Short Paper

Temporal Tolerance of Larval *Theora fragilis*
(Bivalvia: Semelidae) to Hypoxic ConditionsKyoichi Tamai[†]

Nansei National Fisheries Research Institute, Ohno Saeki Hiroshima, 739-04, Japan

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In order to evaluate the population dynamics of benthic animals living in eutrophic embayments, where the ambient oxygen concentration frequently falls to fairly low levels, it is of great interest to examine the ability of the benthic animals to withstand such temporal adverse conditions. Most previous studies on the hypoxic tolerance of benthic animals have dealt with adult stage animals. Despite the fact that larval hypoxic tolerance is one of the key factors that determine the successful recruitment size, little is currently available on larval stages. Presently available information about the hypoxic tolerance of the early developmental stages is limited to that of crustacean larvae including blue crab *Portunus trituberculatus*¹⁾ and white shrimp *Metapenaeus monoceros*.²⁾ This paper is the first report on the hypoxic tolerance of the molluscan larva of *Theora fragilis*, a small bivalve which is well-known as an indicator species of organic pollution characterized by low dissolved oxygen concentration in bottom water and high organic contents in sediments.³⁾

The maintenance temperature of mature bivalves (8–10 mm in shell length) was raised from 21.3 to 24.4°C to induce spawning. After artificial fertilization, newly hatched larvae thus obtained were maintained at 23–24°C providing a flagellate, *Pavlova* sp. as food. Larvae reached the umbo-stage (115 µm in mean shell length) in 6 days and full-grown stage (160 µm in mean shell length) in 13 days after fertilization. Larvae of these two stages were used for the experiments.

Seawater used for the experiments was collected just before each experiment because it is desirable to keep larvae in fresh seawater. Five 12 l acrylic test aquaria filled with 10 l filtered (1 µm mesh) seawater containing 5 mg/l of antibiotic reagent (streptomycin) were used. The salinity of the seawater was 28.3 for the umbo-larva stage and 25.2 for the full-grown larva stage. Five dissolved oxygen concentration levels (0–4.9 ml O₂/l) were established in respective aquaria by bubbling N₂ gas, a mixture gas of 3% O₂ and 97% N₂, that of 6.3% O₂ and 93.7% N₂, that of 13% O₂ and 87% N₂ or pure air continuously during the experiments, as practiced by Llanos⁴⁾ and Tamai.⁵⁾ Dissolved oxygen concentrations were monitored using an LG Nester DO meter (type 8500) for the anoxic aquarium and the Winkler titration method for the other aquaria; the concen-

trations varied little within and between the experiments (Table 1). Two small test chambers (ca 80 cm³) with two windows covered with 40 µm mesh screen were immersed in each of five test aquaria. Each small chamber contained 50 test larvae. At the start of the experiments, 11,000 cells/ml (umbo-larva stage) or 23,000 cells/ml (full-grown larva stage) of *Pavlova* sp. were added to each aquarium as food. The experiments were run for 48 h under continuous light at 24.1°C, the temperature close to that of the natural habitat of this bivalve in summer. In the present study, death of the larvae was judged by cessation of ciliary movement of the gills.

The results of tolerance test of *Theora* larvae to anoxic and hypoxic conditions are summarized in Fig. 1. This figure also includes the data of adult *Theora* reported by Tamai.⁵⁾ In the anoxic condition, all umbo-larvae died within 24 h, but 95% of full-grown larvae survived for 24 h and then 98% of them died in 48 h. In very severe hypoxic condition (1.0 ml O₂/l), mortality of the umbo-larva was 10–20% but almost all full-grown larvae survived for 48 h at this oxygen level. At the oxygen concentration higher than 1.7 ml O₂/l, few specimens died for both larval stages in 48 h. Comparing the present results on umbo- and full-grown larvae with those of Tamai⁵⁾ on adults, it is clear that tolerance against anoxic and hypoxic conditions is the lowest at the umbo-stage, and those of both full-grown larvae and adults are highly resistant to reduced oxygen conditions.

Figure 2 shows the shell length composition of full-grown larvae at the beginning and after the exposure to five different oxygen conditions for 48 h. Differences in

Table 1. Dissolved oxygen concentration (ml/l) during 48-hour experiments on two larval stages of *Theora fragilis*

Stages	Experiments				
	1	2	3	4	5
Umbo-larva	0.0 ~ 0.0*	0.97 ~ 0.98	1.63 ~ 1.89	3.20 ~ 3.22	4.81 ~ 4.85
Full-grown larva	0.0 ~ 0.0	0.93 ~ 0.99	1.64 ~ 1.75	3.25 ~ 3.41	4.83 ~ 4.87

* Range (n=3)

[†] Present address: National Research Institute of Fisheries Science, Fukuura, Kanazawa, Yokohama 236, Japan.

Fig. 1. Survivals of umbo-larvae, full-grown larvae and adults of *Theora fragilis* exposed to four or five different oxygen concentrations. Data of adults were from Tamai.⁵⁾

shell length composition of these five groups exposed to different oxygen concentrations were significant (one factor ANOVA, $p < 0.05$). The results of multiple comparison tests (Scheffe's F) revealed that the larvae exposed to 0 or 1.0 ml O_2/l grew little but those exposed to 1.7, 3.3 and 4.9 ml O_2/l grew substantially ($p < 0.05$), with daily growth rates of 12–14 μm . For adults, Tamai⁵⁾ noted that feeding activity was reduced at the oxygen concentration below 0.9 ml O_2/l but remained active at ≥ 1.5 ml O_2/l .

According to Kang *et al.*¹⁾ and Kang and Matsuda,²⁾ both blue crab and white shrimp exhibited higher hypoxic tolerance at the benthic juvenile stage than at the pelagic larval stage. On the other hand, the present results showed that while the umbo-larvae exhibited somewhat lower tolerance than adults, the full-grown larvae showed the same ability to withstand anoxic and very severe hypoxic conditions (0.9–1.0 ml O_2/l) as adults. The full-grown larvae also showed an ability to grow in severe hypoxic conditions (1.7 ml O_2/l). These facts show that the full-grown larvae and the adults have fairly high hypoxic tolerance. The tolerance level is nearly equal to that of other indicator species of pollution in Japan: polychaetes *Capitella capitata*⁶⁾ and *Paraprionospio* sp. (type A).⁷⁾ Anoxic tolerance of *T. fragilis* is shorter than 48 h, the hypoxic tolerance is, however, high enough for the bivalve to live in the most organically polluted areas, since anoxic conditions occur only in limited areas in Japan. It must be an advantageous physiological characteristic for the prosperity of this bivalve in polluted areas that the hypoxic tolerance of larvae quickly attains the level of adults.

Fig. 2. Changes in the frequency distribution of shell length of *Theora fragilis* before (0 h) and after (48 h) exposure to five different oxygen concentrations.
n: number of individuals.

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