Aquacult Int (2014) 22:195–203
DOI 10.1007/s10499-013-9670-5

Determination of the optimal feeding rate and light regime conditions in juvenile burbot, *Lota lota* (L.), under intensive aquaculture

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Received: 29 January 2013 / Accepted: 26 June 2013 / Published online: 10 July 2013

Abstract The aim of the study was to optimize burbot juveniles rearing in recirculating aquaculture system. In experiment 1 (17 °C, photoperiod 24L:0D), the fish (initial: body weight [W] = 15.36 ± 3.72 g, standard length [SL] = 12.48 ± 1.09 cm) were divided into four groups (I, II, III and IV). Different feeding levels were applied: 1, 2, 3 and 4 % of biomass daily (counted based on dry feed weight). The feed conversion ratio (FCR) and specific growth rate (SGR) were recorded. In experiment 2 (17 °C, feeding level of 2 % of biomass day^{-1}), fish (W = 5.24 ± 2.43 g and SL = 8.54 ± 1.24 cm) were divided into two groups where different light conditions were applied (I: 24 h light [1,800 lx] and II: 24 h darkness [4 lx]). In experiment 1, the highest SGR was recorded in group II (1.93 % day^{-1}), whereas the lowest SGR (1.27 % day^{-1}) and final W ($P < 0.05$) was in I group. The lowest ($P < 0.05$) FCR (0.63) was in group II. In the remaining groups, FCR was similar (0.68–0.70, $P > 0.05$). The feed consumption in group I reached 100 %, in group II, it was 71.3 % ($P < 0.05$) and it was the lowest in groups III (39.26 %) and IV (36.93 %). In experiment 2 no differences in the growth and survival rate were recorded (final SL between 14.16 and 14.19 cm, $P > 0.05$; W between 23.33–23.35 g; $P > 0.05$). The results from experiment 1 indicate that the feeding 2 % of biomass day^{-1} was the most efficient. Also, it was proven, for the first time, that there was no effect of using different constant light conditions.

Keywords Burbot (*Lota lota* L.) · Feed consumption · Feed conversion ratio (FCR) · Intensive production · Photoperiod · Specific growth rate (SGR)

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Introduction

Burbot (*Lota lota* L.) is the only representative of the *Gadidae* family that inhabits freshwater reservoirs of the Holarctic ecozone (Nelson 1994). This species is endangered in almost all of its habitats (Wolnicki 2001), and it has become important candidates selected for intensive aquaculture (Wocher et al. 2011a, b). It has thus become a focus of extensive scientific ventures aimed at elaborating intensive methods of burbot production both for restocking purposes and aquaculture (Wolnicki 2001; Żarski et al. 2009; Jensen et al. 2011; Trabelsi et al. 2011). However, there is a lack of effective rearing procedures of larvae and juvenile fish in controlled conditions (Wolnicki 2001; Jensen et al. 2011; Trabelsi et al. 2011; Wocher et al. 2011a, b).

In freshwater aquaculture, recirculating aquaculture systems (RAS) are most often used to produce species such as African sharptooth catfish (*Clarias gariepinus* Burchell) (Vandecan et al. 2011), tilapia (*Oreochromis niloticus* L.) (Martins et al. 2011) or barramundi (*Lates calcarifer* Bloch) (Worrall et al. 2011). In recent years, there has been a growing interest in diversification of European freshwater aquaculture by the intensification of production of native species such as pikeperch, *Sander lucioperca* (L.) (Wang et al. 2009; Policar et al. 2013) or Eurasian perch, *Perca fluviatilis* L. (Strand et al. 2007; Blaha et al. 2013). The most recently growing interest in aquaculture of species with much lower thermal requirements which is burbot (Jensen et al. 2011; Wocher et al. 2011a, b) is observed. The advantage of RAS is the possibility to control physical and chemical water parameters such as temperature, pH, oxygen content as well as to monitor the health status of fish and to accurately determine feed rations (Blancheton 2000; Remen et al. 2008; Żarski et al. 2008, 2010a).

Feed costs constitute a significant part of the financial input for production (Losordo and Westerman 1994), and it is therefore very important to precisely determine the feed ration to yield a beneficial level of feed conversion ratio (FCR) and high rate of growth to ensure cost-effective production. A carefully selected feed ration helps maintain the physical and chemical water parameters, such as ammonia, nitrites and suspension concentration, at the lowest level possible (Pedersen et al. 2012). However, there is still a lack of data on the optimal feeding ration for juvenile burbot during intensive rearing.

Photoperiod is a factor which has a direct effect on growth and survival rate (Puvanendran and Brown 2002). The light intensity as well as photoperiod duration has been recognized to affect growth rate of larvae in many fish species; however, there is still limited number of data regarding the effect of light conditions on older fish (Boeuf and Le Bail 1999) including burbot.

The aim of the study was to determine the optimal feed ration and light conditions on burbot juveniles reared under intensive controlled conditions.

Materials and methods

Pre-experimental rearing

The larvae of burbot originated from wild spawners from the lower Oder River which were reproduced in the fish farm "Czarci Jar" near Olsztynek (the province of Warmia and Mazury, north-eastern Poland). The spawners were stimulated only by manipulating thermal conditions in accordance with the method described by Żarski et al. (2010b). The eggs were manually stripped from three females with an average body weight of 720 (±108) g and fertilized in vitro with sperm collected from three males with a mean body weight of 332 (±79) g.

The initial rearing of larvae was carried out for 40 days in semi-closed RAS in a 100-L aerated tank with controlled temperature (±0.1 °C) and photoperiod regime. The initial stocking density was 150 specimens L^{-1}. During the first 15 days, the temperature was 12 ± 0.1 °C, and in the next stage, it was increased to 15 ± 0.1 °C. The larvae were fed ad libitum with *Artemia* sp. nauplii (San Francisco origin). When the fish reached the average individual weight of 0.11 (±0.02) g, they were transported to the semi-closed RAS, which consisted of twelve 50-L aquaria. The water temperature was 17 ± 0.1 °C, and the stock initial density during this phase was 10 individuals per L. Water was supplied gravitationally with the upper inflow, and each tank was equipped with additional aeration. The fish were initially fed with *Artemia* sp. nauplii and commercial feed; mixed granulation of feed ranged from 0.3 to 0.9 mm, for trout (Skretting, Norway), was introduced (54 % protein, 18 % fat and 8 % carbohydrates) after two weeks. In these experiments, the feed for rainbow trout was used because there was no specific feed for burbot on the market and this species is a coldwater and freshwater predator similarly to trout. The feed was administered continuously with the belt feeders. The tanks were thoroughly cleaned before each feeding. This pre-experimental rearing on trout feed was carried out three (in experiment 1) and two (in experiment 2) months. The photoperiod was maintained at 24 h (24 L:0 D) during the whole rearing period (of larvae and juvenile stages), and light intensity was 1,800 lx at the water surface. The detailed procedure of initial rearing as well as weaning protocol used in this study was described by Palińska-Żarska et al. (2013).

Experimental rearing

Experiment 1—feeding rate

The experimental stock consisted of fish with an average body weight $W = 15.36 \pm 3.7$ g and the average standard length SL = 124.8 ± 10.9 mm. The rearing was carried out for 5 weeks in semi-closed RAS in 50-L aquaria. The stock density was 0.9 individuals per L. The measurements of SL were made with a caliper (±1 mm) at the beginning and at the end of the experiment. Determination of *W* was made at the beginning and the end of the experiment as well as in every 7 day of the experiment (in order to evaluate the biomass of the fish) with precise balance (±0.1 g, Kern PBS 6200–2 M, Germany). The measurements were taken on the fish under short-term anaesthesia in a Propiscin 0.2 % (IRS–ZPiIR Żabieniec, Poland) (0.5 ml L^{-1}). At the beginning of the experiment, four experimental groups were created (I, II, III and IV), with three replications. In the group I, the daily feed ration was set at 1 % of the biomass, and in groups II, III and IV at 2, 3 and 4 % of biomass, respectively. The feed ration was calculated in feed dry matter according to the formula: $F_A = (X + [X\ 0.01\ M_O])\ 0.01\ B$; where F_A—feed amount per day (g), X—required feeding rate (%), M_O—moisture of the feed (%), B—biomass (g). The moisture of the feed was determined by drying a feed sample at 103 °C for 12 h. The new feed ration was evaluated every 7 days based on the actual biomass updated on the base of the weight measurements made every 7 days. In this experiment, fish were fed with the same feed as in pre-experimental rearing, but with higher granulation of 1.5 mm. The feed was distributed with belt automatic feeders for 24 h day^{-1} (with 2 breaks—1 h each break every 12 h), and its residues were removed from the tanks twice a day. The feed residues were then dried, and the content of dry matter was measured in order to determine the actual feed consumption and the feed conversion ratio (FCR). The consumed feed was calculated according to the formula: $C_F = D_F - U_F$; where: C_F—consumed feed, D_F—distributed feed to the tank (g), U_F—uneaten feed (g). The concentration of ammonia and nitrites was controlled twice a week with LF–205 photometer (Slandi, Poland). The content of oxygen was controlled every two days with an oxygen probe (HI 91410, Hanna Instruments, Italy). The photoperiod was maintained constant throughout the experiment at 24 h (24 L : 0 D; 1,800 lx). The temperature was 17 ± 0.1 °C and stayed in the optimal thermal range (15–18 °C) for juvenile burbot (Wolnicki 2001).

Oxygen content during the entire experiment always exceeds 80 % of saturation. The concentration of ammonia ranged from 0.66 to 1.35 mg L^{-1}, whereas the content of nitrites was 0.095–0.14 mg L^{-1}, dependently on the treatment group (Table 1).

Experiment 2—constant light intensity

This experiment was carried out in the same semi-closed RAS, with the same feeding and thermal regime as in experiment 1. For this experiment, fish had average *W* of 5.24 ± 2.43 g and SL of 85.4 ± 12.4 mm. Initial stocking density was 0.6 fish per L. Fish were randomly divided into two experimental groups with triplicates. In group I, 24 h light with intensity of 1,800 lx was used, whereas in group II, 24 h darkness (4 lx) was applied (Luxmeter MS6610, V&A Instrument, China). Light intensity was measured at the level of water surface. According to the findings of experiment 1, feeding rate was 2 % of biomass a day. Fish biomass was evaluated every 7 days where all the fish from each of the tank were weighted. Feed was distributed with the belt feeders 24 h day^{-1} (with 2 breaks—1 h each break every 12 h) allowing cleaning and reloading the feeders. Experiment lasted

Table 1 The results obtained during 35 days of rearing of burbot juveniles fed with different feeding ratios (experiment 1)

Parameter	Feeding level (% of biomass per day)			
	1 Group I	2 Group II	3 Group III	4 Group IV
Concentration of nitrites mg L^{-1}	0.14 ± 0.01[a]	0.095 ± 0.02[a]	0.14 ± 0.02[a]	0.11 ± 0.03[a]
Concentration of ammonia mg L^{-1}	0.66 ± 0.08[a]	0.79 ± 0.02[a]	1.35 ± 0.23[b]	1.24 ± 0.27[b]
FCR	0.68 ± 0.06[b]	0.63 ± 0.00[a]	0.7 ± 0.02[b]	0.69 ± 0.25[b]
Consumed feed (% of supplied feed)	100[a]	71.3 ± 15.90[b]	39.26 ± 8.6[c]	26.93 ± 6.62[c]
SGR	1.27 ± 0.11[b]	1.93 ± 0.02[a]	1.57 ± 0.07[ab]	1.89 ± 0.16[a]
K	0.6 ± 0.00[b]	0.77 ± 0.01[a]	0.76 ± 0.00[a]	0.76 ± 0.02[a]
Survival rate	100 %	100 %	100 %	100 %
Average standard length SL (cm)				
Start of the experiment (SL_1)	12.48 ± 1.09[a]	12.48 ± 1.09[a]	12.48 ± 1.09[a]	12.48 ± 1.09[a]
End of the experiment (SL_2)	14.75 ± 1.29[a]	15.42 ± 1.29[a]	15.12 ± 1.45[a]	15.57 ± 1.33[a]
Average weight *W* (g)				
Start of the experiment (W_1)	15.36 ± 3.72[a]	15.36 ± 3.72[a]	15.36 ± 3.72[a]	15.36 ± 3.72[a]
End of the experiment (W_2)	25.6 ± 7.42[b]	29.8 ± 8.54[a]	27.9 ± 7.83[ab]	30.6 ± 7.63[a]

Data are expressed as mean ± SD

Data in rows marked with different superscripts were statistically different (Tukey's post hoc, $P < 0.05$)

35 days, and it was followed by 5 days acclimation period. All the measurements and handling procedures were made such as in experiment 1. Oxygen saturation during this experiment did not drop below 80 %. The content of ammonia and nitrites ranged between 0.125–0.193 and 0.033–0.142 mg L^{-1}, respectively.

Data analysis and statistics

Based on the recorded data, the relative specific growth rate (SGR) was determined with Brown's formula (1957).

$$\mathrm{SGR} = 100\left((\ln W_2 - \ln W_1)\,\Delta t^{-1}\right)$$

where W_1—average initial weight (g); W_2—average final weight (g); Δt—rearing period (days).

The individual condition of fish was determined with Fulton's condition factor (K) in accordance with the following formula:

$$K = \left(W\ \mathrm{SL}^{-3}\right) 100$$

where W—body weight (g); SL—standard length (mm).

The feed conversion ratio (FCR) was calculated with the following formula:

$$\mathrm{FCR} = P\,(W + M)^{-1}$$

where P—amount of consumed feed (g); W—increase in fish biomass (g); M—weight of dead fish (g).

The survival rate was determined on the base of initial and final number of fish in each experimental treatment. All the data expressed in percentages were subjected to arcsine transformation prior statistical analysis. In experiment 1, the statistical differences between individual experimental groups regarding W, SL, SGR, FCR and feed utilization were analysed with one-way analysis of variance (ANOVA) and Tuckey's post hoc test at a significance level of 5 % ($\alpha = 0.05$). In experiment 2, data regarding W, SL and SGR were analysed with t test at a significance level of 5 % ($\alpha = 0.05$). The statistical analysis was performed with Microsoft Excel and STATISTICA (data analysis software system), version 9 (StatSoft, USA).

Results

Experiment 1

Mortality was not recorded during the experiment. The highest ($P < 0.05$) specific growth rate (SGR) was recorded in the groups II, III and IV (from 1.93 to 1.57 % day^{-1}), whereas the lowest ($P < 0.05$) was in group I (1.27 % day^{-1}) which was also comparable ($P < 0.05$) to group III (Table 1). The lowest ($P < 0.05$) FCR at 0.63 was recorded in the group II. In the other groups, FCR was on a similar level (0.68–0.70) (Table 1). During rearing, 100 % of the administered feed was consumed by the fish from group I. In the group II, the feed consumption was significantly lower ($P < 0.05$), at 71.3 %. The lowest ($P < 0.05$) feed consumption was recorded in the group III (39.26 %) and group IV (26.93 %) (Table 1).

Table 2 The results obtained during 35 days of rearing of burbot juveniles reared at different constant light conditions (I: 1,800 lx, II: 4 lx) (experiment 2)

Experimental group	I	II
Light intensity	1,800 lx	4 lx
SGR	4.26 ± 1.26	4.26 ± 0.97
K	0.81 ± 0.07	0.82 ± 0.07
Survival	100 %	100 %
Standard length SL (cm)		
At the beginning of the experiment (SL_1)	8.54 ± 1.24	8.54 ± 1.24
At the end of the experiment (SL_2)	14.18 ± 1.48	14.16 ± 1.4
Body weight *W* (g)		
At the beginning of the experiment (W_1)	5.24 ± 2.43	5.24 ± 2.43
At the end of the experiment (W_2)	23.33 ± 8.67	23.34 ± 7.32

Data are expressed as mean ± SD

Data in rows (between different treatment groups) were not statistically different (*t* test, $P < 0.05$)

At the end of the experiment, no statistically significant differences ($P > 0.05$) in SL of burbots from the different experimental groups were recorded. The body weight (W) of individuals from groups II and IV was higher ($P < 0.05$) than the weight of fish in group I. The *W* of fish recorded in group III was comparable to those from groups I, II and IV (Table 1).

Experiment 2

Fish in each of the experimental group were characterized by similar *W*, SL, SGR and *K* ($P > 0.05$). No differences between the treatment groups were recorded as considered content of ammonia and nitrites ($P > 0.05$). Also, no mortality was recorded (Table 2).

Discussion

The paper has presented, for the first time, the optimal feeding rate of juvenile burbot in an intensive culture. Additionally, it was for the first time reported that light intensity (1,800 vs 4 lx) had no effect on the rearing parameters.

Wocher et al. (2011a) recorded SGR at 0.65–0.70 % day^{-1} for burbot fry under laboratory conditions, whereas in the present study, the highest SGR (observed in the group II of the experiment 1) was 1.93 % day^{-1}. However, SGR of fry of other species representing *Gadidae* family, Atlantic cod *Gadus morhua* (L.), ranged between 2.26 and 2.59 % day^{-1} in the intensive rearing conditions (Fulberth et al. 2009). The highest Fulton's condition factor (*K*) was recorded in the fish from the groups II, III and IV of the experiment 1 (0.76–0.77), and it was slightly higher than the value reported by Wocher et al. (2011a) (0.64–0.69). The survival rate of 100 % and lack of cannibalism symptoms, which was reported as a considerable problem in the case of smaller individuals of this species (Jensen et al. 2011), confirm the optimal conditions provided in the present study during burbot rearing. Especially, when it was recently reported that cannibalism in this species coincided mostly with the changes in the feeding regime (during weaning protocols) rather than with other parameter (for details see: Palińska-Żarska et al. 2013). The data presented indicate that carefully prepared rearing of larger burbot specimens (as in our study) and feeding exclusively with compound feed generates a high survival rate of fish and a satisfactory growth rate.

In our study, the best FCR (0.63) was recorded in group II of the experiment 1, and it was lower than the values reported for burbot by Wocher et al. (2011a) (0.73–0.84) and lower than the ratio calculated by Rosenlund et al. (2004) for cod (0.74–0.88) during a 28-week rearing period. Such a low value of the FCR is thus a very satisfactory result. In addition, it should be emphasized that feed consumption in the group II (in the present study) was very high (over 71 %) and it coincided with the highest growth rate. Based on these results, it may be concluded that the feed ration administered in the group II (2 % of biomass day^{-1}) was the most effective of the rations tested. This ration was twofold higher than the dose used by Wocher et al. (2011a). It therefore explains why these authors recorded lower SGR (0.65–0.70 % day^{-1}) in comparison with our study and indicates that the doses used in that experiment were probably insufficient. However, a potential impact of temperature cannot be excluded, since Wocher et al. (2011a) provided a temperature lower by 4 °C than in our study. The negative impact of temperature at 12 °C compared to 15 °C on the growth rate of juvenile burbot has been so far reported (Wolnicki 2001). It has already been proven that temperature and fish size influence feed consumption and conversion (Handeland et al. 2008). Those factors most probably had an impact on a lower degree of feed consumption by burbots in a study by Wocher et al. (2011a) (those authors reared fish that weighed 120 and 360 g). The inclusion of these two variables therefore necessitates further and more detailed research.

During the study in groups III and IV in the experiment 1, elevated ammonia level was certified (Table 1). This parameter was elevated probably due to higher amount of feed ingested by the fish what affected higher FCR recorded. Negative effect of ammonia on growth rate was already reported (Brinkman et al. 2009). Higher amount of ammonia excreted by the fish in relation to feed utilization was previously reported, among others, for the common tench, *Tinca tinca* (L.) (Nowosad et al. 2012). It may be then suggested that higher feeding ratio affected higher feed ingestion by the fish, but in the stocking density applied, it caused elevated ammonia excretion and consequently lower growth rate. This additionally justifies the choice of the feeding ratio at 2 % of the biomass.

The findings of this study for the first time indicate that there was no effect of light conditions, where constant light conditions were applied. This supplements already published data, where light conditions for burbot juveniles remained unclear (Jensen et al. 2011; Trabelsi et al. 2011; Wocher et al. 2011b). In comparison with the larval stage, where light allowed fish to use the eyes to find a food in the rearing system (Boeuf and Le Bail 1999; Harzevili et al. 2004), older fish seems to have no particular preferences as considering light conditions when applied constantly the same. It could stem from the fact that fish have become adapted to the photoperiod provided.

High growth and survival rates and a beneficial FCR indicate considerable productive potential of burbot which has been so far also reported by Jensen et al. (2011), Trabelsi et al. (2011) and Wocher et al. (2011a). The advantage of burbot production in RAS is low rearing temperature (e.g. 17 °C in our study) in comparison with other species. It may positively influence economical efficacy and significantly reduce production costs in the conditions of moderate climate. In our experiments, neither hiding places nor shading of tanks (24 h photoperiod) was provided, which had been previously regarded as necessary elements at this stage of burbot rearing (Wocher et al. 2011a). Thus, it may be concluded that for burbot reared in captivity, the constant conditions (especially light and feeding conditions) are suitable for growth. The fact that burbot is a nocturnal fish (Wocher et al. 2011a) does not matter in fish at the first (stage 1) domestication level (Teletchea and Fontaine 2012) since the environment provided from the very beginning is the environment which fish tolerates despite the biological preferences. However, it may not be excluded that any change provided (e.g. daily shifts of photoperiod) may affect changes in fish preferences and its behaviour (e.g. feeding activity). Nevertheless, it has to be more closely studied to verify such hypothesis.

This paper presents, for the first time, the highest growth rate of burbot fry in intensive rearing conditions with high stock density. It indicates that feeding at 2 % of biomass of stock provides the optimal daily feed ration for commercial rearing in this period. However, the development of comprehensive commercial fish production technology requires determining numerous factors that may have an impact on the efficacy of rearing in the intensive culture conditions, such as, e.g., feed composition.

Acknowledgments This study was financed by the project "Innovations in finfish aquaculture with special references to reproduction" (InnovaFish), Operational Programme Sustainable Development of the Fisheries Sector and Coastal Fishing Areas 2007–2013" (OR14-61724-OR1400003/09/10/11).

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