Effects of glucose during the yolk-sac larvae and the pre-leptocephalus stages in Freshwater eel *Anguilla japonica*

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Introduction

- Freshwater eel are commercially important in Northeast Asia, but mass seed production has not been successful, in part because of their very unique life history.
- Broodstock are known to growth in freshwater during 5-10 years and to spawn at a depth of about 200 m near the Mariana Trench.
- The hatched larvae feed on marine snow and are known to migrate along the currents for about 150 days after hatching (DAH) before metamorphosing into juvenile eels (glass eel).
- These unique ecological characteristics are make it difficult to adapt to aquaculture, resulting in low quality of artificial seed.
- Therefore, this study aimed to provide a new method to enrich nutrition of seeds before the first feeding, and to confirm the effect of glucose as a nutrient source for eel larvae.

Materials and Methods

Yolk-sac larval stage:

Pre-leptocephalus stage:

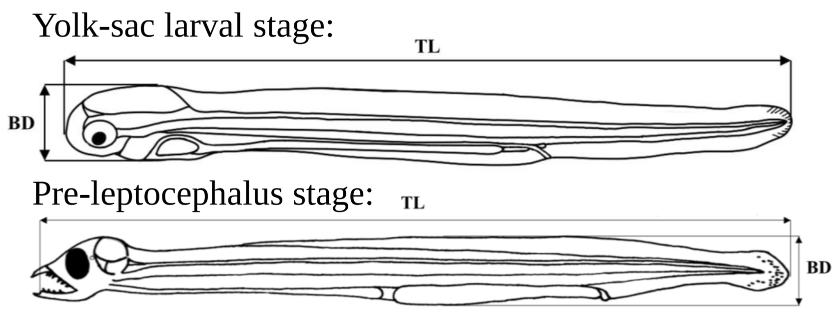


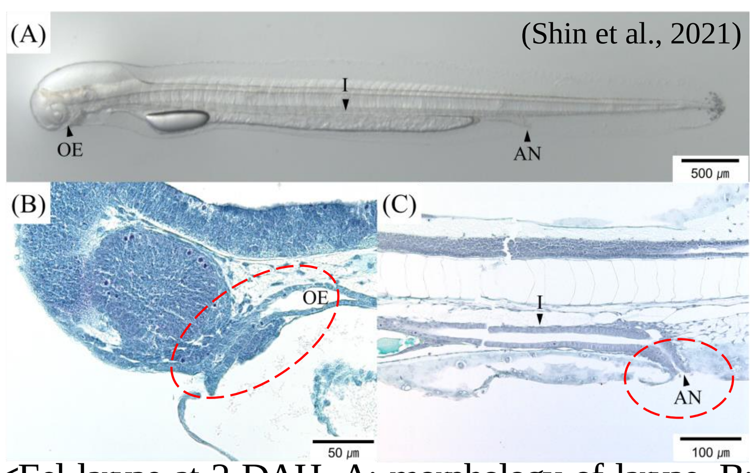
Fig. 1 Morphometric measurements of eel larvae. Abbreviations: TL, total length; BD, body depth.

- **Exp. 1 Rearing**: No feed, 300 L cylindrical tank (rotation system), approx. 23 ℃, glucose addition from 1 DAH to 6 DAH.
- **Exp. 1 Design**: We confirmed the effect of glucose addition in seawater by comparing the control group with no glucose addition and the experimental groups with glucose addition by concentration (0.5%, 1% and 2%) in the yolk-sac larvae stage.
- **Exp. 2 Rearing**: Feed [slurry-type diet {shark egg (50g), fish soluble protein(3g), soybean peptide(3g), krill extract (6g), vitamin mix(0.3g)}; 09:00, 11:00, 13:00, 15:00, 17:00], 20 L round acrylic resin tank (flow through system), approx. 23 ℃, Fed from 7 DAH to 30 DAH.
- **Exp. 2 Design**: We added glucose (0, 0.1, 0.3, 0.5%) to slurry-type feed to understand the importance of an appropriate amount glucose in growth and survival during the pre-leptosephalus stage.

- **Physiological analysis**: The hyaluronan content was measured using the hyaluronan quantikine ELISA kit (Bio-techne, USA) according to manufacturer instructions.
- **Histological analysis**: As paraffin sectioning method, eel larvae have done serial sagittal sections (4–5 μm thick) from each paraffin block. And then, the sections were stained by ab-PAS stain method.
- **Genetic analysis**: The expression levels of genes involved in target genes were assessed by qPCR. Relative gene quantification was performed using the $2^{-\Delta\Delta Ct}$ method. *Slc7a8* (large neutral amino acids transporter small subunit 2) : a transporter of all amino acids except proline in the intestinal basal membrane; *sglt1* (sodium/glucose co-transporter member 1) : a transporter of all amino acids except proline in the intestinal basal membrane; *npc1l1* (Niemann-Pick C1-like 1) : a transporter of sterols of micelles in the apical membranes of enterocyte. (Karasov and Douglas, 2013)

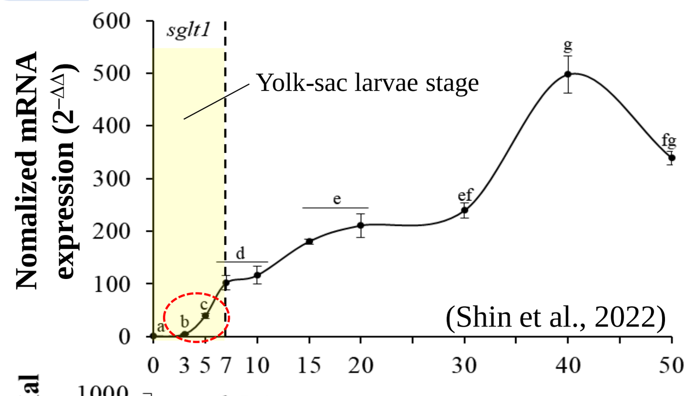
Results and Discussion

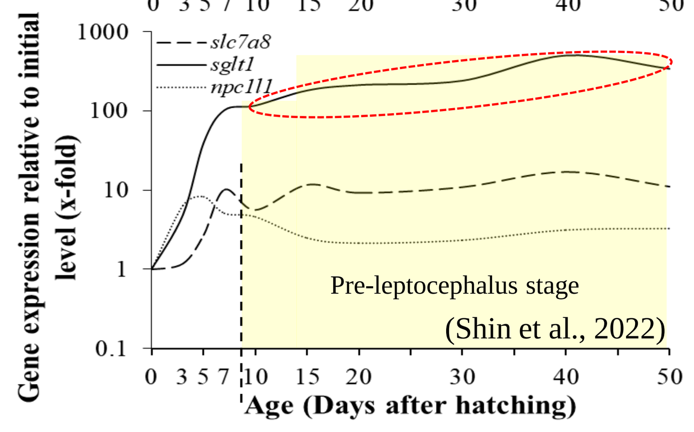
Previous studies



<Eel larvae at 2 DAH. A: morphology of larvae. B: Longitudinal histological sections of the buccopharynx. C: Longitudinal histological sections of the intestine. Abbreviations: DAH, days after hatching; OE, oesophagus; I, intestine; AN, anus>

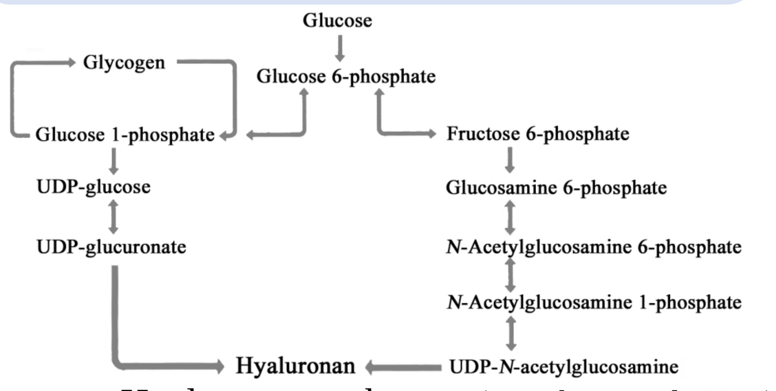
A hole-like shape mouse and anus of eel larvae open at 2 DAH. This is presumed to be able to eat water-soluble substances.





<mRNA levels of nutrient transporters in eel larvae>

We hypothesized that eels can absorb glucose during the Yolk-sac larvae stage, and may be required glucose during the Pre-leptosephalus stage.



<Hyaluronan pathway> (Kawakami et al., 2014)

We predicted that supplied glucose would increase the amount of hyaluronan, which makes up the main of the eel larval body.

The role of hyaluronan : ① composition of bodies, ② energy stores, ③ water homeostasis etc.

Experiment 1 (Yolk-sac larvae stage: 1-6 DAH)

Morphological analysis

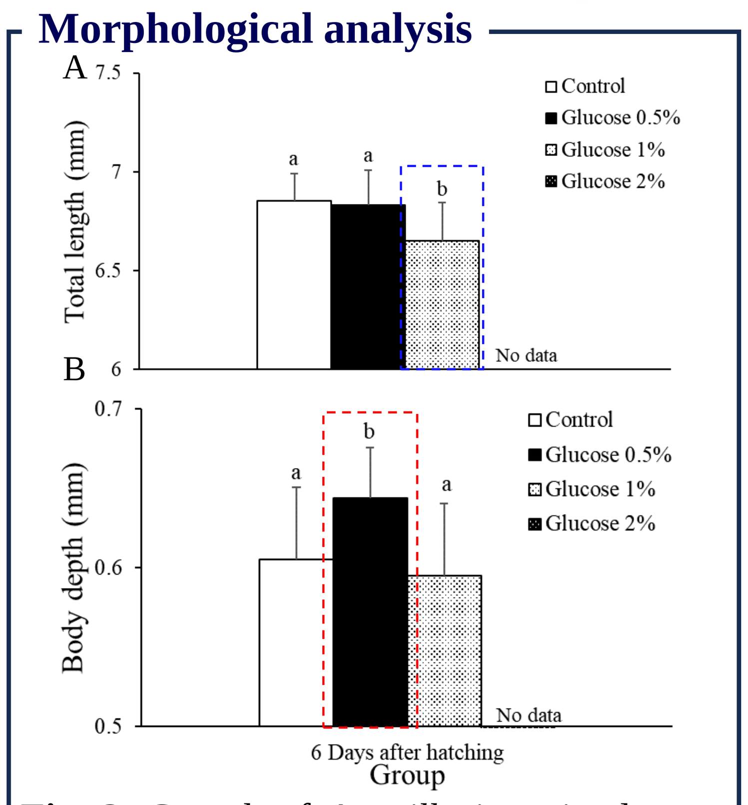


Fig. 2. Growth of *Anguilla japonica* larvae. (A) Total length. (B) Body depth. Values are means ± SD. Different letters represent significant differences ($P < 0.05$).

Physiological analysis

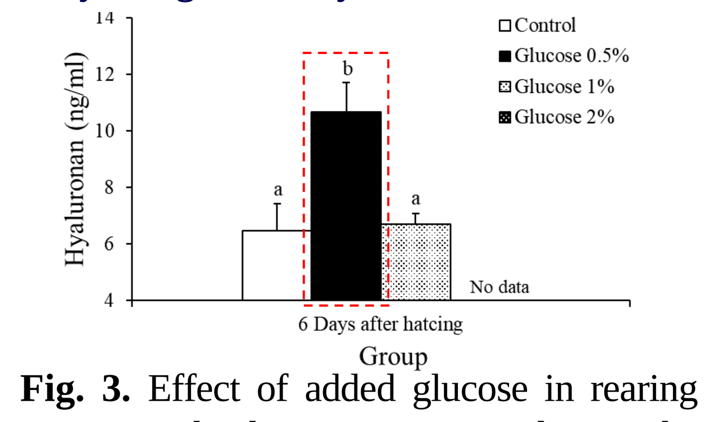
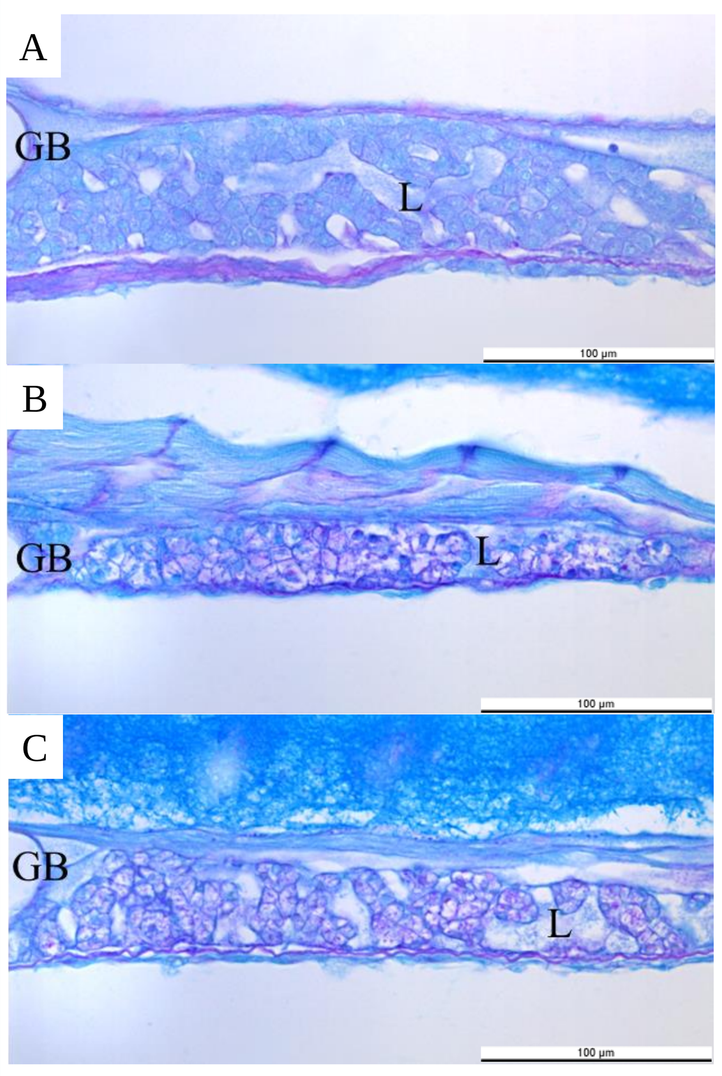


Fig. 3. Effect of added glucose in rearing water on hyaluronan content during the yolk-sac larval stage of *Anguilla japonica*. Values are means ± SD. Different letters represent significant differences ($P < 0.05$).

Histological analysis



Purple : Polysaccharides, Neutral mucopolysaccharides, **Glycogen**
Brightly light-blue : Acid mucosubstances, **Hyaluronan**
Blue : Nuclei

Fig. 4. Longitudinal histological sections in 6 DAH. A: Control Group. B: Glucose-0.5% added Group. C: Glucose-1% added Group. Abbreviations: DAH, days after hatching; GB, gall bladder; L, liver.

Genetical analysis

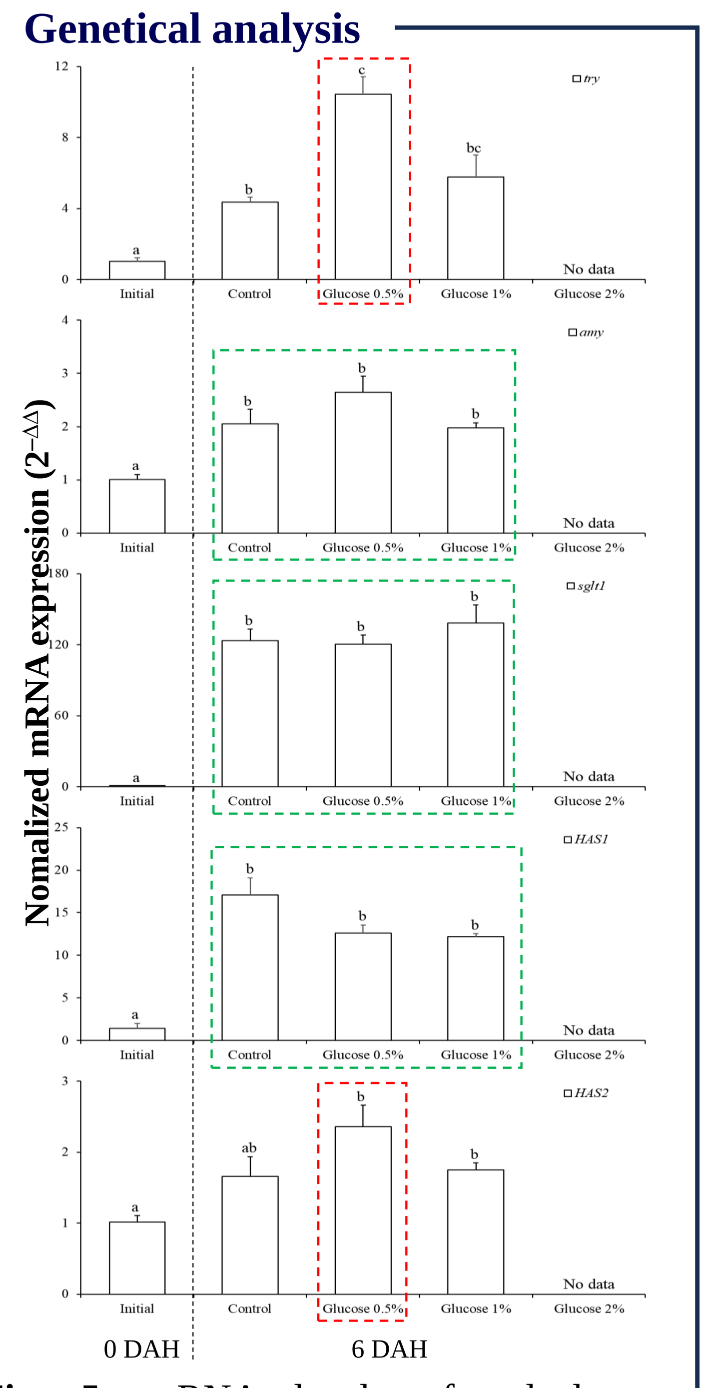


Fig. 5. mRNA levels of eel larvae. Abbreviations: *try*: pancreatic trypsin; *amy*: α-amylase; *HAS1*: Hyaluronan synthase 1; *HAS2*: Hyaluronan synthase 2. Values are means ± SEMs. Different letters represent significant differences ($P < 0.05$).

Experiment 2 (Pre-leptocephalus stage: 7-30 DAH)

Morphological analysis

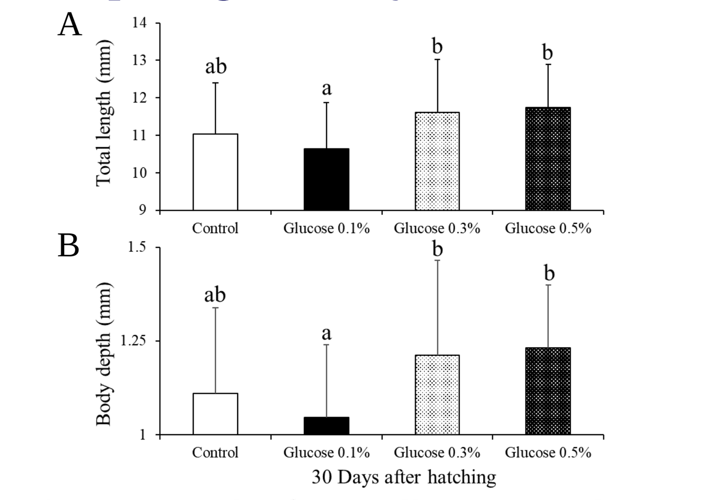


Fig. 6. Growth of *Anguilla japonica* larvae. (A) Total length. (B) Body depth. Values are means ± SD. Different letters represent significant differences ($P < 0.05$).

Survival rate

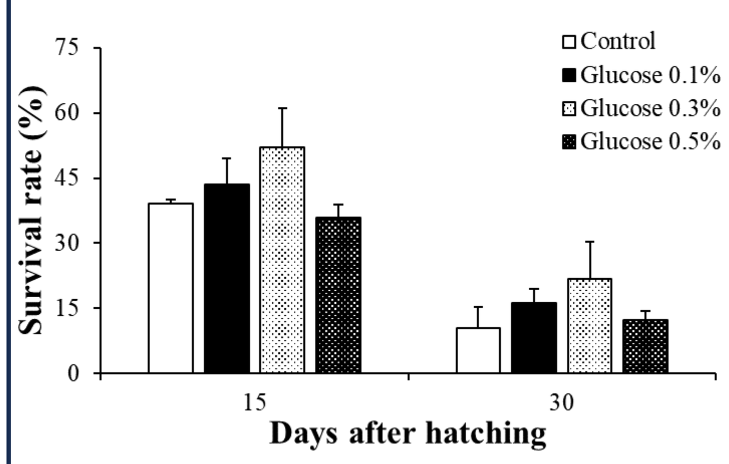


Fig. 7. Changes in survival of *Anguilla japonica* larvae in response to glucose addition in feed. Values are means ± SD.

Physiological analysis

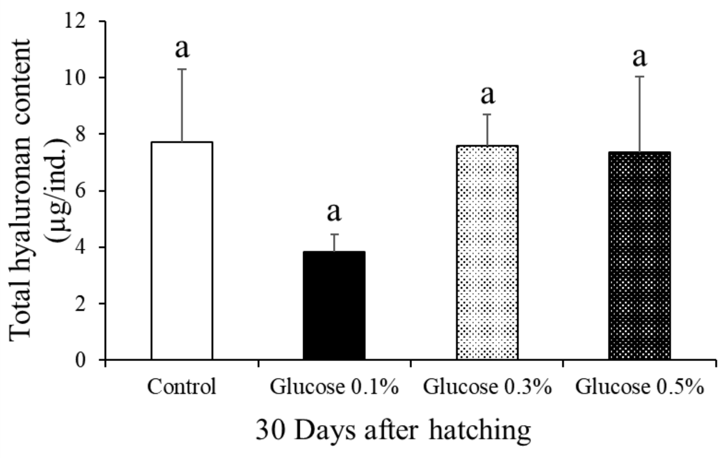


Fig. 8. Effect of added glucose in feed on hyaluronan content of *Anguilla japonica*. Values are means ± SD. Different letters represent significant differences ($P < 0.05$).

◆ **Morphological analysis**

Exp. 1: Significantly increased the body depth ($P<0.05$), when supplied suitable glucose concentration (0.5%).
Exp. 2: Growth was relatively high in the glucose 0.3 and 0.5% groups.

☞ We suggest that adding glucose is effective for growth.

◆ **Survival rate**

Exp. 2: Survival increased with increasing glucose addition up to 0.3%, but glucose 0.5% group had lower survival than 0.3%.

☞ This suggests that suitable glucose addition may help improve survival.

◆ **Physiological analysis**

Exp. 1: 60% increase in the amount of hyaluronan in the body compared to the control group ($P<0.05$), when supplied suitable glucose concentration (0.5%).
Exp. 2: There was no significant difference based on glucose concentration ($P>0.05$). This is probably due to the effect of starvation from 18:00 to 09:00.

☞ Hyaluronan is expected to enhance resistance to starvation through its role in energy storage during larval stages.

◆ **Histological analysis**

Exp. 1: Increased accumulation of estimated substances as glycogen and lipid in the liver when glucose is added.

☞ Accumulation of nutritional components in the liver of eel larvae is expected to enhance resistance to starvation.

◆ **Genetical analysis**

Exp. 1: *try*, which can be used as a marker of nutritional status (Khoa et al., 2019), increases at suitable glucose concentration (0.5%). In *HAS2*, the addition of 0.5% glucose resulted in a relative increase compared to the other groups. This trend was consistent with the pattern of hyaluronan content.

☞ The addition of glucose in seawater may improve the health and promote hyaluronan synthesis during yolk-sac larvae stage.

Highlights

Exp. 1

1. We suggest nutritional enrichment possibilities before first feeding. (**0.5%↓ glucose** was determined as suitable concentration)
2. We confirmed the effect of suitable glucose supply during Yolk-sac larvae stage. (Growth ▲, Glycogen in liver ▲, Hyaluronan ▲, Nutritional status ▲)

Exp. 2

1. We have confirmed that the addition of glucose to the feed improves the growth of eel larvae, as reported by Okamura et al. (2014).
2. We determined the suitable glucose concentration for feed and suggests that it may also contribute to increased survival rates. (**0.3% glucose** was determined as suitable concentration)