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Short Communication

Using the nematode *Panagrolaimus* sp. in larval rearing of longfin yellowtail *Seriola rivoliana*: preliminary results

Laurence Mercier[1], Itzi Y. Guzmán de las Nieves[2], Laurent H. Seychelles[3] Mauricio Contreras-Olguín[2] & Silvie Dumas[2]

[1]Centro de Investigaciones Biológicas del Noroeste (CIBNOR), S.C., La Paz, México
[2]Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Instituto Politécnico Nacional La Paz, México
[3]e~nema, Gesellschaft für Biotechnologie und biologischen Pflanzenschutz mbH Schwentinental, Germany
Corresponding author: Silvie Dumas (sdumas@ipn.mx)

ABSTRACT. The potential of *Panagrolaimus* sp. nematode as a live prey was assessed in longfin yellowtail *Seriola rivoliana* larval rearing. Larvae were reared from 3-11 days post-hatching (dph) in a 2000 L tank and fed on copepods and rotifers. On 12 dph, larvae were transferred to eight 200 L tanks at a density of 12 larvae L^{-1} to start the feeding trial, consisting of two treatments (*Artemia* or nematodes) using four replicates each. Rotifers were offered on 12 dph with a "co-feeding" from 14 to 16 dph (rotifer/enriched *Artemia* or rotifer/nematode). Only *Artemia* or nematodes were supplied from 17 to 19 dph. Feeding incidence was 80-90% on 14 dph, but more larvae were capturing rotifers than *Artemia* or nematodes. *Artemia* consumption increased more than nematodes at 19 dph, though it was not significantly different. Significantly lower enterocyte and villus heights in the posterior intestine were observed at 19 dph in larvae fed on nematodes but only in enterocyte height in the anterior one. No significant differences were observed between treatments in total larval length and body depth on 16 dph, but a significantly higher total length in the nematode one was present on 19 dph. A significantly higher survival was observed in treatment *Artemia* compared to nematodes. These preliminary results showed a lower nutritional condition in larvae fed nematodes; however, technical improvements need to be performed to increase *Panagrolaimus* sp. availability to larvae in the water column.

Keywords: *Panagrolaimus* sp.; *Seriola rivoliana*; *Artemia*; live food; larval feeding; feeding efficiency; aquaculture

The search for new-candidate live prey for feeding marine fish larvae is undeniably important to compensate for some disadvantages of the most commonly used in larval rearing -rotifers and *Artemia*- since both do not entirely satisfy the nutritional requirements of larvae (Dhont et al. 2013). Because of their low content in highly unsaturated fatty acids (HUFA), they need to be enriched (Léger et al. 1986, Dhert et al. 2001). *Artemia* also shows variations in cyst yield, price, nauplius hatching, and nutritional quality fluctuations (Lavens & Sorgeloos 2000) and can be a harmful vector of larval fish pathogens (Dhont et al. 2013). All these factors impact larviculture operations and costs.

On the other hand, the nematode *Panagrolaimus* sp. strain NFS 24-5 naturally synthesizes eicosapentaenoic (EPA, 20:5n-3) and arachidonic (ARA, 20:4n-6; Honnens et al. 2014) acids and it can be enriched in docosahexaenoic acid (DHA) with microalgae *Crythecodinium cohnii* (Seychelles et al. 2017, 2018) just after its production in a bioreactor. This nematode has a suitable size (length: 180-1380 µm; diameter: 10-60 µm), tolerates seawater, and can be mass-produced in liquid culture with *Escherichia coli* (strain K12, DSM 498) (Honnens & Ehlers 2013). *Panagrolaimus* sp. is tolerant to dehydration and can be rehydrated 30-60 min before being offered alive to larvae. Moreover, they can be stored at 4°C for at least 10 months (Seychelles et al. 2018).

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Therefore, the objective of this study was to assess the potential of the nematode *Panagrolaimus* sp. in longfin yellowtail *Seriola rivoliana* larval rearing. This fish species has been identified as a great candidate for aquaculture (Roo et al. 2014, Sicuro & Luzzana 2016) and is commercially produced in the USA and Mexico. However, a constraint impacting juvenile production is the high mortality during early larval development (Roo et al. 2014, Teles et al. 2019). Live foods commonly used in longfin yellowtail larval culture are the rotifer *Brachionus plicatilis* and *Artemia*. To the best of our knowledge, this is the first study using *Panagrolaimus* sp. for larval rearing of a marine fish species.

Fertilized eggs of *S. rivoliana* were donated by Kampachi Farms Mexico (La Paz, Mexico) and transported to the Centro Interdisciplinario de Ciencias Marinas. Embryos were disinfected with formalin (10 ppm) for one hour and incubated in a 130 L fiberglass tank until hatching (26°C, salinity of 37). Newly hatched larvae were transferred to a 2000 L fiberglass tank at a density of 50 larvae L^{-1} (25.7 ± 0.5°C, salinity of 36.7 ± 0.5, dissolved oxygen at 6.1 ± 0.7 mg L^{-1}). The photoperiod was 11.5:12.5 h (light:darkness) with an approximately 2000-2300 lux light intensity at the water surface. Water replacement was performed at night (3 L min^{-1}).

From 3 (mouth opening) to 11 days post-hatching (dph), larvae were fed on *B. plicatilis* at 20 rotifers mL^{-1}, and a mixture of two copepod nauplius species (*Pseudiaptomus euryhalinus*, 2-4 mL^{-1} and *Parvocalanus crassirostris*, 5-6 mL^{-1}) (Fig. 1); *Nannochloropsis oculata* was added to the larval tank (300,000 cell mL^{-1}).

At 12 dph, larvae were transferred to eight 200 L conical fiberglass tanks at a density of 12 larvae L^{-1} to begin the feeding trial with *Panagrolaimus* sp. Tanks were provided with filtered (5 µm) and chlorinated marine water, neutralized with thiosulphate, and sterilized with ultraviolet light. On 12 and 13 dph, larvae were fed only rotifers at 20 ind mL^{-1}. Co-feeding with *Artemia* spp. (control) or *Panagrolaimus* sp. started from 14 dph using four replicate tanks for each dietary treatment and was performed for three days: 75-25, 50-50, 25-75%, decreasing the number of rotifers added to the tanks each day. At 17-19 dph, *Artemia* or nematodes were offered to larvae using an equivalent prey supply according to dry weight. In each tank, *N. oculata* was added daily (600,000 cell mL^{-1}). Rearing conditions were 24 ± 0.2°C, salinity of 35.5 ± 0.1, dissolved oxygen at 5.4 ± 0.4 mg L^{-1}. Water flow and photoperiod were the same as described above for initial rearing.

Rotifers were fed RotiGrow® (Reed Mariculture, USA) and harvested every four days (27°C, salinity of 37, dissolved oxygen at 7 ± 1 mg mL^{-1}). Copepods were produced at 26°C using the microalgae *Chaetoceros calcitrans* and *Tetraselmis suecica*, as well as *Isochrysis galbana* for *P. crassirostris*. *Artemia* cysts (Biogrow®, *Artemia* 80%, Mexico) were incubated for 24 h at 15 of salinity and 25°C. Nauplii were disinfected with iodine (Argentyne®, Argent aquaculture, USA; 2 mL L^{-1}) for 10 min and enriched with S.presso® (INVE Aquaculture, Belgium; 5 mg L^{-1}) for 14 h. Nematodes were produced according to Seychelles et al. (2018) and rehydrated 2 h before larval feeding.

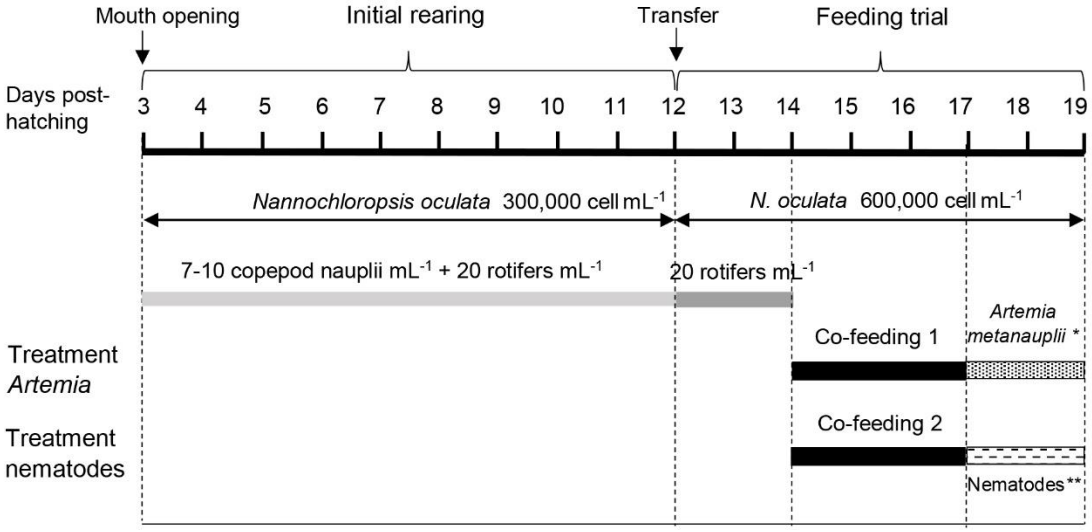

Figure 1. Feeding scheme of *Seriola rivoliana* larvae during the bioassay. *1 *Artemia* mL^{-1}, **170 nematodes mL^{-1}, Co-feeding 1: rotifer-*Artemia* metanauplii, Co-feeding 2: rotifer-nematodes.

On 14 and 19 dph, 10 larvae per replicate were sampled and anesthetized with 2-phenoxyethanol (4 ppm) to determine feeding incidence (FI) and intensity (FT) under a stereoscope (Yin & Blaxter 1987).

$$\text{Feeding incidence} = \frac{\text{Larvae with food in the digestive tract}}{\text{Number of larvae examined}} \times 100$$

$$\text{Feeding intensity} = \frac{\sum\text{Number of preys per larvae}}{\text{Number of larvae with food}}$$

Total length (TL) and body depth (BD) at the anal height of larvae were evaluated on 10 larvae per replicate at 16, and 19 dph with Image-Pro® Plus software (Media Cybernetics, USA) from photos taken with a digital camera (Lumerera® Infinity 1, Canada) coupled to a stereoscope (Olympus® SZ40, Japan). Final survival was determined at 19 dph by counting all remaining larvae. Approximately 13 larvae per treatment were fixed in Davidson's solution and histologically processed as described by Rodríguez-Jaramillo et al. (2008). Height of villi (from the base to the apical part of the fold) and enterocytes (from the basal to apical part, including microvilli) of the anterior and posterior intestines were measured from photographs (Nikon DS-Ri1, Japan) analyzed with Image-Pro® Plus.

All statistical analyses were performed with Statistica 10.0 (StatSoft, USA). Percentage data were transformed using the arcsine square root. Variables were checked for normality and homogeneity, and when these conditions were fulfilled, a Student's *t*-test was performed. Otherwise, a non-parametric Kruskal-Wallis test was applied. The level of significance was $\alpha = 0.05$.

Fish larvae fed the control diet or nematodes showed similar TL and BD at the end of the co-feeding period (16 dph), but a significantly higher TL ($P = 0.04$) was observed in larvae fed on nematodes on the last day of the experiment (19 dph) (Table 1). The survival rate for control larvae at the end of the experiment was 4-fold higher (8%; $P < 0.05$) than those fed on nematodes (Table 1). On the first co-feeding day (14 dph), FI was high (80-90%), and larvae were feeding mostly on rotifers in both treatments (Table 2). At 19 dph, FI was still elevated, and 80% of control larvae switched diets by consuming more *Artemia* alone or combined with rotifers. However, it was not the case for those in the nematode treatment where only 40% of larvae fed on nematodes only or combined with rotifers. The detection of rotifers in the diet of larvae from both dietary treatments at 19 dph would indicate a problem of water renewal in tanks. Nevertheless, this problem was assumed to be present in all tanks of both treatments since they were equipped with the same water renewal system. The lower consumption of nematodes was probably due to their shorter suspension time in the water column due to settling, making them less available for predation, unlike *Artemia* nauplii which swim.

Table 1. Total length (TL, mm), body depth (BD, mm), and survival (%) (mean ± standard deviation) of *Seriola rivoliana* larvae fed on *Artemia* (*Ar*) and *Panagrolaimus* sp. (*Ne)* on 16 and 19 days post-hatching (dph). Means with different superscript letters in each line are significantly different ($P < 0.05$). For TL and BD, $n = 10$ larvae/replicate at 16 dph and n = 30 larvae/replicate at 19 dph.

	dph	*Ar*	*Ne*
TL	16	5.2 ± 0.5	5.2 ± 0.3
	19	7.7 ± 1.7[b]	8.2 ± 0.9[a]
BD	16	0.6 ± 0.1	0.6 ± 0.0
	19	1.1 ± 0.4	1.2 ± 0.2
Survival	19	8.0 ± 1.5[a]	1.9 ± 1.0[b]

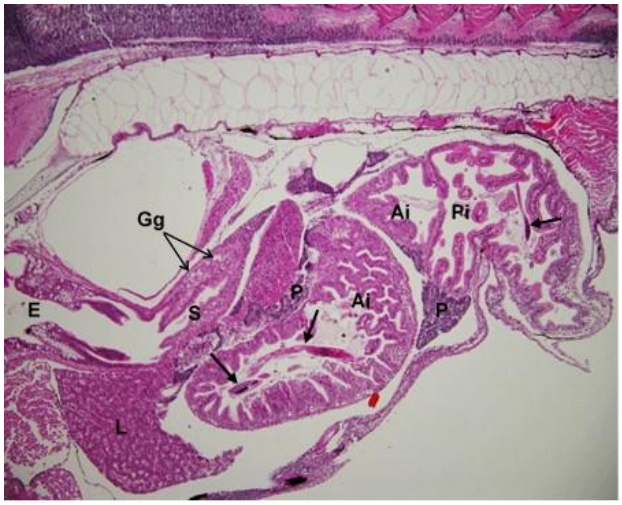

Figure 2. Sagittal sections of *Seriola rivoliana* larvae fed on *Panagrolaimus* sp. at 19 days post-hatching (dph) (10x). Ai: anterior intestine, E: esophagus, Gg: gastric glands, L: liver, P: pancreas, Pi: posterior intestine, S: stomach, ⟶: Nematodes.

During the feeding trial, larvae were observed actively hunting nematodes. According to histological analyses (Fig. 2), the detection of identifiable nematode fragments found in the anterior and posterior intestines of the nematode treatment larvae demonstrated that larvae could ingest and break down nematodes. Previous studies have shown that the nematode *Panagrellus redivivus* can be used as live food in the larval rearing of marine (Reyes et al. 2011) and freshwater fish larvae (Santiago et al. 2003, Schlechtriem et al. 2004, Sautter et al. 2007). The nematode body is surrounded by a cuticle composed mainly of collagen and insoluble proteins called cuti-

Table 2. Feeding incidence (FI) and intensity (FT) (mean ± standard deviation) of *Seriola rivoliana* larvae on the first co-feeding day (14 days post-hatching, dph) and on day 19 dph. Treatments *Artemia* (*Ar*) and *Panagrolaimus* sp. (*Ne*) started on 14 dph. Means with different superscript letters in each line are significantly different ($P < 0.05$). For FI and FT, n = 10 larvae/replicate at 14 and 19 dph. *Larvae showing a mixture of [1]Rotifer-*Artemia* or [2]Rotifer-Nematode in their digestive tract.

		Ar			*Ne*		
	dph	Rotifer	*Artemia*	*Mixture[1]	Rotifer	Nematode	*Mixture[2]
FI	14	70 ± 14.1	10 ± 14.1	0 ± 0.0	80 ± 0.0	10 ± 14.1	0 ± 0.0
	19	10 ± 14.1	20 ± 0.0	60 ± 0.0	50 ± 14.1	10 ± 14.1	30 ± 14.1
FT	14	1.5 ± 1.6^{ab}	0.2 ± 0.6^{b}	-----	7.3 ± 9.2^{a}	0.1 ± 0.3^{b}	-----
	19	1.9 ± 3.5^{ab}	4.4 ± 6.3^{ab}	-----	5.9 ± 6.1^{a}	0.7 ± 0.9^{b}	-----

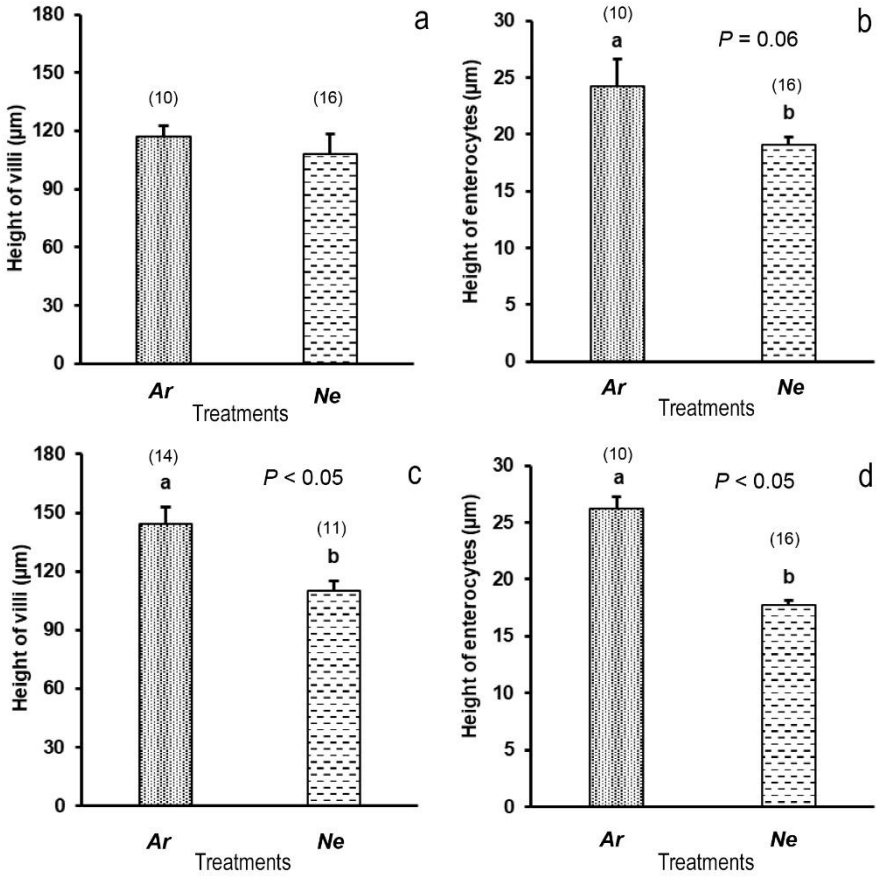

Figure 3. Height of villi and enterocytes (mean ± standard error) in the a-b) anterior and c-d) posterior intestine of *Seriola rivoliana* larvae fed on *Artemia* (*Ar*) and *Panagrolaimus* sp. (*Ne*) at day 19 post-hatching. Numbers in parentheses indicate the number of larvae examined.

clins, associated with glycoproteins and lipids (Page & Johnstone 2007). Arndt et al. (2015) showed *in vitro* evidence of *Panagrolaimus* sp. digestion by bovine trypsin when the nematode cuticle was previously mechanically damaged. In *S. rivoliana* larvae, trypsin activity was reported from 3 to 25 dph (Teles et al. 2019). Additionally, a pepsin-like activity was detected at 15 dph (Teles et al. 2019), and pepsin could digest collagenous connective tissues (Gildberg 2004).

Nevertheless, larvae in the nematode treatment were probably underfed. The enterocyte height in the anterior ($P = 0.06$) and posterior intestine ($P < 0.05$) as well as villus height in the posterior intestine ($P < 0.05$) of larvae fed on nematodes were significantly reduced compared to those of *Artemia* treatment (Fig. 3), indicating a deficient nutritional status in these larvae. Enterocyte height has been widely used as a biomarker

of nutritional condition in several fish species (Gisbert et al. 2008).

This study showed that *S. rivoliana* larvae could ingest nematodes, but a lower survival rate and reduced height of enterocytes suggest they were underfed. Furthermore, this work demonstrated that the supply of nematodes to the rearing tanks must be improved to maintain an optimal density in the water column. A higher frequency in nematode distribution and a drip-feeding supply method described by Reyes et al. (2011) should be tested.

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