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*Research Article*

# Evaluation of Microalgal Diet to Culture Adult *Oithona oculata* Farran (Copepoda, Cyclopoida)

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While copepods are important prey for many wild marine fish larvae and are preferred as a live feed for culturing larval fish, their low productivity and the costs associated with culturing them typically limit their use as a live food source in all but experimental situations. In a series of experiments with four readily and commercially available microalgae (*Thalassiosira weissflogii* Grunow, *Rhodomonas salina* Wislouch, *Tetraselmis suecica* Kylin Butch, and *Isochrysis galbana* Parke), we determine which of them, when exclusively fed to a commonly occurring copepod, *Oithona oculata*, over a 15-day period, results in the greatest copepod survival and egg production rates. We posit that species of *Oithona* might be an ideal candidate for copepod taxa for commercial-scale copepod culture and that the microalga *Rhodomonas salina* is an ideal food source to culture them.

## 1. Introduction

Copepods are natural prey for most marine fish larvae and comprise nearly 80% of their stomach contents [1, 2]. In aquaculture and ornamental fish industries, copepods are preferred over other commonly used species (e.g., *Artemia*, rotifers) as a live feed for marine fish larvae [3]. Marine fish larvae fed copepods survive better [4] and have both better pigmentation [5] and growth [6]. However, despite the obvious advantages of using copepods as a food source, their low productivity and cost of culture limit their use for this purpose.

Species of *Oithona*, marine planktonic copepods that occur widely, can dominate coastal waters [7], provide important links between primary producers and fish larvae [8], and are preyed upon by commercially significant marine species [9]. Consequently, some *Oithona* species are used as experimental live feed in marine aquaculture [10]. *Oithona oculata* Farran, an annually dominant species that occurs in Sagami Bay at high densities [11], has small nauplii that are suitable as a first food item for many small-mouthed larval marine fish species, including ornamentals [10].

Food quality is a major bottleneck in the mass cultivation of planktonic copepods. Diet affects copepod egg production, survival and growth rates, hatching success, and population growth. One difficulty with the mass cultivation of copepods is, however, the varied dietary requirements of individual species [12, 13]. Because of the potential suitability of *O. oculata* as a prey source for larval fishes, we fed adult females four different microalgal diets to determine which of them was the most favourable for the survival and reproduction of this copepod in culture.

## 2. Materials and Methods

Cultures of four often-used and readily available microalgae (*Thalassiosira weissflogii* Grunow, *Rhodomonas salina* Wislouch, *Tetraselmis suecica* Kylin Butch, and *Isochrysis galbana* Parke) were established in *f*/2 medium [14] in 50 mL conical flasks maintained at 20°C in an incubator (FLI-301N, EYELA), with a 12 : 12 h light: dark cycle and light intensity 120 $\mu$mol·m$^{-2}$·s$^{-1}$. Microalgae fed to copepods were harvested during the mid-to-late logarithmic growth phase.

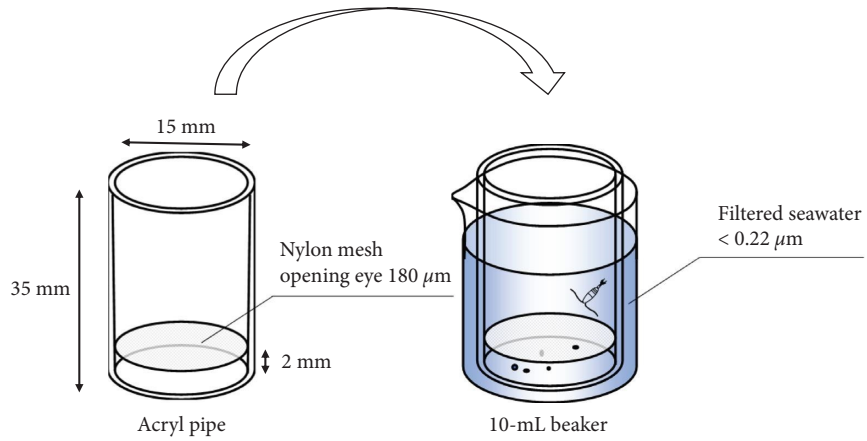


Figure 1: Culture chamber used in egg production experiments.

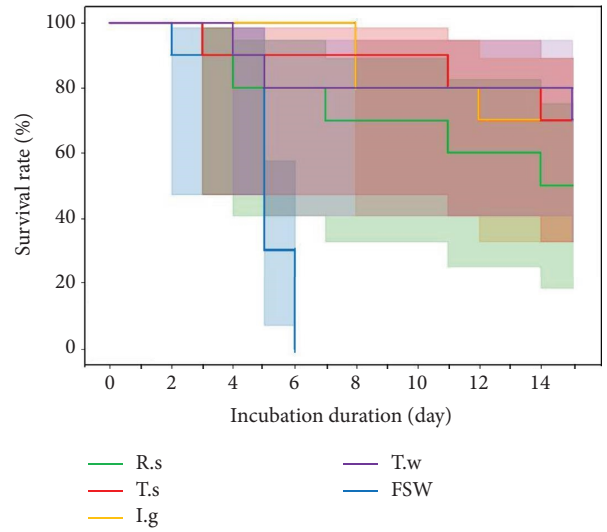


Figure 2: Kaplan–Meier curve with 95% confidence bands displaying the estimated survival probability for female *Oithona oculata* fed different microalgal diets for 15 d. R.s: *Rhodomonas salina*; T.s: *Tetraselmis suecica*; I.g: *Isochrysis galbana*; T.w: *Thalassiosira weissflogii*; FSW: filtered sea water (starvation condition).

Zooplankton was collected on 14 August 2020, by a plankton net (180 $\mu$m mesh) towed gently and obliquely from the seabed to the surface at Manazuru Port (35°09′49″N, 139°10′33″E), northwestern Sagami Bay, Japan. Surface seawater was also collected by the bucket for copepod culture. Within 20 min of sampling, zooplankton samples were transferred to the laboratory, where adult female *O. oculata* with egg sacs, identified following Nishida [15], were sorted under a dissecting microscope (WILD M10, Leica Co., Ltd.). Sixty female *O. oculata* were placed into a bottle containing 2 L of filtered (65 $\mu$m) ambient seawater, then acclimatised at 25°C (near-ambient seawater temperature), fed sufficient (1000 $\mu$g C·L$^{-1}$) microalgal food comprising a 1 : 1 : 1 : 1 carbon ratio of *T. weissflogii*, *R. salina*, *T. suecica*, and *I. galbana*, and incubated (CN-25C, Mitsubishi) in darkness for 24 h to negate the effects of the prior natural food environment [16]. After 24 h, 10 females carrying egg sacs were fixed in a 5% buffered formalin-seawater solution, and the number of eggs within each egg sac was counted under the microscope to estimate the initial egg sac size (eggs sac$^{-1}$).

After acclimatisation, 10 healthy female *O. oculata* were placed into separate culture chambers, within beakers with 10 mL filtered (0.22 $\mu$m membrane (Merck Millipore)) seawater (Figure 1). Each culture chamber had a 180 $\mu$m nylon mesh placed 2 mm above its base to allow nauplii to pass through and minimise their possible cannibalism [17]. Copepods were fed a monomicroalgal diet at 1000 $\mu$g C·L$^{-1}$ (10 $\mu$g C ind.·$^{-1}$) daily and kept in darkness at 25°C in an incubator for 15 d. The culture period was based on the average survival time of *O. oculata* at the adult stage collected from the sampling site. Because females can prey on nauplii [18], 10 mL seawater in each chamber was replaced daily with fresh filtered seawater (FSW) to remove hatched nauplii. The numbers of dead females and egg sacs were recorded daily. The survival rate (%) was calculated from the total number of individuals in each food treatment and the number of individuals that died. Survival analysis was

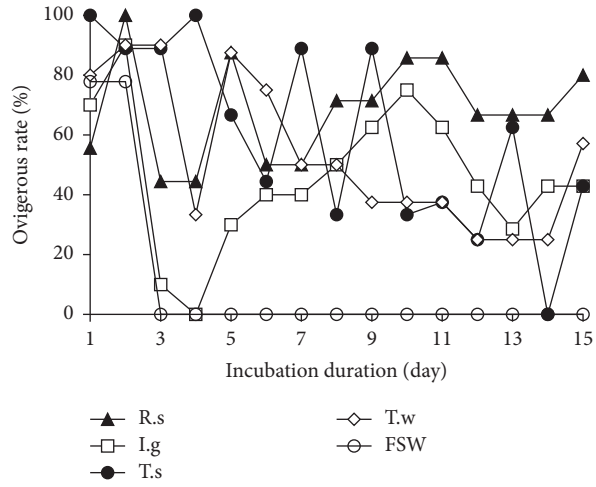


FIGURE 3: Temporal variation in ovigerous rate of female *Oithona oculata* fed different microalgal diets for 15 d, R.s: *Rhodomonas salina*; I.g: *Isochrysis galbana*; T.s: *Tetraselmis suecica*; T.w: *Thalassiosira weissflogii*; FSW: filtered sea water (starvation condition).

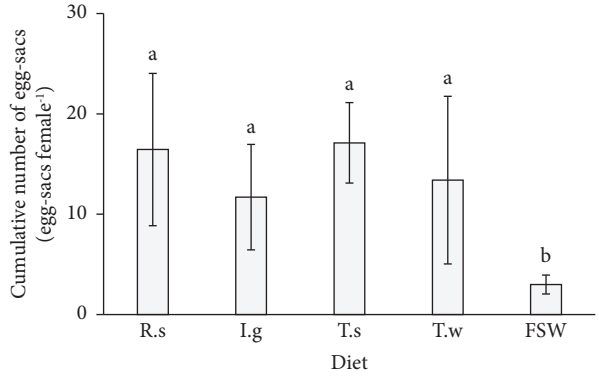


FIGURE 4: Cumulative number of egg sacs spawned by female *Oithona oculata* fed different microalgal diets for 15 d. R.s: *Rhodomonas salina*; T.s: *Tetraselmis suecica*; I.g: *Isochrysis galbana*; T.w: *Thalassiosira weissflogii*; FSW: filtered sea water (starvation condition). Error bars show standard deviations ($N = 10$). Letters above bars indicate significant differences (one-way ANOVA, Tukey–Kramer, $p < 0.05$).

conducted by the Kaplan–Meier method. The ovigerous rate ($O$, %) was calculated as

$$O = \frac{F_{\text{ovi}}}{F_{\text{all}}} \times 100, \quad (1)$$

where $F_{\text{ovi}}$ is the abundance of ovigerous females and $F_{\text{all}}$ is the abundance of all females (ovigerous and not ovigerous). After day 15 of culture, the egg sac size in each diet condition was measured.

All the data met the parametric test assumptions. Differences in egg production between dietary treatments were analysed using one-way analysis of variance (ANOVA). A Tukey–Kramer post hoc test was performed when ANOVA revealed significant differences at $p < 0.05$.

## 3. Results and Discussion

Some planktonic copepods cease producing eggs when fed mono-microalgal diets [19–21], possibly because of nutrient limitations in (for example) elemental ratios [22], highly unsaturated fatty acids [23], sterols [24], and/or amino acids [25]. While we do not use the number of nauplii produced as a metric of copepod production (because they could be underestimated due to cannibalism), we removed nauplii from cultures because they could contribute to the diet of cannibalistic parents. After 15 d of culture on each diet, female survival rates were 70% when fed *T. suecica*, *I. galbana*, and *T. weissflogii* and 50% when fed *R. salina* (Figure 2). The ovigerous rate varied for each diet (Figure 3). There was no significant difference in the numbers of egg sacs produced per female over 15 d among diets, except for starvation (FSW) conditions (Figure 4). Similarly, there were no significant differences in egg sac size among diets (Figure 5). *Oithona oculata* continued to produce eggs in all diet treatments. Because the culture of this species does not require the preparation of any specific diet, it may be a candidate species for mass culture [26]. Populations of the congeneric *O. nana* can also be maintained on a wide range of phytoplankton, microzooplankton, and alternative diets such as soybeans, yeast, rice bran, and corn starch [26, 27].

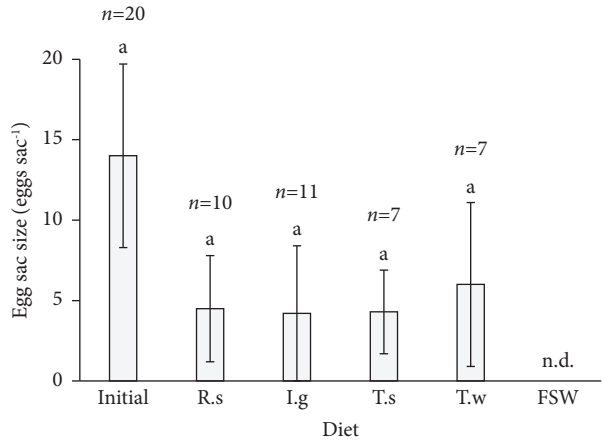


Figure 5: *Oithona oculata* egg sacs at 15 d after being fed different microalgal diets. R.s: *Rhodomonas salina*; T.s: *Tetraselmis suecica*; I.g: *Isochrysis galbana*; T.w: *Thalassiosira weissflogii*; FSW: filtered sea water (starvation condition). Initial egg sac size was measured before incubation. *n* indicates the number of egg sacs measured. Error bars show standard deviations. Letters above bars indicate significant differences (one-way ANOVA, Tukey–Kramer, $p < 0.05$).

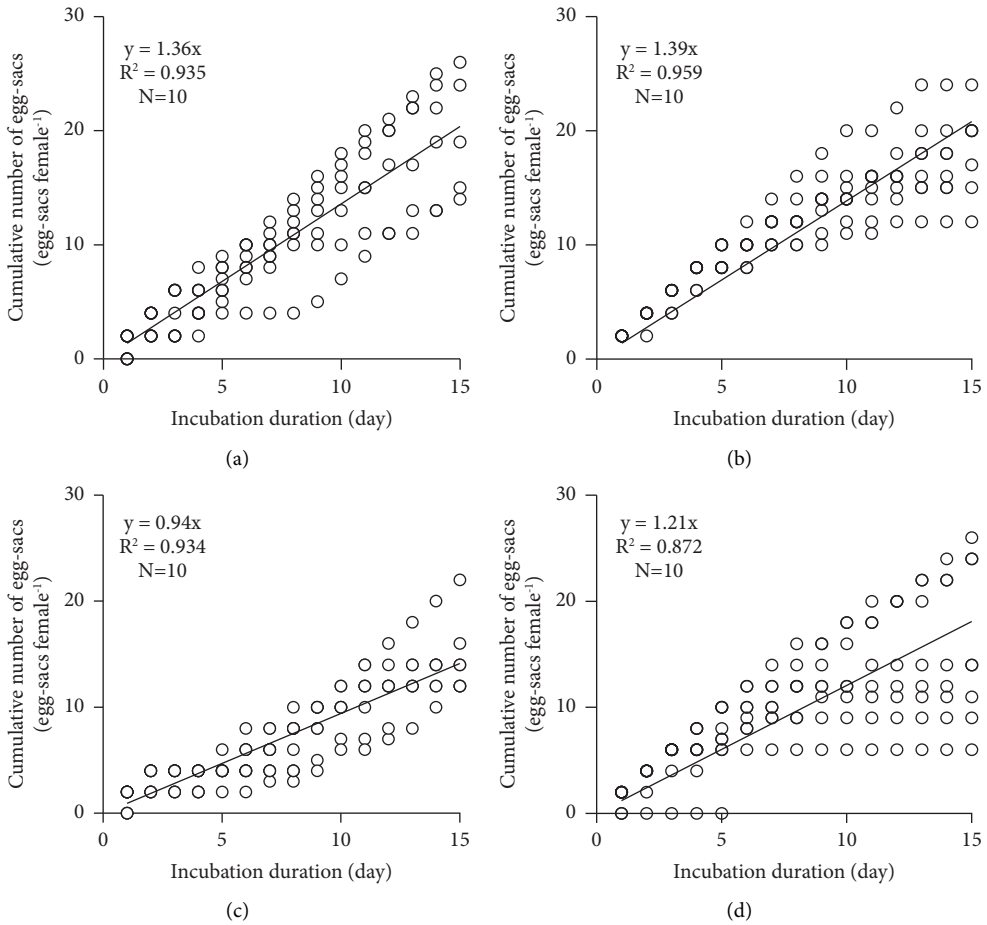


Figure 6: Relationship between incubation duration and the cumulative number of egg sacs spawned by female *Oithona oculata* fed different microalgal diets for 15 d R.s: *Rhodomonas salina*; T.s: *Tetraselmis suecica*; I.g: *Isochrysis galbana*; T.w: *Thalassiosira weissflogii*. *N* = the number of females in each experiment. (a) R.s, (b) T.s, (c) I.g, and (d) T.w.

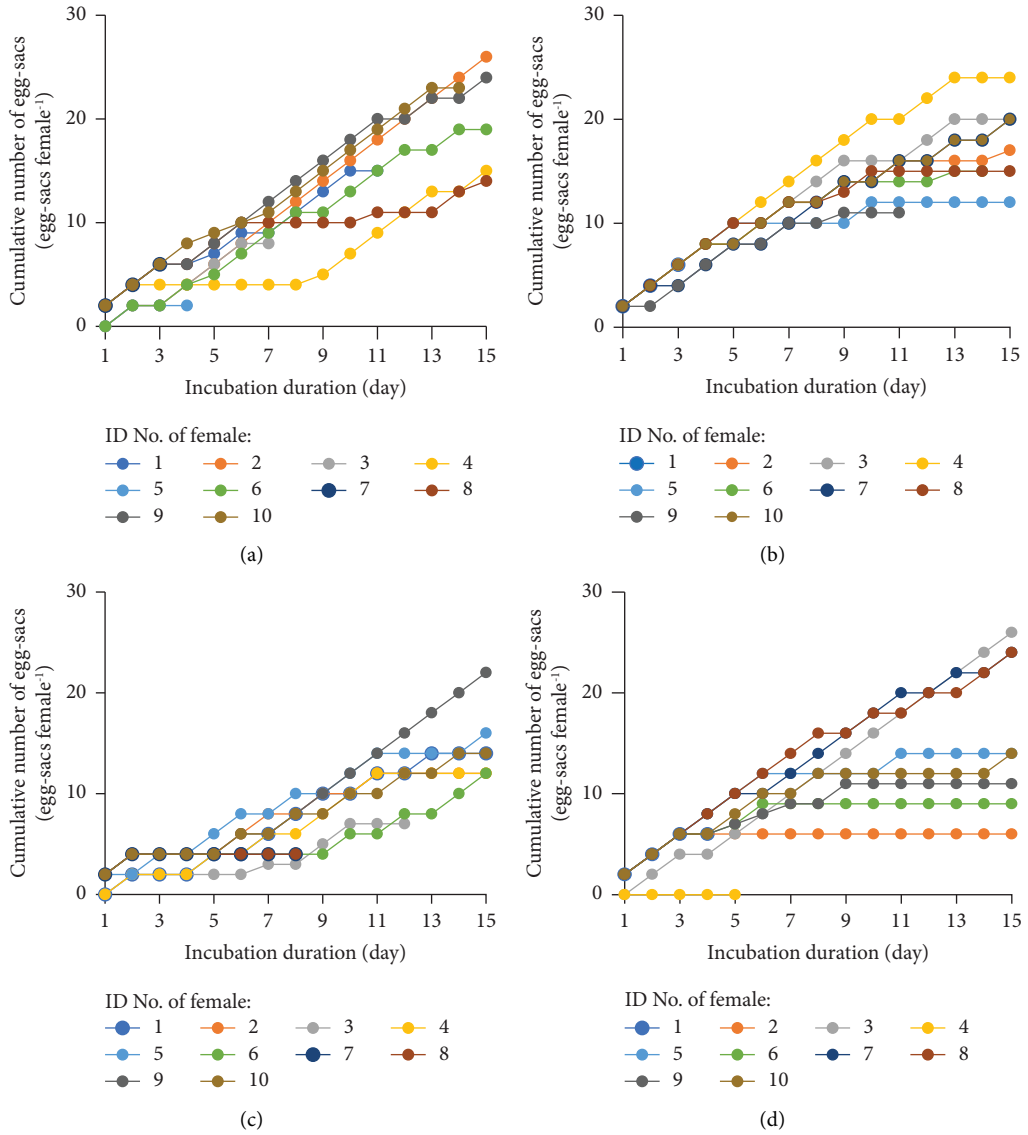


Figure 7: Individual female *Oithona oculata* cumulative egg production rate when fed different microalgal diets for 15 d. R.s: *Rhodomonas salina*; T.s: *Tetraselmis suecica*; I.g: *Isochrysis galbana*; T.w: *Thalassiosira weissflogii*; "No" indicates an individual female number. (a) R.s, (b) T.s, (c) I.g, and (d) T.w.

Therefore, *Oithona* copepods may be highly adaptable to a variety of diets and therefore suitable for mass culture.

To estimate egg sac production rates in females fed each diet, linear regressions were performed between incubation duration and cumulative egg sac number. Egg sac production rates (slopes) are 1.36 (*R. salina*), 1.39 (*T. suecica*), 0.94 (*I. galbana*), and 1.21 (*T. weissflogii*) sacs female$^{-1}$·d$^{-1}$ (Figures 6(a)–6(d)). Production rates are greatest for females fed *R. salina* and *T. suecica*. To identify differences in egg production in females fed different diets, females were both reared and monitored separately (Figures 7(a)–7(d)). Almost all females fed *R. salina* produced egg sacs throughout the 15 d culture period (Figure 7(a)), whereas half of the females fed *T. suecica* and (especially) *T. weissflogii* stopped producing egg sacs late in the culture period (Figures 7(b) and 7(d)). Because *R. salina* was accepted by many individual copepods and resulted in continuous egg production, we conclude that it represents the most appropriate of the four trialled microalgae to feed *O. oculata*.

## Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Ethical Approval

Copepods were collected in accordance with national legislation in Japan, with all necessary permits obtained prior to conducting research.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Yoshiki Takayama, Taisei Yamasaki, and Tatsuki Toda designed the study; Yoshiki Takayama and Taisei Yamasaki conducted field sampling, the experiment, and sample measurements; and Yoshiki Takayama drafted the manuscript. All authors have read and accepted the final manuscript before submission.

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