Procedure for harmless estimation of fish larvae weight

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Abstract

The aim of this study was to evaluate a procedure of weighing live fish larvae and to determine its impact on the survival and growth rates based on the example of ide Leuciscus idus (L.). A 14-day rearing was conducted. Larvae were randomly divided into two groups – control (C) and experimental (E). During the experiment, 10% of larvae from the E group were sampled and weighed (with experimental method) daily. An integral part of weighing method was to place the anaesthetized larvae onto a nylon net platform. The platform with larvae was then dried on the blotting paper and next weighed together with larvae. The weight of the larvae was calculated by deducting the weight of the platform from the total weight recorded. After weighing, the fish were returned to the rearing tank. Fish from the control group were not manipulated during the whole experiment. At the end of the experiment 90 randomly chosen fish from each group were measured and weighted. No significant differences were recorded in larval survival, weight and length (91.33±4.73%; 41.69±10.70 mg; 17.56±1.44 mm and 94.00±4.00%; 49.40±10.79 mg; 18.41±1.24 mm in C group and E group, respectively). Significant differences were recorded in case of SGR that reached 15.80±0.29 in C group and 14.60±0.17 in E group. The described method may be a useful tool for determining fish larvae weight. Although it requires conducting further researches in order to establish the influence of accompanying factors such as e.g. kind and concentration of anaesthetic.

Introduction

Stage of development and dissimilar nutritional requirements from adult specimens (Dabrowski, 1984) and numerous factors influence their survival and growth rate. The rearing of larvae is, thus, the most difficult phase of fish culture in recirculating aquaculture systems (RAS). The role of scientists is to develop protocols to produce high quality stock material for different production purposes (Philipart, 1995; Trusty, 2002; Kestemont et al., 2007; Lawrence, 2007). In view of the procedure improving larvae rearing phenomena and factors like e.g. swim bladder inflation, introduction of exogenous feeding, replacement of live food with artificial feed (weaning), cannibalism, temperature, light (intensity, quality and photoperiod), type, amount and quality of food, number of reared individuals per water volume (stock density), salinity, concentration of ammonia etc. are studied (Marty et al., 1995; Yüfera and Darias, 2007; Trabelsi et al., 2011; Kestemont et al., 2003; Watanabe et al., 1995; Boeuf and Le Bail, 1999; Qin et al., 1997; Sahoo et al., 2004; Lein et al., 1997; Gomulka et al., 2011; Novosad et al. 2013). The impact of examined phenomena and factors on the efficiency of rearing is most often determined by evaluating their influence on survival rate and increases in body weight and length. The accuracy and reproducibility of the methods used to measure survival rates, body length and dry body weight are high and do not raise any objections. Estimation of mortality requires meticulous counting of dead individuals on each day of rearing or determining the stock density at specific time intervals or at the end of rearing (Appelbaum and Kamler, 2000; Morris et al., 2004; Kupren et al., 2011). Measurements of body length are taken with a slide calliper or with microscopes that may be directly connected to a computer yielding very accurate results (Karakatsouli et al., 2010; Kupren et al., 2011). Dry body weight is determined with one of several precisely described methods (Tandler et al., 1995; Steinarsson and Björnsson, 1999; Fielder et al., 2005; Villalta et al., 2005; Catavate et al., 2006; Wocher et al., 2013).

Some problems are encountered when an attempt is made to measure wet body weight (WBW). In general, fish larvae are tiny and immediately after hatching their body weight ranges from a few to several milligrams. The first problem related to measurements of WBW during this period is the safety of the procedure (it should not inflict injuries and cause mortality). The second problem is associated with the measurement precision. A given method should yield the most reproducible results possible. In the available literature, methods of measuring WBW are rarely discussed. Our experience and limited data (Sahoo et al., 2004; Fielder et al., 2005) indicate that WBW is most often determined by drying larvae on a blotting paper or a paper filter and then placing them on a balance. The mortality rate due to this manipulation is very high and may even reach 100%, although the data in the literature is rarely presented (Szkudlarek and Zakęś, 2007). Appelbaum and Kamler (2000) reported that successful measurements of body weight were taken when a nylon net was placed between the blotting paper and fish, which prevented body injuries. However, there is a lack of detailed procedure of larvae weighing with this method and the actual impact of this manipulation on survival and growth rates has not yet been determined. Furthermore, any single (at least partially) standardised method of determining WBW has not been developed. Reproducibility and safety (lack of negative impact on survival rate) of the methods that are currently used are controversial. The aim of the study was to develop a procedure of weighing live fish larvae and to determine its impact on the survival and growth rate. The rearing of larvae is, thus, the most difficult phase of fish culture in recirculating aquaculture systems (RAS). The role of scientists is to develop protocols to produce high quality stock material for different production purposes. The aim of this study was to evaluate a procedure of weighing live fish larvae and to determine its impact on the survival and growth rates based on the example of ide Leuciscus idus (L.). A 14-day rearing was conducted. Larvae were randomly divided into two groups – control (C) and experimental (E). During the experiment, 10% of larvae from the E group were sampled and weighed (with experimental method) daily. An integral part of weighing method was to place the anaesthetized larvae onto a nylon net platform. The platform with larvae was then dried on the blotting paper and next weighed together with larvae. The weight of the larvae was calculated by deducting the weight of the platform from the total weight recorded. After weighing, the fish were returned to the rearing tank. Fish from the control group were not manipulated during the whole experiment. At the end of the experiment 90 randomly chosen fish from each group were measured and weighted. No significant differences were recorded in larval survival, weight and length (91.33±4.73%; 41.69±10.70 mg; 17.56±1.44 mm and 94.00±4.00%; 49.40±10.79 mg; 18.41±1.24 mm in C group and E group, respectively). Significant differences were recorded in case of SGR that reached 15.80±0.29 in C group and 14.60±0.17 in E group. The described method may be a useful tool for determining fish larvae weight. Although it requires conducting further researches in order to establish the influence of accompanying factors such as e.g. kind and concentration of anaesthetic.
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Materials and methods

Description of weighing procedure

A platform constructed with nylon milling gauze with 200 µm mesh formed a crucial element of the weighing procedure (Figure 1a). In the first stage, the platform weight (PW) is determined (Figure 1b). The weighed platform is then placed on a blotting paper which absorbs water transferred on the platform with the larva. Next, the larva is put on the platform (Figure 1c). Non-absorbed water is eliminated by lightly pressing the platform to the blotting paper with a metal or plastic object in a way that prevents damage to the larva (Figure 1d). The dried platform with the larva is then again weighed (Figure 1e). Following measurement of WBW of the larva with the platform, the platform with the larva placed on it is immersed in the tank in order to release the larva (Figure 1f). The WBW of the larva is the difference between the WBW of the larva with the platform and the PW.

The weighing procedure should be carried out following induction of larvae into general anaesthesia, i.e. phase IV (as given by Velisek et al., 2007). After the measurement of the WBW, the larvae should be placed in a transitional tank and when they start swimming freely they should be transferred to a rearing tank.

Estimation of the impact of procedure on survival and growth rates of larvae

The rearing of ide larvae was carried out in order to determine the impact of weighing method on their survival and growth rates. The larvae originated from artificial reproduction. Reproduction of spawners and eggs incubation were carried out according to the methods described by Krejszeff et al. (2009), Targófska et al. (2011), Čejko et al. (2010) Kupren et al. (2011). The rearing was carried out in an experimental device for spawn, incubation and larvae rearing under laboratory conditions. It consisted of one large 50 L tank which served as a water bath and six small 1 L rearing tanks. The water bath was equipped with an aeration system, temperature and photoperiod control devices, a mechanical-biological filter and fluorescent lightning. The small tanks were supplied with water by a sprinkling machine with a regulated water flow. A detailed description of the construction and mechanism of this device was presented by Krejszeff et al. (2010).

The rearing started a day after the larvae began exogenous feeding, which was determined in the following way. After hatching, the larvae were kept together. When the swim bladder was filled, 30 larvae were caught each day and transferred to a 1 L beaker. The larvae were then administered newly-hatched Artemia nauplii. The day on which <50% of individuals consumed the feed was day 0. On that day, the rearing tanks were stocked. The larvae were randomly divided into two groups (in three replications): control group (C) and experimental group (E). The next day was the first day of rearing. The rearing lasted for 14 days. The larvae were reared at a stock density of 100 individuals per 1 L water which was within the range optimal for ide (Kupren et al., 2011). Throughout the experiment, the larvae were fed three times a day (at 10 am, 2 pm and 6 pm) ad libitum with newly-hatched Artemia nauplii (Ocean Nutrition™, Belgium).

The rearing was carried out at 25°C ±0.1°C. The photoperiod was set at 12 h of light and 12 h of darkness (12L:12D). The saturation of water with oxygen did not fall below 85%, and pH ranged between 8.0 and 8.5. The concentration of total ammonia and nitrates did not exceed 0.2 and 0.05 mg L⁻¹, respectively. The water flow through the rearing tanks was 8 L h⁻¹. The rearing tanks were cleaned each day before feeding and 30% of water volume was replaced in the circulation. The dead individuals were counted and then removed. All procedures and measurements of water parameters were performed in accordance with the methods described by Kupren et al. (2011).

Sampling

Ten larvae were randomly selected from each tank (10% of initial stock) of the experimental group on each day of rearing. These larvae were anaesthetized in a 2-phenoxyethanol solution at a concentration of 0.4 mL dm⁻³ (Sigma-Aldrich, Muenchen, Germany). In the next stage, weight was measured in accordance with the above-described procedure with an analytical balance (±0.1 mg). The fish recovered from anaesthesia in the transitional tank following the measurements. When the fish started swimming actively, they were transferred back to the rearing tanks from which they were caught. In any of the cases, the time of exposure to anaesthetic and the time of handling did not exceed 15 min in total. In order to perform comparative analysis at the end of the experiment 90 fish from group E and group C (30 fish from each tank) were sampled. In addition, the total length of larvae (TL, ±0.01 mm) was measured under stereomicroscope (MZ 12.5, Leica, Wetzlar, Germany) with the software for image acquisition and processing ProgRes® Capture Pro 2.5 (Jenoptic,

Figure 1. Photographic description of weighing procedure.
Jena, Germany) on the first and final day of the experiment. Specific growth rate (SGR; % day\textsuperscript{-1}) was calculated according to the following formula: $S\text{GR}=100(\text{Ln BW}_f–\text{Ln BW}_i)/\text{T}$ where BW\textsubscript{f}/BW\textsubscript{i} is the initial and final average larval WBW (±0.01 mg), and T is the duration of the experiment (days) (Brown, 1957).

**Statistical analyses**

All the data expressed in percentage were arc-sine transformed before the statistical analysis. The data were analysed with the t-test at the significance level of 5% ($\alpha=0.05$). The statistical analysis was performed using STATISTICA 9.1 software (StatSoft Inc.) for Windows.

**Results**

In both groups, the survival rate of larvae after 14-day rearing was very high and reached 91.33±4.73% and 94.00±4.00% in groups E and C, respectively. These results did not differ statistically (Table 1). The final body weight of fish from the experimental group was 41.69±10.70 mg and was not statistically different from the final body weight of larvae in the control group (49.40±10.79 mg). The same pattern was recorded for the total length of larvae. The final length of the fish from the experimental group was 17.56±1.44 mm and did not differ statistically from the parameters measured in the control group (18.41±1.24 mm) (Table 1). The SGRs calculated based on the initial and final body weight were 15.80±0.29 and 14.60±0.17 in groups C and E, respectively. Both values were significantly different (Table 1). The growth rate estimated on the basis of daily measurements is shown in Figure 2. The exponential formula ($y=ae^{bx}$) was best fitted to experimental data ($R^2=0.9842$).

**Discussion**

The results of this study clearly indicate that the proposed method of determination of WBW in larvae did not exert any impact on the survival of the larvae during the 14-day rearing period despite daily (and potentially very harmful) handling procedures. Applied method could have a slight effect on the growth rate of larvae, that affected SGR. However, the negative effect of anaesthetic or manipulation itself cannot be excluded. In spite of this, the results indicate that the proposed method of WBW determination is very useful in controlled rearing of larvae.

The reduction of mortality in larvae during measurements of body weight enabled us to increase the frequency of sampling. It allowed us to collect more data to better determine the impact of a given factor. However, comparison of the final WBWs between examined groups (as in the papers already published, e.g. Kupren et al., 2011) depicts the best one, but it does not allow for determination of the day of rearing on which a given factor started to significantly influence the growth rate.

The analysis of published data on initial WBW of ide larvae reveals considerable discrepancies. Depending on the author, WBWs of larvae recorded on the day when exogenous feeding started ranged from 1.64 to 5.40 mg with body lengths from 7.65 to 8.75 mm (Wolnicki and Górny, 1995; Kujawa, 2004; Kupren et al., 2011). Table 2 illustrates the published data on initial WBW of ide larvae.

**Table 1. Initial and final characteristics of the rearing of larval ide Leuciscus idus (L.).**

|                        | Control group | Experimental group |
|------------------------|---------------|--------------------|
| Mean initial weight, mg| 5.40±0.68     | 5.40±0.68          |
| Mean final weight, mg  | 49.40±10.79   | 41.69±10.70        |
| Mean initial length, mm| 8.75±0.32     | 8.75±0.32          |
| Mean final length, mm  | 18.41±1.24    | 17.56±1.44         |
| Survival, %            | 94.00±4.00    | 91.33±4.73         |
| Specific growth rate, % | 15.80±0.29a   | 14.60±0.17b        |

Data are presented as mean ±SD. a,bResults in the same row with different letter differ significantly (P<0.05).

**Table 2. Published data on initial wet body weight (mg) and length (mm) of ide larvae.**

| Initial wet body weight | Initial length | Reference                  |
|-------------------------|----------------|----------------------------|
| 3.0                     | 8.1            | Wolnicki and Górny, 1995   |
| 3.0                     | 8.3            | Kujawa et al., 2000        |
| 3.0±0.2                 | 8.3±0.1        | Kujawa, 2004               |
| 2.2                     | 8.29±0.29      | Shiri Harzevili et al., 2004|
| 1.64±0.253             | 7.65±0.557     | Hanáčková et al., 2007     |
| 1.8±0.2                 | 8.1±0.24       | Kowiatkowski et al., 2008  |
| 1.67±0.32              | 8.57±0.23      | Kupren et al., 2011        |
| 5.40±0.68              | 8.75±0.32      | Present publication        |
The obtained results clearly suggest that there is high need for further studies on the effect of anaesthesia (kind and doses of anaesthetic used) as well as the impact of handling procedures on fish larvae growth rate.

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