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Short communication

A comparative in-vitro-test on the digestibility of live prey for fish larvae under specific consideration of trypsin

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ABSTRACT

When evaluating live prey for the successful rearing of fish larvae, general availability and nutritional quality of the prey are mainly in the major focus. However, the digestibility of prey items is also crucial as it directly affects the accessibility of the nutrients inside the prey item. Harpacticoid copepods, as well as nematodes, are considered as potential live feed for fish larvae but their digestibility has not yet been identified. Therefore, a comparative in-vitro evaluation of the digestibility of several prey organisms for larval fish was conducted under specific consideration of the efficiency of the proteolytic enzyme trypsin. This endoprotease plays a major role in the digestion process in the early stages of marine species. Common (*Artemia* sp., *Brachionus plicatilis*) and candidate prey organisms for commercial larval rearing (*Acartia tonsa* (Calanoida), *Tachidius discipes*, *Tisbe* sp. (both Harpacticoida), *Panagrolaimus* sp. (Nematoda)) were exposed to a trypsin solution. Photos of trypsin-treated prey organisms were taken and compared with controls to quantify the effect of trypsin on the inner body disintegration. Additionally, the effects of the larval ring muscles in the gut and the pharyngeal teeth were imitated by mechanical treatment. While *Artemia* sp. showed the highest digestibility, *Panagrolaimus* sp. was the opposite with very low degradation. The calanoid copepod *A. tonsa* and the harpacticoid copepod *Tisbe* sp. were more digestible than *T. discipes* and the nematode *Panagrolaimus* sp.

1. Introduction

Marine fish larvae feed primarily on copepods of which calanoids are the dominant food source in open marine waters (Turner, 1984, 2004), whereas harpacticoids are an important link between primary producers and fish larvae in coastal areas (Alheit and Scheibel, 1982; Sibert et al., 1977). For aquaculture purposes, calanoid copepods were reared in open ponds and used in semi-extensive systems for the cultivation of flat fish (Engell-Sørensen et al., 2004), but also intensive rearing techniques were developed (reviewed by Drillet et al., 2011; Støttrup, 2003). For harpacticoid copepods different batch and continuous culture systems were evolved (Kahan et al., 1982; Rhodes, 2003; Støttrup and Norsker, 1997). However, copepods are rarely used in professional hatcheries since the production of pelagic copepods requires a much higher effort than the production of rotifers and *Artemia* nauplii in artificial rearing of marine fish larvae. Therefore, the rearing of marine fish larvae relies mostly on a consecutive administration of rotifers and *Artemia*, which are relatively easy to culture, but both live feeds have deficiencies in their nutritional value compared to natural feeding organisms such as copepods. This results in weaker growth performance, higher mortalities or malpigmentation of fish larvae (Busch et al., 2010; Payne et al., 2001; Schipp, 2006; Shields et al., 1999). The deficiencies of the traditional live feed are a reasonable justification to consider the replacement of the commonly used live feed by copepods. Benthic harpacticoids are considered as promising since they have some advantages compared to calanoid copepods; including higher rearing densities (Støttrup, 2000), higher tolerance to salinity and temperature changes and their ability to feed on diverse food sources (Hicks and Coull, 1983; McIntyre, 1969). However, Fleeger (2005) assumed a lower digestibility of harpacticoids and accordingly, herring larvae showed a high mortality, associated with a low RNA/DNA-ratio, when fed with harpacticoid copepods (Arndt, 2013).

Nematodes were discussed as another promising potential live feed for fish larvae (Schlechtriem et al., 2004) due to their small size, high reproduction rate and the option to charge nematodes prior to preservation with a favourable fatty acid profile (Brüggemann, 2012). In addition, some species, such as *Panagrolaimus* sp., can be stored in a desiccated mode for up to 10 weeks (Honnens et al., 2013) providing an off-the-shelf product similar to *Artemia*. However, larvae fed with nematodes had a lower body weight at the end of the experiment compared to those fed with *Artemia* sp. (Santiago et al., 2003; Schlechtriem et al., 2004) and it was argued that possibly poor digestibility was the major

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reason. To evaluate the digestibility of calanoid, harpacticoid copepods and nematodes as live feed, an in-vitro test with a trypsin solution was conducted comparing common (*Artemia* sp., *Brachionus plicatilis*) and potential live feed organisms (*Tachidius discipes*, *Tisbe* sp., *Acartia tonsa*, *Panagrolaimus* sp.) to estimate their relative susceptibility to proteolytic digestion. Trypsin was chosen since it is the major pancreatic enzyme to degrade protein in the early stages of fish larvae and plays a key role in activating other proteolytic enzymes (Rønnestad et al., 2013).

2. Material and methods

Six prey items were tested for their digestibility: *Artemia* sp., *B. plicatilis*, *Tisbe* sp. and *T. discipes* (both Harpacticoida), *A. tonsa* (Calanoida), and *Panagrolaimus* sp. (Nematoda). *Artemia* eggs (Premium Artemia, Sanders, USA) were incubated for 24 h in filtered sea water (FSW, 0.2 μm filtered, 17 PSU) at 30 °C and the newly hatched nauplii (Instar I) were used for the digestibility test. Rotifers were reared in 10 L-vessels in FSW at 22 ± 1 °C and fed with resuspended *Nannochloropsis* sp. concentrate (BlueBiotech GmbH, Büsum, Germany). The harpacticoid copepods *T. discipes* and *Tisbe* sp., as well as the calanoid copepod *A. tonsa*, were cultivated in 5 L-vessels in FSW at 18 ± 1 °C and fed with *Rhodomonas* sp. Their copepodite stages were used for the digestibility test. The organisms were cultivated at a 16:8-L:D-cycle. The nematode *Panagrolaimus* sp. was obtained alive from the company e-nema GmbH (Schwentinental, Germany).

The protein digestibility of these prey organisms was investigated by incubating the organisms in a trypsin solution for 3 h. This time was chosen in accordance with food retention times in the gut of fish larvae which range from 1.5 h (Fossum, 1983) to 5 h (Blaxter, 1965).

Lyophilized trypsin (10 mg mL^{-1} ≈ 28,000 nmol hydrolyzed substrate min^{-1} $larva^{-1}$) from bovine pancreas (1:250, SERVA GmbH, Germany) was dissolved in TRIS-buffer (0.1 M, pH 8) with $CaCl_2 \cdot H_2O$ (0.02 M). TRIS-buffer without trypsin was used as control. Since the relative comparison of the digestibility of different organism was the focus of this experiment, the tryptic enzyme concentration was adjusted significantly higher than the natural concentration in the gut of fish larvae (0.5–50 nmol hydrolyzed substrate min^{-1} $larva^{-1}$) (Rønnestad et al., 2013). In a preliminary experiment no changes in the physical structure of all prey items were visible compared to the control when natural trypsin concentrations were chosen. The prey items were handled in two different ways prior to exposing them to trypsin: (1) live prey items were not treated mechanically and (2) prey items were shock-frozen at −80 °C for 2 min and then squeezed once with a sharp tweezer to imitate the possible damage of the prey item by peristaltic movement of the larval ring muscles (Rønnestad et al., 2003) and by pharyngeal teeth (Walford and Lam, 1993).

Five individuals of each species were put in 1.5 mL vials filled with the trypsin or control solution. The vials were agitated (Mixer 5432, Eppendorf GmbH, Germany) in a climate cabinet at 30 °C for 3 h. The rotifer *B. plicatilis* was not agitated, because of its tendency to stick on the surface once in contact with air. The temperature of 30 °C was chosen, because bovine trypsin needs higher temperature than trypsin from cold adapted fish to show similar catalytic efficiency (Outzen et al., 1996) and in order to maximise the visibility of a potential effect of the treatment. To evaluate digestibility, photos of prey items were taken after 3 h using a microscope camera (AxioCam MRc, Zeiss GmbH, Germany) mounted on a microscope (Axio Observer.A1, Zeiss GmbH, Germany).

The total body area surrounded by the cuticle and the inner body area of the specimen in the control and the trypsin treatment were measured using the software ImageJ (v1.46r) to quantify the effect of trypsin solution on the prey organisms (Fig. 1).

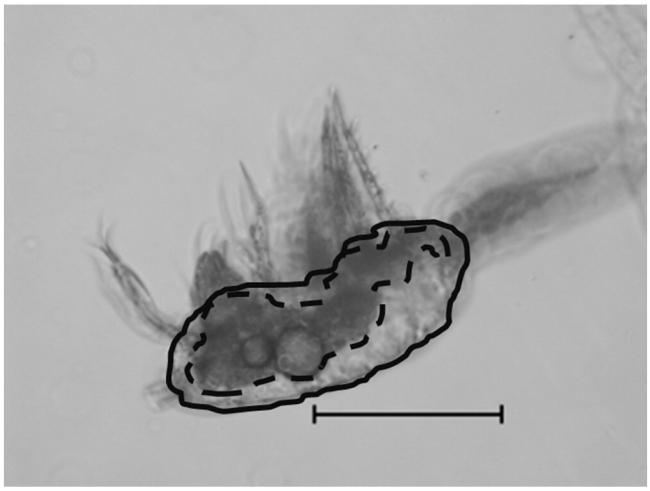

Fig. 1. Exemplary visualisation of body area calculation. Inner body area (dashed line), total body area surrounded by the cuticle (solid line).

Subsequently, the percentage of inner body disintegration (D) was calculated:

$$\overline{A_c} = \frac{1}{n}\sum_{i=0}^{n}(A_{ic}/A_{tc}) \quad (1)$$

$$D = 100 \cdot \left(1 - \frac{A_{it}/A_{tt}}{\overline{A_c}}\right) \quad (2)$$

where $\overline{A_c}$ = mean ratio of the inner body area (A_{ic}) and the total body area surrounded by the cuticle (A_{tc}) of the specimen in the control treatment, n = number of specimens, A_{it} = area of the inner body of the specimen treated with trypsin, A_{tt} = total body area surrounded by the cuticle of the specimen treated with trypsin.

The assumptions of normality and homogeneity of variances were examined and data were square-transformed. Differences in digestibility of the prey items were analysed by a two-factorial-ANOVA with 'prey type' and 'pre-treatment' as fixed factors. Post-hoc comparisons (Unequal N HSD, $\alpha = 0.5$) were performed using STATISTICA 8.

3. Results

All prey items, except the nematode *Panagrolaimus* sp. were affected by trypsin after 3 h, regardless of pre-treatment (Fig. 2). The exoskeletons of the three copepod species were intact but the inner part of the body was reduced compared to the control (Fig. 2.1–2.3). *A. tonsa* and *Tisbe* sp. showed a higher inner disintegration than *T. discipes*. *B. plicatilis* was partly still alive in the trypsin solution without mechanical treatment (Fig. 2.4). *Artemia* sp. was affected the most by trypsin (Fig. 2.5). Only parts of the antennae were left and the thin cuticle was still visible. *Panagrolaimus* sp. were still alive after 3 h trypsin solution without mechanical pre-treatment (Fig. 2.6B). They only showed evidence of digestion if a damage of the cuticle occurred by mechanical treatment prior to being treated with trypsin (Fig. 2.6C).

The disintegration differed significantly between prey types (ANOVA: $F = 10.99$, $p < 0.001$) (Fig. 3). *Artemia* sp. had the highest digestibility, which was significantly higher than that of *T. discipes* ($p < 0.001$), *B. plicatilis* ($p < 0.01$) and *Panagrolaimus* sp. ($p < 0.001$). Furthermore, there were differences between copepod species with *A. tonsa* and *Tisbe* sp., being more digestible than the harpacticoid copepod *T. discipes* ($p < 0.01$ and $p < 0.05$, respectively) and the nematode *Panagrolaimus* sp. ($p < 0.01$, both). *T. discipes* and the nematode *Panagrolaimus* sp. were the least affected by the treatments.