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## Predatory reactions of juvenile stages of sea trout (*Salmo trutta trutta* L., 1758) fed with three feeding regimens

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### Abstract

Restocking is used in rivers to restore fish populations. The biological value of stocking material derived from hatcheries is low, which is reflected in low survival rate in natural environment after stocking. For this reason, in pre-rearing, attention is increasingly paid to minimize the undesired hatchery-rearing effects, which greatly reduces the biological value. The quality of stocking material can be improved by introducing live food to the diet. The aim of the study was an attempt to determine the biological quality of trout larvae reared in three feeding regimens by determining the predatory behavior. The experiment started with a 30-day rearing of fish in three feeding variants: starter feed and algae, starter feed, and live brine shrimp larvae. Best weight and length gain of the fish and the survival rate were recorded in the group of fish fed exclusively Nutra HP 0.3 starter feed (Skretting). Pre-reared larvae of sea trout from three feeding variants were released in reservoirs, in which ide *Leuciscus idus* larvae were introduced as live food. It was observed that sea trouts most effectively caught their prey in a variant, in which sea trout larvae had been previously fed brine shrimp larvae, *Artemia* sp. The results indicate that the type of first food can model the predatory behavior of juvenile stages of sea trout.

**Keywords:** Sea trout *Salmo trutta trutta*, Fish larvae rearing, Foraging ability, Behavior

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## Introduction

Stocking with various forms of stocking material is one of the methods commonly used to maintain population equilibrium or to restore economically valuable fish species. In Poland, sea trout *Salmo trutta trutta* is considered a valuable fish in terms of fishing and fishery (Chełkowski, 1990). Sea trout stocking material in the form of juvenile stages is produced in artificial conditions in fish hatcheries. Unfortunately, it is presumed that the mortality of fish derived from such rearing is twice higher than wild fish (Svasand *et al.*, 1989; Olla *et al.*, 1998; Lindeyer and Reader, 2010; Manassa and McCormic, 2012; Oulton *et al.*, 2013). The reasons for the high mortality of pre-reared juvenile salmonids after releasing into the water resource are unknown (Brown *et al.*, 1997). However, this may result from the worse behavioral adaptation of juvenile stages to feeding on organisms naturally occurring in the watercourse (Chełkowski, 1990; Brown and Day, 2002; Oulton *et al.*, 2013). In addition, the fish from hatchery rearing are often not able to recognize a predator or react improperly in its presence (Suboski and Tepletton, 1989; Svasand *et al.*, 1989; Suboski *et al.*, 1990; Gliwicz and Jachner, 1992; Hall and Suboski, 1995; Jachner, 1995; Jachner, 1996; Weber and Fausch, 2003; Augustyn *et al.*, 2006; Oulton *et al.*, 2013; Thonhauser *et al.*, 2013). Many authors indicated that rearing on live food, which in consequence provides better adaptation of larvae to prey on live food after stocking is an important factor that can

increase the success of stocking (Lindeyer and Reader, 2010). Newly hatched larvae are characterized by immature state of many organs and tissues. The success of a complex process of larvae ontogeny depends on many factors, including digestive tract efficiency (Blaxter, 1986). The metabolism compounds, phyto- and zooplankton metabolites, such as methionine, inosine-5'-phosphate or glycine are chemical signals received by larvae chemoreceptors causing a higher activity of feeding behavior (Knutsen, 1992). Feeding with algae and/or live food induces in larvae visual and chemical stimuli, activates prey catching and supports ontogeny (Svasand *et al.*, 1989; Brown *et al.*, 1997; Brown and Day, 2002). Both bacteria and algae are the first organisms caught by fish larvae starting to feed exogenously, which is often overlooked and omitted (Zakęś *et al.*, 2015). Algae are very valuable fish diet, due to the high nutrient content, including approximately 35% of protein reach in amino acids, such as arginine, histidine, isoleucine, leucine, valine, methionine or  $\beta$ -alanine, 23% of fat, vitamins, including vitamin E, ascorbic acid, thiamine, and cobalamin. Moreover, algae are a source of antioxidants and are increasingly used in fish larvae rearing (Rocha *et al.*, 2008). There are 16 species known of unicellular algae, ranging in size from 3 to 6  $\mu\text{m}$ , which are successfully applied in fish rearing; their presence favorably changes the microflora of the environment, improves foraging and digestive system functioning; they are

also readily available in lyophylized form (Zakęś *et al.*, 2015). Training fish to search for and identify prey is a key element that often determines the survival of an individual after its release to the watercourse (Laland and Williams, 1997; Brown *et al.*, 2003; Warburton, 2003; Wadekind *et al.*, 2007; Oulton *et al.*, 2013). The aim of this study was to determine the breeding parameters during a 30-day rearing of sea trout larvae and to determine the impact of administered food on predatory skills of Sea trout juveniles.

### Materials and methods

The experiment was conducted in the laboratory of Fisheries Department, West Pomeranian University of Technology in Szczecin. Larvae were obtained from the hatchery of Polish Angling Association in Goleniów. Twenty-day-old larvae of sea trout with an average total length ( $L_t$ ) and an average unit weight (W) of 23.9 mm and 152 mg, respectively, were introduced to tanks with aeration (20  $\text{dm}^3$  volume). The first stocking density of 30 larvae for each tank and 9 individual tanks were set up for entire experiment. The first food was administered after free swimming observed and exogenous feeding began to start. The first stage of the experiment lasted for 30 days. After larvae started to forage, they were fed in three variants, each in triplicate. In the first variant **A** ( $V_A$ ), larvae were fed a  $0.5 \text{ dm}^3 \text{ day}^{-1}$  *Monoraphidium* sp. algae at a density of 700 thsd ind.  $\text{dm}^{-3}$ , density of algae estimated using method

microscopic, algae fed with the addition of Skretting Nutra HP 0.3 starter feed in an amount of  $0.2 \text{ g day}^{-1}$ ; starter feed accounted for 2–5% of the stocking mass. In the second variant **B** ( $V_B$ ), *Artemia* sp. cysts were hatched under light, hatchability, salinity, aeration, and temperature parameters recommended by the manufacturer (Ocean Nutrition), hatched brine shrimp larvae *Artemia* sp. (nauplii stage) were provided *ad libitum*, and in variant **C** ( $V_C$ ) sea trout larvae were fed only Nutra HP 0.3 (Skretting) starter feed in an amount of  $0.4 \text{ g day}^{-1}$ , which represented 4–10% of the stocking mass. The nutritional composition of the feed, according to the manufacturer, is shown in Table 1. Larvae of variant A and C were fed daily every 3–4 hours, from 7.00 to 17.00. Larvae of variant B were administered once *ad libitum* live *Artemia* sp. larvae (nauplii stage). Water temperature in each tank during the experiment was kept at  $17.2^\circ\text{C} \pm 0.21$ . In the experiment, the average content of ammonium ions  $\text{NH}^4$  in individual tanks was on average  $0.6 \text{ mg dm}^{-3} \pm 0.02$ , and the dissolved oxygen in the water did not fall below 7  $\text{mg O}_2 \text{ dm}^{-3}$ ; pH was maintained at  $7.5 \pm 0.2$ . Thirty larvae were randomly harvested from each variant every 7 days, and then larvae were short-term anesthetized in an aqueous solution of anesthetic (Propiscin at  $1.5 \text{ ml dm}^{-3}$ ) in order to measure the total length and weight. Furthermore, for each variant, for the first part of the experiment, Fulton's condition coefficient (K) (Bolger and Connolly, 1989; Fulton, 1902), specific growth rate (SGR), the

final coefficient of weight variation (CV) and survival (S) were calculated and additionally feed conversion ratio (FCR) (Soosen *et al.*, 2010) was

calculated for fish from variants A and C. The sample size was calculated using the Fleiss equation (Fleiss, 1981).

**Table 1: Feed characteristics used in the experiment.**

Parameter	Test feeds			Variant C Skretting Nutra HP 0.3*
	Variant A Skretting Nutra HP 0.3*	Variant A <i>Monoraphidium</i> sp.*	Variant B brine shrimp larvae <i>Artemia</i> sp.*	
Protein % wet weight ww	59	19.3	55	59
Fat ww	17	40	20	17
Carbohydrates % ww	7.5	13.1	15	7.5
Pellet size ww	0.20-0.60	-	-	0.20-0.60

\*Manufacturer's data: Skretting (Skretting Nutra HP), PhytoBloon (*Monoraphidium* sp.), Ocean Nutrition (brine shrimp larvae)

After 30 days of rearing, 5 larvae were randomly selected from each variant and individually released to three tanks. Then, 10 seven-day-old larvae of ide *Leuciscus idus*, which served as live food, were introduced to each tank. Ide larvae swam in the water column, which facilitated the natural feeding of juvenile sea trouts. The time was measured from release to catch of all 10 ide larvae by sea trout, and behavior of these fish was also observed. The number of attacks before consumption of all prey was calculated based on observations, observation was done method proposed by Huse and Skiftesvik, (1990), including the number of failed attacks and the resignation from the prey. Moreover, the type of attack was specified: from the head, from the tail, from the side. When the juveniles of sea trout catch all 10 ide individuals, it was substituted with another one from the same variant

and another 10 ide larvae were introduced.

The significance of breeding parameter differences between sea trout from particular feeding variants was determined using general linear models of one-way ANOVA ( $p=0.05$ ). Tukey test was used as a post hoc test (Sokal and Rohlf, 2012).

## Results

### Feeding experiment

In all variants, only isolated mortality cases were recorded during sea trout larvae feeding in the first 15 days of the experiment, and the survival rate was over 90% (Fig. 1). During the following 5 days, the survival rate was similar and on day 20<sup>th</sup> of the experiment it ranged in different variants from 82.4% (V<sub>A</sub>) to 85.9% (V<sub>B</sub>). It should be noted that survival in variant C remained at a similar level to the end of the experiment and after a 30-days rearing period it amounted to 85.7%. However,

variants A and B, where the fish were fed *Monoraphidium* sp. algae with the addition of Skretting Nutra HP 0.3 starter feed and brine shrimp larvae *ad libitum*, respectively, the survival rate of fish from day 20<sup>th</sup> to 30<sup>th</sup> of the experiment decreased to 56.6% and 59.7%, respectively. (Fig. 1, Table 2). In addition, fish of which received variant C were characterized by the highest final unit weight ( $p<0.05$ ) at a high homogeneity of the sample, as evidenced by the lowest value of the coefficient of weight variation (CV), and the highest overall length (Table 2). This indicates the highest increase in weight of fish fed Skretting starter feed ( $V_C$ ), in particular in the last 14 days of the experiment (Fig. 2). The lowest weight gain was observed in variant A where larvae were fed Skretting starter

feed and *Monoaphidium* sp. (Fig. 2). Moreover, specific growth rate (SGR), Fulton's coefficient (K) and the final unit weight of fish in this variant were statistically significantly lower compared to other variants ( $p<0.05$ ). Probably the low Fulton's coefficient in this variant was caused by feeding the fish insufficient feed quantity (2–5% of fish biomass), while it was 5–10% of fish mass in variant C. Feed conversion ratio (FCR) was calculated for variants A and C fed with the starter feed, and its value for these variants was 0.53 and 1.63, respectively. More favorable, in terms of culture, lower value of K coefficient, in addition to a lower feed dose in variant C, was caused by the presence of algae.

**Table 2: Breeding parameters of sea trout larvae fed feed mixed with algae *Monoraphidium* sp. and Skretting Nutra HS 0.3 starter feed - variant A, brine shrimp larvae *Artemia* sp. - variant B, commercial starter Skretting Nutra HS 0.3 - variant C (mean±SD).**

Specification	Feeding variant		
	Variant A	Variant B	Variant C
Survival S %	56.6	59.7	82.5
Initial body weight mg	152 <sup>a</sup> ±3.0	152 <sup>a</sup> ±3.0	152 <sup>a</sup> ±3.0
Final body weight in variants mg	218 <sup>a</sup> ±40.0	264 <sup>b</sup> ±80.0	327 <sup>c</sup> ±11.5
Specific growth rate SGR % d <sup>-1</sup>	0.36 <sup>a</sup> ±0.05	0.55 <sup>b</sup> ±0.03	0.65 <sup>c</sup> ±0.06
Final coefficient of weight variation CV %	33.66 <sup>a</sup> ±0.16	33.47 <sup>b</sup> ±0.08	31.00 <sup>c</sup> ±0.19
Initial total length L <sub>t</sub> mm	23.9 <sup>a</sup> ±1.21	23.9 <sup>a</sup> ±1.21	23.9 <sup>a</sup> ±1.21
Final total length L <sub>t</sub> mm	33.0 <sup>a</sup> ±3.60	30.7 <sup>b</sup> ±4.0	34.6 <sup>a</sup> ±7.50
Initial Fulton's condition coefficient K	0.67 <sup>a</sup> ±0.21	0.67 <sup>a</sup> ±0.21	0.67 <sup>a</sup> ±0.21
Final Fulton's condition coefficient K	0.72 <sup>a</sup> ±0.34	0.77 <sup>b</sup> ±0.32	0.76 <sup>b</sup> ±0.22
Feed conversion ratio FCR for Nutra HP Skretting feed	0.53	—	1.63

Groups marked with the same letter index in the same row do not differ significantly statistically ( $p=0.05$ )

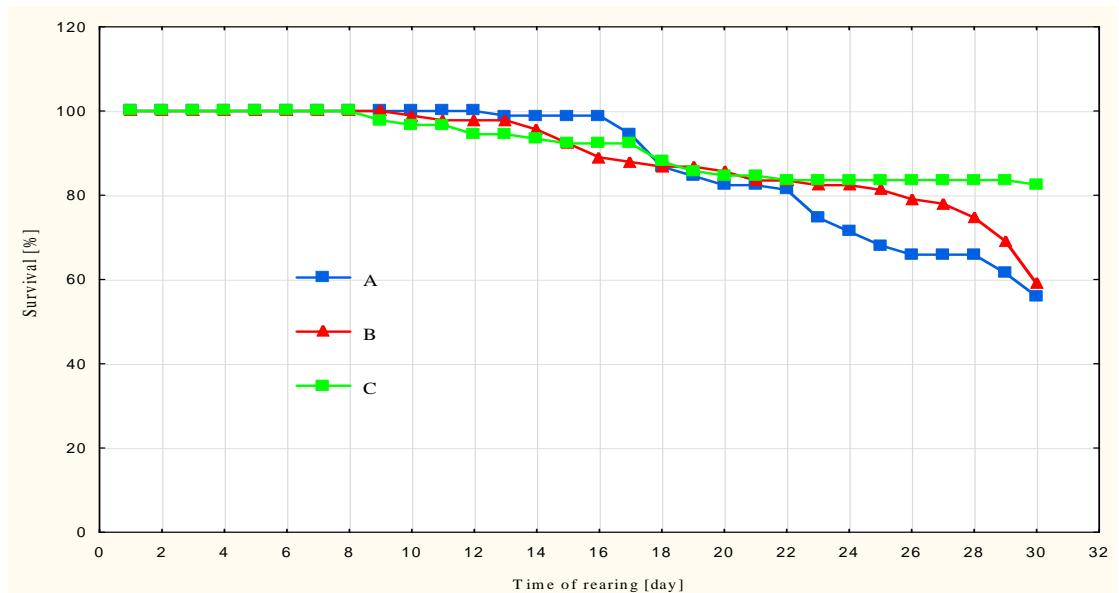


Figure 1: Survival of sea trout larvae in particular feeding variants (A, B, C).

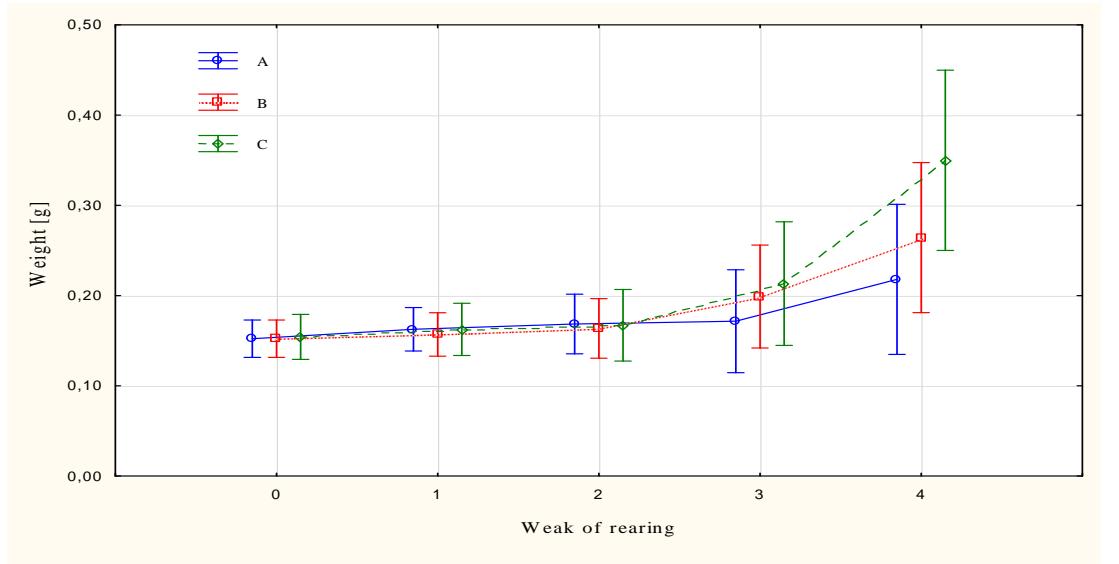


Figure 2: The growth rate of juvenile sea trout mass in particular feeding variants.

#### Behavioral analyses

Fish pre-reared on brine shrimp *Artemia* sp. demonstrated the highest foraging efficiency, measured by the lowest number of attacks a fish needed to eat 10 ide larvae ( $V_B$ , an average of 17.3 attacks). Moreover, fish from this variant were characterized by the lowest number of failed attacks and resignation from the prey (Fig. 3, Table 3).  $V_A$  sea trout, with an average of 31.2 (UNIT gr. Or cm), showed the lowest

statistically significant efficacy ( $p<0.05$ ) and most frequently resigned from the prey (Table 3). During the study period, several specific attacking methods of sea trout larvae to catch the prey could be distinguished in all variants: from the head, tail or side. Fish from variant A, previously fed on algae *Monoraphidium* sp. with the addition of Skretting Nutra HP 0.3 starter feed, with attacked the prey most frequently from the side. Fish fed only

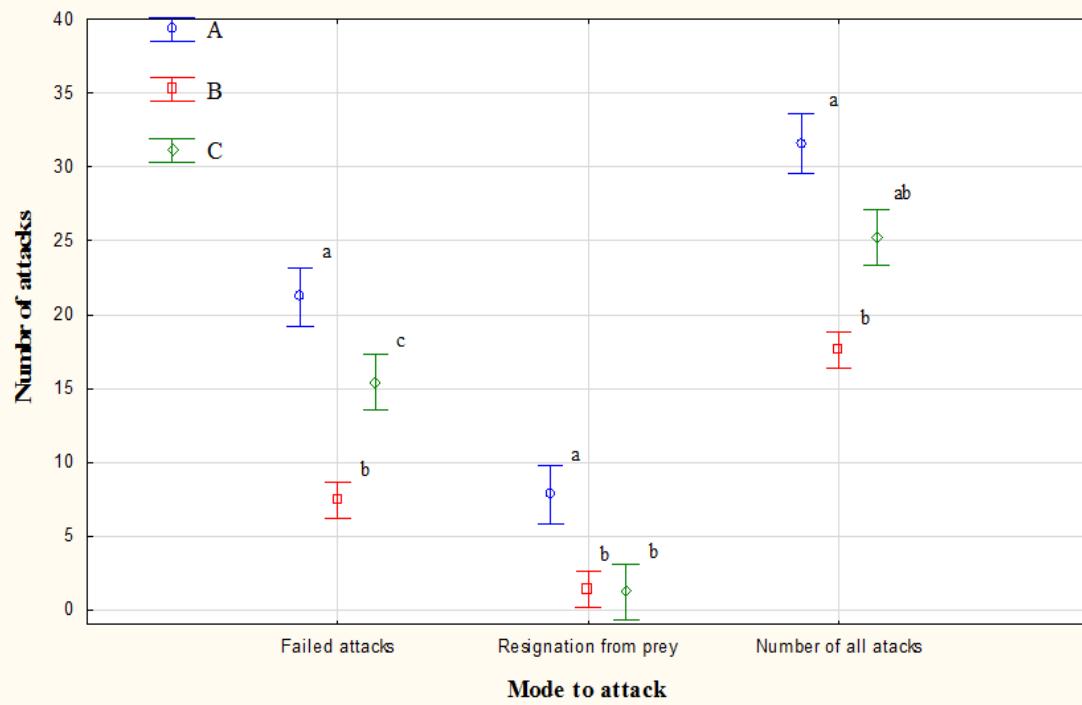
Skretting Nutra HP 0.3 starter feed (Variant C) executed most attacks from the tail. In contrast, fish previously fed brine shrimp larvae showed no explicit trend of prey capture (Table 3, Fig. 3). This probably results from sea trout training by feeding live food at the larval stage.

Attacks on the prey were preceded by swimming after it or waiting followed by a sudden attack. These are characteristic behaviors of sea trout during hunting in the wild. The fastest first attempts to catch ide larvae, 60 seconds after introduction to the experimental tanks, were observed in juvenile sea trouts reared on brine

shrimp larvae *Artemia* sp. (Variant B). It seems that this results from the prior administration of live brine shrimp (*Artemia* sp.). Feeding brine shrimp causes an increased feeding behavior, which in turn increases the chances of survival after stocking natural waters with this material. In variant A, sea trout larvae previously fed *Monoraphidium* sp. and Skretting Nutra HP 0.3 starter feed, began feeding on ide larvae 36 minutes after the introduction of ide larvae, while sea trouts in variant C were the last to identify their prey (60 minutes after ide larvae introduction).

**Table 3: Evaluation of attack effectiveness of juvenile sea trouts (mean $\pm$ SD).**

Variant	Number of attacks	Failed attacks	Resignation from prey	Mode of attack		
				from the head	from the tail	from the side
A	31.2 <sup>a</sup> $\pm$ 8.25	21.2 <sup>a</sup> $\pm$ 8.25	7.8 <sup>a</sup> $\pm$ 5.35	2.2 $\pm$ 0.44	2.6 $\pm$ 0.54	5.2 $\pm$ 0.44
B	17.3 <sup>b</sup> $\pm$ 0.89	7.3 <sup>b</sup> $\pm$ 0.89	1.4 <sup>b</sup> $\pm$ 0.21	3.2 $\pm$ 0.83	3.2 $\pm$ 0.83	3.6 $\pm$ 1.34
C	25.4 <sup>ab</sup> $\pm$ 3.25	15.4 <sup>c</sup> $\pm$ 3.25	1.3 $\pm$ 1.75	1.4 $\pm$ 0.54	5.2 $\pm$ 2.04	3.4 $\pm$ 1.94



**Figure 3: The effectiveness of catching prey by juvenile trouts in particular variants.**

## Discussion

In the natural environment, the first exogenous feed of sea trout larvae is live food (Watanabe and Kiron, 1994; Girri *et al.*, 2002). However, many studies indicate the possibility of using artificially produced feed during fish rearing (Jones *et al.*, 1993). Despite this, hatcheries in addition to feed apply natural feed: algae, phytoplankton, zooplankton or nekton (Szlauer, 1977; Brown *et al.*, 1997; Rocha *et al.*, 2008; Czerniawski *et al.*, 2011; Zakeś *et al.*, 2015). Phytoplankton is an underrated and a unique feed for fishes larvae, and its popularity continues to grow (Brown *et al.*, 1997; Zakeś *et al.*, 2015). However, it is still rarely used feed in commercial rearing of salmonids, mainly because of its limited quantities at the time of sea trout larvae hatching (Czerniawski *et al.*, 2009). In addition, as indicated by the breeding parameters obtained in this experiment, rearing using this type of food feed mixed with artificially produced extruded feed does not yield the intended results. For example, fish in this variant ( $V_A$ ) during the 30-days rearing gained the lowest average unit weight, specific growth rate (SGR), condition coefficient and the lowest survival rate (Table 2).

Artificially produced starter feed, due to its availability and nutritional composition adapted to the cultured species, is most commonly used in the industrial rearing of juvenile sea trout stages (Czerniawski *et al.*, 2009). As demonstrated by our experiment, it allows obtaining a high survival rate and the significantly highest average unit weight and specific growth rate

(SGR). However, satisfactory results in the form of breeding indicators were also obtained for fish larvae fed brine shrimp (Table 2), which is consistent with the studies of Czerniawski *et al.* (2011) and Akbary *et al.* (2010). The high value of live food recorded in the previous studies by Czerniawski *et al.* (2009, 2011) and Akbary *et al.* (2010), probably stems from the fact that the live food is readily accepted because of its constant movement that stimulates interest in fish (Morrison, 1983). Zooplanktons are also a valuable source of proteins, amino acids, lipids and enzymes (Ogino, 1963), and the protein level may even reach up to 65% (Kibria *et al.*, 1999). For example, the research conducted by Girri *et al.* (2002) showed that the nutritional value of brine shrimp larvae is higher than of the artificial food feed. In addition, an advantage of brine shrimp larvae is their availability throughout the year and active movements in the entire water volume in rearing tanks (Akbary *et al.*, 2010).

It should be noted that the high breeding parameters of juvenile sea trout introduced into watercourses cannot be the only element in determining the quality of juvenile fish stages during restocking. An important parameter for economic reasons is survival rate in the watercourse, which is higher in the pre-reared and fed fish fry than in the non-fed hatch (Stanfield and Jones, 2003). The survival rate of the non-fed hatch after release into surface waters, according to research of McNeil (1991) and Salvanes (2001), is only approximately 10%. In contrast,

the survival of sea trout reared on feeds in the first year reaches 20–30%, although some sources say that it may be significantly lower (Paszkowski and Olla, 1985; Arai *et al.*, 2007; Lindeyer and Reader, 2010; Oulton *et al.*, 2013). At the same time it should be stressed that positive results of rearing on extruded feeds do not translate into the high survival rate directly after stocking (Gliwicz and Jachner, 1992; Hall and Suboski, 1995; Jachner, 1995; Jachner 1996). Koskela *et al.* (1997) suggested that it might be related to several-week acclimatization to the new environment, and learning to catch live food during rearing may reduce mortality after stocking (Paszkowski and Olla, 1985; Brown and Lalande 2001; Brown *et al.*, 2003). In addition, on the basis of these research results, it can be concluded that fish having earlier contact with a live, moving food feed, subsequently can more effectively forage in the watercourse than fish reared only on dry food. This confirms the suggestion of Lazzaro (1987) and Czerniawski *et al.* (2011) that fish fed with live food acquire habits of typical predators already during rearing, which may translate into their subsequent behavior in the watercourse. The effects, observed on the basis of the current behavioral experiments, were more effective attacks, fewer failed attacks and resignation from prey. The reason for this was probably the repeated stimulation of visual and chemical stimuli while hunting for actively moving live food (Suboski *et al.*, 1990; Brown *et al.*, 1997; Brown and Day 2002).

In the present study, fish fed with extruded feed at an early stage of the rearing behaved differently than fish fed live food (brine shrimp larvae). They were searching for feed near the bottom or on the bottom and moved up to forage only during the intensive feeding, whereas the group of fish fed with live food continuously penetrated the entire volume of the tank. Therefore, on one hand, although fish fed artificial feed used less energy in search of food and reached the highest length and weight gains, the group feeding on live brine shrimp larvae was better prepared to capture ide larvae in the second part of the experiment. It was evidenced by the higher efficacy of attacks on prey by fish reared on live food and a markedly shorter time needed to locate and attack the prey compared to fish reared on feed. This is likely related to the better-developed predatory instinct, which results in a lower mortality and higher growth rates of fish pre-reared on live food and introduced into surface waters compared to fish reared on feed (Czerniawski *et al.*, 2011).

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