), 20 (FW20), and 25 (FW25) days post-hatching were assessed on survival, growth, intestine development, liver status. Significantly higher total length ($P < 0.05$) was observed in FW15 and FW20 compared to LF, AF, FW5, and FW10. Significantly higher body mass ($P < 0.05$) was observed in FW15 compared to LF, AF, FW5, FW10, and in FW20 in comparison with AF and FW5. Significantly higher larval yield ($P < 0.05$) was observed in FW15 than in LF, AF, FW5, and FW10, and in FW20 compared to AF. Intestine diameter, villi length, and villi thickness ($P < 0.05$) were significantly higher in LF. The AF regime was associated with severe villi oedema and moderate exfoliation of intestine epithelium. The greatest hepatocyte nucleus diameter was observed in AF, and the greatest hepatocyte diameter in LF (days 5 and 10), FW10 (day 15), and AF (day 20).

Keywords: *Artificial diet, coregonids, intestine development, larviculture, live feed, weaning*

INTRODUCTION

The maraena whitefish *Coregonus maraena* (Bloch 1779) is a species promising for inland freshwater aquaculture throughout East and Central Europe (Mukhachev & Guni 1999) as well as in northern Europe, particularly Finland (Jobling et al., 2010) and Norway (Siikavuopio, Knudsen, Amundsen, Sæther, & James, 2011). Several decades ago, due to predation by the great cormorant *Phalacrocorax carbo* (L.), the population declined dramatically (Suter, 1997). Overfishing (Jackson et al., 2001), hybridization (Luczynski, Falkowski, Vuorinen, & Jankun, 1992), eutrophication (Thomas & Eckmann, 2007), degradation of natural spawning sites (Winfield, Fletecher, & James, 2004), pollution, and environmental changes (Walther et al., 2002) have also contributed to the whitefish stock depletion. At present, it is important that re-establishment of whitefish natural production be accompanied by the culture in intensive aquasystems. Establishment of whitefish production in recirculating aquaculture systems (RAS) requires identification of optimal larviculture conditions, including water physicochemical parameters, stocking density, nutrition, and feeding regimens (Goddard, 1996).

It is standard practice to wean from live food to a commercial formulated diet. Brine shrimp *Artemia salina* (L.) nauplii comprise approximately 40% of the live feed used in aquaculture

and are particularly suitable for hatchery operations, as they can be stored over long time periods and are readily available when needed (Lavens & Sorgeloos, 2000). Feeding on *Artemia* is essential to many fish species, for instance in the early development of lake whitefish *Coregonus clupeaformis* (Mitchill, 1818) (Harris, 1992). Alternatively, commercial dry feed only can be used for the first exogenous feeding of coregonids (Enz et al., 2001; Leithner & Wanzenbock, 2015), usually with nutritional supplementation, for instance, with propionic acid, as the case with maraena whitefish (Lahnsteiner & Kletzl, 2015). In general, it is known that feeding can influence welfare and fish health. Fish health, which is closely tied to ontogenesis, is a crucial factor in profitable aquaculture. Organ function is associated with feeding, with the intestine and liver directly related to digestion and nutrition utilization. High fat diets may lead to increased fat deposits in fish (Lee, Jeon & Lee, 2002), abnormal oxidative status (Rueda-Jasso et al., 2004), and metabolic alterations including fatty liver (Dos Santos, Burkow, & Jobling, 1993) as well as deterioration of nutritional value and impairment of organoleptic and physical properties (Gjedrem, 1997). The fatty liver constitutes wasted energy, as there is little point in supplying an energy-yielding nutrient that is simply deposited and stored unused in adipose tissue (Hansen et al., 2008).

Research into effects of diet and feeding regimen on intestine and liver development of coregonids is scarce. This investigation aimed to identify feeding strategies optimal for survival, growth, intestine development and liver status of maraena whitefish larvae.

MATERIALS AND METHODS

Eggs and larvae

Maraena whitefish broodstock were obtained from lagoons in Szczecin in the River Odra, north-western Poland. Gametes of 35 female and 35 male naturally spawning (no hormone stimulation) maraena whitefish were stripped manually by commercial fishermen in December 2016 shortly after capture and transported to local hatcheries for fertilization and incubation. Eggs (100 mg) were fertilised with 0.5 mL 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 = ~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, ~750 000 eggs were incubated. After 60 days, eggs were transferred to a second set of 8 L Zug jars and incubated at 8–9°C to accelerate development and hatching. After 5 days, temperature was increased to 10°C for mass hatching. Hatching success was estimated at 90%, and ~675 000 larvae were available for the experiment. Hatched larvae swam across to a tank (total volume 1 m^3) underlain with 0.2 mm mesh. Larvae (2 dph) were transferred to tanks in the RAS.

Experimental system and rearing conditions

Seven groups of larvae in three repetitions were transferred to the experimental aquasystem consisting of twenty-one 2L aquaria, 96 × 154 × 200 mm. Two-hundred-fifty larvae (initial mass, 7.4 ± 0.1 mg, mean ± SEM; initial total length, 13.0 ± 0.1 mm) were placed into each aquarium. A total of 5250 larvae were used in the experiment. The experiment lasted 30 days.

The oxygen level, water temperature, and pH were checked daily at 8.00 and 16.00. The pH range was monitored using an OXYGUARD H04PP Handy pH meter (OXYGUARD International, Denmark). The initial temperature without supplemental heat was 10°C. Temperature was elevated to 15°C by 24 hours, 19°C at 48 hours, and maintained at ~19°C by a HAILEA HC-1000A cooler (China). Oxygenation was maintained using two SICCE Syncra 5.0 pumps

(5000 L/h) (Italy). Temperature and oxygenation were monitored using probes connected to central electronic software OXYGUARD Pacific Insatech A/S (Denmark). Ammonia, nitrate, and nitrite concentrations were analysed using HACH, LCK 304, LCK 339, and LCK 341 (Germany) with a HACH DR5000 spectrophotometer (Germany). Disinfection used a 30 W UV MCT Transformatoren GmbH steriliser (Germany). Sodium chloride 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_2 level was maintained via aeration and keeping alkalinity stable. During the 30 day trial, basic physico-chemical parameters were temperature = 19.1 ± 0.0°C, pH = 8.7 ± 0.0, O_2 saturation = 85.8 ± 0.9%, O_2 concentration = 7.9 ± 0.1 mg/L, NH_3^+ = 0.1 ± 0.0 mg/L, NO_2 = 0.8 ± 0.1 mg/L, NO_3 = 21.2 ± 5.4 mg/L.

Feeding

Larvae were fed to excess during the light phase (8.30 to 17.30). Group AF fish were fed artificial feed (SKRETTING PERLA LARVA PROACTIVE 4.0, Nutreco, Netherlands), ground to suitable sized particles, 0.1 and 0.2 mm. The live feed (LF) group of larvae were fed fresh *Artemia* metanauplii (Ocean Nutrition, HE > 230 000 NPG, Belgium) (20-24 h old, 0.4-0.5 mm) at 10 mL of *Artemia* homogenous suspension/tank at three-hour intervals, four times per day. Composition of commercial diet and *Artemia* is provided in Table 1.

The experimental feeding regime was as follows: AF – artificial feed during the 30 days; LF – live feed provided during the 30 days; FW5 – first weaning from live food to artificial diet after 5 days; FW10 – first weaning from live food to artificial diet after 10 days; FW15 – first weaning from live food to artificial diet after 15 days; FW20 – first weaning from live food to artificial diet after 20 days; FW25 – first weaning from live food to artificial diet after 25 days (Table 2). The type of diet determined the feeding schedule. Live feed was supplied only once during a three-hour feeding period, whereas AF was provided seven times per hour during the feeding intervals (Table 3). This feeding practise was based on the character of the diet, as *Artemia* metanauplii, with its swimming ability, colour, and enzyme secretions acting as visual and chemical stimuli, extend feeding activity. On the contrary, artificial food has limited attraction and it is advised to present it more frequently.

Table 1. *Nutritional composition of Skretting feed and Artemia (manufacturer's data) used for intensive culture of maraena whitefish larvae Coregonus maraena in a 30day trial.*

Skretting			***Artemia***		
Fish mass	g	0.007–0.1	Fish size	g	0.007–0.1
Age	dph	2–31	Age	dph	2-31
Particle size	mm	0.1–0.2	*Artemia* size	NPG	HE > 230 000
Crude proteins	g/kg	620	Crude proteins	g/kg	540
Crude lipids	g/kg	110	Crude lipids	g/kg	110
Crude ash	g/kg	90	Crude ash	g/kg	50
Crude cellulose	g/kg	11	Moisture	g/kg	80
Vit A	IU/kg	672			
Vit D3	IU/kg	671			
Na	g/kg	8			
Ca	g/kg	22			
P	g/kg	17			
$MnSO_4 \times H_2O$	mg/kg	69.3			
$FeSO_4 \times H_2O$	mg/kg	182.4			
$ZnSO_4 \times H_2O$	mg/kg	369.8			
$CuSO_4 \times 5H_2O$	mg/kg	29.5			
KI	mg/kg	3.9			

Table 2. *Feeding strategy of maraena whitefish larvae Coregonus maraena in a 30 day trial.*

Group	**Days**						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Artificial feed	*+	*+	*+	*+	*+	*+	*+
Live feed	+	+	+	+	+	+	+
FW5			*+	*+	*+	*+	*+
FW10				*+	*+	*+	*+
FW15					*+	*+	*+
FW20						*+	*+
FW25							*+

First weaning (FW) from live to artificial diet at 5 days (FW5), 10 days (FW10), 15 days (FW15), 20 days (FW20), 25 days (FW25). Light grey blocks with * indicate application of artificial diet, dark grey blocks indicate live diet. The plus (+) sign indicates histological analysis conducted.

Table 3. *Feeding frequency of live feed and artificial feed applied to maraena whitefish larvae Coregonus maraena in a 30 day trial.*

Diet type	Time (feeding frequency)			
Live *	8:30 (1)	11:30 (1)	14:30 (1)	17:30 (1)
	Day time and (feeding frequency)			
Artificial **	8:30-9:30 (7)	11:00-12:00 (7)	14:00-15:00 (7)	16:30-17:30 (7)

*10 mL of *Artemia*/tank; **feeding *ad libitum*/tank consistent in each tank

Sampling and measuring

The survival rate (SR), size heterogeneity (SH), larval yield (LY), and condition factor (K), was assessed as follows:

SR (%) = 100 × (Nf/Ni)

in which Ni and Nf = initial and final number of larvae, respectively.

LY (g/group) = ((Ni/100) × SR) × mass

with SR and mass = % surviving and mean mass (g) of larva groups.

SH (%) = 100 × (SD/W_m)

in which SH = size heterogeneity; SD = mean standard deviation of mass of 10 randomly selected larvae/tank; W_m = mean mass (mg) of 10 larvae/tank.

K = 100 000 × $W/(TL^3)$

in which W = mean mass (g) of 10 larvae/tank; TL = mean total length (mm) of 10 larvae/tank.

Ten larvae from each tank (30 of each group) were anaesthetized (Propiscin - 0.4 ml/L; IRS, Poland), weighed on a digital microbalance (ABJ 220-4M KERN, Germany), and measured manually from images taken with Leica MZ16 A stereomicroscope and a digital colour camera with 5 Mp resolution for Leica DFW420 image analysis. Total length (TL, ± 0.01 mm) and body mass (BW, ± 0.1 mg) were measured on days 0, 5, 10, 15, 20, 25, and 30 of rearing.

A sample size of 10 larvae/tank and 30 larvae/treatment was also used by Laczynska et al. (2016); Celada, Aguilera, Carral, Saez-Royuela & Melendre (2008); Fletcher et al. (2007); Kaiser, Endemann & Paulet (2003); Mahmood, Ali & Anwar-ul-Haque (2004); Nowosad et al. (2013); and Palinska-Zarska et al. (2014).

Histology

Five larvae from each group were sampled for histology on days 0, 5, 10, 15, 20, 25, 30. Whole larvae were fixed in Bouin's fluid for 24 to 48 h depending on size. The fixed material was washed in an ethanol series (75, 80, 90, 95%), acetone, xylene, and liquid paraffin at 54°C. The obtained material was embedded in paraffin blocks and cut into 6 µm sections on a rotating microtome (Leica RM 2155), and sections were placed onto protein-coated slides. The preparations were made with Mayer's haematoxylin and eosin (H&E) (Baginski, 1965). Subsequently, the stained preparations were sealed in Histokitt mounting medium (Glaswarenfabrik Karl Hecht GmbH & Co KG, Germany). After drying, the preparations were analysed microscopically (Axio Scope A1, Zeiss, Germany) with AxioVs40 v. 4.8. 2.0 software (Carl Zeiss MicroImaging GmbH, Germany).

Five larvae from each group were photographed, and intestine diameter, villi length, villi thickness, hepatocyte nucleus diameter, and hepatocyte diameter were measured. The measurements were compared among groups on days 5, 10, 15, and 20. At the completion of trial, presence of intestine and liver pathology was assessed and compared using criteria of McFadzen *et al.* (1997) to categorise liver condition. Each specimen was assigned a grade (1–3), a healthy specimen scoring 1 to degraded liver scoring 3 (Table 4). Intestine degradation score was evaluated and each fish was assigned a grade (-, +, ++, +++), a healthy fish scoring '-' to severe degradation '+++' (Table 5).

Table 4. *Classification of liver and intestine degradation of maraena whitefish Coregonus maraena larvae in a 30 day growing trial.*

	Grade		
Tissue part	**1. Healthy**	**2. Intermediate**	**3. Degraded**
Liver nuclei	Nuclei lightly granular, small and indistinct	Nuclei with abundant dark granules; nucleoli	Nuclei small dark and pyknotic
Liver hepatocyte cytoplasm	Structured: varied texture, scattered granules with eosin positive patches	Homogenous, granular, slight variability in staining property	Hyaline, lacking texture, dark small and often separated from the cell boundary
Intestine mucosa	Enterocytes intact, villi with deep, longitudinal folds, cytoplasm homogenous, no vacuolation, microvilli intact	Separation of enterocytes in basal region, coarse dark cytoplasm, frequent areas of microvilli degeneration	Enterocytes small dark and separated, extensive intercellular cells may be present, microvilli often indistinct

Adapted from McFadzen *et al*. (1997)

Statistical analysis

The data are presented as mean ± standard error of mean (SEM). Statistical analyses were conducted using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). The effects of feeding regime on body mass (BW), total length (TL), survival rate (SR), larval yield (LY), size heterogeneity (SH), condition factor (K), intestine diameter (ID), villi length (VL), villi thickness (VT), hepatocyte nucleus diameter (ND), hepatocyte diameter (HD), and intestine degradation score (IDS) were analysed by one-way ANOVA with feeding as fixed variables. The level of significance used for all tests was P = 0.05 (Zar, 1999). Prior to ANOVA, survival percentages were arcsin-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. Tukey's test was used for identifying significant differences among groups.

Table 6. *One-way ANOVA results for feeding strategy on total length, body mass, size heterogeneity, condition factor, larva yield, and survival rate of maraena whitefish Coregonus maraena larvae.*

Parameters	**Source of variation**	**SS**	**DF**	**F**	**MS**	***P***
Total length	FS	196	6	33	9	0.00
Body mass	FS	57144	6	9524	8	0.00
Size heterogeneity	FS	168	6	28	1	0.32
Condition factor	FS	0	6	0	2	0.13
Larva yield	FS	127	6	21	7	0.00
Survival rate	FS	42	6	7	0	0.66

SS, sum of square; DF, degrees of freedom; F = distribution fitting; MS, mean square, *P* = probability; Factor parameter: FS, feeding strategy

RESULTS

Growth performance, survival, size heterogeneity, condition factor, yield

At the conclusion of the trial, the highest BW (171.4 ± 8.9 mg), TL (32.2 ± 0.3 mm), LY (23.1 ± 1.24 g/tank), SH (28.4 ± 2.0%), and K (0.5 ± 0.01) was observed in the FW15 group (Table 7). Body mass, TL, and LY differed significantly ($P < 0.05$) among some groups, while no significant differences were observed in SH, K, and SR (Table 6). Significantly higher BW ($P < 0.05$) was observed in FW15 compared to LF, AF, FW5, and FW10 and in FW20 compared to the AF and FW5 groups. Significantly higher TL ($P < 0.05$) was observed in FW15 and FW20 in comparison with the LF, AF, FW5, and FW10 groups. The LF group exhibited the highest growth/mass in the first 20 days of rearing, and, in contrast, AF showed poorest results over the course of the trial. Body mass and TL increments in 5 day periods are shown in Fig. 2 and 3. Significantly higher LY ($P < 0.05$) was obtained in FW15 compared to LF, AF, FW5, and FW10 and in FW20 compared to AF (Fig. 4).

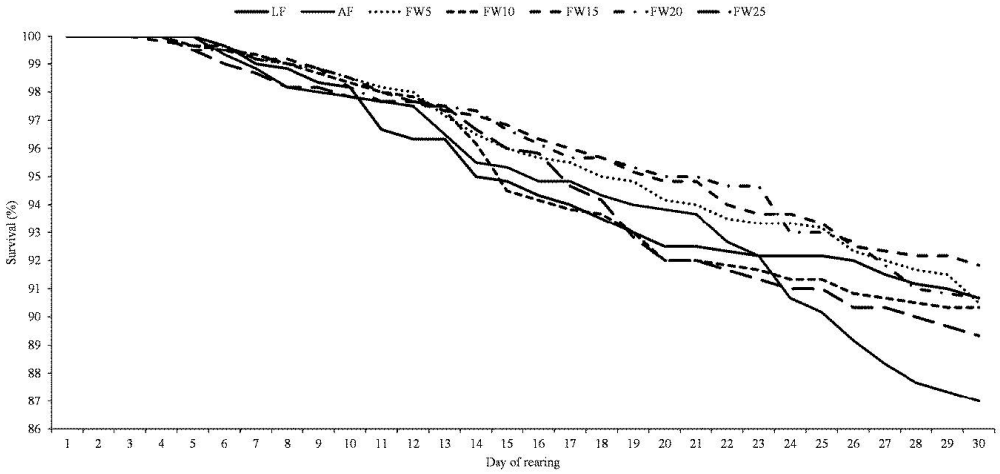

Figure 1. *Cumulative survival of maraena whitefish larvae Coregonus maraena (Bloch 1779) in a 30-day feeding trial. Line is mean of triplicate trials (n=3).*

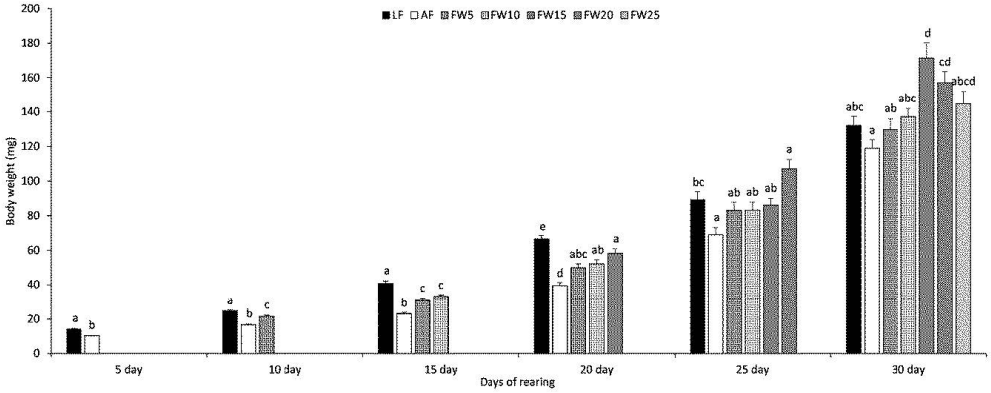

Figure 2. *Mean body mass (mg) of maraena whitefish Coregonus maraena (Bloch 1779) at 5-day intervals during the 30-day feeding regimen trial. Different letters indicate significant differences ($P < 0.05$). Bars represent means, and whiskers indicate standard error of mean (SEM) of three replicates (n = 3).*

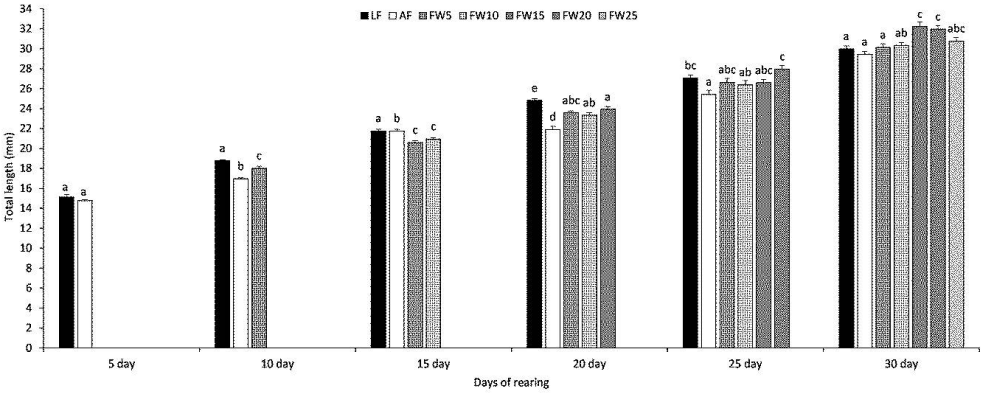

Figure 3. *Mean total length (mm) of maraena whitefish Coregonus maraena (Bloch 1779) measured at 5-day intervals during the during the 30-day feeding regimen trial. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM of three replicates (n = 3).*

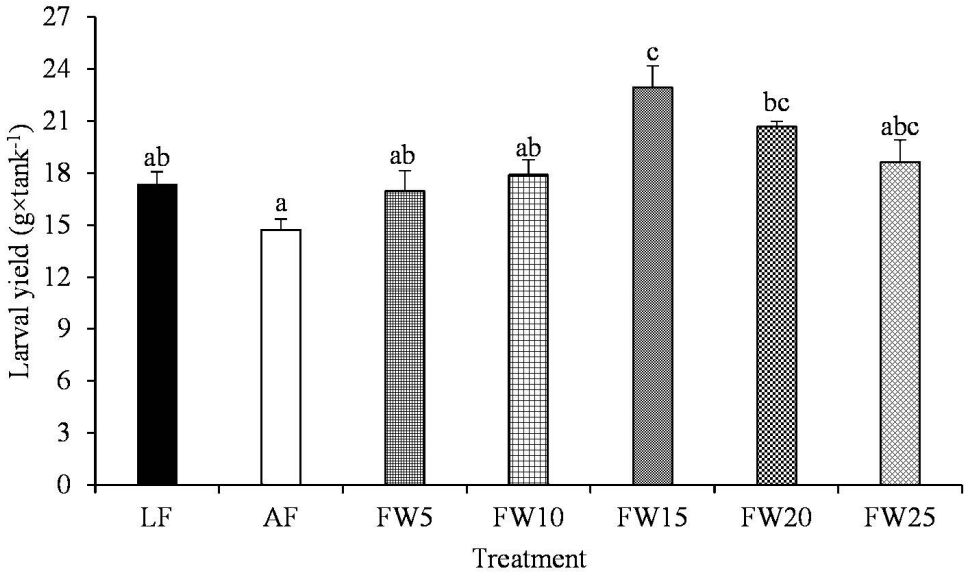

Figure 4. *Mean larval yield (g/tank) in maraena whitefish Coregonus maraena (Bloch 1779) among tested feeding strategies. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM of three replicates (n = 3).*

Table 7. *Effects of feeding strategy on growth and survival of maraena whitefish Coregonus maraena larvae in a 30 day growing trial. Table indicates mean ± S.E.M. Parameters with no significant differences (P > 0.05) among groups.*

Group	FSH (%)	K	SR (%)
Live diet	22.5 ± 1.0	0.5 ± 0.01	90.5 ± 0.3
Artificial diet	22.7 ± 3.1	0.5 ± 0.02	85.8 ± 1.1
FW5	23.9 ± 2.5	0.5 ± 0.01	90.0 ± 2.1
FW10	18.4 ± 1.5	0.5 ± 0.01	90.2 ± 0.4
FW15	28.4 ± 2.0	0.5 ± 0.01	90.8 ± 2.0
FW20	23.0 ± 1.2	0.5 ± 0.01	89.7 ± 1.0
FW25	25.7 ± 5.0	0.5 ± 0.01	88.2 ± 2.9

FSH = final size heterogeneity; K = condition factor; SR = survival rate
First weaning (FW) from live diet to a commercial diet at 5 days (FW5), 10 days (FW10), 15 days (FW15), 20 days (FW20), 25 days (FW25).

Intestine

Significantly smaller intestine diameter ($P < 0.05$) was observed in AF compared to LF and FW5 on day 10, and in AF compared to LF, FW5, and FW10 on day 15 (Fig. 5). Significantly higher villi length ($P < 0.05$) was observed in LF compared to AF, FW5, and FW10 and in FW5 and FW10 compared to AF on day 15 (Fig. 6). Significantly higher villi thickness ($P < 0.05$) was observed in LF compared to AF and FW15 (day 20) (Fig. 7). Over the 30-day trial, the LF, FW5, FW10, and FW15 groups exhibited grade 1 intestine degeneration. The AF group showed grade 3 intestine degeneration (Table 5, Fig. 8).

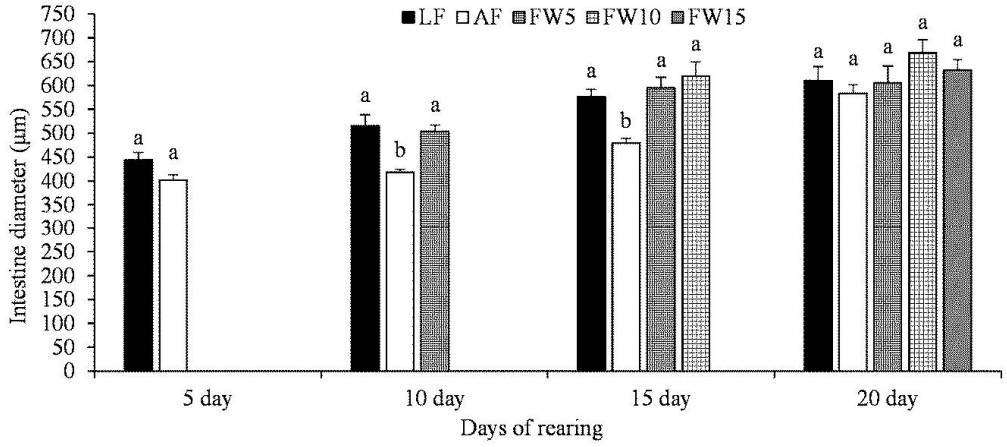

Figure 5. *Mean intestine diameter (µm) in maraena whitefish Coregonus maraena (Bloch 1779) measured at 5-day intervals during the 30-day feeding trial. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM (n = 3).*

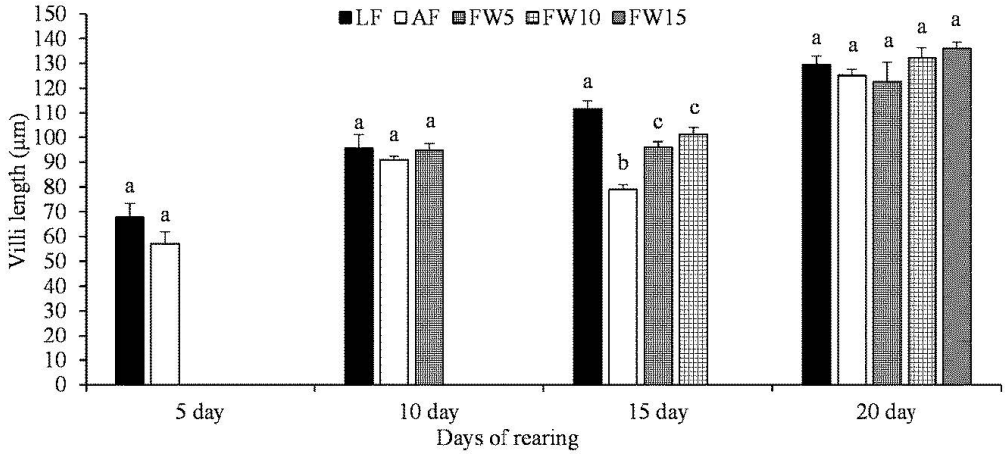

Figure 6. *Mean villi length (µm) in maraena whitefish Coregonus maraena (Bloch 1779) measured at 5-day intervals during the 30-day feeding trial. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM (n = 3).*

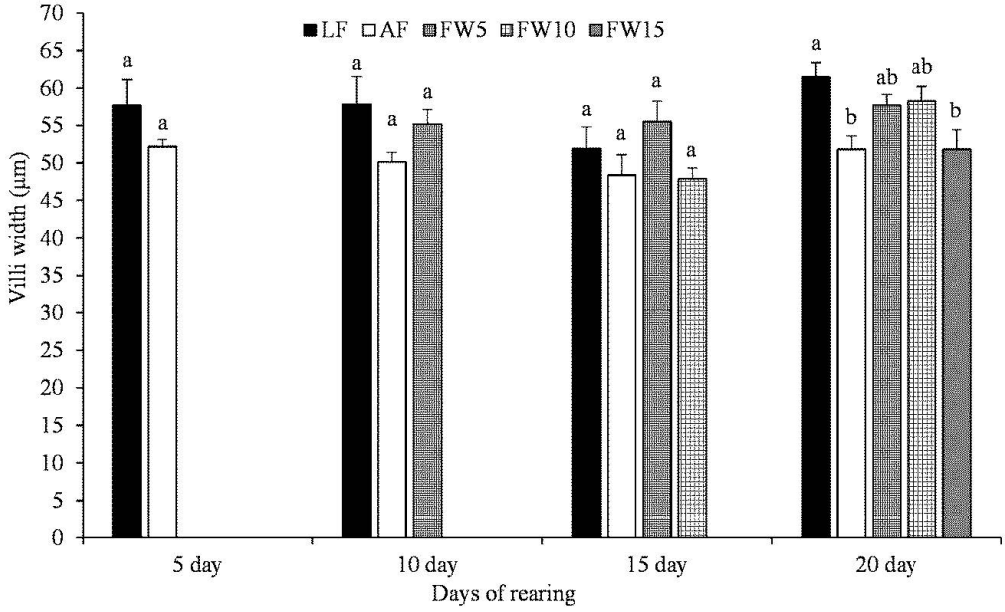

Figure 7. *Mean villi width (µm) in maraena whitefish Coregonus maraena (Bloch 1779) measured at 5-day intervals during the 30-day feeding trial. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM (n = 3).*

Liver

Significantly higher hepatocyte nucleus diameter was observed in AF compared to FW5 on day 10 and in LF compared to FW10 on day 20 (Fig. 9). Significantly greater hepatocyte diameter was observed in LF compared to AF on day 5, and in AF compared to LF, FW5, FW10 and FW15 on day 20 (Fig. 10). Over the 30-day trial, the liver degradation score was grade 1 in all groups (Table 4).

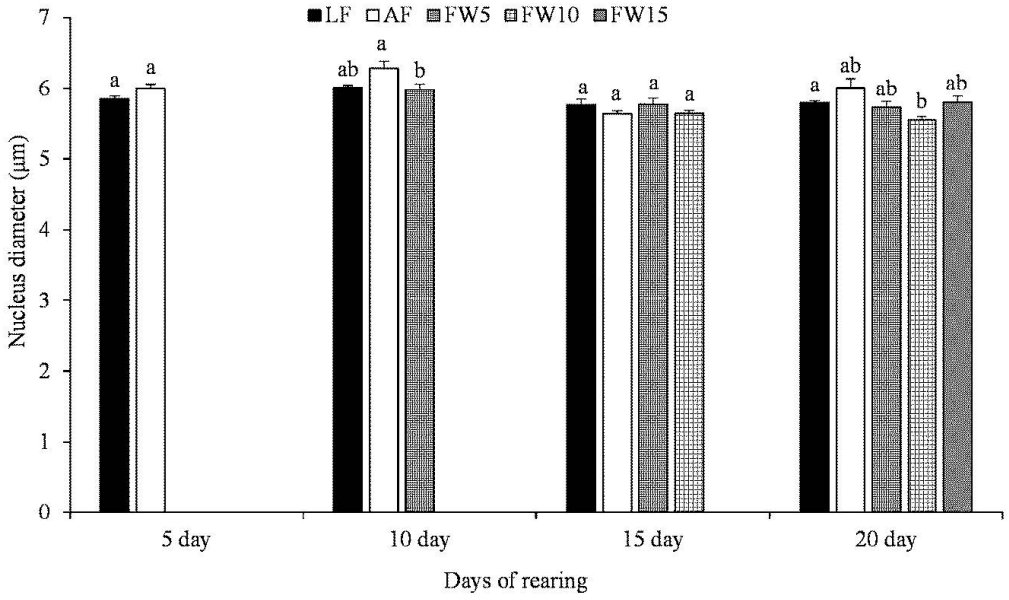

Figure 9. *Mean nucleus diameter (µm) in maraena whitefish Coregonus maraena (Bloch 1779) measured at 5-day intervals during the 30-day feeding trial. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM (n = 3).*

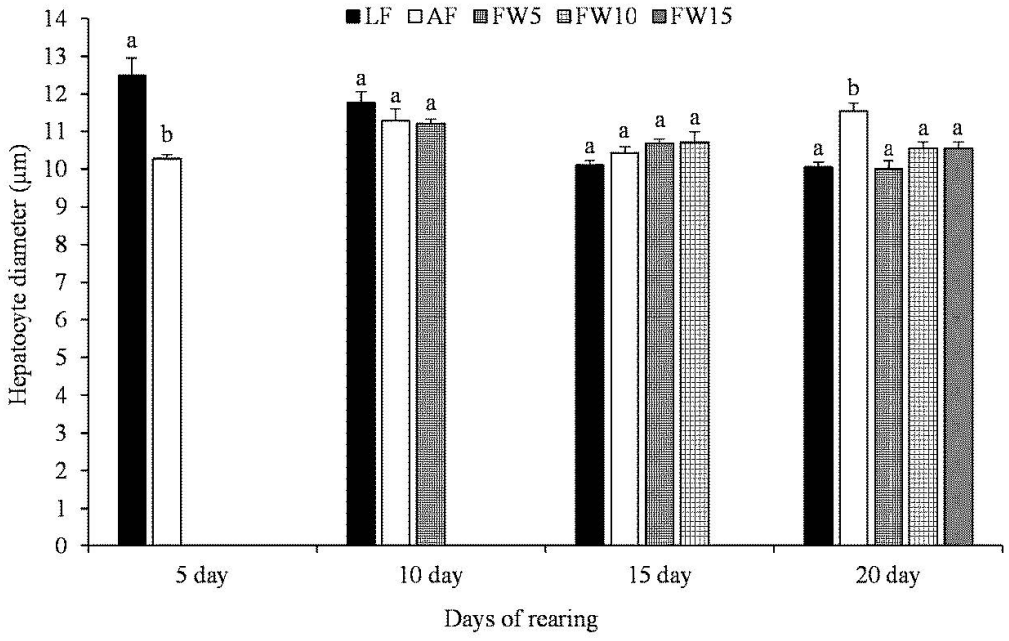

Figure 10. *Mean hepatocyte diameter (µm) in maraena whitefish Coregonus maraena (Bloch 1779) measured at 5-day intervals during the 30-day feeding trial. Different letters indicate significant differences (P < 0.05). Bars represent means and whiskers indicate SEM (n = 3).*

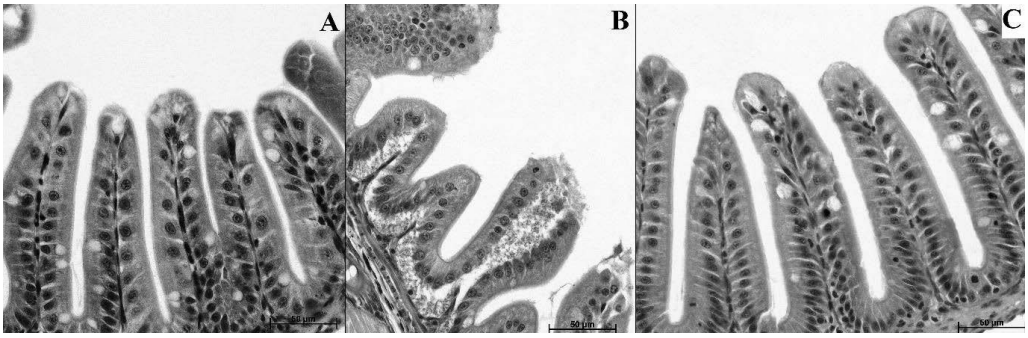

***Figure 8**. Definitions of intestine degradation score in maraena whitefish Coregonus maraena (Bloch 1779) larvae in 30-day feeding trial. A = LF, B = AF, C = FW5.*

***Table 5.** Classification of degradation and histomorphometry of intestine of maraena whitefish Coregonus maraena larvae in a 30 day trial. Histomorphometry parameters indicate mean ± S.E.M. (n = 3). Different letters indicate significant differences (P < 0.05). Degradation score starts with – (none) and ends with +++ (severe)*

Lesion	Groups						
	LF	AF	FW5	FW10	FW15	FW20	FW25
Hyperplasia of mucosa	+	+	++	++	+	+	+
Villus oedema	-	+++	++	+	+	+	-
Exfoliation of intestine epithelium	+	++	++	+	+	+	-
Intestine diameter (μm)	629.3 ± 18.30	690.1 ± 23.24	717.6 ± 32.42	744.9 ± 58.31	686.7 ± 82.21	646.7 ± 11.92	672.0 ±22.46
Length of villi (μm)	148.4 ± 1.83^{ab}	133.9 ± 4.10^{a}	151.4 ± 5.21^{ab}	136.2 ± 5.53^{a}	163.5 ± 9.48^{ab}	152.9 ± 9.71^{ab}	176.6 ± 9.03^{b}
Width of villi (μm)	54.5 ± 2.42	52.4 ± 3.01	58.2 ± 1.79	56.5 ± 3.04	54.5 ± 1.12	51.1 ± 3.39	53.1 ± 0.74
Intestine injury score	0.18 ± 0.05^{a}	2.03 ± 0.41^{b}	0.49 ± 0.05^{a}	0.22 ± 0.05^{a}	0.18 ± 0.04^{a}	0.17 ± 0.03^{a}	0.06 ± 0.02^{a}

- none, + mild, ++ moderate, +++ severe
First weaning (FW) from live diet to a commercial diet at 5 days (FW5), 10 days (FW10), 15 days (FW15), 20 days (FW20), 25 days (FW25).

DISCUSSION

Growth and survival

Larva survival and growth is affected by starter feed, which must satisfy nutritional needs immediately after depletion of the yolk sac (Puvanendran & Brown, 1999). Therefore optimal feed composition and feeding regime to decrease larva mortality and ensure growth are of critical importance (Lee, 2003). The timing of weaning is considered to be the most important factor in successful larval feeding in peled *Coregonus peled* (Gmelin) (Stejskal et al., 2017), pikeperch *Sander lucioperca* (L.) (Hamza, Mhetli, & Kestemont, 2007), totoaba *Totoaba macdonaldi* (Gilbert) (Mata-Sotres, Lazo, & Baron-Sevilla, 2015), burbot *Lota lota* (L.) (Palinska-Zarska et al., 2014), golden pompano *Trachinotus ovatus* (L.) (Ma et al., 2015), and butter catfish *Ompok bimaculatus* (Bloch) (Pradhan, Jena, Mitra, Sood, & Gisbert, 2014).

We found no significant ($P < 0.05$) differences in SR, SH, and K among the feeding treatments. The SR of larvae fed the commercial diet was lower than in the other treatments but not significantly. This was also observed by Mahmoudzadeh, Ahmadi & Shamsaei (2009), who reported that larvae fed dry feed showed comparable SR to those fed a live and a live/artificial mixed diet during the first four weeks. At the end of our trial, significantly higher larva TL, BW, and LY ($P < 0.05$) was observed in FW15 and FW20 compared to other treatments. The LF group showed the greatest length and mass growth during the first 20 days of the trial, and AF produced inferior results throughout the trial. Our results are similar to those of Bochert, Horn & Luft (2017) at thirty days, who reported enhanced growth in European whitefish larvae fed with live *Artemia* nauplii at first feeding: fish fed *Artemia* at 6–16 dph and artificial feed 17–42 dph displayed the highest TL and BW from 7 day to day 42. Hundt et al. (2015) confirmed the highest growth in European whitefish larvae fed with *Artemia* compared to dry diet or live nematodes at 17 dph. Stejskal et al. (2017) observed the lowest body mass with artificial feed in all weekly increments from 7–35 dph, with live feed producing the highest body mass values except at 28 and 35 dph.

Histology

Interaction of intestine and liver function is assumed to be a key factor for growth and welfare of farmed fish. Histological examination revealed the most severe intestine degradation (grade 3) (Table 5) in the AF group, corresponding to the lowest intestine diameter, villi length, and villi thickness, as well as the lowest growth, survival, and larval yield in this group. The intestine diameter, as well as villi length and thickness, displayed a trend to higher values with LF and later weaning time, producing higher growth and survival in these groups. This fact may be attributed to the digestive enzymes obtained from live food. However, it remains to be clarified whether the digestibility of dry diets is comparable to that of live diets.

In our investigation, none of the experimental groups presented evidence of liver pathology (Table 4), and all showed a level of fat deposit within the normal range. Escaffre and Bergot (1986), in a study of rainbow trout *Oncorhynchus mykiss* (Walbaum) reported that the diameter of hepatocyte nuclei reflects the nutritional status of the fish. Segner et al. (1988) stated that European whitefish *Coregonus lararetus* (L.) larvae fed on zooplankton exhibited the largest nuclei; with the hepatocyte nuclei of larvae reared on dry diets being significantly smaller. In our study, hepatocyte nucleus diameter was similar in tested groups, and no significant differences ($P > 0.05$) were found in LF compared to AF. On the other hand, live food may stimulate liver metabolic activity, in particular protein metabolism, which enhances growth increments of maraena whitefish larvae. This was observed by Segner et al. (1988) who reported that an artificial diet did not meet the nutritional requirements of European whitefish larvae or trigger intensive hepatic metabolism, which was projected to result in lower growth compared to live feed.

This 30-day investigation showed initial weaning from live feed to artificial diet after 15 days (FW15) to be the optimal feeding strategy, with beneficial effects on maraena whitefish larva growth, mass, and yield. Efficacy of other tested feeding regimes can be ranked FW20>FW25>FW10>LF>FW5>AF. Live feed and appropriate weaning time to artificial diet was beneficial for intestine development, while artificial feed was associated with severe intestine impairment. The assessed feeding strategies were not related to liver pathology in any group.

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CHAPTER 6

COMBINED EFFECT OF WEANING TIME AND CO-FEEDING DURATION ON GROWTH AND SURVIVAL OF PELED *COREGONUS PELED* (GMELIN) LARVAE

Stejskal, V., Matousek, J., Prokesova, M., Podhorec, P., Sebesta, R., Drozd, B., 2017. Combined effect of weaning time and co-feeding duration on growth and survival of peled *Coregonus peled* (Gmelin) larvae. Aquaculture Nutrition 24, 434–441.

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Combined effect of weaning time and co-feeding duration on growth and survival of peled *Coregonus peled* (Gmelin) larvae

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Abstract

The study investigated the combined effect of weaning from live feed to a commercial dry pellet at 10, 15, 20, 25 or 30 days posthatching (dph) and co-feeding for 1, 3 or 6 days on survival and growth of *Coregonus peled* larvae. Additional groups fed only live *Artemia* sp. nauplii (ART), and only Biomar LARVIVA ProWean 100 (DRY) were included. A final survival rate of 66.4%–85.5% was observed in groups weaned after 20 dph. Final body weight (BW) and total length (TL) were significantly lower in groups weaned at 10 and 15 dph, regardless of the duration of co-feeding. Larvae reached 29–37 mg BW and TL of 17.7–19.0 mm in groups weaned at 20, 25 and 30 dph. The recommended minimum duration of feeding with live food, based on these results, is 20 days. Based on the significantly higher yield of larvae weaned after 20 dph irrespective of co-feeding duration, it can be concluded that abrupt weaning to dry food after 20 days of feeding with live prey can provide adequate production while reducing the effort and costs associated with live feed.

KEYWORDS
co-feeding, feeding cost, formulated diets, larviculture, macronutrient analysis, whitefish

1 | INTRODUCTION

Peled *Coregonus peled* (Gmelin 1789) has the potential for inland freshwater aquaculture, particularly in local markets in central and east Europe (Turkowski 1999; Mukhachev & Gunin, 1999). Its rapid growth and palatable flesh make it a strong candidate for diversification of intensively cultured fish species. As peled is a candidate for intensive aquaculture, there is some information about its intensive culture in different systems (Furgala-Selezniow, Mamcarz, & Skrzypczak, 2005; Mamcarz & Szczerbowski, 1984; Matousek, Prokesova, Stejskal, & Kouril, 2014; Stejskal et al., 2013).

Rearing of peled in recirculating aquaculture systems is a recent innovation, and optimization is necessary to standardize aspects of culture, including weaning of larvae from live feed to artificial diet (Matousek et al., 2014; Stejskal et al., 2013); however, there are no standard rearing protocols for this species. Some previous studies reported survival in range from 20% to 80% in protocols using different dry feed (Dabrowski, Takashima, Strüssmann, & Yamazaki, 1986; Luczynski, Majkowski, Berdega, & Dabrowski, 1986).

Peled is naturally fed mainly on copepods, cladocerans and rotifers (Furgala-Selezniow et al., 2005). Combined feeding of live prey, chiefly *Artemia* sp, and commercially formulated feed, referred to as co-feeding, is a strategy used to enhance larval performance beyond that obtained by feeding either type of feed alone (Canavate & Díaz, 1999; Chepkirui-Boit et al., 2011; Rónyai & Feledi, 2013; Rosenlund, Stoss, & Talbot, 1997; Van, Dierckens, & Nguyen, 2010). Co-feeding is intended to improve larvae nutrition and condition to more readily accept commercial diets when live feed is withdrawn. Several weaning protocols implemented with a co-feeding period have proved successful in significantly reducing time to complete weaning in some marine fish like totoaba *Totoaba macdonaldi* (Mata-Sotres, Lazo, & Baron-Sevilla, 2015), Atlantic cod *Gadus morhua* (Baskerville-Bridges & Kling, 2000), *Trachiotus ovatus* (Ma et al., 2015) or freshwater species like barramundi *Lates calcarifer* (Curnow, King, Partridge, & Kolkovski, 2006), butter catfish *Ompok bimaculatus* (Pradhan, Jena, Mitra, Sood, & Gisbert, 2014) and Chinese longsnout catfish *Leiocassis longirostris* (Liu et al., 2012). Moreover, in some species co-feeding techniques lead to improved growth and survival (Wilcox, Tracy, & Marcus, 2006).

Quality of fish fry cultured is assessed using different approaches differing among authors. The traditional methods are based on reached morphometrical and gravimetrical parameters combined with survival rate (Palinska-Zarska et al., 2014); more advanced ones are grounded either on stress resistance ability or evaluation of biochemical indicators such as fatty acids (Abi-ayad, Boutiba, Melard, & Kestemont, 2004; Hamza, Mhetli, & Kestemont, 2007) and/or macronutrients (Kamler et al., 1994; Kamler, Keckeis & Bauer-Nemeschkal 1998; Kamiński, Kamler, Korwin-Kossakowski, Myszkowski, & Volnicki, 2006) compositions.

The aim of this study was to investigate the effects of weaning age and duration of co-feeding on survival, growth, quality and yield of peled larvae.

2 | MATERIALS AND METHODS

2.1 | Fish

Newly hatched peled larvae (n = 18,900) obtained from captive broodstock (hatchery Rybářství Kinský s.r.o.) were randomly allocated to fifty-one 3.5 L at a density of 100 larvae per L. The initial total length, standard length, body height and body weight (BW) of the larvae (mean ± *SD*) were 9.7 ± 0.6 mm, 9.2 ± 0.6 mm, 0.9 ± 0.2 mm and 3.1 ± 0.5 mg, respectively. The recirculating system (1,800 L total water volume) included fifty-one aquaria and a tank with series of filtration sections (total biofilter volume 900 L), a settling tank (300 L water volume) and a UV treatment unit, which was incorporated into the direct recirculation flow. The glass aquaria have dimensions 120 × 135 × 215 mm, flat bottom and overflow with mesh size of 0.31 mm. The flow rate in each tank was approximately 4 L/hr with light aeration.

2.2 | Feeding

Fish were manually fed the commercial diet Larviva ProWean 100 (BioMar, France) and metanauplii of *Artemia* (24–32 hr old, 0.4–0.5 mm, 210,000 nauplii per g, Ocean Nutrition Europe, Belgium) twelve times during daylight, 8.00–20.00. Feeding levels were fixed at 0.3–0.5 g tank^{-1} day^{-1} for formulated feed and 500–700 *Artemia* sp. metanauplii fish^{-1} day^{-1}. The daily rations were based on previous experiment (unpublished data). Moreover, these rations were in slight excess as some of uneaten metanauplii as well as dry feed were observed in tanks at the end of day. Composition of commercial feed was crude protein 580 g/kg, crude lipid 120 g/kg, crude ash 111 g/kg, crude cellulose 5 g/kg, vitamin C 1,000 mg/kg, vitamin E 800 mg/kg, vitamin A 2.6 mg/kg, vitamin D3 0.044 mg/kg, phosphorus 16.4 g/kg and n-3 HUFA 25 g/kg (manufacturer data). Particle size was in the range of 80–200 μm.

2.3 | Culture conditions

Photoperiod was set at 12:12 hr (dark:light) with light intensity of 200–400 Lx at the surface. Dead larvae were removed and counted daily. Uneaten feed and faeces were siphoned out daily. Oxygen level (8.4 ± 0.5 mg/L), pH (7.3 ± 0.3), water temperature (14.5 ± 0.5°C) (HACH HQ 40) and larvae mortality monitored twice daily at 8.00 and 16.00. Rearing conditions were based on previous study on peled (Žil'ukas, Penaz, & Prokes, 1983). Ammonia, nitrate and nitrite concentrations were analysed by kits (HACH, LCK 304, LCK 339, LCK 341), using a HACH DR2800 Spectrophotometer. The concentration of nitrite-N, nitrate-N and ammonia-N was 0.041 ± 0.21, 5.70 ± 1.84, and 0.067 ± 0.035 mg/L, respectively.

2.4 | Experimental design

Fifteen experimental co-fed groups differing in weaning age (W), 10, 15, 20, 25 and 30 dph), and co-feeding duration (C), 1, 3 and 6, days were established. The groups combined five weaning times (10, 15, 20, 25 and 30 days posthatch) labelled W10, W15, W20, W25, W30 with three co-feeding durations (1, 3 and 6 days) labelled C1, C3, C6. Two additional groups of larvae were fed only live *Artemia* prey (ART) and dry commercial feed (DRY) as is shown in diagram (Figure 1). All groups were conducted in triplicate in a 35-day trial.

2.5 | Evaluation of larvae growth, survival and quality

At the beginning of the trial, 30 larvae and, at the end of the trial, all larvae were weighed and microscopically examined to assess development. Thirty larvae from each tank were randomly sampled at 7, 14, 21, 28 and 35 dph for growth evaluation. The anaesthetized (0.3 ml/L of clove oil) larvae were preserved in 10% buffered formalin until the biometric analysis was performed. The total length (TL, mm), standard length (SL, mm) and body height (mm) were measured within 0.1 mm using digital image analysis software MicroImage 4.0 (Olympus, Hamburg, Germany) (manual measurement mode), while wet body weight (BW, mg) of preserved larvae was measured using a digital balance (Pioneer, Ohaus Corporation, USA, d = 0.0001 g). Survival was assessed as follows:

$$\text{Survival}\,(\%) = 100 \times \text{Nf}\,(\text{Ni} - \text{Ns})^{-1}$$

where Ni and Nf = initial and final number of fish per tank, Ns = number of sampled fish per tank.

The overall success was based on larvae yield at 35 dph and was expressed as fish weight per unit of water volume (g/tank).

Fish fry quality was investigated using survival rate, reached morphometric and gravimetric parameters as well as macronutrients composition. Samples designated for chemical analyses collected at 35 dph 150 were stored for 180 days in 4% paraformaldehyde solution and then dissected, and body tissue excluding head and viscera was examined. Samples were dried to constant weight overnight at t = 105°C. The percentages of macronutrients carbon (C), hydrogen (H) and nitrogen (N) were determined using the CHNS-O element analyser (Flash 2000 organic elemental analyzer; Thermo Fisher Scientific Inc., Germany) with methionine as a reference, according to Kamler et al. (1994, 1998).

Experimentation was carried out in accordance the European Communities Council Directive of 24 November 1986 (86/609/EEC).

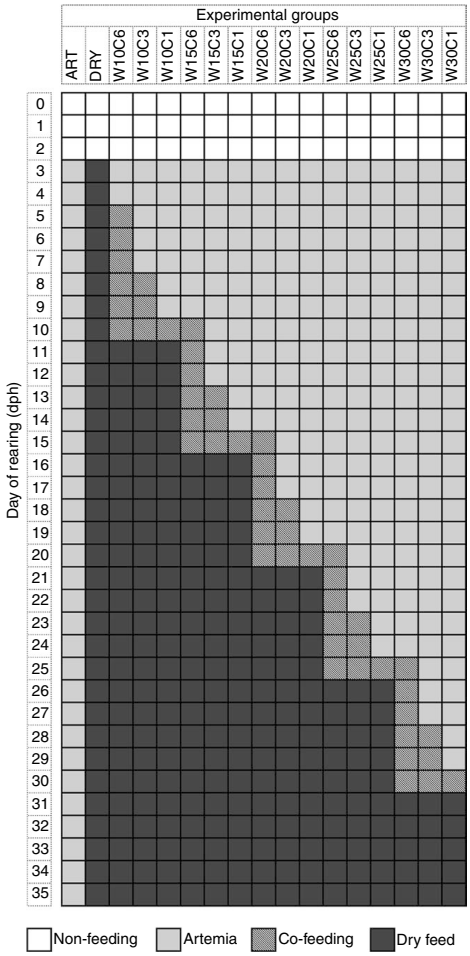

FIGURE 1 Experimental groups of *Coregonus peled* larvae and associated feeding protocols. Groups combined five weaning times (10, 15, 20, 25 and 30 days posthatch) labelled W10, W15, W20, W25, W30 with three co-feeding durations (1, 3 and 6 days) labelled C1, C3, C6. Two additional groups of larvae were fed only live *Artemia* prey (ART) and dry commercial feed (DRY). Groups were in triplicate

2.6 | Feed cost analysis

To determine the relative efficacy of weaning strategies used and their resulting growth benefit for peled larvae, the total feed cost for each tested group was calculated. The per kilogram cost (excluding labour and taxes) of both feeds used at the time of purchase (March 2014) from commercial retailers was as follows: EUR 34.1 per kg for Larviva ProWean 100 and EUR 115 per kg for *Artemia* sp. (210,000 nauplii per g).

2.7 | Statistical analysis

The effects of the weaning treatments on body weight and final total length, survival and larvae yield were analysed separately by two-way ANOVA with weaning age and co-feeding duration as fixed factors. The effects of the weaning treatments on hydrogen, carbon and nitrogen content were compared by Kruskal–Wallis non-parametric analysis with median test, and multiple pairwise comparisons by ranks was used. Differences were considered significant at $p < .05$. One-way ANOVA was used for comparison of groups with same weaning time with control groups (ART and DRY). Prior to ANOVA, survival percentage values and condition factor data were arcsine-transformed as well as length data were log-transformed. All data were tested for homogeneity of variance using Cochran, Hartley, Bartlett test. The data were expressed as mean ± *SD*, and statistical analyses were performed using STATISTICA 12.0 (Prague, Czech Republic).

3 | RESULTS

3.1 | Peled larvae growth

Both final larval BW and TL at the end of the experimental period were significantly affected by weaning age, co-feeding duration and their interaction (Table 1). Weaning age was the factor more important, explaining a large proportion of the variation of both variables: 82% and 79% for BW and TL, respectively. Thus, in treatments W10 and W15, mean final BW and TL of the larvae were lower in those co-fed for 6 days (C6W10 and C6W15) and higher in those co-fed for 1 day (C1W10 and C1W15) (Table 1 and Figure 2). However, only in the W10 group the co-fed larvae for 6 days had significantly lower weight than those co-fed for 1 day (Table 1). In both W10 and W15 groups, the co-fed larvae for 6 and 3 days had significantly lower body weight co-fed for 1 day (Figure 2). There were no significant difference in final BW between DRY group and groups W10 C6, W10C3, W15C6 and W15C3 (one-way ANOVA, Tukey test). All co-fed groups weaned after 20 dph showed BW similar to the ART group (one-way ANOVA, Tukey test).

3.2 | Survival of larvae

The final survival was also significantly affected by weaning age, co-feeding duration and their interaction (Table 2). The effect of co-feeding duration by reducing the survival in co-fed larvae for 6 days was dependent on the age of weaning, as indicated by a significant interaction between weaning age and co-feeding duration. Thus, in larvae early weaned at 10 and 15 dph (W10 and W15) the mean final survival was the lowest when co-fed for 6 days (C6W10 and C6W15), but only in W10 group the co-fed larvae for 6 days had survival rate significantly lower than those co-fed for 1 and 3 days (Figure 3). Survival was not significantly different between DRY group and groups W10C6, W10C3, W15C6, W15C3 and W15C1 (one-way ANOVA, Tukey test).

TABLE 1 Body weight (mg) of *Coregonus peled* larvae during experiment with different weaning times (W10, W15, W20, W25 and W30) and co-feeding durations (C1, C3 and C6)

Group	Co-feeding duration (days)	Weaning age (day)	7 dph	14 dph	21 dph	28 dph	35 dph
W10C6	6	10	4.3 ± 0.1 a	5.6 ± 0.5 ef	8.9 ± 1.3 e	10.6 ± 0.5 e	12.3 ± 2.0 f
W10C3	3	10	4.2 ± 0.1 a	4.5 ± 0.2 f	9.1 ± 1.4 e	11.3 ± 0.9 e	14.4 ± 1.5 ef
W10C1	1	10	4.3 ± 0.1 a	5.7 ± 1.2 df	11.1 ± 1.0 e	16.3 ± 1.9 de	22.5 ± 2.0 de
W15C6	6	15	4.3 ± 0.1 a	5.7 ± 0.2 df	9.8 ± 0.6 e	10.0 ± 3.9 e	15.5 ± 3.0 ef
W15C3	3	15	4.5 ± 0.2 a	5.3 ± 0.3 ef	11.2 ± 0.6 e	13.4 ± 1.5 e	19.5 ± 1.6 ef
W15C1	1	15	4.4 ± 0.2 a	5.9 ± 0.6 cef	11.7 ± 0.8 e	15.5 ± 0.3 de	23.4 ± 2.2 cde
W20C6	6	20	4.6 ± 0.2 a	8.4 ± 0.2 ab	17.3 ± 1.8 abcd	28.0 ± 5.6 abc	31.9 ± 6.1 bc
W20C3	3	20	4.4 ± 0.1 a	7.3 ± 0.4 bdef	15.3 ± 0.3 cd	22.8 ± 1.5 cd	29.5 ± 3.5 bcd
W20C1	1	20	4.6 ± 0.3 a	8.4 ± 0.4 ab	16.1 ± 1.1 bcd	26.0 ± 2.5 bc	30.2 ± 3.9 bcd
W25C6	6	25	4.6 ± 0.1 a	10.0 ± 0.6 a	19.8 ± 0.4 a	29.7 ± 3.6 abc	33.6 ± 2.8 ab
W25C3	3	25	4.5 ± 0.1 a	8.0 ± 0.4 abc	14.9 ± 1.5 d	26.3 ± 1.7 bc	35.2 ± 4.0 ab
W25C1	1	25	4.5 ± 0.1 a	8.0 ± 1.6 ab	17.0 ± 1.7 abcd	25.2 ± 4.2 bc	32.3 ± 3.0 bc
W30C6	6	30	4.4 ± 0.1 a	8.8 ± 0.6 ab	19.5 ± 0.9 ab	33.2 ± 3.3 ab	41.7 ± 3.1 a
W30C3	3	30	4.6 ± 0.2 a	7.8 ± 0.8 abcd	18.4 ± 1.7 abcd	27.4 ± 2.0 abc	33.7 ± 2.6 ab
W30C1	1	30	4.7 ± 0.2 a	7.8 ± 1.bcd	18.5 ± 0.7 abc	35.5 ± 2.1 a	42.2 ± 2.3 a
ART			4.4 ± 0.8	7.2 ± 2.2	18.6 ± 5.8	32.7 ± 13.0	33.1 ± 15.1
DRY			2.9 ± 0.6	4.1 ± 1.4	5.7 ± 1.6	6.8 ± 2.1	9.9 ± 4.0

Data are expressed as mean ± standard deviation (n = 3). Different letters indicate significant differences.

3.3 | Larvae yield

As a consequence of larval survival rate and BW, the larval yield at the end of the experiment was also influenced by weaning age, co-feeding duration and their interaction (Table 2). Only in groups W10 and W15, mean larval yield was significantly lower in larvae co-fed for 6 days (W10C6 and W15C6) than in those co-fed for 1 day (W10C1 and W15C1) (Figure 4), as indicated by a significant interaction between weaning age and duration of co-feeding. Co-fed groups weaned after 20 dph showed similar larvae yield to the ART group (one-way ANOVA, Tukey test, F = 0.115, p = .925).

3.4 | Carbon, hydrogen and nitrogen analysis

There were no significant differences in larvae carbon, hydrogen or nitrogen content in groups receiving live prey. Content of carbon, hydrogen and nitrogen was significantly lower in the DRY group than in other groups (Figure 5.).

3.5 | Feed cost analysis

Feeding cost was significantly affected by weaning age and interaction, whereas co-feeding duration had no significant effect (Table 2). A proportion of 68% of the variation of feeding costs was explained by age of weaning, with means values of 9.1 ± 2.4, 10.6 ± 1.7, 11.3 ± 0.9, 12.8 ± 0.7 and 15.4 ± 1.4 EUR per 1,000 larvae for W10, W15, W20, W25 and W30, respectively. Larvae weaned at 10, 15 and 20 dph had feeding costs significantly (one-way ANOVA, Tukey test, F = 4.060, p = .024) lower than those weaned at 30 dph. Only in group W10, mean feeding cost was significantly higher in larvae co-fed for 6 days (C6W10) than in those co-fed for 1 and 3 days (C1W10 and C3W10) (Figure 6), as indicated by a significant interaction between weaning age and duration of co-feeding. The relationship between average feeding cost and larval yield shows that groups of larvae weaned after 20 dph (W20, W25 and W30) were about 1.6 times better than the larvae groups weaned earlier (W10 and W15). Weaning peled larvae at 20 dph was shown to be the most economical approach as larval growth and survival (and hence yield) was equivalent to late weaning, but superior to early weaning, and food cost less than the late weaning, but equivalent to early weaning.

4 | DISCUSSION

Larviculture is an important step in the fish production cycle (Alves, Cerqueira, & Brown, 2006; Cahu & Zambonino Infante, 2001; Canavate & Díaz, 1999; Fletcher et al., 2007; Ribeiro, Forsythe, & Qin, 2015). There is close link between larvae quality in early stages of rearing and survival and growth in further stages of production (Cahu & Zambonino Infante, 2001).

Live prey (*Artemia* sp.) provides a highly digestible protein source for fish larvae, while other protein sources such as fish meal have low digestibility. Overall success of weaning using a co-feeding strategy depends on the suitability of the diet and the development of the

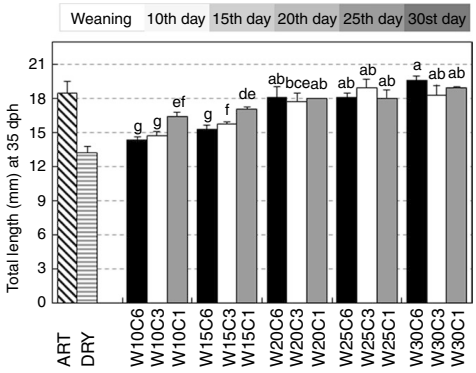

FIGURE 2 Total length of *Coregonus peled* larvae after 35 days, with different weaning times (W10, W15, W20, W25 and W30) and co-feeding durations (C6—black bars, C3—white bars and C1—grey bars). Data are expressed as mean ± standard deviation (whiskers) (n = 3). Different letters indicate significant differences

larval digestive system (Cahu & Zambonino Infante, 1994; Hamza et al., 2007; Pradhan et al., 2014). The development of the digestive system is related to larvae size or to age (Cahu & Zambonino Infante, 1994).

Under the experimental conditions employed (including foods), the duration of the co-feeding had influence on younger larvae (negative effect of longer co-feeding) (Table 1 and Figure 1). However, co-feeding duration may play role in other fish species, such as percids, with a tendency to develop high size heterogeneity (Curnow et al., 2006; Hamza et al., 2007; Ribeiro et al., 2015). The timing of weaning was found to be the most important factor for peled larvae feeding. Timing of the start of the weaning phase has also been found to be major factor in other fish species like pikeperch *Sander lucioperca*, totoaba *Totoaba macdonaldi*, burbot *Lota lota*, golden pompano *Trachinotus ovatus* or butter catfish *Ompok bimaculatus* (Hamza et al., 2007; Ma et al., 2015; Mata-Sotres et al., 2015; Palinska-Zarska et al., 2014, 2015; Pradhan et al., 2014). It should be concluded that co-feeding duration does not bring any advantage to peled larviculture. On the other hand, the weaning timing was confirmed as most important factor during early rearing of peled.

There were found differences in growth pattern of the larvae weaned at 10 and 15 dph from the age of 21 dph. Larvae co-fed for 6 days (C6W10 and C6W15) had lower body weight and those co-fed for 1 day (C1W10 and C1W15) had higher body weight. At the end of the experiment (35 dph), only fish in the group weaned at 10 dph and co-fed for 6 days were significantly smaller than those co-fed for 1 day. Therefore, it seems that there was a negative effect to provide dry food early, even in co-feeding regime (at 5 and 10 dph, respectively, for C6W10 and C6W15 groups). It is possible to speculate that larvae start to prefer dry feed in conditions of excessive feed rate and small particle size even if dry feed is not better nutritionally. Same speculation could be made in case of survival rate.

It was observed from 21 dph that larvae of C1W10 and C6W15 groups which started receiving the dry food at the same time at 10 dph showed different growth pattern. Larvae with shorter co-feeding and early weaning (C1W10) had generally higher final body weight than those with longer co-feeding and late weaning (C6W15). However, no significant difference was found between these groups (C1W10 and C6W15) at the end of experiment. Therefore, it is possible to assume that the negative effect of early supply by dry food (maximum before 14 dph) is closely connected with a longer co-feeding.

The effect on larval growth is an important parameter to consider in evaluating weaning strategies (Mata-Sotres et al., 2015; Pradhan et al., 2014; Rónyai & Feledi, 2013; Rosenlund et al., 1997). However, at this stage of production, larvae growth may not be as critical as survival, as differences in larvae size may be compensated for later in the rearing process (Cahu & Zambonino Infante, 2001; Rosenlund

TABLE 2 Two-way ANOVA results for the factors co-feeding duration, weaning age and their interaction on final BW, survival, yield and feeds cost of *Coregonus peled* larvae

Variables	Source of variation	DF	SS	MS	*F*	*p*
Body length of larvae at 35 dph (mm)	Co-feeding duration	2	0.003	0.001	8.65	.001
	Weaning age	4	0.063	0.016	93.20	<.001
	Weaning age × co-feeding duration	8	0.009	0.001	6.56	<.001
Survival rate at 35 dph (%)	Co-feeding duration	2	0.174	0.087	11.61	<.001
	Weaning age	4	0.334	0.084	11.14	<.001
	Weaning age × co-feeding duration	8	0.280	0.035	4.67	.001
Larvae yield at 35 dph (g/tank)	Co-feeding duration	2	0.001	0.001	6.3	.005
	Weaning age	4	0.033	0.008	76.18	<.001
	Weaning age × co-feeding duration	8	0.004	0.000	4.44	.001
Total feed cost (EUR 1,000 per larvae)	Co-feeding duration	2	7.9	3.94	2.66	.086
	Weaning age	4	205.7	51.42	34.76	<.001
	Weaning age × co-feeding duration	8	42.9	5.37	3.63	.005

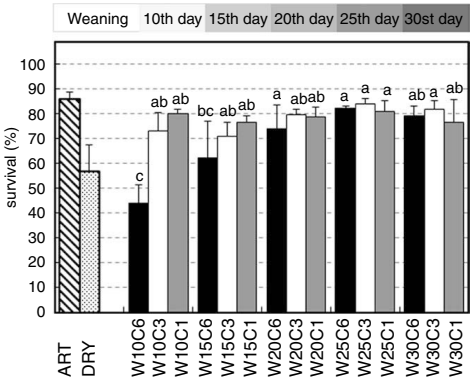

FIGURE 3 Survival of *Coregonus peled* larvae after 35 days, with different weaning times (W10, W15, W20, W25 and W30) and co-feeding durations (C6—black bars, C3—white bars and C1—grey bars). Data are expressed as mean (bar) ± standard deviation (whiskers) (n = 3). Different letters indicate significant differences

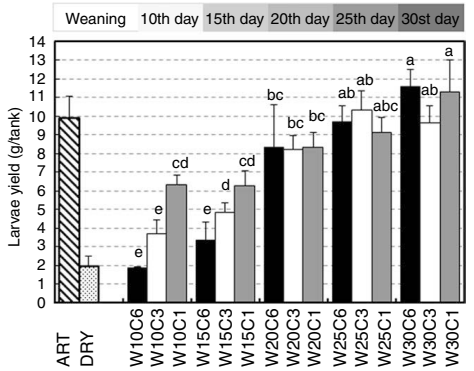

FIGURE 4 Yield of *Coregonus peled* larvae after 35 days, with different weaning times (W10, W15, W20, W25 and W30) and co-feeding durations (C6—black bars, C3—white bars and C1—grey bars). Data are expressed as mean (bar) ± standard deviation (whiskers) (n = 3). Different letters indicate significant differences

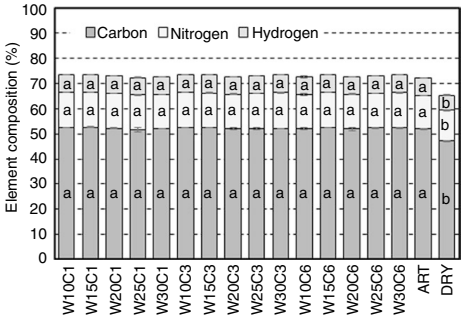

FIGURE 5 Carbon, hydrogen and nitrogen content of *Coregonus peled* larvae after 35 days under different weaning strategies. Data are expressed as mean (column) ± standard deviation (whiskers) (n = 3). Different letters indicate significant differences (p < .05)

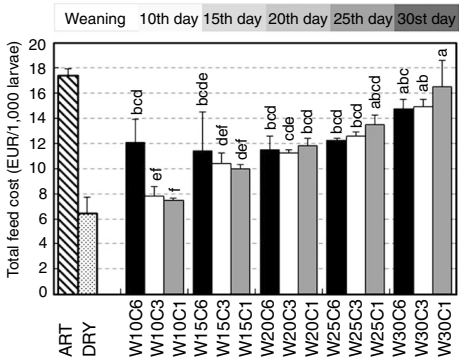

FIGURE 6 Feeding costs incurred using different weaning strategies in *Coregonus peled* larvae. Data are expressed as mean (column) ± standard deviation (whiskers) (n = 3). Different letters indicate significant differences

et al., 1997). On the other hand, larvae of lower body weight could be more susceptible to stress and mortality postmetamorphosis in some species (Liu et al., 2012). In contrast, some species showed higher survival in smaller larvae (Curnow et al., 2006; Palinska-Zarska et al., 2014; Rónyai & Feledi, 2013). In the present study, peled larvae fed solely on *Artemia* nauplii, along with all groups weaned after 20 dph, showed the best results on final BW. In contrast, all larvae offered dry feed before 20 dph showed lower final BW and TL. The lowest growth rate was observed in the group receiving dry feed only. Luczynski et al. (1986) reported higher final body weight (up to 100 mg), but with weak survival rate (ranged from 20%) after comparable period of rearing by artificial feed and zooplankton (35 days). On the other hand, Dabrowski et al. (1986) reported similar final body weight (47.1 mg) after comparable period of rearing by artificial feed (35 days).

In the present study, peled larvae were able to ingest dry feed as early as 10 dph, but growth performance of early weaned larvae was weak. Withdrawal of live food at 10 and 15 dph, as well as feeding solely on dry diets, resulted in reduced growth, supporting the hypothesis that the peled digestive system may not be fully developed at these stages. Present results indirectly confirmed conclusions of Cahu and Zambonino Infante (2001) that the digestive enzyme activity pattern is age-dependent, but can be modified by diet. It has been previously reported that co-feeding with live feed enhanced the efficacy of commercial diets by promoting assimilation and utilization of nutrients (Baskerville-Bridges & Kling, 2000; Hamza et al., 2007; Parma

et al., 2013). However, the present results on peled larvae are not in agreement with these conclusions as peled larvae were not affected by the duration of co-feeding excluding negative influence of longer co-feeding in early weaned larvae (W10 and W15, 10 and 15 dph).

Slightly higher (non-significant) growth was observed in peled larvae weaned after 30 dph. Significantly higher larvae yield (g/tank) was seen in larvae weaned after 30 dph compared to groups weaned at 20 dph, indicating that later weaning could be superior. However, prolonged live feeding is costly (Cahu & Zambonino Infante, 2001), and live food for an extended period may lead to nutritional deficiencies (Hamza et al., 2007; Parma et al., 2013). Weaning of peled larvae to a dry diet at 20 dph is recommended and is supported by calculation of feeding costs. In this relation, some other live feeds such as copepods or nematodes could reduce costs for early rearing (Abate, Nielsen, Nielsen, Jepsen, & Hansen, 2016; Hundt et al., 2015; Piasecki, Goodwin, Eiras, & Nowak, 2004; Wilcox et al., 2006).

Larval survival is generally the most important parameter in evaluation of the success of any weaning strategy. It is commonly accepted that the weaning of fish larvae to dry feed requires protocols to facilitate adaptation during a period of extensive morphological and physiological change. In the present study, larvae weaned after 10 dph with co-feeding for 1 or 3 days, as well as groups weaned after 15 dph (irrespective confiding duration), showed survival rates similar to that of the group fed only on *Artemia* nauplii (62.4%–86.0%). Feeding of peled larvae on dry feed only or early weaning with 6 days of co-feeding resulted in survival values ranging from 43.9% to 56.9%. While high mortality is reported after cessation of live feeding in some fish species (Van et al., 2010), this was not observed in the present study. Luczynski et al. (1986) reported much lower survival rate in peled (20%) after comparable period of rearing (35 days). On the other hand, Dabrowski et al. (1986) reported wide range of survival rate (from 20% to 94%) as results of using of different artificial diets in peled.

Some studies on African catfish *Clarias gariepinus*, common nase *Chondrostoma nasus* and lake minnow *Eupallasella percnurus* reported that rearing conditions (mainly temperature) should influence elemental analyses and quality of larvae (Kamler et al., 1994; 1998; Kamiński et al., 2006). In the present study, no difference was observed in the content of carbon, hydrogen and nitrogen in any group of peled larvae reared with live feed for part of their life. Decreased levels of these elements were found in peled larvae reared solely on dry feed. Generally, biogenic elements decrease in somatic tissues, mainly C and N both participating predominantly in proteins synthesis for rapid muscles formation in early fish life stages (Aragão, Conceição, Dinis, & Fyhn, 2004; Pedersen, 1997; Rønnestad, Thorsen, & Finn, 1999), which occurs in unfavourable culture condition as a consequence of elevated basal metabolism requirements and/or lowered nutrients transformation ability (Kamler et al., 1994; 1998; Kamiński et al., 2006) when proteins even serve as a main energy source (Holt, 2011). That results in growth rate retardation and individual size decrease (Kamler et al., 1994; 1998; Kamiński et al., 2006). Moreover, a presence of live feed during initial phase of rearing is highly required in coregonids as an essential source of autolytic enzymes which help larvae to properly digest as long as a stomach is developed. It happens not earlier than in age of 50 dPH (Lauff & Hofer, 1984).

Our results thus suggest that firstly, timing and co-feeding period duration do not affect macronutrients composition in peled early life stages, and secondly, rearing of peled larvae only on dry feed is possible, but the quality of larvae produced is low.

5 | CONCLUSION

Based on the significantly higher yield of larvae weaned after 20 dph irrespective of co-feeding duration, it can be concluded that abrupt weaning (one day of co-feeding) to dry food after 20 days of feeding with live prey can provide adequate production while reducing the effort and costs associated with live feed. For further development of larval rearing technology, feed formulations and relationships to abiotic factors should be assessed in peled larvae.

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CHAPTER 7

THE EFFECT OF WATER OXYGEN SATURATION ON GROWTH AND HAEMATOLOGICAL PROFILE OF JUVENILE PELED *COREGONUS PELED* (GMELIN)

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The effect of water oxygen saturation on growth and haematological profile of juvenile peled *Coregonus peled* (Gmelin)

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Abstract

The effect of varying oxygen saturation regimes on growth and haematological profiles of peled *Coregonus peled* was investigated on fish of initial age 90 days post hatching. Eighty-five juveniles per group (initial body weight 3.09 ± 0.80 g) were submitted to a 63-day experiment with one of four water saturation regimes: normoxia (NORm, 80%–90%), hypoxia (HYPo, 50%–60%), hyperoxia (HYPe, 150%–160%) and intermittent hyperoxia (iHYPe, 150%–160% - 80%–90%). Survival rate in NORm, HYPe and iHYPe ranged from 96.3 ± 2.1% to 97.7 ± 2.7, but survival 87.5 ± 3.0 was significantly lower in the HYPo group. No differences were observed in feed conversion ratio. The highest final body weight of 18.2 ± 4.6 g and a specific growth rate of 2.81 ± 0.01%/day were seen in the NORm group. Significant differences were found in haemoglobin concentration with increased saturation. The fish had lower haemoglobin 55.00 ± 5.72 and 51.35 ± 10.89 g/L in treatments HYPe, iHYPe with compared to the normoxia (64.22 ± 5.78 g/L). Haematocrit was similar in the groups HYPo, NORm and iHYPe (0.55 ± 0.04, 0.58 ± 0.05 and 0.54 ± 0.09) with the exception of HYPe, which was significantly lower (0.48 ± 0.06). Significantly lower count of erythrocyte was observed in iHYPe group (0.88 ± 0.20) with compared to the normoxia (1.06 ± 0.13). The supersaturation level was not associated with effects on growth and survival, and adding oxygen is not recommended for intensive rearing of peled. The results showed normoxia oxygen level to be the most suitable conditions for peled.

KEYWORDS

feed conversion ratio, food intake, hyperoxia, hypoxia, recirculating aquaculture systems, specific growth rate

1 | INTRODUCTION

Whitefish *Coregonus* sp. as well as peled provide high-quality meat with high polyunsaturated fatty acid content (Orban et al., 2006) and show potential for rearing in RAS systems (Siikavuopio, Knudsen, Amundsen & Sæther, 2012). Peled has been a valuable commercial species in Russia. Its importance increased with introduction to another countries such as Estonia, Lithuania, Latvia, Byelorussia, Poland, Germany, Finland, the Czech Republic, France and Japan. There were stocked in lakes or produced in ponds and cage systems (Gordeeva, Karmanova & Shitova, 2008; Luczynski, Mamcarz, Brzuzan, & Demska-Zakes, 1999). A new intensive way of whitefish production is in recirculation systems (RAS) (Jobling et al., 2010; Szczepkowski, Sczepkowska & Krzywosz, 2006). This efficient

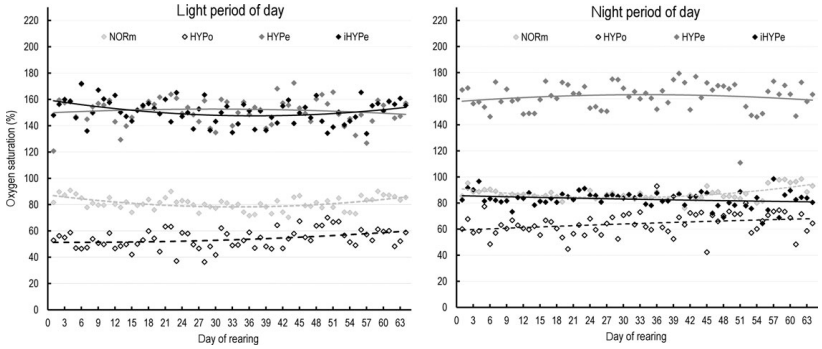

FIGURE 1 Oxygen saturation levels during the 63-day trial: normoxia (NORm), hypoxia (HYPo), hyperoxia (HYPe) and intermittent hyperoxia (iHYPe). Values are mean of three measures per day

farming of whitefish is relatively new method which can product high amount of marketable fish or stocked fish. The intensive farms have been gradually developing in countries where whitefish have high potential like Finland, Germany, Poland, Sweden, Japan and Italy (Jobling et al., 2010). Despite the fact of developing, it is necessary to solve a lot of zootechnical aspects.

Technology for regulating water oxygen content has considerable potential for improving water quality in aquaculture, especially in RAS systems (Dwyer, Colt & Owsley, 1991; Wajsbrot, Gasith, Krom & Popper, 1991). Hypoxia, an insufficient level of dissolved oxygen in water, produces stress in aquatic organisms and has negative effects on fish survival and growth, decreasing production in intensive fish farming (Batiuk et al., 2009). Hyperoxia can increase fish feed intake and growth of rainbow trout (*Oncorhynchus mykiss*) but may generate negative effects such as health problems and it can cause death of fish (Ritola, Tossavainen, Kiuru, Lindström-Seppä & Mölsä, 2002): Consistent oxygen saturation of 140%–150% may cause stress at Atlantic salmon (*Salmo salar* L.) which leading to increased susceptibility to diseases and reduced growth of fish as well as increased mortality (Fridell et al., 2007; Lygren, Hamre & Waagbø, 2000). Dabrowski, Lee, Guz, Verlhac and Gabaudan (2004) presented positive effect of oxygen supersaturation (180%) on growth of rainbow trout. In another study which was focused on effect of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L.) smolts, reports on positive effect of hyperoxia (123%) on growth of smolts. However, the study recommends next investigations effect of hyperoxia and their interaction among with regard to the total gas pressure (Hosfeld et al., 2008).

The aim of this study was to investigate the effects of selected oxygen regimes on growth, production and haematological parameters of juvenile peled whitefish *Coregonus peled* reared in intensive RAS. The hyperoxia could improve rearing environment and it could lead to increasing of food intake and growth of peled as was caused at rainbow trout in study of Dabrowski et al. (2004).

2 | MATERIALS AND METHODS

Newly hatched *Coregonus peled* larvae were obtained from farm Kinský Žďár a.s. on 27 March and the experiment was finished 26 August. Fish were fed *Artemia* for 20 days post hatching (dph) and then weaned to a dry diet, Larviva ProWean 100 (BioMar, France). Commercial diets Larviva ProWean 300 and BioMar Inicio Plus G 0.6 GR (BioMar, France) were fed to initiation of the experiment. Eighty-five 90 dph juveniles of mean body weight (BW) 3.09 $\pm$ 0.80 g were stocked into each of 12 tanks. Initial mean biomass per tank was 255.0 $\pm$ 2.9 g. Four treatments (three tanks per treatment) differing in oxygen regimes (normoxia [NORm], hypoxia [HYPo], hyperoxia [HYPe] and intermittent hyperoxia [iHYPe]) were established (Figure 1). Target oxygen water saturation was achieved by mixing pure oxygen (O_2) or pure nitrogen (N_2) with water in a mixing towers measuring 3 m in height and 250 mm diameter, with a volume of 147 L, containing Bioakvacit PP30 medium, and equipped with a flowmeter for regulation of gas inflow. The HYPo group with 50%–60% O_2 saturation was created by adding N_2. The water in groups HYPe and iHYPe was enriched with O_2 to 150%–160%. In the HYPe group, this level was continuously maintained, while in the iHYPe group, hyperoxia was maintained during daylight hours (07.00–19.00), with normoxia during darkness. The intermittent hyperoxia iHYPe was regulated by switching of inflow with normoxia water (80%–90%) and supersaturated water (140%–150%). The speed of change of level saturation took 30 min between daylight and darkness period. Oxygen saturation of 80%–90% in the NORm group was created by aeration using an air pump (SECOH) in a header tank as well as in the culture tank. Water parameters, excluding O_2 saturation, are summarized in Table 1.

The experiment continued for 63 days (from 90 to 153 dph). At time 0, 21, 42 and 63 days of experiment, 50 fish per tank were anaesthetized (0.02 ml/L clove oil), weighed and individually photographed for later measurement of total length, TL (mm). Survival

TABLE 1 Water quality parameters: temperature, pH, ammonia, nitrite, nitrate at selected O2 saturation levels

	Unit	HYPo	NORm	HYPe	iHYPe (L)	iHYPe (D)
Temperature	°C	20.0 ± 0.9	20.0 ± 0.9	20.0 ± 0.9	20.0 ± 0.9	20.0 ± 0.9
pH	mg/L	7.00 ± 0.15	7.00 ± 0.16	6.99 ± 0.14	6.97 ± 0.14	6.97 ± 0.15
NH_4^+	mg/L	0.91 ± 0.20	0.87 ± 0.18	0.90 ± 0.18	0.93 ± 0.4	0.93 ± 0.4
NH_3^-	mg/L	0.003 ± 0.001	0.004 ± 0.002	0.005 ± 0.002	0.006 ± 0.003	0.006 ± 0.003
NO_2^-	mg/L	0.54 ± 0.40	0.59 ± 0.41	0.51 ± 0.30	0.60 ± 0.20	0.60 ± 0.21
NO_3^-	mg/L	17.2 ± 1.9	12.1 ± 3.2	15.1 ± 4.2	14.4 ± 4.5	14.4 ± 4.6

Dates are presented as mean + *SD* maintained throughout the experiment.

and fish biomass were recorded. Photographs were processed using Micro-Image 4.0 (Olympus, Germany).

The fish were fed by hand to obvious saturation at 2-h interval during daylight on commercial food Biomar Inicio Plus G 0.6 mm (62.0% protein, 13.0% lipid, 8.9% carbohydrate). In the point of saturation, the fish did not intake any food. Water temperature, pH and O_2 level were monitored three times per day using the Handy Multimeter (Hach HQ40d multi, Germany). Ammonium, nitrite and nitrate were measured weekly by commercial Kits and spectrophotometer HACH LANGE DR 280 (Table 1). Water temperature was maintained at 20.0 ± 0.9 with a flow-through cooler.

At the end of the experiment, blood samples were taken from 15 fish in each treatment for haematology. Complete blood count (CBC) was conducted according to Svobodova, Pravda and Palackova (1991). The CBC included erythrocyte count (RBC), leucocyte count (WBC), haematocrit (Htc), haemoglobin concentration (Hb) and parameters of erythrocyte: mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Haematocrit was investigated from blood which was taken into capillaries and centrifuged. A solid part of blood was measured and calculated ration in percentages. The RBC and the WBC were investigated from sample of blood which was coloured by a solution of Natt Herrick. Erytrocyty and leukocyty were calculated under microscope. Haemoglobin was investigated from samples which were diluted by a solution according van Kampena and Zijlstra. There erythrocyte secreted haemoglobin into the solution. Concentration of haemoglobin was measured in a spectrophotometer.

The photoperiod with light intensity of 110–140 lux was set at 12:12 L:D, with light from 07.00 to 19.00 hours. Inflow was regulated at 80 L/hr providing water exchange twice per hour.

Data were used to calculate the following parameters:

cumulative survival(%) = $(n_0 - n_1/n_0) \times 100$

specific growth rate(%), SGR = $([\ln W_t - \ln W_0] \times d^{-1}) \times 100$

coefficient of weight variation, CV = (*SD*/mean of fish weight) × 100

Fulton's condition factor, K = $(W_t/TL^3) \times 100$

feed conversion ratio, FCR = $F/(Biomass_t - Biomass_0)$

mean cell volume, MCV = (Htc × 1000)/RBC

mean corpuscular haemoglobin, MCH = Hb/RBC

mean corpuscular haemoglobin concentration, MCHC = Hb/(Htc × 1000)

where W_0 (g) is initial average individual weight; W_t (g) is average individual fish at the end of the measuring period; n_0 (individuals) is the initial number of fish; n_1 is number of fish at the end of the experiment; TL (cm) is total length; d is duration of period in days; and F (g) is weight of served feed.

Data are presented as mean ± *SD*. The data were tested for normal distribution and homogeneity of variance (Cochran-Hartley-Bartlett test). One-way analysis ANOVA (STATISTICA 10.0) with Tukey's HSD test was used for comparisons of survival, BW, SGR, K CV, RBC, TL, WBC, Htc, Hb, MCV, MCH, MCHC and the nonparametric Kruskal–Wallis test was conducted to compare FCR. Significance level was $p < .05$.

3 | RESULTS

3.1 | Growth and survival

Significantly lower survival was observed in the HYPo group compared to other groups. No differences were observed in survival in the NORm, HYPe and iHYPe groups (Table 2).

TABLE 2 Survival (%), food conversion ratio (FCR), Fulton condition factor (K), coefficient of weight variance (CV) and body length (mm) of *Coregonus peled* juveniles reared 63 days at selected O_2 saturation levels

	Indicators	HYPo	NORm	HYPe	iHYPe
Day 63	survival (%)	87.5 ± 3.0^b	97.7 ± 2.7^a	96.3 ± 2.1^a	97.2 ± 1.0^a
Day 0–63	FCR	2.06 ± 0.46^a	2.18 ± 0.15^a	1.83 ± 0.09^a	1.74 ± 0.03^a
Day 63	K	0.90 ± 0.02^a	1.03 ± 0.04^b	0.84 ± 0.02^c	1.01 ± 0.01^b
Day 63	CV (%)	28.8 ± 3.0^a	25.3 ± 3.0^a	23.9 ± 2.7^a	25.3 ± 2.7^a
Day 63	total body length (mm)	116.7 ± 6.7^a	124.8 ± 9.0^a	128.3 ± 3.3^a	115.8 ± 12.0^a

Values are presented as mean ± *SD*, $n = 3$.
Different superscript letters indicate significant differences ($p < .05$).

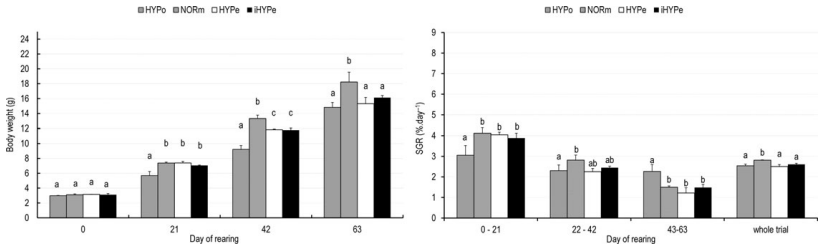

FIGURE 2 Effect of different oxygen saturation on body weight (BW; $n = 3$) on the left and specific growth rate (SGR; $n = 3$) of juvenile *Coregonus peled* on the right in different times of sampling (0th, 21th, 42th, 63th day of experiment). Tested levels of saturation were normoxia (NORm), hypoxia (HYPo), hyperoxia (HYPe) and intermittent hyperoxia (iHYPe). Data are expressed as mean ± standard deviation. Different superscript letters indicate significant differences ($p < .05$) among groups

The FCR values ranged from 1.74 ± 0.03 to 2.18 ± 0.15, and differences among treatments were not significant (Table 2).

Mean K was 1.04 ± 0.48 at the start of the experiment. The highest coefficient K was observed in treatments NORm and iHYPe. Differences among treatments were not significant (Table 2). There were observed significant lower K at groups HYPe and HYPo.

All groups showed similar coefficient of body weight variation (CV) throughout the experiment (Table 2).

Initial mean TL was 68.0 ± 8 mm. The greatest length increase was observed in the HYPe and NORm groups but there were not observed significant differences between all treatments (Table 2).

Initial mean body weight was similar in all groups at 3.1 ± 0.8 g. Weight increased in all groups but weight of fish in HYPo was significantly lower. There were no differences in mean individual weight gain among groups during the first (day 0–21) with the exception of HYPo. In the second period (day 21–42) was growth significant reduced at groups HYPe and iHYPe compared to NORm (Figure 2). At the end of the experiment, weight in the NORm group was significantly higher than in other groups. The HYPe, iHYPe and HYPo groups showed similar growth in weight.

During the first period (0–21 days), SGR was significantly higher in NORm, HYPe and iHYPe (Figure 2). In the final period (43–63 days), the most rapid growth was observed in the NORm. Overall SGR (0–63 days) was significantly higher in the NORm than in the other groups.

3.2 | Haematological profiles

No significant differences among treatments were seen in WBC and parameters of erythrocyte MCH and MCV. Leucocyte count (WBC) was higher in groups with higher saturation (HYPe, iHYPe) compared with the NORm. The differences were not significant. These groups showed high individual variation (Table 3).

Significantly higher RBC count was found in HYPo than in the iHYPe. Volume of Hb was significantly lower in groups with higher oxygen saturation (iHYPe, HYPe) in comparison with NORm, while the difference among NORm and HYPo was not significant. Haematocrit was similar among treatments excluding HYPe. Significantly lower Hct was found in the HYPe in comparison with the other treatments. Differences of Hct were not between treatments the HYPo, NORm and iHYPe. Concentration of haemoglobin in erythrocyte (MCHC) was significantly lower in the iHYPe in comparison with the other treatments.

4 | DISCUSSION

In intensive aquaculture, the general recommended minimum oxygen saturation is 60% as measured in effluent from the tank (Portz, Woodley & Chech, 2006; Timmons & Vinci, 2007). Wedemeyer (1997) recommends oxygen saturation in effluent ranging from 71 to

TABLE 3 Complete blood count (CBC) parameters of juvenile *Coregonus peled* reared 63 days at selected O_2 saturation levels

CBC	Unit	HYPo	NORm	HYPe	iHYPe
RBC	T/L	1.18 ± 0.16^{a}	1.06 ± 0.01^{ab}	1.02 ± 0.11^{ab}	0.88 ± 0.4^{b}
WBC	G/L	15.26 ± 0.90^{a}	16.74 ± 1.08^{a}	23.58 ± 7.58^{a}	23.45 ± 8.64^{a}
Hct	%	55 ± 2^{a}	58 ± 3^{a}	48 ± 4^{b}	54 ± 2^{ab}
Hb	g/L	67.20 ± 0.30^{a}	64.21 ± 2.55^{a}	55.00 ± 2.81^{b}	51.35 ± 3.85^{b}
MCV	fl	517.5 ± 37.4^{a}	561.3 ± 7.6^{a}	493.4 ± 80.1^{a}	615.0 ± 14.2^{a}
MCH	pg	60.0 ± 8.6^{a}	62.2 ± 2.3^{a}	54.6 ± 4.6^{a}	58.4 ± 2.7^{a}
MCHC	L/L	0.121 ± 0.005^{a}	0.110 ± 0.001^{a}	0.115 ± 0.008^{a}	0.093 ± 0.005^{b}

Values are presented as mean ± *SD*, $n = 3$. Different superscript letters indicate significant differences ($p < .05$).
RBC, erythrocyte; WBC, leukocyte; Htc, haematocrit; Hb, haemoglobin; MCV, mean cell volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

81% for farmed fish in the temperature range of 5–15°C. Hypoxia is common in aquaculture systems and can affect survival and production characteristics of fish (Dabrowski et al., 2004). Batiuk et al. (2009) reported that hypoxia increases stress in aquatic organisms and has a negative effect on survival. On the other hand, high hypoxia could cause oxidative stress, decreased food intake and increase in fish disease and mortality of salmonids (Fridell et al., 2007; Lygren et al., 2000; Ritola et al., 2002). We found a significant decrease in fish survival only in the HYPo group in the present study. Remen, Imsland, Stefansson, Jonassen and Foss (2008) tested three oxygen levels (hypoxia 57%–69%, normoxia 83%–88%, and hyperoxia 101%–104%) on juvenile Atlantic cod (*Gadus morhua*), and they reported no differences in mortality. A study of effects of supersaturation (147% and 224%) on juvenile turbot (*Scophthalmus maximus* L.) reported no differences in mortality (Person-Le Ruyet et al., 2002).

Individual body weight as well as SGR of fish was significantly higher in the NORm group at the end of the trial than in other groups, which showed no differences. Excessive total gas could affect fish in a recirculation system. Supersaturated water, most often due to nitrogen and oxygen, may cause gas bubble disease, leading to decreased food intake and lower FCR and growth (Skov, Pedersen & Pedersen, 2013). A study of the effect of supersaturation on rainbow trout found saturation lower than 140% to have no negative effect on growth, feed conversion and this saturation is feasible in farming of rainbow trout (Ritola et al., 1999). Dabrowski et al. (2004) demonstrated in a study with three oxygen levels, hypoxia (50%), normoxia (100%) and hyperoxia (180%) demonstrated positive effects of hyperoxia on growth of juvenile rainbow trout. The authors suggested that positive effects of hypersaturation may appear later in the rearing period. Edsall and Smith (1990) found no significant difference in growth of rainbow trout reared in hyperoxic conditions (180%) from those in normoxia (94%). Schisler, Bergersen and Walker (1999) tested four O_2 saturations from 100% to 110% and observed no effect of saturation levels on growth of juvenile rainbow trout.

Foss, Imsland, Roth, Schram and Stefansson (2007) reported growth in wolf fish *Anarhichas minor* similar to results of the present study. Wolf fish in hyperoxia did not show significantly higher growth from those reared in normoxia but study discovered that supersaturation is associated with a reduction in ammonium toxicity.

The FCR in the present study was higher than is usually seen in salmonids (from 0.96 to 1.16), in tilapia (*Oreochromis niloticus*) (from 1.20 to 1.47) and in perch (*Perca fluviatilis* L.) (from 0.9 to 1.7) reared in intensive aquaculture (Luo et al., 2014; Stejskal, Kouril, Policar, Hamackova & Musil, 2009; Sun et al., 2016). Small particles of uneaten food remaining in the aquaria may have led to inaccurate assessment. Differences in FCR among groups were not significant. Similar results have been obtained in juvenile turbot, where oxygen saturation in interaction with different concentrations of ammonia had no effect on FCR, which was found to range from 1.28 ± 0.11 to 1.41 ± 0.08 without significant differences between treatments (Foss et al., 2007). Tran-Duy, Schrama, van Dam and Verreth (2008) reported in Nile tilapia *Oreochromis niloticus* that increased oxygen concentration ranging from 3.0 mg/L (22% saturation) to 5.6 mg/L (76% saturation) had a positive effect on food intake and growth rates but not on FCR. The effect of hypoxic levels (40%–86% O_2) on feed conversion was tested in European sea bass (*Dicentrarchus labrax* L.). Feed conversion ratio was 0.62 ± 0.13 in hypoxia compared to 0.69 ± 0.06 in normoxia, without being significantly different (Thetmeyer, Waller, Black, Inselmann & Rosenthal, 1999).

Remen et al. (2008) studied effects of three oxygen saturation levels, 57%–69%, 83%–88% and 101%–104% in combination with three levels of ammonia (1–2 μg/L [control], 31–34 μg/L and 115–120 μg/L) on growth and condition of juvenile Atlantic cod (*Gadus morhua*). Higher dissolved oxygen decreased the negative effects of ammonia. In groups with the highest ammonia content, highest condition factor (K) was seen with hyperoxia (0.86), followed by normoxia (0.77) and hypoxia (0.76). Significant difference was between hyperoxia and group of normoxia. In the control group (1–2 μg/L), a significantly lower K was observed with hypoxia (0.86) compared with hyperoxia (0.92).

Oxygen saturation was associated with some aspects of the haematological profile. The physiological response of fish to hyperoxic conditions may lead to lower RBC count, lower concentration of Hb and reduced haematocrit. In the present study, lower volume Htc in HYPe group and Hb concentration was observed in groups under hyperoxic condition. A similar decrease of Htc in supersaturated conditions was reported by Dabrowski et al. (2004) in juvenile rainbow trout. According to Jewett, Behmer and Johnson (1991), hyperoxia may decrease Hb and haematocrit. In contrast, Person-Le Ruyet et al. (2002) found no significant differences of haematocrit, Hb or RBC in juvenile turbot held in supersaturation conditions. A similar trend of RBC was observed in the present study with erythrocyte count showing no differences when group iHYPe was excluded. Count of leucocyte (WBC) can indicate health condition of fish as well as stress of fish (Modra, Svobodova & Kolarova, 1998). In the present study, there was high individual variation in count of WBC at groups HYPe and iHYPe but leucocyte count was not significantly different among tested treatments. The used hyperoxia had no effect on indicators of health condition and stress of fish.

Whitefish, and the majority of other intensively cultured fish species, show increased metabolism and oxygen consumption during feeding period in daylight hours (Stejskal, Kouril, Valentova, Hamackova & Policar, 2009; Zakęś, Demska-Zakęś & Kata, 2003); hence, used of hyperoxia only during this period may be seen as an economical approach. The present study did not reveal a positive effect of such treatment on growth of peled, but it may be useful with other cultured species. Dabrowski et al. (2004) suggested a positive effect of supersaturation on growth of fish during the later rearing stages.

Oxygen saturation levels of 50%–70% have negative impact on growth of juvenile peled. Supersaturation had no positive effect on SGR and growth of peled. Intermittent hyperoxia did not create neither negative nor positive a growth effect on peled in comparison with permanent hyperoxia. Studies of the effects of weak hyperoxia (110%–120%) may be valuable in future research.

Assessment of oxidative stress in fish and biochemical analysis of blood related to water oxygen levels ought to be adding in the next study. These investigating could enrich results about an impact of oxygen saturation on stress and welfare of fish.

Hyperoxia (150%–160% saturation) had no effect on growth and survival, so supersaturation is not recommended in intensive rearing of peled, and normal oxygen levels (normoxia: 80%–100% saturation) are adequate for growth.

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CHAPTER 8

PREVALENCE OF DEFORMITIES IN INTENSIVELY REARED PELED *COREGONUS PELED* AND COMPARATIVE MORPHOMETRY WITH POND-REARED FISH

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SHORT COMMUNICATION

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Prevalence of deformities in intensively reared peled *Coregonus peled* and comparative morphometry with pond-reared fish

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Keywords: abnormalities, fin damage, opercular deformity, radiography, spinal deformity, vertebrae

Peled *Coregonus peled* (Gmelin 1789) is candidate for inland freshwater aquaculture, particularly in local markets in central and east Europe (Turkowski 1999). Skeletal and opercular deformities, often reported in fish reared in intensive systems, can seriously affect efficient culture (Koumoundouros, Oran, Divanach, Stefanakis, & Kentouri, 1997; Policar et al., 2016), negatively affecting fish physiology and welfare, growth performance, and quality and value of product (Fjelldal et al., 2012). The aetiology, frequency of occurrence and biological significance of deformities vary among fish species (Chin, Loh, Hong, & Gibson-Kueh, 2017; Georgakopoulou, Katharios, Divanach, & Koumoundouros, 2010; Lü et al., 2015; Nguyen, Whatmore, Miller, & Knibb, 2016). The aim of this study

TABLE 1 Nutritional composition of BioMar feed (manufacturer's data) used for intensive culture of Coregonus peled

		Larviva ProWean	Larviva ProWean	Inicio plus G	Inicio plus	Inicio plus	Inicio plus
Fish size	g	0.004–0.05	0.05–0.4	0.4–1.0	1.0–5.0	5.0–20.0	20.0–80.0
Age	dph	4–35	36–60	61–80	81–120	121–150	151–276
Particle size	mm	0.08–0.20	0.20–0.40	0.6	1.1	1.5	2
Crude proteins	%	58	58	63	56	54	52
Crude lipids	%	10	10	11	18	18	23
Crude ash	%	11.7	11.7	10.7	11	9.4	8.7
Crude cellulose	%	0.4	0.4	1.5	0.3	1.1	0.9
Nitrogen-free extract	%	x	x	10	10.1	13.1	12
vitC added	mg/kg	1,000	1,000	1,000	1,000	500	150
vitE	mg/kg	700	700	500	380	150	150
vitA	IU/kg	17,500	17,500	17,500	15,000	15,000	15,000
vitD3	IU/kg	875	875	875	750	750	750
P	%	1.77	1.77	1.5	1.7	1.3	1.2
Gross energy	Mj/kg	x	x	21.2	22.1	22	23.5
Digestible energy	Mj/kg	x	x	18.8	19.9	19.2	20.6
n3—HUFA	%	2.3	2.3	x	x	x	x

was to record the frequency of vertebral, opercular and fin deformities in intensively reared peled *Coregonus peled* (Gmelin 1879). The second aim was to compare morphometry of intensively cultured and pond-reared fish.

Eggs of 15 female peled were pooled and fertilized with pooled sperm of 15 males at Rybářství Kinský s.r.o. (Zdar nad Sazavou, Czech Republic) and incubated in hatchery at 4–10°C during 124 days. The newly hatched larvae were transported to the wet laboratory of the Institute of Aquaculture and Protection of Waters in České Budějovice. Water temperature was gradually increased from 10°C to 14°C over the course of 4 days. From 4 to 20 days post-hatching, larvae were fed with *Artemia* sp. and then weaned to dry feed. The nutritional composition of feed is shown in Table 1. Culture conditions and system design at different stages are presented in Table 2.

At 276 days, the incidence of opercular, fin and spinal deformities was visually evaluated by one experienced person in intensively cultured fish held in 12 tanks, each containing from 185 to 221 fish (n = 2436).

Randomly selected, normally (without macroscopically visible deformities) developing intensively cultured (IN, 72.1 ± 10.2 g, n = 33), visibly deformed intensively cultured (ID, 65.4 ± 12.2 g, n = 33) and pond-reared control (CP, 70.5 ± 9.2 g, n = 33) fish were compared in a radiographic and morphometric study. Pond-reared peled (without supplemental feed) were obtained from the fish farm Kinský Žďár, a.s.

Fish were anaesthetized with 0.2 ml/L of clove oil and examined by radiography using direct digitization with a GIERTH RHF 200 (GIERTH X-Ray International GmbH, Germany). Fish were classified as exhibiting normal or abnormal vertebral columns. In those with abnormal vertebral column curvature, the number of deformed or fused vertebrae was determined. Prevalence of deformed vertebrae was evaluated in cranial trunk (R1), caudal trunk (R2), tail region (R3) and caudal fin region (R4) according to methodology Kacem, Meunier, and Baglinière (1998). Compressed, fused or 'K'-shaped vertebrae were classified as deformed. The number of vertebrae was determined from radiographs. The ratio of cranio-caudal length to dorsal-ventral height (R_{cd}) of each vertebra was calculated as:

TABLE 2 Culture conditions and system design for intensive culture of Coregonus peled at different ages

	Larval rearing	Younger juveniles	Older juveniles
Culture parameters related to fish			
Duration (days)	45	63	168
Age (dph)	0–45	45–108	108–276
Number of stocked fish	10,000	3,500	2,560
Survival	81 ± 4.4	92.3 ± 3.1	95.3 ± 3.3
Initial BW (g)	0.004 ± 0.001	0.8 ± 0.5	15.2 ± 4.7
Final BW (g)	0.9 ± 0.6	14.1 ± 5.8	68.1 ± 12.3
Initial biomass (kg m^{-3})	x	4.2 ± 0.1	10.8 ± 0.2
Final biomass (kg m^{-3})	x	25 ± 1.4	46 ± 5.1
Culture system design			
Number of tanks	9	18	12
Volume of tank (L)	60	60	300
Volume biofilter (L)	500	1,500	4,000
Volume of sump tank (L)	500	1,500	2,100
Drum filter	KC-10[a]	AEM[b]	AEM[b]
Biofilter media	RATZ BT10[c]	RATZ BT10[c]	RATZ BT10[c]
Additional equipment	Flow-through cooler[d]	x	x
Rearing conditions			
Temperature (°C)	14.0 ± 0.5	18.9 ± 0.5	19.1 ± 0.7
pH	7.22 ± 0.30	6.90 ± 0.40	6.97 ± 0.55
oxygen (%)	>88%	>85%	>75%
NH_4^+ (mg L^{-1})	0.09 ± 0.05	0.96 ± 0.89	0.89 ± 0.49
NH_3^- (mg L^{-1})	0.001 ± 0.001	0.004 ± 0.004	0.002 ± 0.002
NO_2^- (mg L^{-1})	0.35 ± 0.15	0.91 ± 1.05	0.61 ± 0.55
NO_3^- (mg L^{-1})	5.7 ± 2.5	12.1 ± 2.2	17.7 ± 3.1

[a]Koi-Collection, Malaysia.
[b]AEM-Products V.O.F., Netherlands.
[c]Ratz aquaculture GmbH, Remscheid, Germany.
[d]Hailea Group Co. Ltd, Guangdong, China.

$$R_{cd} = L_{dv}/L_{cc}$$

where L_{dv} is vertebra dorso-ventral height, and L_{cc} is vertebra cranio-caudal length.

Relative size of intervertebral space (RS_{in}) was calculated as

$$RS_{in} = 100 - (100/L_{vc} \times S_{ccl})$$

where L_{vc} is the length of vertebral column, and S_{ccl} is the sum of cranio-caudal length of vertebrae.

Morphometric characters were measured on radiographs using MicroImage 4.0 software and included head length, predorsal distance, preventral distance, pre-anal distance, distance between pectoral and ventral fins, and distance between ventral and anal fins. Maximum and minimum body height was expressed as per cent of standard length. The morphometric characters pre-orbital distance, post-orbital head length, eye diameter and occipital head height were expressed as per cent of head length according to methodology of Specizár, Berscényi, and Müller (2009). All data were analysed for normality by the Cochran, Hartley and Bartlett test. Relative size of intervertebral space showed normal distribution and was analysed by one-way ANOVA and Tukey's post hoc test. Vertebrae count and morphometry data were *arcsin* transformed, and the nonparametric Kruskal–Wallis test was used. All analysis was conducted using Statistica 7.0 (StatSoft CR).

Mean frequency (n = 12) of opercular deformities in intensively cultured peled was 12.9 ± 4.2% (Figure 1). Differences in head profile were also observed (Figure 1), possibly an effect of the rectangular tank. Walling behaviour, known to be a cause of such deformities (Negm, Cobcroft, Brown, Nowak, & Battaglene, 2014), was observed during the larval phase. Frequency of opercular deformities has been

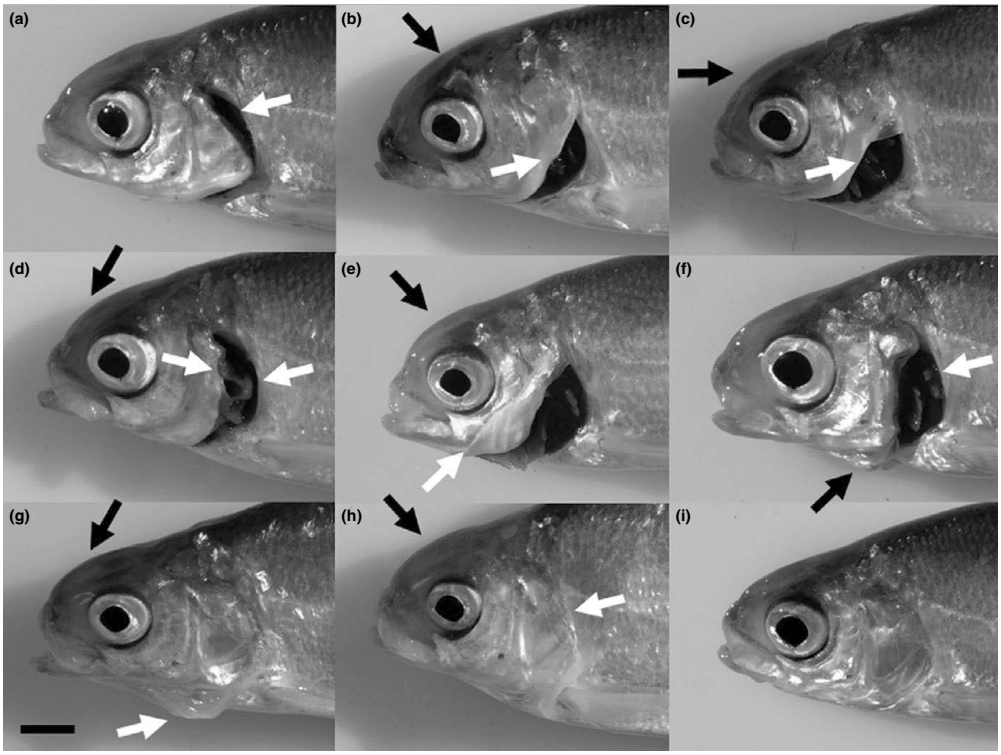

FIGURE 1 Opreculum in deformed intensively (ID) reared peled Coregonus peled. (a) Portions of suboperculum (white arrow) branchiostegal rays missing; (b) larger area of suboperculum and branchiostegal rays missing (white arrow), head profile atypical (black arrow); (c) portion of operculum and suboperculum, as well as branchiostegal rays, missing (white arrow), head profile atypical (black arrow); (d) portion of operculum (white arrow) missing, head profile atypical (black arrow); (e) branchiostegal rays twisted outward (white arrow), shape of front line changed (black arrow); (f) large portion of operculum and suboperculum missing (white arrow), branchiostegal rays twisted outward (black arrow), head profile unchanged; (g) branchiostegal rays twisted outward (white arrow) operculum normal, head profile atypical (black arrow); (h) operculum deformed and recovered (white arrow), head profile atypical (black arrow); (i) normally developed head and operculum

reported to reach 80% in intensively cultured sea bream, negatively influencing morphology, growth, survival and vulnerability to disease (Koumoundouros et al., 1997). The causes of opercular deformities may include dietary ascorbic acid deficiency or unfavourable culture conditions during the larval stage (Galeotti et al., 2000; Georgakopoulou, Angelopoulou, Kaspiris, Divanach, & Koumoundouros, 2007).

Spinal deformities were visually observed in 8.2 ± 2.8% of the intensively cultured peled. Additionally, using a radiology we observed additional deformities in IN group (slightly examples of deformities are shown in Figure 2). Intensively cultured Atlantic salmon *Salmo salar* and European sea bass *Dicentrarchus labrax* have been reported to show a higher prevalence of such deformities than the peled examined in the present study (Boglione, Gagliardi, Scardi, & Cataudella, 2001; Fjelldal, Glover, Skaala, Imsland, & Hansen, 2009). The consequences of vertebral deformities for body shape depended on position along the vertebral column (Figure 2). Frequency of spinal deformities varies among cultured fish species, with reports ranging from 7.7% in barramundi *Lates calcarifer* to 19% in Atlantic salmon (Fraser, Anderson, & de Nys, 2004; Fraser, Hansen, Fleming, & Fjelldal, 2015; Lü et al., 2015).

We found higher numbers of affected vertebrae in the R1 and R3 regions of the ID group (Figure 3). Deformities in the R4 region were minimal. Fraser et al. (2015) reported R1 and R4 to be the most affected spinal areas in intensively cultured Atlantic salmon *Salmo salar*. Amoroso, Adams, Ventura, Carter, and Cobcroft (2016) and de Azevedo et al. (2016), in Atlantic salmon and Senegalese sole *Solea senegalensis*, respectively, reported R4 to be the most affected region. Grini, Hansen, Berg, Wargelius, and Fjelldal (2011) found up to 50% of vertebrae in R3 to be deformed in Atlantic salmon held at 16°C.

The pattern of R_{cd} in individual vertebra varies among groups or along the vertebral column, especially in some parts (Figure 3). Grini

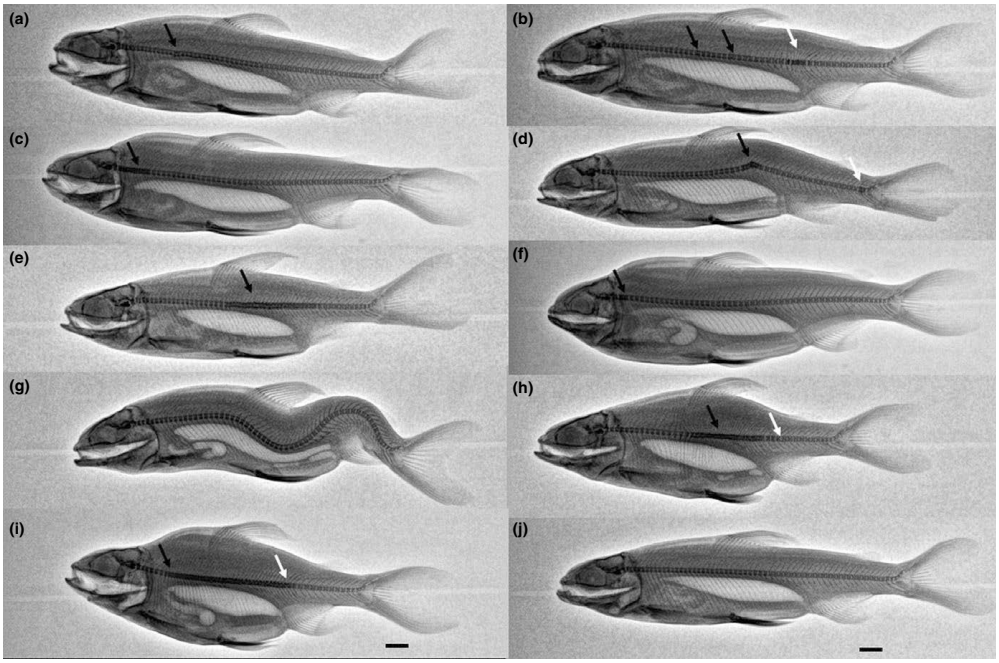

FIGURE 2 Radiographs of vertebral columns of deformed (ID) and normal (IN) intensively reared peled Coregonus peled. (a) Vertebrae displaced ventrally (black arrow) no atypical body shape; (b) fusion of two (black arrows) or more vertebrae (white arrow) along spine, no atypical body shape; (c) fusion of several vertebrae in R1 (black arrow) with body kyphosis in R2; (d) fusion and dorsal displacement of several vertebrae in R3 (black arrow), body kyphosis in R3 combined with fusion of vertebrae in R4 (white arrow); (e) fusion of numerous of vertebrae in R2 and R3 without creating atypical body shape; (f) fusion of two vertebrae in R1 (black arrow), body kyphosis in R2; (g) severe lordosis and scoliosis of vertebral column with no fusion of vertebrae; (h) fusion of numerous vertebrae in R2 and R3 (black arrow), humpback morphology associated with increased dorso-ventral body height relative to body length and wider intervertebral space (white arrow); (i) fusion of numerous vertebrae in R2 (black and white arrows), humpback morphology associated with increased dorso-ventral body height relative to body length; (j) morphologically normal (IN) fish. Scale bars = 1 cm

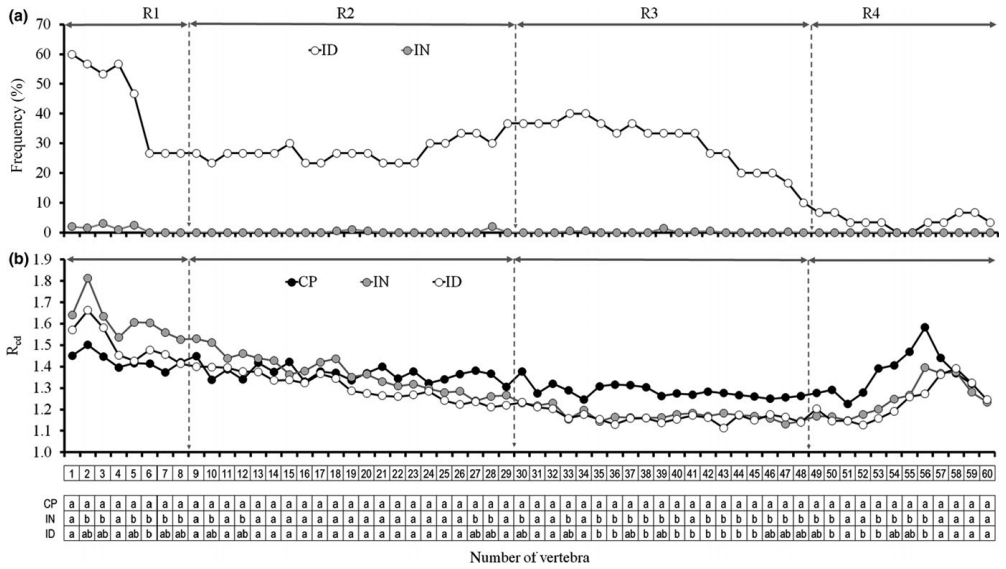

FIGURE 3 Frequency of deformed (ID) vertebrae in intensively reared Coregonus peled (a), and pattern of mean cranio-caudal length and dorsal-ventral height ratio (b) in individual vertebrae along the vertebral column in control (CP) (black spot), normally developed (IN) (grey spot) and ID (white spot) (n = 15–33 per vertebra per group). Different letters in table indicate significant differences for appropriate vertebrae

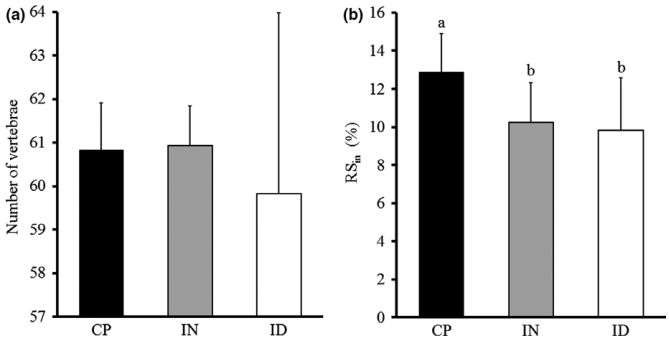

FIGURE 4 Number of vertebrae (a) and relative size of intervertebral space (b) in intensively reared deformed (ID) and normal (IN), and pond-reared (CP) Coregonus peled (n = 33 per group)

et al. (2011) and Fraser et al. (2015) showed that R_{cd} could be influenced by ploidy level and by thermal regimes during rearing in Atlantic salmon.

Studies of Atlantic salmon and European seabass have found increased incidence of skeletal deformities associated with inappropriately high water temperature during egg incubation and larva rearing (Fraser et al., 2015; Georgakopoulou et al., 2007; Grini et al., 2011). Water temperature during larval rearing in the present study was comparable to that used by Žil'ukas, Penaz, and Prokes (1983), who did not report deformities in peled reared on live food. Matousek, Stejskal, Prokesova, and Kouril (2017) used water temperatures of 13, 16, 19, 22 and 25°C in peled with initial body weight 0.6 g and reported no deformities during growth trials to body weight of 30 g. Temperature-induced deformities were not observed in the similar species *Coregonus lavaretus* (Siikavuopio, Knudsen, Amundsen, Sæther, & James, 2013).

No difference among groups was found in vertebra count (Figure 4). Significantly ($p < .001$) larger RS_{in} was found in the CP group compared to IN and ID groups. Reduction in intervertebral space has also been described in deformed Atlantic salmon (Fjelldal et al., 2016; Fraser et al., 2015). No authors have compared RS_{in} in tested groups of fish. We assume that this parameter provides an indication of vertebral column development.

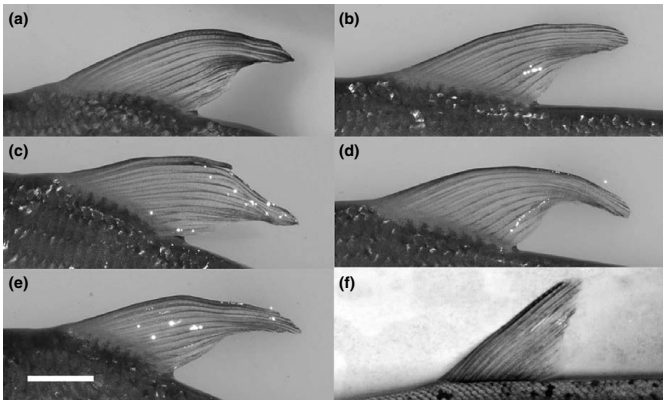

FIGURE 5 Examples of dorsal fins in deformed (ID) Coregonus peled. (a–e) Dorsal fins from ID group (f) normally developed dorsal fin of pond-reared group control pond-reared (CP). Scale bars = 1 cm

TABLE 3 Morphometric characters (mean±*SD*) in control pond-reared (CP), intensively cultured normally developed (IN) and intensively cultured deformed peled (ID) Coregonus peled

Morphometric characters	CP	IN	ID
HL	23.9 ± 0.7^{a}	23.8 ± 0.8^{a}	24.4 ± 2.0^{a}
PDD	48.4 ± 0.6^{b}	48.8 ± 1.0^{ab}	50.1 ± 2.9^{a}
PVD	51.4 ± 0.6^{a}	50.6 ± 1.0^{b}	51.8 ± 3.0^{ab}
PAD	76.3 ± 0.9^{a}	75.3 ± 1.5^{b}	75.7 ± 2.0^{ab}
DVA	25.5 ± 0.9^{a}	25.3 ± 1.2^{a}	25.2 ± 2.0^{a}
DPV	27.4 ± 0.7^{a}	26.1 ± 1.2^{b}	27.0 ± 1.9^{ab}
Hmax	23.4 ± 0.8^{c}	26.9 ± 0.9^{b}	30.3 ± 3.8^{a}
Hmin	9.0 ± 0.3^{b}	9.2 ± 0.4^{b}	9.7 ± 0.5^{a}
PRD	21.8 ± 1.1^{b}	25.6 ± 0.9^{a}	25.5 ± 2.0^{a}
POD	49.3 ± 1.9^{a}	46.6 ± 2.0^{b}	45.3 ± 2.6^{b}
ED	27.2 ± 2.4^{b}	28.0 ± 1.3^{ab}	28.8 ± 2.5^{a}
OCH	72.6 ± 1.8^{b}	84.3 ± 3.3^{a}	86.3 ± 5.2^{a}

Data are presented as mean ± *SD* (*n* = 33 per group). Different superscripts indicate significant differences.
Head length (HL), predorsal distance (PDD), preventral distance (PVD), pre-anal distance (PAD), distance between ventral and anal fins (DVA), distance between pectoral and ventral fins (DPV), maximum body height (Hmax) and minimum body height (Hmin) are expressed as % of standard length. Pre-orbital distance (PRD), post-orbital head length (POD), eye diameter (ED) and occipital height of head (OCH) are expressed as per cent head length

Phosphorus levels in feed up to 16 mg/kg have been shown to prevent vertebral deformities (Fjelldal et al., 2016). This was not borne out by our study, in which dietary phosphorus ranged from 12.0 to 17.7 mg/kg.

Deformities of the dorsal fin characterized by curvature of fin rays were observed infrequency of 51.5 ± 10.4% (Figure 5). Other fins showed normal development. In intensively reared salmonids and percids, pectoral and ventral fins are affected (Fragkoulis et al., 2017; Policar et al., 2016; Stejskal, Policar, Křišťan, Kouril, & Hamackova, 2011). It is presumed that fin ray curvature in percids and salmonids is related to intraspecific aggression. No deformities of fin were found in pond-reared fish.

Intensively reared peled, both ID and IN, tended to have higher body (Hmax) than the pond-reared fish, and differences from pond-reared were seen in the majority of measured morphometric characters (Table 3). These results are particularly in accordance with Koumoundouros et al. (1997) who reported differences in post-orbital distance between normally developing and deformed sea bream.

This study showed the intensive rearing system to be associated with skeletal, opercular and fin deformities in peled, and suggests potential factors that require investigation in future controlled experiments. However, the factors the affected normal development were not identified. We can speculate on potential effects of inappropriately high water temperature during early rearing and/or inadequacy of the commercial feed. Good husbandry and diets are crucial to establishing a rearing protocol that minimizes abnormalities in this species.

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CHAPTER 9

GENERAL DISCUSSION

The fish of the family Coregonidae provided a lot of problems for researchers related to study of their evolution, zoogeography and taxonomy. They display confusing morphological variation and plasticity, as well as unusual distribution. The distributional patterns which is associated with recent glaciation of much of their ranges, and instances of species flocks and siblings species with highly restricted distributions (Bodaly et al., 1991). The systematics of *Coregonidae* is traditionally regarded as a real chaos. In reality, the main issue is that coregonid systematics has been complicated by outdated and poor scientific publications which resulted in general confusion in systematic classification. In addition, the literature is complex, heterogenous and of very variable quality. A lot of studies are affected by ideas and theories that coregonid systematics should be different from the systematics of other fishes. The typical representative fish species of this universal systematic chaos is European whitefish (*Coregonus lavaretus*) which is also object of this thesis. At the same time, many authors have called most whitefish populations *C. lavaretus*. Some scientists have used the name *C. lavaretus* for one of the Baltic species. Correctly, the name applies only to the species of Lake Bourget in France where the fish was already reported by Guillaume Rondelet in 1555 (Kottelat and Freyhof, 2007).

Apart from systematics problems, situation is even worse because some coregonid species considered as a clear species could be hybrids of two different species with "contaminated" genes. (Luczynski et al., 1992). This can lead to rapid degradation or even extinction of some coregonid species. Further factors affecting coregonids decline are activity of great cormorants (*Phalacrocorax carbo*) (Suter, 1997), overfishing (Jackson et al., 2001), eutrophication (Thomas and Eckmann, 2007), degradation of natural spawning sites (Winfield et al., 2004), pollution, and environmental changes (Walther et al., 2002). On the other hand, thanks to re-stocking programmes, especially in Baltic Sea basins, whitefish production has increased in Poland (Fopp-Bayat et al., 2015) and Finland (Säisä et al., 2008). Whitefish larvae are stocked to increase year-class strength (Leskela et al., 1995) and production (Turkowski and Bonar, 1995), to sustain populations without natural reproduction (Steffens, 1995), and to restore extinct populations (Luczynski et al., 1998). Nowadays, it is important that re-establishment of whitefish natural production be accompanied by the culture in intensive aquasystems. Production of whitefish in recirculating aquaculture systems (RAS) requires identification of optimal larviculture conditions, containing optimal light regime, water temperature, stocking density, feeding regime, oxygen requirements and other important rearing aspects to avoid some factors which can cause for instance fish deformities etc.

In the present study, effect of selected rearing aspect on maraena whitefish and peled early development in intensive aquaculture systems was investigated.

Effect of light intensity and tank wall color in peled larvae (Paper I.)

Light in its intensity, spectrum and photoperiod is extremely variable factor which can rapidly change over a tremendous range. Furthermore, light shows interesting characteristics in the aquatic environment. In fact, quality (represented by different wavelengths absorbed by water to various extents), quantity (characterised by different intensities) and 'periodicity' (dependant on daily cycles, which vary seasonally according to latitude) should be considered. The status of fish receptors must also be taken into consideration, on the other hand very little information is available on this problematic. Fish are very sensitive to illumination and their eyes together with the pineal gland are the main light receptor organs. It is well known that most species need light for food detection and prey capture. Thus, apart from light effect

itself, its effect on fish performance is often in connection with other factors such as feeding activity (Sumpter, 1992). The setting, maintenance and control of the optimal light condition could be really difficult, even though it is much easier to control and regulate light regimes in the laboratory, or in intensive indoor aquaculture systems, where it is possible to examine fish under fixed conditions. Numerous and extensive experiments have been carried out in this way (Boeuf and Le Bail, 1999).

Many experiments deal with single effect of light intensity (Mortensen and Damsgard, 1993), tank wall colour (Monk et al., 2008), light spectrum (Stefansson and Hansen, 1989), and photoperiod (Duray and Kohno, 1988) on growth and development of various fish species. In our study we focused on the combined effect of light intensity (low, intermediate, and high) and tank wall colour (black, white, grey, green, red, blue, and clear) on peled larvae growth performance and survival. This complexity and combinations makes our study quite unique. It is very important to establish an optimal light intensity because the ideal light intensity for fish survival could not correspond with this beneficial for fish growth or development and vice versa. This claim is confirmed by Ronzani Cerqueira et al. (1991) focused on sea bass larvae (*Dicentrarchus labrax*). A compromise needs to be found because too bright light can have stressful or even lethal effect on fish as was seen in study Barahona-Fernandes (1979) in sea bass larvae (*Dicentrarchus labrax*).

In relation to light intensity in our investigation, peled larvae were independent of light intensity when no significant differences in survival and growth were observed among all examined groups. Slightly higher (non-significant) survival was observed within intermediate illumination. On the contrary, tank wall colour effect was apparent when larvae in black and white tanks showed significantly higher survival rate compared to grey tanks. No walling behaviour (larvae vigorously swim into the walls of the tank) was observed during the experiment. Jaw deformities can be affected by mechanical strikes against the walls as was the case of study Cobcroft and Battaglene (2009), when both walling behaviour and jaw deformities were associated with red tanks at striped trumpeter (*Latris lineata*). Based on our investigation, intermediate LI combined with black TWC can be recommended for successful rearing of peled larvae, but other interactive factors accompaining light conditions need to be tested.

Effect of water temperature in maraene whitefish larvae (Paper II.)

Temperature changes can affect survival, growth, feeding and the maturation of fish (Jobling, 1994). Optimum thermal conditions were identified for many fish species and their certain developmental periods with respect to development and mortality rates (Martell et al., 2005), but scarcely with regard to the other factors such as development of skeletal deformities at advanced ontogenetic stages (Sfakianakis et al., 2004; Ørnsrud et al., 2004). High temperature reduces survival of eggs and newly hatched fry of vendace (*Coregonus albula*) (Luczynski and Kirklewska, 1984), increases frequencies of vertebral (Brooke, 1975), eye (Rajagopal, 1979) and embryo (Cingi et al., 2010) deformities in newly hatched mountain whitefish (*Prosopium williamsoni*) and lake whitefish (*Coregonus clupeaformis*). Too high incubation temperature could produce shorter and smaller post-hatch larvae (Blaxter, 1992), as well damage yolk-sac fry what could affect swimming behaviour. As a consequence, chances of deformed smaller larvae to survive decrease (Cingi et al., 2010).

Determination of optimal temperature requirements is also essential for those who rear whitefish larvae in lake cages (Mamcarz and Nowak, 1987), hatcheries (Champigneulle, 1988), or recirculating systems (Szczepkowski, 2006). In hatchery, and intensive aquaculture systems the rearing temperature can be regulated for support of fast growth rate and the

best utilization of expensive dry diet (Luczynski et al., 1986 a,b). The food intake requirements increase simultaneously with increasing temperature and the efficiency of food which is converted to growth is reduced (Goolish and Adelman, 1984). Consequently, as food becomes limiting (by reduced abundance, energetic value, utilization by competing fish species, shorter photoperiod causing reduced feeding times), the temperature for optimum growth is gradually lowered (Brett et al., 1969). This situation was seen in study Szczepkowski (2006) when increasing water temperature above 22°C resulted in less efficient food utilization although it was served in quantities that were fully consumed by the fish.

It is obvious that not only temperature but also other accompaining factors decide about successful larvae breeding. In the case of Siikavuopio et al. (2013) the highest European whitefish mortality was observed in the treatment with maximal temperature used in the test. Contrastly, in study Siiakuvopio et al. (2010) the European whitefish survival decreased with temperature decline. Our results demonstrates that water temperature during larval phase has a significant effect on maraena whitefish growth performance and survival. Fish reared in 19 °C displayed the lowest survival after 28 days of rearing. The reverse situation was observed in association with growth, when larvae which were reared in 19°C showed the highest growth-weight parameters. It could be emphasised that both survival rate and growth rate must be considered in judging a suitable temperature which will be used in the rearing system for the best larvae performance. It is apparent that various whitefish species displays different (more or less) thermal tolerance. On the other hand, differences in optimal temperature within one fish species are presumably connected to the differences in population, feeding regime, photoperiod and other factors used in the test. There is still space for study of an optimal temperature of different coregonid species and developmental stages.

Effect of stocking density in maraene whitefish larvae (Paper III.)

The capability to rear larvae at higher stocking densities could have a significant effect on total production productivity (Turkowski et al., 2008b). Despite this fact, an improper stocking density in combination with other above mentioned factors (deterioration of water quality, welfare) can influence the survival and growth of larvae. A lot of studies indicate the evidence of a negative correlation between growth rate and inappropriate stocking density (Żarski et al., 2008; Rowland et al., 2006; Saoud et al., 2008), as well as negative relationship between survival and inconvenient stocking density (Molnár et al., 2004; Szkudlarek and Zakes, 2007; Ellis et al., 2012). This was not case of our study, because stocking density used in our experiment (25, 50, 100, and 200 larvae $\cdot L^{-1}$) had no significant effect on growth rate and survival of maraena whitefish larvae. A slightly better (non-significant) larvae growth rate and survival in stocking density group is negligible. In the studies of most of the fish species, it was demonstrated that the stocking density 25 larvae $\cdot L^{-1}$ might have a significant impact on the fish size heterogeneity that might have contributed to increased cannibalism (Baras et al. 2000a; Shields, 2001; Kestemont et al., 2003). In our test, size heterogeneity did not significantly differ and cannibalism did not appeared because maraena larvae are not piscivorous. The present study demonstrates that larvae rearing at higher stocking densities can be successfully managed, as was also found for instance in Kupren et al. (2011). The recommendation of this study for fishery practise is that it is possible to rear maraena whitefish larvae at high stocking density in the same water volume followed by no negative consequences.

Effect of feeding strategy in peled and maraene whitefish larvae (Paper IV. and V.)

Coregonids are currently species of great interest in aquaculture. However, for a long time, their nutritional requirements were little known. Consequently several authors tried to develop formulated practical diets, for instance in Japan (Dabrowski et al., 1984), Europe (Champigneulle, 1988), or USA and Canada (Harris and Hulsman, 1991). For fish intensive aquaculture, the knowledge about nutritional requirements, feeding practise, and feed management strategies are essential (Jørgensen et al., 1996). Prosperous techniques for whitefish rearing require identifying of the optimal feed composition and feeding strategies (Ruohonen et al., 2007).

Both, disproportionate feeding rates (too high or too low) negatively influence production effectivity. Restrictive feed rations are too small to satisfy the nutritional requirements of the fish, which can lead to competition for food and formation of a hierarchy among the fish (Jobling, 1995), followed by increasing size heterogeneity (Alanärä and Brännäs, 1993), decreasing growth rate of stocking, and growing occurrence of cannibalism (Szczepkowski, 2009b). On the other hand, cannibalism among whitefish population is scarce in wild (Tolonen, 1997) or in intensive aquaculture (Wunderlich et al., 2011). No of described negative phenomena related to inadequate feeding were observed in both of our experiments (Paper IV., and V.). We fed larvae slightly in excess because increased feed rations usually have an advantageous impact on fish growth performance (Fiogbe and Kestemont, 2003). Nevertheless, after the maximum feeding level is exceeded in defined conditions, the feed is no longer consumed or converted to the fish growth (Cotton and Walker, 2005).

Apart from an optimal feeding rate, the choice of food with its optimal composition remain important aspect not only for support of fish digestion process but also for rentability of all production. Live *Artemia* and other live organisms are generally used for first larval feeding (Dhont et al., 2013). On the other hand, since the development of commercial marine fish culture in the late 1970s, the demand for *Artemia* cysts has gradually increased from a few metric tons to approximately 800 t per annum worldwide, representing approximately 40% of the total aquaculture feed for early life stages (Lavens and Sorgeloos, 2000). Furthermore, culture of live feed does not ensure safe and stable nutritional quality (Drossou et al., 2006). Some *Artemia* strains, in particular, are relatively low in eicosapentaenoic (EPA, 20:5n-3) and especially docosahexaenoic acid (DHA, 22:6n-3) (Sorgeloos et al., 2001). Alternative live feed such as copepod, nematodes (Abate et al., 2015), and rotifers (Sorgeloos et al., 2001) may reduce costs of early rearing. Another option is dry feed enriched with enzymes to aid digestion (Kuzminski et al., 1996). On the other hand, the low cost, ready availability, and ease of handling make commercial feeds popular. However, in comparison with live feed, growth may be impaired by lack of required nutrients (Kotani et al., 2006), particularly of enzymes present in natural prey that enhance the digestive process in first-feeding larvae (Verreth et al., 1993).

Besides feeding rate and choice of food, feeding strategies including weaning time (Paper IV.), coo-feeding or both combination of different weaning time and co-feeding duration (Paper V.) have to be taken into consideration. Weaning time could be characterized as shift from live food to artificial diet, whereas co-feeding is defined as combined feeding of live prey and commercially formulated diets. Both strategies are used to improve larval growth and survival with regards to right digestive process.

In our first feeding study (Paper IV.) we applied seven feeding strategies (live feed, commercial diet, first weaning from live feed to a commercial diet at 5, 10, 15, 20 and 25 days). First weaning from live feed to a commercial diet showed best growth and survival of maraena whitefish after 30 days, whilst live feed displayed the best results during the first

20 days of rearing. This situation can be justified by the crucial role of enzymes obtained in live feed when *Artemia* sp. is a highly digestive source of protein compared to commercial diet (Cahu and Zambonino Infante, 1994). Enzymes help larvae to properly digest food prior to stomach is development (Lauff and Hofer, 1984). Unfortunately, we did not examined digestive enzymes but their role on the feeding digestibility is undoubted. The object of our study was also evaluation of intestine development and liver alterations. Summarily said the best intestine histomorphometry (intestine diameter, villi length, villi width) was observed within live feed group. The least severe histopathology (the first intestine degeneration grade) displayed all groups excluding commercial diet which showed the third degradation grade. Raskovic et al. (2016) suggest integration of histopathology and histomorphometry as a marker of general fish health. Intestinal epithelium is considered an important site of nutrient absorption, osmotic balance, immunity, and proper function of enzymes and macronutrients (Alvarez-Pellitero, 2011), and the distal intestine is the site of protein endocytosis (Rombout et al., 1985). We did not record any pathological liver alteration, when liver degeneration score showed first grade. On the other hand, it is difficult to determine a threshold for what should be regarded a healthy liver in farmed fish, and it is known that commercial feed causes lipid droplet accumulation, hepatic cell membrane degeneration and vacuolization (Bilen and Bilen, 2013), but it did not corresponded to our study.

In our second study related to larvae feeding (Paper V.) we used five weaning times (10, 15, 20, 25, and 30 days posthatch) and three co-feeding durations (1, 3, and 6 days). Peled growth and survival were significantly affected by weaning age, co-feeding duration and their interaction. It is obvious that feeding techniques including weaning time and co-feeding duration are crucial factors in successful larviculture. At the same time, the cost of feeding used in the test was significantly affected by weaning age and interaction of weaning age and co-feeding duration. Co-feeding duration itself had no significant effect. Feed cost analysis could be one of the clues which affects breeders viewpoint on choice of feeding strategy in fishery practice. On the other hand, digestibility of food and development of larval digestive system which is associated with larvae age and size (Cahu and Zambonino Infante, 1994) is more important aspect which should be considered.

Effect of water oxygen saturation in peled juveniles (Paper VI.)

The oxygen level plays a key role in water quality in aquaculture (Valverde et al., 2006) and optimal oxygen concentrations in water are vital to intensive fish farming (Ritola et al., 2002). The oxygen consumption measurement is an indirect way for estimation of fish metabolism (Pichavant et al., 2001). Transitions between hypoxia/anoxia and normoxia or between normoxia and hyperoxia could cause oxidative stress (Storey, 1996). Not only unsuitable oxygen level but also interaction of handling, transport, confinement, social hierarchies, agonistic behaviour, and poor chemistry in general reflect fish health status (Barton and Iwama, 1991; Davis, 2006). Based on many researches it can be asserted that fish stress response and tolerance is species dependant. Nevertheless, maintenance of excellent water physical-chemical treatment and considerate manipulation could be a basic precondition to successful fish rearing. In our study water quality (excluding oxygen saturation) as well as manipulation corresponded with these rules.

On the other hand, in fishery practise, oxygen concentrations are usually maintained in a mild hypersaturation conditions. The mild hyperoxia could lead to morphological adaptation of the respiratory apparatus as was seen in sea bass (*Dicentrarchus labrax*) (Cecchini et al., 1999), increases serum immunoglobulin concentration (Scapigliati et al., 1999), and specific antibody response (Cecchini and Saroglia, 2002). Contrariwise, low oxygen level produces

a stress in fish (Perry and Gilmour, 1999), as well as increases the susceptibility to infective agents (Bunch and Bejerano, 1997). In our research, we applied four oxygen levels: normoxia (80%–90%), hypoxia (50%–60%), hyperoxia (150–160%) and intermittent hyperoxia (150%–160% – 80%–90%). We did not found any significant differences in FCR among all tested groups. Body weight differed mainly among normoxia compared to other treatments whereas hyperoxia group did not show any elevated growth compare to other groups. It is possible that hyperoxia does not cause physiological disturbances in the acid-base status when compare with normoxia. The same situation was also observed in Cecchini and Caputo (2003). Moreover, condition factor and body weight in some time period were significantly lower in hypoxia conditions compared to other groups. This situation was not attributed to infection or disease but fish stress in hypoxia conditions what corresponded to general worsening of welfare. We did not observed stress hormones but hypoxia conditions were associated with increased mortality compared to other treatments.

The hyperoxia conditions were connected to lower erythrocytes count, concentration of haemoglobin and reduced haematocrit at peled juveniles. Similar situation was also seen in Hosfeld et al. (2010) at Atlantic salmon presmolts, when significantly reduced haematocrit was observed under hyperoxia conditions compared to normoxia. The reverse situation was observed in Lemarié et al. (2011) at European sea bass and Hosfeld et al. (2008) at Atlantic salmon when no significant differences in erythrocytes count, concentration of haemoglobin and haematocrit size were observed at fish reared in different levels of oxygen saturation. It is evident that haematological parameters are valuable indicators which could be taken into account before planning of future experiments.

Effect of culture system on deformities level in peled (Paper VII.)

Frequency of body deformities vary among fish species, as well as rearing conditions. Deformities of the vertebral column in wild and farmed teleosts are represented by spine curvature, as anterior/posterior shortening of the spine, or as a combination of spine curvature and shortening (Kvellestad et al., 2000; Gavaia et al., 2002). Whilst deformities involving bending of the spine (lordotic, kyphotic and skoliotic malformations) might appear obvious at earlier life stages, the anterior/posterior spine shortening is usually much less noticable. Mild cases affecting only part of the vertebral column often remain undetected (unless the fish are X-rayed) or might be erroneously considered as undeformed (McKay and Gjerde, 1986, Madsen et al., 2000). The abnormal changes of the vertebral bodies can be compression, a combination of compression and ankylosis (Fjelldal et al., 2006; Witten et al., 2006) and ankylosis and dislocation of normal vertebral bodies (Fjelldal et al., 2004). Ankylosis and compression is characterised by a fusion of two or more deformed vertebral bodies, (Wargelius et al., 2005).

Our study indicates that mean frequency of opercular deformities in intensively reared deformed peled was 12.9 ± 4.2%. Opercular deformities were accompanied by atypical head profile. We assume that the causes of opercular deformities can be attributed to dietary ascorbic acid, heritability or environmental factors. Next, spinal deformities in intensively reared deformed peled were 8.2 ± 2.8%, followed by lower prevalence at intensively reared group. No differences in fish vertebrae count were observed among all examined treatments whereas significantly larger relative size of intervertebral space was observed in fish originated from pond conditions compared to intensive condition groups. Our assumption is that temperature used in the test was optimal to prevent from vertebral deformitiews. Furthermore, higher content of some minerals such as phosphorus and calcium can prevent from vertebral deformities. This theory is supported by Lall and Lewis-McCrea (2007) who

claim that minerals content has a strong effect on the mechanical strength of vertebral body. Phosphorus is obtained and absorbed from the diet whereas calcium content is covered from the water and the diet at the same time. Dorsal fin deformities characterized by curvature of fin rays were observed in frequency of 51.5 ± 10.4% in intensively deformed fish group whilst no fin deformities were associated with pond conditions. This could be a consequence of abrasion from rough tank surfaces, as was also observed in study Bosakowski and Wagner (1995). The majority of peled morphological parameters at intensively reared fish (both deformed and normal group) differed significantly compared to pond reared (control) group. Based on our own experiences we know that except rearing environment, temperature is an important factor influencing peled morphometry (Sebesta, unpublished data).

Conclusions

This dissertation thesis includes seven impacted papers describing several specific aspects which can enhance performance and rearing success of European whitefish and peled. These publications can be used to improve whitefish intensive aquaculture.

The following conclusions were gained:

1) The light intensity, tank wall colour or their simultaneous effect had no impact on peled growth performance, but tank wall colour significantly affected survival. Hence, rearing of peled in black tanks can be recommended for ensuring of the highest survival.
2) The optimal temperature for maraena whitefish larvae growth acceleration was 19°C, whereas 11°C treatment displayed significantly highest survival in 28-days trial.
3) Stocking density 200 larvae $\cdot L^{-1}$ of maraena whitefish larvae can be used to increase productivity.
4) The initial weaning from live feed to artificial diet after 15 days is the optimal feeding strategy with regards to maraena whitefish larva growth, mass, and yield. Live feed showed to be the most beneficial for intestine development, whereas artificial diet was connected to severe intestine impairment. The tested feeding strategies were not associated with liver pathology in any treatment.
5) Peled larvae weaned after 20 days post hatching regardless co-feeding duration reach significantly higher yield compared to other groups. It can be summarised that abrupt weaning (one day of co-feeding) to dry food after 20 days of feeding with live prey ensure optimal production while reducing the cost and effort related to live feed.
6) Normoxia (80%–100% saturation) is optimal for peled juveniles growth. The hyperoxia conditions were associated with lower erythrocytes count, concentration of haemoglobin and reduced haematocrit. Studies of the effects of mild hyperoxia (110%–120%) may be valuable in future research.
7) Intensive aquaculture systems are related to opercular, skeletal and fin deformities in peled juveniles, as well as variable morphometry. Combination of inappropriate water temperature and inadequate feeding can probably contribute to body shape abnormalities.

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ENGLISH SUMMARY

Selected aspects of intensively cultured European whitefish (*Coregonus maraena*, Bloch) and peled (*Coregonus peled*, Gmelin)

Roman Šebesta

Maraena whitefish (*Coregonus maraena* Bloch, 1779) and peled (*Coregonus peled* Gmelin, 1788) are non-original fish species occurring in Czech Republic. Maraena whitefish origin is Mazurian lakes and origin of peled is Siberia. In past, both species were reared in pond polyculture with carp. Based on their productive, biological, and culinary properties, these fish are considered as economically important. Initially, production of both fish whitefish species reached relative results, consequently it declined dramatically. The production decline was attributed to predatory activity of cormorants, followed by eutrophication, pollution, alteration of water bodies and degradation of natural spawning sites. Situation was worsened by unprofessional releasement of hybrids of both species in to open waters. In order to preclude production decline or even total extinction of peled and maraena whitefish, the re-stocking programmes were established. Whitefish breeding is transitioned from ponds to intensive rearing conditions. In recent years, recirculation systems appear to be perspective for rearing of various fish species. Whitefish rearing in indoor recirculation system eliminates or considerably restricts risks mentioned above. On the other hand, it is essential to maintain optimal rearing conditions obviously in larviculture.

The objectives of present thesis were to test the effect of selected aspects which can improve the quality of larvae or juvenile rearing in recirculation aquaculture systems and ensure higher production of all rearing. Observed factors are following: light regime, temperature, stocking density, feeding and feeding strategy, oxygen, and rearing environment.

The aim of the first study was to find out an optimal combination of light intensity and tank wall colour in rearing of peled larvae. Three light intensities (80, 380, and 3800 lux) and seven coloured variations (black, grey, white, red, green, blue, and clear) were used in the test. The results of rearing revealed that peled larvae are independent of light intensity effect but prosper in black and white tanks. Combined effect of light intensity and tank wall colour did not affect final survival rate but resulted in a big size heterogeneity among examined groups. Rearing of peled larvae in black and white tanks combined to intermediate light intensity (380 lux) could be recommended.

The goal of the second trial was to test a temperature effect on growth and survival of maraena whitefish larvae. Three temperature (11, 15, and 19°C) were applied in the experiment. It was investigated that the best performance of maraena whitefish during the first 21 days was observed at 15, and 19°C. From the period 22–28 days, the best growth was observed at 19°C whereas the highest survival rate was observed at 11, and 15°C. At the end of the experiment, the highest condition factor and larval yield was observed in larvae reared at 19°C compared to larvae reared at 11, and 15°C. Temperature 19°C is recommended for the highest growth, yield and condition factor whilst 11°C is ideal temperature for the best larvae survival.

The objective of the third study was to test stocking density effect in rearing of maraena whitefish larvae. Four initial stocking densities (25, 50, 100, and 200 larvae $\cdot L^{-1}$) were used in the trial. Maraena whitefish displayed no significant differences in growth and survival rate, condition factor, size heterogeneity, and larval yield among all stocking density treatments. Slightly higher (non-significant) parameters were obtained from larvae reared at 25 larvae $\cdot L^{-1}$. On the other hand, stocking density 200 larvae $\cdot L^{-1}$ can be used to gain higher production within the same system and volume capacity accompanied by no negative effects on growth and survival of maraena whitefish larvae.

The aim of the forth study was to find out an adequate feeding strategy enhancing survival, and growth. Intestine development, and liver status of maraena whitefish larvae were also examined parameters. Seven feeding strategies (live food, commercial diet, and first weaning from live feed to a commercial dry diet at 5th, 10th, 15th, 20th, and 25th days post hatch) were applied in the examination. The liver degradation score represented grade 1 in all treatments. The intestine damage score showed solely grade 3 in commercial diet group whereas live feed, and first weaning from live feed to a commercial dry diet at 5th, 10th, 15th, 20th, and 25th days post hatch took grade 1. Based on our results, the first weaning from life feed to a commercial dry diet at 15th day post hatch is recommended optimal feeding strategy of maraena whitefish larvae.

The fifth study aimed to investigate an optimal combined effect of weaning time and co-feeding duration elevating growth and survival of peled larvae. Five weaning times (10th, 15th, 20th, 25th, and 30th days post hatch) and three co-feeding durations (for 1, 3, and 6 days) were applied in the test. It was detected that weaning from live feed to a commercial dry diet at 20 day post hatch combined to 1 day of co-feeding provides an optimal feeding strategy assuring with regards to performance and production of peled larvae. At the same time, this combination reduces effort and price related to live feed and so in practical fishery standpoint this feeding technique is strongly advisable.

In the sixth study we examine the effect of various water oxygen saturation on growth and haematological profile of juvenile peled. Four oxygen regimes (normoxia – 80–90%, hypoxia – 50–60%, hyperoxia – 150–160%, and intermittent hyperoxia – 150–160% – 80–90%) were used in the investigation. It was determined that hypoxia level has a negative impact on peled growth and survival. Supersaturation had neither positive nor negative impact on peled performance. Fish reared in hyperoxia had lower haemoglogin concentration and haematocrit level compared to fish in hypoxia and normoxia group. Fish reared in intermittent hyperoxia showed significantly lower content of erythrocytes, on the contrary, no significant differences were associated with content of leukocytes among all examined group. Thus normoxia conditions are the most optimal for peled juveniles rearing.

The objective of the seventh study was to assess prevalence of deformities in intensively reared peled and comparative morphometry with pond-reared fish. Skeletal, opercular and dorsal fin deformities were evaluated. Intensive rearing led to opercular deformities and head profile changes, as well as dorsal fin curvatures. Spinal deformities were represented mostly by fusion or compression resulted in atypical body shapes like kyphosis, lordosis or scoliosis. There were observed significant differences in most of morphometric parameters in intensively reared fish compared to pond reared fish. It is very important to pay attention to negative factors causing mentioned defects.

This dissertation deals with rearing of maraena whitefish and peled in recirculation system including optimization of some crucial aspects stimulating fish growth, increasing survival, improving organ development and blood profile parameters, and avoiding some negative factors. The majority of the thesis is focused on larviculture as a most critical period in fish rearing. Individual studies can provide some essential and practical advices to fishery practise.

CZECH SUMMARY

Vybrané aspekty intenzivního chovu síha marény (*Coregonus maraena*, Bloch) a peledě (*Coregonus peled*, Gmelin)

Roman Šebesta

Síh maréna (*Coregonus maraena* Bloch, 1779) a síh peleď (*Coregonus peled* Gmelin, 1788) jsou nepůvodní druhy ryb vyskytující se v České republice. Maréna pochází z oblasti Mazurských jezer a domovinou peledě je Sibiř. Oba druhy se v minulosti chovaly v rybniční polykultuře společně s kaprem. Pro své produkční, biologické a kulinářské vlastnosti jsou tyto ryby považovány za hospodářsky významné. Z počátku produkce obou druhů síhů dosahovala relativních výsledků, ale následně rapidně poklesla. Produkční propad byl způsoben převážně predační aktivitou kormoránů, dále pak eurofizací, znečištěním, zásahy v povodí a poškozením výtěrových míst. Situaci zhoršilo neodborné vysazování hybridů obou druhů síhů do volných vod. Ve snaze zamezit produkčnímu úbytku či úplnému vyhynutí druhu, vznikly programy zabývající se znovuobnovením obsádek peledě a marény. Chov síhů se přesouvá z rybníků do intenzivních podmínek chovu. V posledních letech se jeví jako perspektivní chov různých druhů ryb v recirkulačních systémech. Chov síhů v zastřešeném recirkulačním systému vylučuje či značně omezuje výše zmíněná rizika. Na druhou stranu je nezbytné zajištění optimálních podmínek chovu, obzvláště v průběhu odchovu larev.

Cílem této dizertační práce bylo testovat vliv vybraných aspektů, které mohou zlepšit kvalitu chovu larev či juvenilních ryb v recirkulačním akvakulturním systému, a zajistit tak vyšší produkci celého chovu. Zkoumané faktory jsou následující: světelný režim, teplota, hustota obsádky, krmení a krmná strategie, kyslík a prostředí chovu.

Cílem první studie bylo nalézt optimální kombinaci světelné intenzity a barev nádrží v chovu larev peledě. Tři světelné intenzity (80, 380 a 3800 lux) a sedm barev stěn nádrží (černá, šedá, bílá, červená, zelená, modrá a průhledná) byly použity v testu. Výsledky odchovu odhalily, že larvy síha peledě jsou nezávislé na světelné intenzitě, ale prosperují v černých a bílých nádržích. Kombinovaný efekt světelné intenzity a barev stěn nádrží neovlivňuje přežití, ale růstová heterogenita byla velmi variabilní u zkoumaných skupin. Lze doporučit chov larev peledě v černých a bílých nádrží v kombinaci se světelnou intenzitou 380 lux.

Ve druhé studii byl testován efekt teploty na růst a přežití larev síha marény. Tři teploty (11, 15 a 19°C) byly testovány v experimentu. Bylo zjištěno, že v průběhu prvních 21 dní larvy marény nejvíce rostou a přežívají v 15 a 19°C. V období 22–28 dní byl pozorován největší růst v 19°C, zatímco nejvyšší přežití bylo pozorováno v 11 a 15 °C. Na konci experimentu, nejlepší koeficient kondice a nejvyšší výnos larev byl pozorován v odchovu při teplotě 19°C v porovnání s larvami chovanými v 11 a 15°C. Teplota 19°C je doporučena pro dosažení vyššího růstu, výnosu larev a koeficientu kondice, zatímco 11°C je optimální pro nejlepší přežití larev.

Třetí studie se zabývá vlivem hustoty obsádky v chovu larev síha marény. Čtyři počáteční hustoty obsádek (25, 50, 100 a 200 larev $\cdot L^{-1}$) byly použity v našem pokusu. Nebyly nalezeny žádné signifikantní rozdíly v růstu, přežití, kondičním faktoru, růstové heterogenitě a výnosu larev marény u jednotlivých skupin. Nepatrně lepší (bez statistické významnosti) parametry byly získány u larev chovaných při hustotě obsádky 25 larev $\cdot L^{-1}$. Na druhou stranu, hustota obsádky 200 larev $\cdot L^{-1}$ může být použita za účelem získání vyšší produkce v rámci stejného systému a chovného objemu, což není doprovázeno žádnými negativními efekty na růst a přežití larev marény.

Cílem čtvrté studie bylo najít optimální krmnou strategii zlepšující přežití a růst. Dalším zkoumaným parametrem byl vývoj tenkého střeva a jater u larev marény. V testu bylo

aplikováno sedm krmných strategií (živé krmení, komerční suché krmivo a odstavení z živé potravy na suchou krmnou směs v 5., 10., 15., 20. a 25. den experimentu). Poškození jater bylo hodnoceno 1. stupněm ve všech skupinách. Míra narušení tenkého střeva dosáhla 3. stupně u skupiny krmené výhradně komerční směsí, zatímco skupiny krmené živou potravou či technikou odstavení z živé potravy na suchou krmnou směs v 5., 10., 15., 20. a 25. den vykazovaly 1. stupeň poškození. Na základě našich výsledků můžeme u larev marény doporučit optimální techniku krmení spočívající v odstavení z živé potravy na suchou krmnou směs v 15. den.

Pátá studie se snaží najít ideální kombinaci odstavení z živé potravy na suchou krmnou směs a společného krmení živé potravy a komerční směsi u larev peledě. Celkem pět různých časů (v 10., 15., 20., 25. a 30. den) odstavení z živé potravy na suchou krmnou směs a tři časy (1, 3 a 6 dní) společného krmení živé potravy a komerční směsi byly použity v testu. Bylo zjištěno, že odstavení z živé potravy na suchou krmnou směs v 20. den v kombinaci s jedním dnem společného krmení, poskytuje vhodnou krmnou strategii, která zajišťuje optimální růst, přežití a produkci larev peledě. Současně tato kombinace snižuje úsilí a cenu spojenou s použitím živého krmení, a tudíž je velmi doporučena z pohledu rybářské praxe.

V šesté studii jsme zkoumali efekt rozdílného nasycení kyslíkem na růst a hematologický profil juvenilů peledě. Čtyři kyslíkové režimy (normoxie – 80–90%, hypoxie – 50–60%, hyperoxie – 150–160% a přerušovaná hyperoxie – 150–160% – 80–90%) byly použity v testu. Bylo zjištěno, že hypoxie má negativní dopad na růst a přežití peledě. Supersaturace neměla ani negativní, ani pozitivní účinek na růst a přežití peledě. Ryby chované v hyperoxii měly nižší koncentraci hemoglobinu a úroveň hematokritu v porovnání se skupinou ryb chovaných v hypoxii a normoxii. Ryby chované v podmínkách přerušovaná hyperoxie měly signifikantně nižší obsah červených krvinek, naopak žádné změny v obsahu bílých krvinek nebyly zaznamenány mezi jednotlivými skupinami. Podmínky normoxie jsou tedy nejvhodnější pro chov juvenilů peledě.

Cílem sedmé studie bylo vyhodnotit výskyt tělesných deformit u intenzivně chovaných peledí a porovnat morfometri těchto ryb s rybami chovanými v rybničních podmínkách. Byly hodnoceny kosterní, operkulární a deformity hřbetní ploutve. Intenzivní chov zapříčinil operkulární deformity a rovněž tak s nimi spojené změny profilu hlavy a pokřivení hřbetních ploutví. Deformity páteře, které jsou charakterizované fúzí či kompresí, vyústily v atypický tvar těla, a to kyfózu, lordózu nebo skoliózu. Rovněž byly pozorovány signifikantní rozdíly ve většině morfometrických parametrů u intenzivně chovaných ryb v porovnání s rybami chovanými v rybníce. Je třeba věnovat zvýšenou pozornost faktorům, které tyto negativní projevy zapříčiňují.

Tato dizertační práce se zabývá chovem síha marény a peledě v recirkulačním systému, což zahrnuje optimalizaci vybraných klíčových aspektů, které stimulují růst, zvyšují přežití ryb, zlepšují vývoj orgánů, parametry krevního profilu a popřípadě zamezují výskyt některých negativních faktorů. Větší část práce je zaměřena na chov larev, který je nejkritičtější periodou v chovu ryb. Jednotlivé studie mohou poskytnout některé důležité a prakticky využitelné rady v rybářské praxi.

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LIST OF PUBLICATIONS

Peer-reviewed journals with IF

Sebesta, R., Kucharczyk, D., Nowosad, J., Sikora, M., Stejskal, V., 2018. Effect of temperature on growth and survival of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae in controlled conditions. Aquaculture Research 00, 1–7 (IF 2017=1.475). Accepted.

Sebesta, R., Kucharczyk, D., Nowosad, J., Sikora, M., Stejskal, V., 2018. Effect of stocking density on growth and survival of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae in controlled conditions. Aquaculture Research (IF 2017=1.475). Submitted.

Sebesta, R., Nowosad, J., Sikora, Biegaj, M., Kucharczyk, D., Stejskal, V., 2018. Effect of feeding strategy on survival, growth, intestine development, and liver of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae cultured under RAS conditions. Aquaculture Nutrition (IF 2017=2.078). Submitted.

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Sebesta, R., Stejskal, V., Matousek, J., Prokesova, M., Novikava, K., 2016. The combined effect of light intensity and tank wall colour on growth and survival of peled (*Coregonus peled* Gmelin, 1788) larvae. In: Aquaculture Europe 2016, September 20–23, Edinburgh, Scotland, pp. 909–910.	2016

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Prof. Dariusz Kucharczyk, University of Warmia and Mazury in Olsztyn, Department of Lake and River Fisheries, Olsztyn, Poland. (2 months, Effect of temperature on growth and survival of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae in controlled condition; Effect of stocking density on growth and survival of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae in controlled conditions).	2017
Prof. Dariusz Kucharczyk, University of Warmia and Mazury in Olsztyn, Department of Lake and River Fisheries, Olsztyn, Poland. (1 month, Effect of feeding strategy on survival, growth, intestine development, and liver of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae cultured under RAS conditions).	2018

Pedagogical activities	Year
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Announcing the project entitled Fitness in Eurasian perch (*Perca fluviatilis*) – effect of culture conditions at Summer school.	2018
Lecturing of students (RAS subject) of master study. Co-organization of students from Yspertal, propagation of faculty in open days.	2016–2018
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2018 Prof. Dariusz Kucharczyk, University of Warmia and Mazury in Olsztyn, Department of Lake and River Fisheries, Olsztyn, Poland. (1 month, Effect of feeding strategy on survival, growth, intestine development, and liver of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae cultured under RAS conditions).