www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 643-650 (2016) DOI: 10.4194/1303-2712-v16_3_18

RESEARCH PAPER

Substitution of Natural Food with Artificial Feed during Rearing Larvae of Sichel *Pelecus cultratus* (L.) under Controlled Conditions

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Received 15 January 2016
Accepted 31 May 2016

Abstract

Larvae of sichel *Pelecus cultratus* (L.) were reared up on natural food (*Artemia* sp. nauplii), after a certain time replaced by dry feed (Perla – Skretting). The rearing was conducted in aquaria of the capacity of 50 dm^3 each working in a closed, water recirculation system. The sichel larvae were reared up for 21 days in five nutrition groups: P21 – dry feed only, A4P17 – 4 days of *Artemia* larvae followed by 17 days of dry feed; A8P13 – 8 days of Artemia nauplii followed by 13 days of dry feed; A12P9 - 12 days of *Artemia* larvae followed by 9 days of dry feed, and A21–*Artemia* nauplii throughout the whole experiment. The assessment of the effect of substituting natural food with feed was based on the size reached by larvae, survivability, relative growth rate of individual fish larvae and body condition. The larvae fed on the dry feed and the ones which first received *Artemia* nauplii for the first 4 to 8 days presented the smallest gain, which suggests that natural food should be given to sichel larvae for at least the first 12 days of rearing

Keywords: Sichel, *Pelecus cultratus* (L.), natural food, dry feed, growth, survival (cyprinid, juvenile, fodder, transition).

Introduction

Aquaculture goes beyond production of fish eaten by man, encompassing such issues as production of fish stock material to stock open water bodies. In Central and Eastern Europe, production of stock material is a highly profitable part of inland fish aquaculture. It is particularly important nowadays, when the quality of surface inland waters is progressively improved and there are several ongoing programs aimed at the restitution of fish populations which are at risk of disappearance (Nowosad *et al.*, 2014). Reduced stocks of fish and in many cases complete extinction of valuable species were caused by the deteriorated quality of the natural environment at the end of the 20th century. Discharge of untreated household and industrial wastewaters, same as runoffs from intensively fertilized arable fields, contributed to the eutrophication of flowing waters (Penczak, Koszalińska, 1993). Regulation works in river troughs, excavation of stone and gravel, sedimentation of organic substances as well as construction of dams across rivers all led to the silting-up of rivers, which destroyed many natural spawning grounds and sites where juvenile rheophilic cyprinid fish can grow up (Vannote *et al.*, 1980; Backiel, 1985). All these adverse events coincided and caused a considerable decrease in the number and range of occurrence of many rheophilic fish species (Marszał, Przybylski, 1996; Witkowski 1996) to the extent that some have been entered on lists of species threatened with extinction (Witkowski, 1992). Sichel *Pelecus cultratus* (L.), which until recently was mass fished in Poland, e.g. in the Vistula Lagoon, the Bay of Szczecin and the Bay of Kamień, is an example of a fish species now at risk of disappearing. Over the past few years, the total population of sichel in Poland has decreased drastically, as a result of which it is now listed as an endangered species, given all-year complete protection. The Red List of lampreys and fish drawn up for Poland (as of 2009) assigns sichel the status of a critically endangered species (code: CR) (Witkowski *et al.*, 2009). Maintaining the number of sichel on a level that will secure the survival of local populations must involve protection of natural spawning grounds and substitution of natural by controlled spawning. Incubation of eggs under controlled conditions is followed by the rearing-up of hatched larvae to be used for fish stocking of open water. Such methods have been designed for rheophilic cyprinid fish (Kwiatkowski *et al.*, 2008), including asp (Targońska *et al.*, 2010), barbel (Wolnicki, Górny, 1995b; Kujawa *et al.*, 1998b), common nase (Kujawa *et al.*, 2010), ide (Wolnicki, Górny, 1995a; Jamróz *et al.*, 2008; Kucharczyk *et al.*, 2008), dace (Nowosad *et al.*, 2014) and chub (Żarski *et al.*, 2008). Larvae of cyprinid fish are extremely responsive to a number of factors which influence their physiological and health condition (Peňáz *et al.*, 1989; Korwin-Kossakowski, 1992; Nowosad *et al.*, 2013). They are particularly sensitive to any shortage of food during the transient stage between endogenous nutrition (nutrients stored in the yolk) to exogenous foraging (zooplankton). The complex of own digestive enzymes in reophilic cyprinid fish is poor during the first days of life (Dabrowski, 1984b), which is why it partly compensated with the enzymes of the ingested zooplankton. These fish need some species- specific time to acquire the ability to digest dry feed (Dabrowski, 1984a; Hofer, Nasir Uddin, 1985). Any attempts at feeding larvae of reophilic cyprinid fish except larvae of barbel and common nase (Kujawa *et al.*, 1998b; Fiala, Spurny 2001) with dry feed only from the first day of life typically lead to a retarded growth and inferior resistance to diseases, which are consequently responsible for mass deaths of fish (Wolnicki, 1996). The absence of detailed solutions to the above problems in the rearing of sichel larvae has motivated the current research, whose aim was to determine the optimal period of time when larvae should receive natural food before it is replaced with dry feed.

Material and methods

Material

Larvae of sichel *Pelecus cultratus* (L.) originated from artificial reproduction, carried out under controlled conditions according to the protocol described by Kujawa *et al.* (2013). Sichel spawners caught in the wild (the Vistula Lagoon) were transported to a fish hatchery at the University of Warmia and Mazury in Olsztyn, and placed in fish tanks, each of the capacity of 1000 dm3 of water (Kujawa *et al.*, 1999). The eggs and milt were obtained from spawners of a similar size, previously subjected to hormonal stimulation (Kujawa *et al.*, 2013). Ovopel, a preparation with the active GnRH analogue and metoclopramide (Ovopel – Unitrade, Hungary) was administered for hormonal stimulation of fish. Each granule of Ovopel (on average, 25 g) contains 18-20 μg of GnRH analog and 8-10 mg metoclopramide (Horvath *et al.*, 1997). Injections of Ovopel were given under the base of the ventral fin. Germ cells were collected about 12 hours after the injections. Having obtained the sex products from females, semen was collected from males with a syringe, according to the method described by Billard and Marcel (1980). Eggs collected from several females were fertilized with semen from three males. Once the gametes were mixed and some water was poured, the whole mixture was left for about minute. Next, it was transferred to Weiss jars, placing 1.0 dm^3 of swollen spawn in each 7-litre jar. The flow of water in a jar was regulated so that it mixed gently the whole contents of the jar. The spawn was incubated for 5 days in water of the temperature 18.5°C (Kujawa *et al.*, 2015), which was found as an optimal temperature was sichel embryonic development. Larvae were allowed to hatch for one day since the first hatched individuals were spotted. Next, they fell to the bottom of a jar. When larvae had hatched, they were transferred to shallow rearing tanks containing water of the same temperature. There, larvae remained for 3 days, during which they resorbed the contents of yolk sacs.

Methods

Individuals chosen for the proper experiment were those sichel larvae whose swim bladder was already partly filled and which had begun swimming in search for food. The moment they started active foraging, they were placed in tanks of the working capacity of 50 dm^3 each, with three tanks assigned to each nutritional regime. The tanks were operated in a closed recirculation system (Kujawa *et al.*, 2000). The initial density of larvae was 40 $indiv.\cdot dm^{-3}$. Throughout the whole rearing period, the daily light and darkness cycle was fixed, with 12 hours of day and 12 hours of night. The water temperature was 25.0±0.5ºC (± standard deviation) (Kujawa *et al.* 2015), which was found as one from the thermal optimum range for sichel embryonic development. The concentration of water dissolved oxygen ranged from 7.1 to 7.7 mg O_2 dm^{-3}, while the value of pH was from 7.2 to 7.4. No ammonia was detected in water during the whole experiment (Nowosad *et al.*, 2013).

The larvae were first given food 12 hours after being placed in the rearing tanks. Natural food, consisting of live nauplii larvae of the brine shrimp (*Artemia* sp.) was prepared according to the protocol described by Sorgeloos *et al.* (1977). In different experimental groups, natural food was replaced with dry feed gradually after 4, 8 and 12 days of rearing. Natural food was substituted with the feed called Perla, manufactured by Skretting for feeding commercially reared fish larvae. The composition of the natural food and feed given to sichel larvae during the experiment is specified in Table 1.

The control group was composed of larvae which were fed nauplii larvae of the brine shrimp during the whole rearing period (21 days). This approach was motivated by some reports found in relevant literature, where only a ‘positive’ variant was included (larvae fed on natural food throughout the rearing period) (Albrecht *et al.*, 1977). The fish were fed *ad libitum*, four times a day, every three hours.

Every morning, prior to the first feeding, dead individuals were removed and counted. Also, uneaten food remains and any contaminants were removed in order to maintain adequate sanitary conditions and avoid formation of ammonia in water. The daily counting of dead larvae enabled us to determine accumulated mortality, which is the number of dead larvae versus the initial stock in [%].

Collecting and analysis of samples

The control sample was taken on the day the tanks were stocked with larvae, before the first feeding. The subsequent samples (each composed of 30 individuals) were collected every 4 days. Samples were obtained immediately after switching on the lights but before the first morning feeding. Prior to measuring and weighing, sampled larvae were briefly anesthetized in 2-phnoxyethanol solution according to the method described by Weyl *et al.* (1996). The concentration of the solution (0.1-0.4 $cm^3 \cdot dm^{-3}$) was set based on the results of the authors' earlier experiments. Larvae were weighed with up to 0.1 mg accuracy and measured (*longitudo totalis* – TL) with up to 0.1 mm accuracy. Following the measurements, the larvae were returned to the proper tanks.

The recorded measurements served for calculating the relative specific growth rate (SGR) and relative biomass growth rate (SBR) from the onset of feeding to the termination of the experiment (Brown, 1957).

In order to compare the results, the relative final mean length, body weight and biomass of experimental fish were computed, with the calculations based on the assumption that the length, weight and biomass of control fish (a 21-day period of feeding with brine shrimp nauplii) at the end of the experiment equaled 100%. Statistical differences between the experimental groups were assessed with the Duncan's test (1955), at the level of significance $\alpha = 0.05$. The results obtained were processed using two computer software packages: Excel 2010 and Statistica 10.0 for Windows.

Results

The highest body gains and length increases were achieved by sichel larvae fed on natural food (Table 2). The mean weight of fish which received live brine shrimp nauplii all the time was 108.2 mg on the last day of the experiment, while the mean body length was 28.4 mm. On the other hand, fish larvae fed on dry feed since day 5 weighed 48.9 mg and measured 20.8 mm in length on average when the experiment ended. The statistical differences in the average weight and length of the fish body reached by individuals from the particular nutrition groups did not occur until day 12 of the rearing period (Figures 1,

Table 1. Chemical composition of *Artemia nauplii* *and starter diets produced by Skretting ** (% dry weight) (*- dane wg. P. Candreva. Inve Aquaculture NV. Dendermonde. Belgia, ** Sjøhagen 15 4016 Stavanger Norway)

Food	Component				
	protein	fat	carbohydrate	ash	phosphorus
Artemia sp	47.0	21.5	10.6	9.5	-
Perla	62.0	11.0	-	10.0	0.8

Table 2. Results of rearing sichel larvae *Pelecus cultratus* (L.) fed with *Artemia* and transferred to starter after 4, 8 or 12 days

Parameter	Starter	Trout starter			Natural food
	21P	4A17P	8A13P	12A9P	21A
Initial mean body weight (mg)	2.1 ± 0.1^a	2.1 ± 0.1^a	2.1 ± 0.1^a	2.1 ± 0.1^a	2.1 ± 0.1^a
Final mean body weight (mg)	37.38 ± 5.6^a	48.8 ± 8.5^{ab}	68.28 ± 9.6^c	94.3 ± 10.3^d	108.15 ± 12.3^{de}
Initial mean body length (mm)	7.8 ± 0.1^a	7.8 ± 0.1^a	7.8 ± 0.1^a	7.8 ± 0.1^a	7.8 ± 0.1^a
Final mean body length (mm)	19.05 ± 0.7^a	20.8 ± 1.5 ^{ab}	24.3 ± 1.3^c	26.8 ± 1.1^d	28,4 ± 1.6^e
Initial stock (indiv.)	2000	2000	2000	2000	2000
Final stock (indiv.)	1512 ± 3.3^a	1764 ± 2.2^b	1909 ± 4.2^c	1926 ± 3.1^c	1948 ± 3.2^c
Survival (%)	75.6 ± 0.3^a	88.2 ± 0.2 ^{b}	95.5 ± 0.4 ^{c}	96.3 ± 0.3^c	97.4 ± 0.4^c
Increase in total length (ITL) ($mm \cdot d^{-1}$)	0.54 ± 0.01^a	0.62 ± 0.03^b	0.79 ± 0.02^c	0.90 ± 0.03^d	0.98 ± 0.03^e
Relative growth rate (RGR) for weight ($\% \cdot d^{-1}$)	14.69 ± 0.1^a	16.17 ± 0.1^b	18.03 ± 0.2^c	19.86 ± 0.2^d	20.65 ± 0.2^e
Relative grow rate (RGR) for length ($\% \cdot d^{-1}$)	4.34 ± 0.1^a	4.78 ± 0.1^b	5.56 ± 0.2^c	6.05 ± 0.2^d	6.35 ± 0.3^e
Relative growth rate (RBR) for biomass ($\% \cdot d^{-1}$)	13.18 ± 0.2^a	15.48 ± 01.^{b}	17.77 ± 0.2^c	19.65 ± 0.2^d	20.50 ± 0.3^e
Biomass ($g \cdot dm^{-3}$)	1.13 ± 0.1^a	1.72 ± 0.1^b	2.61 ± 0.1^c	3.63 ± 0.1^d	4.21 ± 0.1^e
Fultona K	0.54 ± 0.0^a	0.54 ± 0.0^a	0.48 ± 0.0^b	0.49 ± 0.0^b	0.47 ± 0.0^b

Mean value ± S.D. Results in rows with the same letter index are not statistically significantly different ($p \leq 0.05$)

2). Larvae fed on dry feed from day 8 reached the mean weight of 68.3 mg at the mean body length of 24.3 mm. During the rearing period, deaths of larvae correlated with the time of introducing dry feed occurred (Figure 3). The lowest survivability was achieved by larvae in the group fed exclusively on dry feed, where it equaled 75.6%. The group given dry feed from day 5 attained the survivability rate of 88.2%. In the other groups, the percentage of surviving larvae was approximately 96%. Sichel larvae feeding on brine shrimp nauplii gained body weight and length nearly twice as fast as larvae which received only dry feed. The index of body length increase per time unit (ITL) was within 0.54 a 0.98 $mm \cdot d^{-1}$. The biomass of fish obtained during the rearing period after four days of feeding dry feed was 1.72 $g \cdot dm^{-3}$, whereas the biomass of fish larvae constantly receiving brine shrimp nauplii was 4.21 $g \cdot dm^{-3}$. The biomass of sichel larvae which were put on dry feed after 8 and 12 days of rearing was 2.61 and 3.63 $g \cdot dm^{-3}$, respectively. The Fulton index showing physical condition reached valued from 0.47 to 0.54. The calculated values of RGR of the weight and length of larvae fed on dry feed from day 12 were 19.86 and 6.05, respectively, thus being the closest to the values calculated for control group larvae (Table 2). Regarding the mean relative final length (RFL), for variant A12P9 larvae it reached 87.2% of the mean length of larvae from variant A21, while the mean relative final length of larvae from variant P21

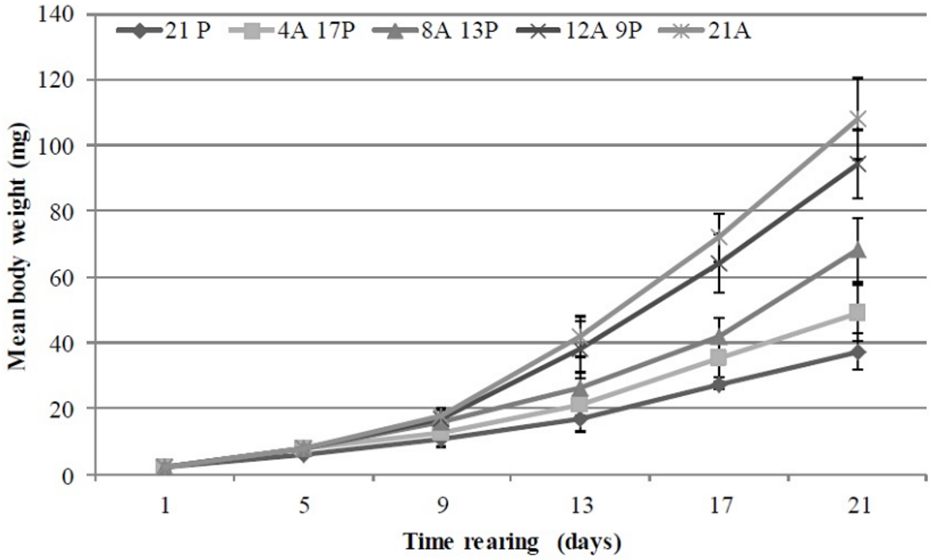

Figure 1. Growth rates body wet weight of sichel larvae fed using different feeding regimes. Groups are described at Material and Methods.

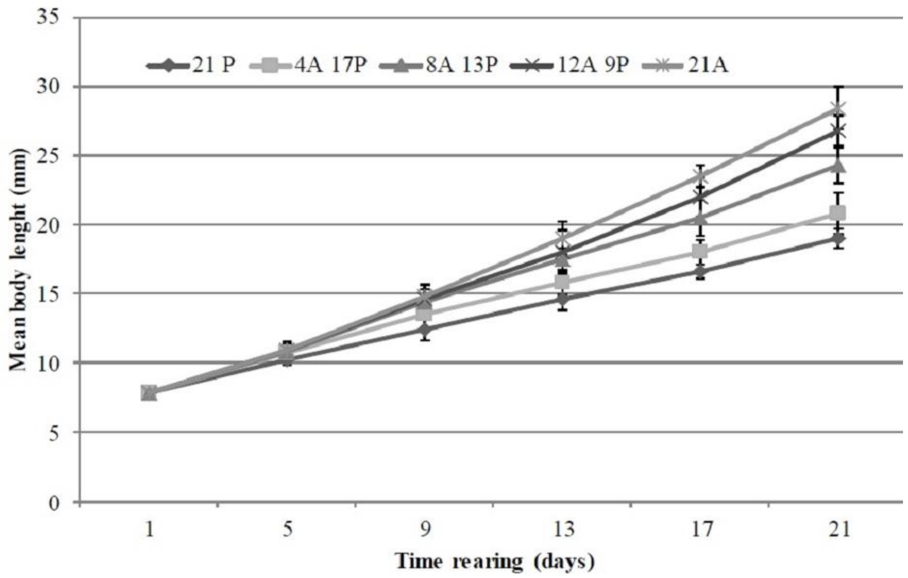

Figure 2. Growth rates total length of sichel larvae fed using different feeding regimes. Groups are described at Material and Methods.

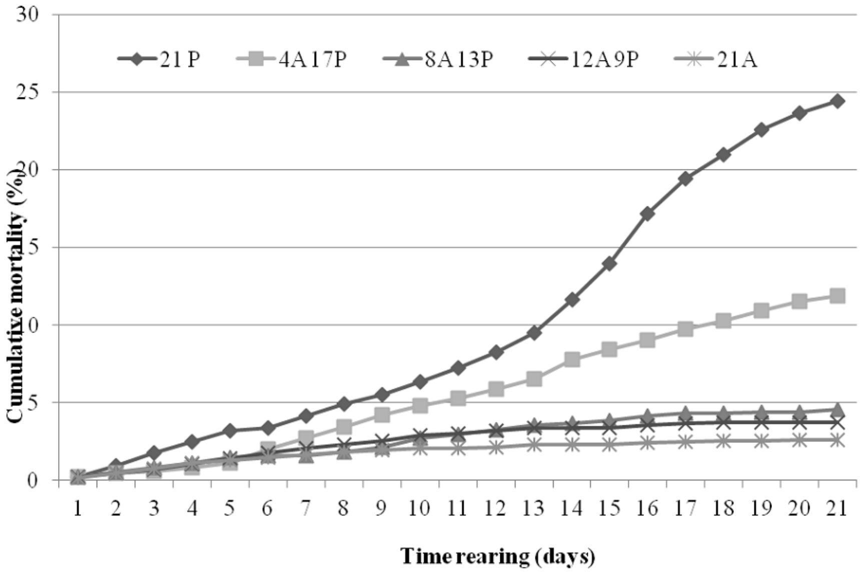

Figure 3. Cumulative mortality of sichel larvae fed using different feeding regimes. Groups are described at Material and Methods.

was 34.6%. The mean relative final weight (RFW) of larvae from variant A12P9 was 94.4%, and from variant P21 – 67.1%, compared to the mean final weight of larvae from variant A21. The mean relative final biomass (RFB) of larvae from variant 12A9P was 86.3% of the mean final biomass of larvae from variant A21, while larvae from variant P21 reached just 26.8% of the RFB (Table 3).

Discussion

Rearing larvae under controlled conditions is a very important stage in production of fish stocking material (Palińska-Żarska *et al.*, 2014a, b). Among the major problems encountered while rearing fish larvae is the question of providing them with an adequate quantity and quality of food. Cyprinid fish larvae, unlike those of the percids, coregonids and gadids, begin to ingest food when they have an almost resorbed yolk sac and partly air-filled swim bladder (May 1974; Malhotra, Munshi, 1985; Balon, 1986; Elliott, 1989). The duration of the period when larvae can use the nutrients stored in the yolk sac before they start ingesting exogenous food supplies depends on a species, origin of spawners (populations of fish), size of eggs and the temperature of water in which the egg incubation takes place and larvae are kept (Blaxter, Hempel, 1963; Dabrowski, 1976ab; Pepin, 1991). The onset of exogenous feeding is a critical time for cyprinid larvae. If the food density falls below a certain threshold figure, larvae restrain foraging and sometimes even discontinue ingesting food.

While rearing, fish larvae can be fed live food and/or dry feed (Schumpberger et a1., 1976; Kamler *et al.*, 1987; Opuszyński *et al.*, 1989; Foresti, 2000). The most common natural food consists of zooplankton captured in natural water bodies or originating from special cultures. Feeds, on the other hand, are a variety of commercial foodstuffs, composed according to strictly designed recipes, so as to contain all essential nutrients used by fish for body growth. While feeding fish larvae on dry feeds, the availability of food depends on how fast feed particles fall downwards to the bottom of a tank because during the first days following resorbtion of the yolk sac most larvae catch food floating in water. Its catchability depends on the amount of food in water, size of food particles and the speed at which it moves in water depths. So far, no suitable feeds for larvae of each species of reophilic cyprinid fish have been designed. Attempts are made to feed such larvae reared commercially with widely available feeds for trout or carp larvae.

Larvae of reophilic cyprinid fish begin to take up food during the final stage of resorbtion of food stores in the yolk sac. The most popular live food, characterized by a wide availability and accessibility to most fish larvae, is freshwater zooplankton or nauplii larvae of the brine shrimp *Artemia* sp. Such live food can be the only food given to fish larvae during the early stage of live or else it can be supplemented with dry feeds (Wiggins *et al.*,1986; Fermin, Recometa, 1988; Kestemont, Stalmans, 1992; Abi-Ayad, Kestemont, 1994; Kwiatkowski *et al.*, 2008). Cyprinid fish larvae should be initially fed on natural food because it takes some time before they are able to absorb efficiently the nutrients enclosed in dry feeds (Dabrowski, 1984a; Hofer, Nasir Uddin, 1985).

There is an immense variety of feeds used for rearing fish larvae, depending on a fish species. Several types of additives have been tested in order to improve the availability of dry feeds, for example hormonal stimulants added to food of salmonid fish (Poczyczyński *et al.*, 1998) or digestive enzymes when feeding marine fish (Kolkovski *et al.*, 1997;

Koven *et al.*, 2001). However, before a suitable feed for reophilic cyprinid fish larvae is designed, we can feed them with commercial feeds prepared for carp or trout larval rearing. This, however, must be preceded by at least a few days of feeding reophilic cyprinid larvae on natural food. Some authors claim that larvae of cyprinid fish can be given dry feeds when they reach an individual weight of 5-15 mg (Bryant, Matty, 1981), but during the first days of life they must be given live food. This enables them to achieve rapid body gains and to reach the subsequent developmental stages of life faster.

When cyprinid fish larvae are given dry feed from the start, their body weight and survivability are low, in contrast to larvae fed on zooplankton (Mookerji, Rao, 1991). This observation is confirmed by studies on rearing cyprinid larvae using dry feeds (Wolnicki, 1996; Wolnicki, Górny, 1995ab). The research results indicate that while some fish species, e.g. barbel or nase, can be fed on dry feed from the first days of life, others require a few days of rearing on natural food. Afterwards, they are able to absorb nutrients from feeds.

This paper presents results of rearing sichel larvae on natural food, replaced with dry feed after a specific number of days. This study was necessary to design a biotechnology of growing sichel fish stocking material. The experiment was conducted under comparable conditions in terms of the origin and type of research material, water temperature, stock density, size of rearing tanks, number of replications, frequency and number of samples, sampling method, type of feed and its chemical composition. Owing to this approach, the designed biotechnique of rearing sichel stock material is repeatable and ensures a breeding success.

The results obtained during the rearing effort described herein support some earlier observations that during the earliest stage of life – different in length in different cyprinid fish – larvae must be given natural food (Kujawa, 1998ab). Supplying them with dry feed alone does not enable larvae to fully exploit their growth potential, the fact also verified by some previous research of Kujawa *et al.* (2010). While rearing larvae of the asp *Aspius aspius* and common nase *Chodrostoma nasus* (L.) on natural food and on a mixed diet (dry feed plus nauplii) given in different time intervals, the best results were obtained when larvae were fed on the brine shrimp alone. Similar values of the biomass growth and survivability of asp and nase larvae were reached on dry feed with added nauplii of *Artemia* sp., and the poorest results were recorded when larvae were fed on dry feed alone. By giving natural food during the first days of rearing it was possible to obtain larvae with the weight similar to the ones from the control group, which were fed brine shrimp nauplii all the time. Even when no deaths of larvae are observed, the body gains obtained by larvae eating only dry feed are much smaller than the ones when natural food is supplied to larvae during the initial period of rearing.

The reported experiment demonstrated that after 12 days of the rearing of sichel larvae on natural food, it can be successfully replaced by dry food, and the obtained body gains and length increases as well as the survivability rate of larvae will not differ substantially from the ones attained by larvae from the control group, which received only natural food. Having established the minimum time when sichel larvae should be supplied with natural food, we ought to be able to decrease costs incurred by purchase of brine shrimp cysts and to raise the capacity of producing sichel stock material under controlled conditions. Besides, replacing natural food (brine shrimp nauplii) with dry feeds means that the feeding process can be automated by using feeders (Charlon, Bergot, 1984, 1986), which will substantially decrease maintenance costs.

Conclusions

The results of sichel larval rearing are analogous with results of experiments on other reophilic cyprinid fish, which implies that sichel, too, can be successfully reared up under controlled conditions. Sichel larvae, like other reophilic cyprinid fish of a similar size, must be fed on natural food in the initial period of rearing, for at least 12 days. In water temperature of 25 °C, the sichel larvae should be fed with natural food for minimum 12 days.

Acknowledgments

This research has been financed by the NCN the Polish National Science Centre under the research project number NN 304 302 640

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