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Effect of Water Temperature on Specific Gravity of North Pacific Giant Octopus *Enteroctopus dofleini* Paralarvae

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ABSTRACT The North Pacific giant octopus *Enteroctopus dofleini* is distributed in coastal areas of the North Pacific Ocean from western North America to Japan. This species is an important commercial marine organism in northern Japan. This study examined the effect of water temperature on the specific gravity of *E. dofleini* in the early paralarval stage under rearing conditions. Specific gravity, which serves as an indicator of larval buoyancy, was examined from egg to six days after hatching (dAH) under starved and dark conditions at 5ºC and 10ºC. After hatching, the specific gravities of *E. dofleini* paralarvae decreased significantly until 2 dAH at 5°C and until 1 dAH at 10°C. After 2 dAH at 5°C and after 1 dAH at 10°C, specific gravity did not fluctuate significantly. Overall, specific gravity values were lower at 5°C than at 10°C. Water temperature during the early paralarval stage could affect the ability of *E. dofleini* paralarvae to gain buoyant force.

KEYWORDS: *Enteroctopus dofleini*; paralarvae; specific gravity; floating adaptation
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INTRODUCTION

The North Pacific giant octopus *Enteroctopus dofleini* is a coleoid cephalopod in the family Octopodidae that can reach up to 3 m in total length and 198.2 kg in weight (McClain et al., 2015). This octopus is distributed in coastal areas of the North Pacific Ocean from western North American to Japanese waters (Scheel, 2002; Sano & Bando, 2015) and is an important commercial marine organism in northern Japan (Sano et al., 2017). Octopuses adopt two life-history strategies (Boletzky, 1977). The first strategy is the permanent adoption of a benthic life after hatching. The second incorporates a free-swimming planktonic life phase during the paralarval stage after hatching. *Enteroctopus dofleini* undergoes this planktonic life phase during its early life stages after hatching (Okubo, 1979) although its hatchlings are of extraordinarily large size. Octopus are believed to be able to diffuse into new natural habitats during the planktonic life phase. Kubodera (1991), for example, has reported finding *E. dofleini* paralarvae that were 3-13 mm in mantle length in the North Pacific and Bering Sea, and suggested that these paralarvae had been transported by the Alaskan stream. In addition, the planktonic paralarvae of *Octopus vulgaris* have been reported to prey on floating decapod zoeae (Villanueva et al., 1996). Floating is thus a very important behaviour for the survival and growth of planktonic octopus paralarvae, in terms of not only feeding but also expansion of their distributional range through diffusion. Yet little is known about the biology of *E. dofleini* during the planktonic phase, especially with regard to their floating situations and mechanisms. In fact, there have been only two reports on their paralarvae in natural environments, one in the Aleutian Archipelago (Kubodera, 1991) and the other in the eastern North Pacific (Yamashita & Torisawa, 1983). Further, no studies have yet clarified their rearing technique during the paralarval planktonic phase. In this study, to understand the floating mechanisms of octopus paralarvae, we used specific gravity as an indicator of paralarval buoyancy. When the specific gravity of a paralarva is close to that of seawater, the buoyant force acting on it is stronger and the sinking force is weaker. To understand the floating mechanism of paralarvae, their specific gravity during the paralarval life stage should be clarified. This study reports the specific gravity of *E.*

dofleini during the early paralarval stage at different water temperatures under starved and dark conditions and discusses the effect of water temperature on specific gravity.

METHODOLOGY

Rearing paralarvae

Eggs of *E. dofleini* were obtained from an adult female reared in the Wakkanai Fisheries Research Institute, Hokkaido Research Organization, Japan (WFRI). The female, which had a body weight of 25.3 kg, had been captured from a coastal area of northern Hokkaido, Japan, in April 2017, and was subsequently reared under conditions of <15°C in temperature, 32-33 psu in salinity and around 10 mg L^{-1} in dissolved oxygen. Spawning of eggs was found in June 2017 and spawned eggs were maintained by the female until April 2018. The eggs were transported from the WFRI on 12 March 2018 and were delivered to the Faculty of Bioindustry, Tokyo University of Agriculture (TUA) on 13 March 2018. Most of the eggs had hatched prior to arrival (0 days after hatching; dAH). Groups of 60 paralarvae were transported by pipet into two 2-L polycarbonate bottles filled with 2 L seawater for examination of specific gravity. One bottle was kept at 5°C and the other at 10°C, both under starved and dark conditions, in a multi-temperature incubator. Air supplemented with 240 ml min^{-1} was supplied through a glass pipet placed above the bottom of the bottle so that it would mix with the rearing water. Water was replenished and 15% of bottle volume was exchanged daily. During the rearing period, temperature (°C), salinity (psu) and dissolved oxygen (mg L^{-1}) were measured with a digital thermometer (SPT-800PT, SANSYO, Japan), a potable salinometer (LAQUAact D-70, HORIBA, Japan) and an optical dissolved oxygen sensor (ODO200, Xylem Japan, Japan), respectively, at 8:00-10:00 every day.

Measurements for specific gravity of paralarvae

To determine the specific gravity of the bodies of *E. dofleini* paralarvae, density of the paralarvae was measured according to a density-gradient column method modified from Coombs (1981) (Figure 1). This method requires a stable column of liquid with a density gradient in a cylinder with scales. This stable column was made by several steps. Firstly, liquids of lower and higher density were prepared. Secondly, the higher-density liquid was diluted continuously by addition of the lower-density liquid. Finally, the diluted liquid was slowly poured into a cylinder such that the density of the upper layer was low and that of the lower layer was high, forming a density-gradient column. In this study, densities of liquids were adjusted with NaCl, and higher- and lower-density liquids contained 10.4% and 1.8% NaCl (w/v), respectively. As an anesthetic for the paralarvae, ethanol (4%, v/v, in final concentration) was included in both liquids. The diluted liquid was continuously poured into a 1^{-L} graduated glass cylinder. After completion of the density-gradient column, standard density floats (SHIBAYAMA SCIENTIFIC Co., Ltd., Japan), which have a specific density at 20°C, were introduced into the cylinder. Positions of the standard floats suspended in the gradient were read according to the scale on the graduated cylinder. A linear regression equation was obtained between the densities of the standard floats and the scale numbers on the graduated cylinder.

Measurements of the density of eggs and paralarvae from 0 to 6 dAH were carried out in the TUA laboratory at around 20°C. Ten paralarvae and eggs were collected from each rearing bottle with a pipette and were anesthetized with 4% ethanol (v/v) for 5 min. Each paralarva or egg in a relaxed state was dropped into the density-gradient column. When the sinking speed of the paralarva or egg suddenly decreased, the position of the paralarva on the scale of the cylinder was recorded. The density of the paralarva or egg was calculated from the regression equation between this scale and the density of the standard floats. Specific gravity was obtained by dividing the density of each paralarva or egg by that of water at 20°C.

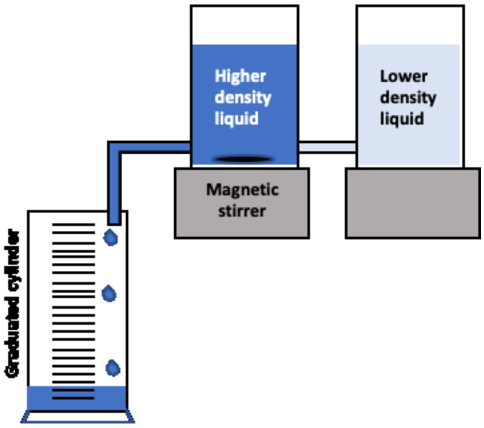

Figure 1. Schematic diagram of our experimental design modified from Coombs (1981).

Statistical analyses

Differences in specific gravity at different dAH were examined by one-way ANOVA followed by Tukey-Kramer test. Statistics were calculated with the "R" free software package and $p < 0.05$ was considered to represent a significant difference.

RESULT AND DISCUSSION

This study is the first report that shows changes in the specific gravities of planktonic octopus paralarvae during the early paralarval stages after hatching. The specific gravity of *Enteroctopus dofleini* eggs was 1.053, which was higher than that of the paralarvae, which ranged from 1.035 to 1.044 (Figure 2, Table 1). Tsukamoto et al. (2009) examined specific gravities of marine invertebrates to understand their floating adaptation (Table 1). The specific gravities of gelatinous zooplankton, such as ctenophorans, salps, cnidarians and chaetognaths, were 1.020–1.040; these were classified as "gelatinous floaters" (Tsukamoto et al., 2009), with a specific gravity relatively close to that of seawater and distributed in the ocean's upper layers. Except for some species of chaetognaths, these gelatinous zooplankton do not engage in large-scale diel vertical migration. Their lower specific gravity could contribute to their buoyant force. In this study, the specific gravities of *E. dofleini* paralarvae were 1.035–1.044, comparable to those of Ctanchiidae cephalopods and gelatinous floaters as reported in Tsukamoto et al (2009). In contrast, the specific gravities of crustacean zooplankton such as copepods, euphausiids and decapods were 1.060–1.080; these were classified as "muscle swimmers" (Tsukamoto et al., 2009). Crustacean zooplankton such as copepods (e.g. Steele & Henderson, 1998) and euphausiids (e.g. Nakagawa et al., 2003; Taki, 2008) have swimming ability and engage in typical diel vertical migration. The sinking forces of muscle swimmers could be higher than those of gelatinous floaters because the specific gravities of muscle swimmers are higher than those of gelatinous floaters. Zooplankton with higher specific gravities could acquire buoyant force by swimming (Tsukamoto et al., 2009). Our results suggest that *E. dofleini* paralarvae are highly buoyant and that unlike muscle swimmers they contribute to their active transportation by flows of various strength.

After hatching, the specific gravities of *E. dofleini* paralarvae decreased significantly until 2 and 1 dAH at 5°C and 10°C, respectively (Figure 2). This means that, after hatching, paralarvae may acquire more buoyant force by reducing their specific gravities, and that the paralarvae can adapt to floating after hatching immediately. After 2 dAH at 5°C and after 1 dAH at 10°C, the specific gravities did not significantly fluctuate and the values at 5°C were lower than those at 10°C (Fig. 2). The specific gravity of octopus paralarvae could be determined by measuring their body volume and weight. Octopus paralarvae after hatching are equipped with yolk reserves that contribute to their survival and growth until the yolk is completely absorbed (Boletzky, 1975). The yolk volume of squid paralarvae is related to their body weight and the absorption of yolk is influenced by temperature (Vidal et al., 2002; 2005). The results of this study suggest that the reduction in body volume after 2 dAH of *E. dofleini* paralarvae at 10°C might be greater relative to body weight than that in paralarvae at 5°C. Therefore, water temperature may affect the body volume of *E. dofleini* paralarvae as well as their body weight. These results suggest that *E. dofleini* paralarvae reared at lower temperatures have higher buoyant force, which contributes to their diffusion and survival in nature, than those reared at higher temperatures.

Table 1. Specific gravities of marine invertebrates.

Marine invertebrate	Specific gravity	Reference
Ctenophorans	1.025	Tsukamoto et al. (2009)
Salps	1.026	Tsukamoto et al. (2009)
Cnidarians	1.027–1.028	Tsukamoto et al. (2009)
Chaetognaths	1.034	Tsukamoto et al. (2009)
Cephalopods	1.024–1.061	Tsukamoto et al. (2009)
	1.035–1.044	**This study**
Copepods	1.059-1.061	Tsukamoto et al. (2009)
Euphausiids	1.092	Tsukamoto et al. (2009)
Decapods	1.065–1.088	Tsukamoto et al. (2009)

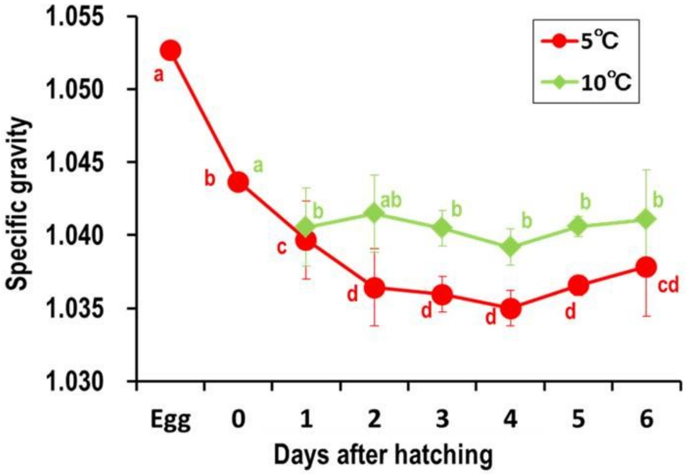

Figure 2. Changes in the specific gravity of *Enteroctopus dofleini* from egg to six days after hatching at 5°C and 10°C under starved and dark conditions. Different letters associated with data points represent significant differences among the specific gravity values recorded on each day after hatching within each rearing water temperature ($p < 0.05$).

Our results may indicate a strategy that benefits octopus paralarvae during the planktonic life phase. The specific gravities of the octopus hatchlings decreased dramatically within 2 to 3 days after hatching and acquired a high buoyancy similar to that seen in gelatinous floaters. This change in buoyancy just after hatching may play an important role in the survival of the planktonic paralarvae of *E. dofleini* in the wild.

CONCLUSION

The specific gravity of *Enteroctopus dofleini* paralarvae was used as an indicator of their buoyancy and was examined from the egg stage to six days after hatching under starved and dark conditions at 5°C and 10°C. Paralarvae raised at both temperatures had specific gravities that would make them highly buoyant in the marine environment. Our results suggest that *E. dofleini* paralarvae reared at lower temperatures develop greater buoyant force than paralarvae reared at higher temperatures, which contributes to their diffusion and survival in nature.

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