



## Effects of Water Temperature and Post-Feeding Period on Postprandial Ammonia Excretion and Oxygen Consumption by Larval Pacific Cod (*Gadus macrocephalus*) Fed Rotifers (*Brachionus plicatilis*)

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### Abstract

Postprandial ammonia excretion and oxygen consumption of 15-d post-hatch larval Pacific cod (*Gadus macrocephalus*), rotifer-fed (*Brachionus plicatilis*) since their first feeding, were studied in stationary cultures at 10°C, 13°C, and 16°C for 48 h. Postprandial ammonia excretion and oxygen consumption rates were determined after 2, 4, 6, 8, 10, 12, 18, 24, 36 and 48 hours. The 48-h post-feeding weight-specific ammonia excretion and oxygen consumption rates increased until 4 h post-feeding, decreased to 18 h post-feeding, and remained relatively stable until 48 h post-feeding. The highest weight-specific ammonia excretion and oxygen consumption rates were  $215 \pm 19.8 \mu\text{g NH}_3\text{-N g}^{-1} \text{h}^{-1}$  and  $1,778 \pm 55.6 \mu\text{g O}_2 \text{g}^{-1} \text{h}^{-1}$  2–4 h post-feeding at 13°C. The higher ( $P < 0.05$ ) resting ammonia and oxygen consumption at 16°C 12-h post-feeding indicate that this temperature is not appropriate for inducing higher growth of rotifer-fed Pacific cod larvae.

**Keywords:** Pacific cod, larvae, ammonia-nitrogen excretion, and oxygen consumption.

### Introduction

Over 50% of the nitrogen input into a marine fish culture system may be lost to the water as excretion (Gowen and Bradbury, 1987). Fish may excrete nitrogen in the form of ammonia, urea, amines, and amino acids (Wood, 1958; Davenport and Sayer, 1986; Porter *et al.*, 1987; Sayer and Davenport, 1987). Most marine teleosts are predominantly ammonotelic and reportedly excrete 20–75% of ingested nitrogen, with 70–90% of nitrogen excretion in the form of ammonia nitrogen (Randall and Wright, 1987; Dosdat *et al.*, 1996). Ammonia nitrogen is harmful to fish and is considered to be a major factor limiting fish biomass and stocking densities in intensive culture systems (Cai and Summerfelt, 1992; Forsberg and Summerfelt, 1992). Benthic fish species excrete higher urea-N rates compare to pelagic species in defence against ammonia toxicity (Dosdat *et al.*, 1996; Engin and Carter, 2001; Smutna *et al.*, 2002; Ip *et al.*, 2007; Merino *et al.*, 2007). Quantification of ammonia excretion is important for estimating optimum carrying capacities in hatchery systems, especially in a larvae production tank managed with stationary culture conditions.

The Pacific cod, *Gadus macrocephalus*, is an important cold-water fisheries species, but is suffering

from declining catches. Some efforts to artificially produce this species for fisheries management and aquaculture have been attempted. Most hatchery production of Pacific cod begins at December when water temperature is declined to 11–12°C and hatchery produced Pacific cod are held in indoor facilities until April. In this period, water temperature is ranging 10–15°C. During the hatchery period, especially in a larvae production, water temperature is kept at 12°C higher than natural water temperature to enhance the cod larvae growth by heating. In order to save energy and reduce the amount of water discharged, cod larvae are usually kept at high densities and under a stationary culture conditions. At this condition, ammonia and oxygen might be limits to the growth and survival of cod larvae (Foss *et al.*, 2004; Remen *et al.*, 2008). However, there is a lack of information about ammonia excretion by larval cod that have been fed rotifers, *Brachionus plicatilis*. Water temperature is an important factor controlling larval fish stocking densities in relation to ammonia nitrogen excretion and oxygen consumption rates (Paulson, 1980; Cai and Summerfelt, 1992; Forsberg and Summerfelt, 1992; Ruyet *et al.*, 2004). Therefore, this study examined postprandial ammonia excretion and oxygen consumption rates of rotifer-fed larval cod as a function of water temperature and post-

feeding period to provide useful data for hatchery management.

## Materials and Methods

### Larvae Preparation and Experimental Conditions

The experiment was carried out at the Gyeongsangnam-Do Fisheries Resources and Research Institute, Tongyong, Korea. Eggs were artificially fertilized ( $1.13 \pm 0.038$  mm, diameter  $\pm$  SD) and incubated in a hatching jar at  $10^\circ\text{C}$ . Larvae that hatched within 24 h ( $4.03 \pm 0.325$  mm, TL  $\pm$  SD) were randomly transported to a stock tank ( $15 \text{ m}^3$ ). The water temperature was gradually adjusted to  $12^\circ\text{C}$ . The 3-d to 14-d post-hatch cod larvae were fed rotifers (*Brachionus plicatilis*) at a concentration of 10–22 individuals  $\text{ml}^{-1}$  plus greenwater (*Nannochloropsis gaditana* and *Isochrysis galbana*; 450,000–500,000 cells  $\text{ml}^{-1}$ ). From 9-d post-hatch the larvae in the stock tank were randomly sampled with water and transported to three substock tanks ( $15 \text{ m}^3$ ). The water temperatures in three substock tanks were gradually adjusted to  $10^\circ\text{C}$ ,  $13^\circ\text{C}$ , and  $16^\circ\text{C}$  until 14-d post-hatch. Larvae were maintained under natural photoperiod and salinity (34 ppt). During acclimation period, salinity, pH, ammonia, and dissolved oxygen (DO) were maintained at  $33 \pm 1$  psu,  $8.1 \pm 0.2$ ,  $< 0.005$   $\text{mg L}^{-1}$ , and  $> 7$   $\text{mg L}^{-1}$ , respectively. Light intensities at the water surface of the experimental tanks ranged from 170 lux at night to 300 lux at daytime.

### Sampling and Analysis

The 15-d post-hatch cod larvae ( $1.3 \pm 0.06$  mg, mean  $\pm$  SD) fed rotifers were drawn randomly from the substock populations by siphoning and counted. Rotifers were separated from the larvae by siphoning. Bunches of larvae (437 to 513 individuals) were distributed into 6 L water volume plastic bags filled with filtered ( $0.45 \mu\text{m}$ , Millipore) seawater at  $10^\circ\text{C}$ ,  $13^\circ\text{C}$ , and  $16^\circ\text{C}$ , respectively. This stocking number and water volume were decided from previous experiments that allowed larvae alive and water quality detectable. One hundred and twenty plastic bags were used in the experiment with three temperature regions, ten time periods, and three replicates with one control held sea water only without larvae. Before stocking the larvae, DO was measured using a DO meter (Handy Polaris, OxyGuard, Birkerød, Denmark) and water samples were collected to analyze ammonia nitrogen. The plastic bags were sealed and returned to the experimental tanks, which were maintained at  $\pm 0.2^\circ\text{C}$  of the desired experimental temperature.

After 2, 4, 6, 8, 10, 12, 18, 24, 36 and 48 hours, DO and ammonia nitrogen concentrations were determined immediately after the plastic bags were opened for the three replicate treatments and controls

(without larvae) of each water temperature. The larvae in each container were then removed with a net, weighed and counted again. The water remaining in the containers was homogenized and filtered using a  $0.45\text{-}\mu\text{m}$  filter (Millipore) for the analysis of ammonia nitrogen. Ammonia nitrogen was determined according to Verbeeten *et al.* (1999) and Merino *et al.* (2007).

Weight-specific ammonia excretion ( $\mu\text{g NH}_3\text{-N g}^{-1} \text{ h}^{-1}$ ) and oxygen consumption ( $\mu\text{g O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) were calculated as:  $\{(C_{\text{Nf}} - C_{\text{Ni}})_t \cdot W_t^{-1} - (C_{\text{Nf}} - C_{\text{Ni}})_c\} \cdot Wv^{-1}$  and  $\{(C_{\text{Of}} - C_{\text{Oi}})_t \cdot W_t^{-1} - (C_{\text{Of}} - C_{\text{Oi}})_c\} \cdot Wv^{-1}$  where  $C_{\text{Nf}}$  and  $C_{\text{Ni}}$  are the ammonia concentration at the end and the beginning of the experiment, respectively, and  $C_{\text{Of}}$  and  $C_{\text{Oi}}$  are the oxygen concentration at the end and the beginning of the experiment, respectively.  $W_t$  is the total wet larvae weight (g),  $Wv$  is the water volume (l), and the subscripts t and c stand for treatment and control that hold or do not hold larvae, respectively.

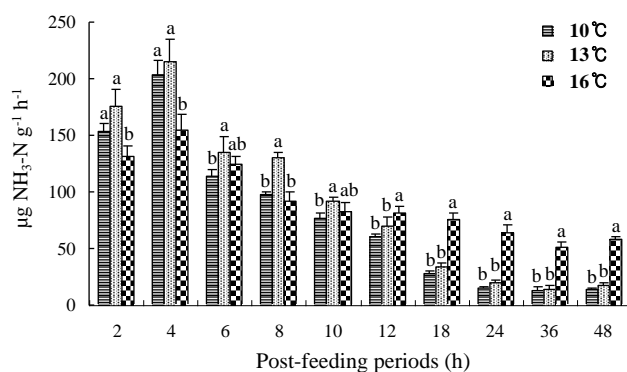
### Statistical Analysis

The data on ammonia excretion and oxygen consumption levels are expressed as means  $\pm$  SD,  $n=3$ . Effects of post-feeding period and water temperature on ammonia excretion and oxygen consumption were tested using a two-way nested ANOVA. Significant ANOVAs were followed by a Student-Newman-Keuls multiple comparison test to identify differences among treatments. The relationship between ammonia excretion rates and post-feeding period as well as oxygen consumption rates and post-feeding period was described by linear regression of the  $y = a + bx$ , where  $y$  is the ammonia excretion rates and oxygen consumption rates and  $x$  is the post-feeding period for three temperatures, depending on the computed values of  $R^2$  and the significance of regression parameters. Significance was accepted at  $P < 0.05$ . All statistical analyses were conducted using the SAS program (SAS Institute Inc., Cary, North Carolina, USA).

## Results

### Postprandial Ammonia Excretion

Weight-specific ammonia excretion by temperature and post-feeding period is presented in Figure 1. Interaction between post-feeding period and water temperature on ammonia excretion was detected until 18 h post-feeding (two-way nested ANOVA,  $P < 0.0001$ ; Figure 1). Mean excretion rates were higher at  $13^\circ\text{C}$  than at the other two temperatures until 10 h post-feeding. The peak excretion rates occurred at 2–4 h post-feeding for all temperatures. At 12–48 h post-feeding, the excretion rates at  $16^\circ\text{C}$  were significantly greater than those at  $10^\circ\text{C}$  and  $13^\circ\text{C}$  ( $P < 0.05$ ). At 1–48 h post-feeding, the excretion patterns were similar at  $10^\circ\text{C}$  and  $13^\circ\text{C}$ , but



**Figure 1.** Weight-specific ammonia ( $\text{NH}_3\text{-N}$ ) excretion by Pacific cod larvae fed *Brachionus plicatilis* for the variables of water temperatures and post-feeding periods. Values are means  $\pm$  SD ( $n = 3$ ) for each treatment.

showed a lower excretion rate and a slower rate of decline at 16°C. About 76%, 75%, and 62% of the 24-h post-feeding ammonia excretions were excreted within 8 h post-feeding at 10°C, 13°C, and 16°C, respectively. The highest weight-specific hourly excretion rate ( $215 \pm 19.8 \mu\text{g NH}_3\text{-N g}^{-1} \text{h}^{-1}$ ) was detected in cod larvae stocked at 13°C within 2 to 4 h post-feeding.

The regression between mass-specific ammonia excretion ( $A_m$ ) and post-feeding period ( $pf$ ) was diphasic at the 4-h post-feeding point (Figure 2). Regression lines for the 10°C, 13°C, and 16°C temperature treatments were  $A_{m10^\circ\text{C}} = 104.33 + 24.83 pf$  ( $F_{1,4} = 34.69$ ,  $r^2 = 0.896$ ,  $P < 0.01$ ),  $A_{m13^\circ\text{C}} = 136.26 + 19.7 pf$  ( $F_{1,4} = 7.53$ ,  $r^2 = 0.653$ ,  $P > 0.05$ ),  $A_{m16^\circ\text{C}} = 108.33 + 11.5 pf$  ( $F_{1,4} = 5.21$ ,  $r^2 = 0.565$ ,  $P < 0.05$ ) in the first phase at the 2-4h post-feeding period, and  $A_{m10^\circ\text{C}} = 97.82 - 2.25 pf$  ( $F_{1,22} = 47.55$ ,  $r^2 = 0.683$ ,  $P < 0.001$ ),  $A_{m13^\circ\text{C}} = 120.23 - 2.77 pf$  ( $F_{1,22} = 44.41$ ,  $r^2 = 0.668$ ,  $P < 0.001$ ),  $A_{m16^\circ\text{C}} = 103.69 - 1.23 pf$  ( $F_{1,22} = 34.79$ ,  $r^2 = 0.612$ ,  $P < 0.001$ ) in the second phase at the 6-48h post-feeding period (Figure 2).

### Postprandial Oxygen Consumption

Weight-specific hourly oxygen consumption by temperature and post-feeding period is presented in Figure 3. Interactions between post-feeding period and water temperature on ammonia excretion were detected until 12 h post-feeding (two-way nested ANOVA,  $P < 0.0001$ ; Figure 3). Mean oxygen consumption rates at 13°C were significantly ( $P < 0.05$ ) higher than those at 10°C until 12 h post-feeding. The peak consumption rate occurred 2–4 h post-feeding at all temperatures. In 12–48 h post-feeding, the oxygen consumption rates at 16°C were higher than those at 10°C and 13°C ( $P < 0.05$ ). Rapidly decreasing oxygen consumption rates at 10°C and 13°C were seen at 12–18 h post-feeding. The oxygen consumption rates in 18–48 h post-feeding were not significantly different between the 10°C and 13°C treatments ( $P > 0.05$ ). The highest weight-specific hourly consumption rate was  $1,778 \pm 55.6 \mu\text{g O}_2 \text{g}^{-1} \text{h}^{-1}$

for cod larvae at 13°C and 2–4 h post-feeding.

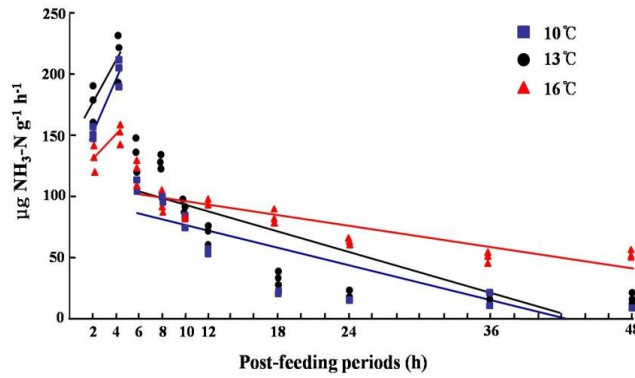
The regression between mass-specific oxygen consumption ( $OC$ ) and post-feeding period ( $pf$ ) was diphasic at the 4-h post-feeding point (Figure 4). Regression lines for the 10°C, 13°C, and 16°C treatments were:  $OC_{10^\circ\text{C}} = 219.26 + 347.5 pf$  ( $F_{1,4} = 62.21$ ,  $r^2 = 0.93$ ,  $P < 0.01$ ),  $OC_{13^\circ\text{C}} = 698.46 + 269.9 pf$  ( $F_{1,4} = 30.78$ ,  $r^2 = 0.885$ ,  $P < 0.01$ ),  $OC_{16^\circ\text{C}} = 843.63 + 148.98 pf$  ( $F_{1,4} = 19.86$ ,  $r^2 = 0.832$ ,  $P < 0.01$ ) in the first phase at the 2-4h post-feeding period, and  $OC_{10^\circ\text{C}} = 610.35 - 15.17 pf$  ( $F_{1,22} = 36.27$ ,  $r^2 = 0.6225$ ,  $P < 0.01$ ),  $OC_{13^\circ\text{C}} = 810.54 - 20.18 pf$  ( $F_{1,22} = 34.18$ ,  $r^2 = 0.608$ ,  $P < 0.01$ ),  $OC_{16^\circ\text{C}} = 832.72 - 14.204 pf$  ( $F_{1,22} = 26.69$ ,  $r^2 = 0.548$ ,  $P < 0.01$ ) in the second phase at the 6-48 h post-feeding period (Figure 4).

## Discussion

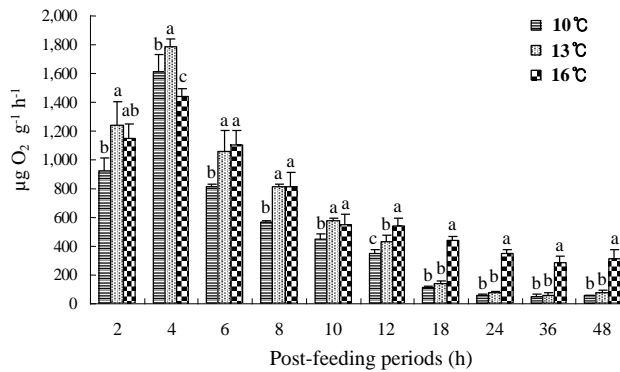
### Postprandial Ammonia Excretion

Due to the difficulties in detecting ammonia excretion by sensitive larvae (Okamoto, 1969; Ishibashi *et al.*, 2003), few studies have examined larval ammonia excretion rates (Buckely and Dillman, 1982; Klumpp and Westernhagen, 1986). As noted by Klumpp and Westernhagen (1986), it is difficult to confine larvae to the relatively small volumes of water needed to detect nitrogenous wastes. Cod larvae younger than 9-d post-hatch are very sensitive to physical stress from handling and are easily killed. Thus, in this study, cod larvae from 9-d post-hatch were handled and 15-d post-hatch larvae allowed to actively move in the treatment plastic bags filled with 6 L water. All cod larvae were alive during and after experimental periods.

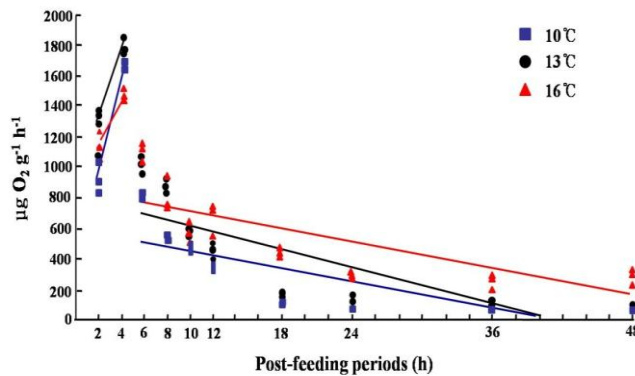
Ammonia excretion and energy budget are usually temperature dependent (Finn *et al.*, 2002; Sun and Chen, 2009), as is generally found in fish. In warm water species, ammonia excretion usually increases with temperature (Claireaux and Lagardère, 1999; Ruyet *et al.*, 2004). In the present study, however, the mean values of postprandial weight-specific ammonia excretions were higher at 13°C than



**Figure 2.** The relationship between ammonia excretion rates and post-feeding period for the Pacific cod larvae fed *Brachionus plicatilis* at three different water temperatures. The regressions for the first phase at 2-4 h post-feeding period:  $y_{10^{\circ}\text{C}} = 104.33 + 24.83 x$  ( $r^2 = 0.896$ ),  $y_{13^{\circ}\text{C}} = 136.26 + 19.7 x$  ( $R^2 = 0.653$ ),  $y_{16^{\circ}\text{C}} = 108.33 + 11.5 x$  ( $r^2 = 0.565$ ), and the regressions for the second phase at 6-48 h post-feeding period:  $y_{10^{\circ}\text{C}} = 97.82 - 2.25 x$  ( $r^2 = 0.683$ ,  $P < 0.001$ ),  $y_{13^{\circ}\text{C}} = 120.23 - 2.77 x$  ( $r^2 = 0.668$ ),  $y_{16^{\circ}\text{C}} = 103.69 - 1.23 x$  ( $r^2 = 0.612$ ).



**Figure 3.** Weight-specific oxygen consumption by Pacific cod larvae fed *Brachionus plicatilis* for the variables of water temperatures and post-feeding periods. Values are means  $\pm$  SD ( $n = 3$ ) for each treatment.



**Figure 4.** The relationship between oxygen consumption rates and post-feeding period for the Pacific cod larvae fed *Brachionus plicatilis* at three different water temperatures. The regressions for the first phase at 2-4h post-feeding period:  $y_{10^{\circ}\text{C}} = 219.26 + 347.5 x$  ( $r^2 = 0.93$ ),  $y_{13^{\circ}\text{C}} = 698.46 + 269.9 x$  ( $r^2 = 0.885$ ),  $y_{16^{\circ}\text{C}} = 843.63 + 148.98 x$  ( $r^2 = 0.832$ ), and the regressions for the second phase at 6-48h post-feeding period:  $y_{10^{\circ}\text{C}} = 610.35 - 15.17 x$  ( $r^2 = 0.6225$ ),  $y_{13^{\circ}\text{C}} = 810.54 - 20.18 x$  ( $r^2 = 0.608$ ),  $y_{16^{\circ}\text{C}} = 832.72 - 14.204 x$  ( $r^2 = 0.548$ ).

at 16°C until 10-h post-feeding. Moreover, excretions at 10°C were also higher than those at 16°C until 4-h post-feeding. This might be caused by the feeding rate; cod larvae stocked at 16°C might not have eaten

as many rotifers as the larvae stocked at 10°C and 13°C (Park, 2008). Because cod is stenothermal and feeding rate and ammonia excretion might be affected by abnormal temperatures such as 16°C. The higher

resting ammonia excretions at 16°C than at 10°C and 13°C also support this so that a water temperature of 16°C might not be conducive to mass culture of cod larvae.

According to Klumpp and Westernhagen (1986), the basal ammonia excretion rate in plaice larvae (*Pleuronectes platessa*) reared at 6°C was around 120  $\mu\text{g NH}_3\text{-N g}^{-1} \text{h}^{-1}$ . It is difficult to compare excretion rates among different species and temperatures, but this rate is very high compared to those of the cod larvae in this study stocked at 10°C, 13°C, and 16°C 12 and 24 h post-feeding. The 419% and 341% higher ammonia excretion rate at 16°C 24 h post-feeding relative to those at 10°C and 13°C might have led to weight loss and elevated maintenance costs.

A higher weight-specific rate of postprandial ammonia excretion is usually seen at the early stage of the postprandial period (Dosdat *et al.*, 1996; Ip *et al.*, 2004). However, this rate varies with fish species and culture conditions. According to Klumpp and Westernhagen (1986), the highest levels of nitrogen wastes for three marine fish larvae fed *Artemia* sp. occurred 2-3 h after feeding and returned slowly to the baseline 5-10 h post-feeding. The trends were similar for peak excretion, but the time periods to reach resting excretion rates were shorter than those for the cod larvae in the present study.

### Postprandial Oxygen Consumption

Among cold-water fish species, oxygen consumption usually increases with temperature in a limited temperature zone (Saunders, 1963; Paul, 1986). According to Paul *et al.* (1988), oxygen consumption rates for Pacific cod sized from juvenile to adult are similar to those of Atlantic cod and other cold water gadidus. For the cod with their size ranging from 150 to 7,100 g at temperatures between 3°C and 15°C, small cod consume oxygen at a greater rate per unit weight than large ones (Saunders, 1963). Because none of data are shown on oxygen consumption for cod larvae and their respiratory responses according to temperatures, no direct comparison can be made.

Like ammonia excretion, the higher oxygen consumption rates were detected at 16°C from 10 h to 48 h post-feeding. This might be caused by the effects of higher water temperatures on activity and resting metabolism. Much higher oxygen consumption at 10°C and 13°C than 16°C in 2-4 h post-feeding might be caused by higher feeding rate. The 610% and 460% higher oxygen consumption at 16°C relative to those at 10°C and 13°C at 24-h post-feeding might have resulted from elevated maintenance costs at 16°C. This increment is enormous high compared to adult Atlantic cod (Saunders, 1963) and Pacific cod (Paul *et al.*, 1988).

While many studies have examined live foods for the growth of fish larvae, few reports have been

published on the effect of temperature on ammonia excretion and oxygen consumption from larvae fed live food, though these data are necessary for fish larviculture. In nursery systems, fish larvae are usually reared under stationary conditions with a slow rate of water replacement that would accumulate ammonia in culture tanks. Therefore, data of ammonia excretion and oxygen consumption rates from fish larvae fed live food according to temperatures are precious for the carrying capacity estimation and better growth. Based on the results of the present study, we recommend rearing cod larvae at temperatures below 16°C.

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