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Notes on the Dormancy in the Adults of *Tigriopus japonicus*

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(Figs. 1–4, Tables 1–4)

The neritic harpacticoid copepod, *Tigriopus japonicus*, has repeatedly proved to be one of the key food organisms for sea-dwelling fish larvae in the recent development of artificial mass production of marine-fish fry in this country. Now this copepod is successfully cultivated on a large scale at several fisheries experimental stations, and is routinely used as food for the fry of the red sea bream, *Chrysophrys major*, and the sweet-fish, *Plecoglossus altivelis*.

Tigriopus japonicus was described as a new species by MORI[1] in 1938. HANAOKA[2] observed in detail the metamorphosis of its nauplii, and ITO[3] described its life history. This species has been frequently used as an experimental material in physiological and ecological studies.[4)–8)]

By the way, *Tigriopus fulvus*, a closely allied species, is known for suspending its mobility for a long time in concentrated sea water in rock pools. During the basic experiments on artificial mass culture of *Tigriopus japonicus*, the present authors had the opportunity of observing salinity-dependent dormancy occurring in the adults of this species as well as in *T. fulvus*. Furthermore, it was observed that this copepod is induced into a dormant state by low temperatures.

This study has been undertaken to determine the salinity and temperature conditions causing dormancy in *T. japonicus*. A few additional experiments have also been performed to get some information on the effective conditions for storing cultured individuals as a live food organism.

MATERIALS AND METHODS

Live specimens of *Tigriopus japonicus* were obtained from the stock cultured at Tomo Fisheries Laboratory, Hiroshima University, which in turn was the progeny of the specimens collected from rock pools in Misaki, Kanagawa Prefecture. In view of

the experiments, the animals were allowed to propagate for several months in tanks of each 30 liter capacity in our laboratory at temperatures between 15~32°C. Ammonium sulfate and superphosphate of lime were added to the sea-water medium in concentrations of 0.25g/1 and 0.05g/1 respectively as the nutrient salts for the growth of *Chlorella* sp., which served as food for the copepod. In addition to live *Chlorella*, dried *Chlorella* was occasionally given in concentrations of 0.5 ~ 1.0g/1, and the sea-water culture medium was renewed at intervals of one or two months. The adult animals recovered from one of the tanks were each time used for experiments.

In the experiments on the dormancy induced by low temperatures, 10~20 adult specimens were placed each in a small plastic vial containing about 20 ml of normal sea-water (salinity, 32.5 ~ 34‰). After the vials were kept at temperatures ranging from 10°C to −5°C for 24 hours, the animals were checked for their motility and the vials were transferred back to the original temperatures to investigate revival rates of dormant individuals. In the experiments on the dormancy induced by concentrating the sea water, 30~50 ml of normal sea water containing a large number of adults was evaporated by using an electric fan until all the animals fell into a state of dormancy, and then their revival rates were observed. In the experiments on the survival in dormancy, dormant animals were kept under conditions combining different temperatures and salinities, and their revival rates were recorded at definite time intervals. In the revival experiments, those individuals which regained their normal activity within 24 hours after being transferred to a favorable condition were regarded as 'revived individuals'.

More details of the experimental procedures will be described in the following section.

RESULTS

Temperature-dependent dormancy

The experiments on the occurrence of temperature-dependent dormancy were carried out using the specimens cultured at 15~20°C and 25°C. As shown in Table 1, the animals cultured at 15~20°C were active at temperatures above 4°C for 24 hours.

Table 1. Occurrence of the inactive individuals and their revival rates at various temperatures

1-1. On the animals cultured at 25°C

Temperature (°C)	10	8	6	4	2	0	-2	-4--5
Inactive inds. (%)	0	100	100	100	100	100	100	100
Revival rate (%)	–	100	100	100	35	25	5	0

1-2. On the animals cultured at 20-15°C.

Temperature (°C)	6	4	2	1-0	-1--2	-2--3	-3--4	-4--5
Inactive inds. (%)	0	0	100	100	100	100	100	100
Revival rate (%)	–	–	100	100	100	60	45	0

At temperatures below 2°C the animals lost their locomotive power, sank to the bottom of the vial with their limbs still moving ineffectively and finally lay motionless. As all the individuals that became motionless at temperatures between 2°C and −2°C could be revived, it may be said that they fell into a state of dormancy. However, about half of the animals having been made motionless at −2~−4°C and all those made motionless at temperatures below −4°C could not be revived, and consequently were considered as being dead. The animals cultured at 25°C became motionless at and below 8°C. All the animals made motionless at 8~4°C revived, while the majority of those made motionless at 2~0°C did not revive. Among those made motionless at −2°C a few females but no males revived.

Then, the survival of the animals made dormant by a low temperature was investigated. The experiment was performed using the specimens cultured at 16°C. In each vial containing normal sea water 5 male specimens or 20 female specimens were placed. The vials were kept at 0°C for periods up to two months after the animals had lost their activity at that temperature. The revival rates investigated at definite intervals are shown in Fig. 1. A revival rate of 80% was obtained in both sexes after 30 days.

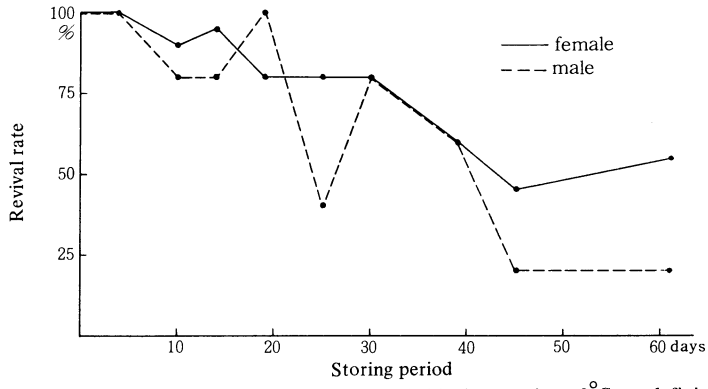

Fig. 1. Revival rates of the dormant individuals stored at 0°C at definite time intervals.

However, the revival rate after 60 days was only 20% for the males but 55% for the females. From the experiments described above, it may be said that the temperature causing dormancy is correlated to the temperature to which the animals have been acclimatized, and that the female tend to survive the dormancy induced by low temperatures for longer periods than male.

Salinity-dependent dormancy

A preliminary experiment was carried out in order to find out the range of salinity within which the animals could survive when they were directly transferred from normal sea water. As shown in Tables 2 and 3, all the animals transferred to salinities ranging 3.6~72.2‰ were normally alive after 4 days. In contrast, all locomotive individuals disappeared within a few minutes after being transferred to salinities of 114‰ or above.

Table 2. Motility of the animals directly transferred from the sea water (salinity, 33.3‰) into various salinity solutions at 25°C

Salinity (‰)	0	3.6	7.3	10.9	14.5	18.1	27.1	36.1	72.2	108	144	181
After 2 hours	–	+	+	+	+	+	+	+	+	–	–	–
24	–	+	+	+	+	+	+	+	+	±*	–	–
48	–	+	+	+	+	+	+	+	+	±*	–	–
96	–	+	+	+	+	+	+	+	+	–	–	–

+ : Active individuals only (swimming or crawling).
– : Inactive individuals only (laying motionless).
± : Both active and inactive individuals.
* Some of the inactive individuals recover.

Table 3. Revival rates of the inactive individuals shown in Table 2

Salinity (‰)		0	108	144	181
After 2 hours	Inactive inds. (%)	100	100	100	100
	Revival rate (%)	0	100	60	20
24 hours	Inactive inds. (%)	100	40	100	100
	Revival rate (%)	0	75	0	0
48 hours	Inactive inds. (%)	–	85	–	–
	Revival rate (%)	–	65	–	–

When transferred to fresh water, all animals died in 2 hours. At a salinity of 108‰, only 15% of the animals were observed to be active after 2 days, and their mortality attained 30%. In summary, the animal could not survive high salinities above 108‰ into which they were directly transferred.

Then an experiment was done in order to investigate the salinities causing dormancy in the copepod when the environmental water was gradually concentrated. The animals cultured at 31~32°C were placed in vessels containing normal sea water, and then the sea water was concentrated by evaporation at one of the following rates: (a) about 20 hours and (b) about 90 hours were required to attain at a salinity of about 150‰. During the experiment the temperature varied between 23 and 26°C. As shown in Figs. 2 and 3, the animals successively became incapable of locomotion (i.e., swimming or crawling) with their limbs moving ineffectively, and then shifted to the state of dormancy, the limb movement ceasing completely. According to the (a) or (b) evaporation rates, no individuals were locomotive at salinities of some 101‰ or 137‰ for males and at 127‰ or 151‰ for females. And all individuals fell into dormancy at salinities of some 123‰ or 150‰ for males and at 140‰ or 153‰ for females. From the obtained results, we may say roughly that the lowest salinities required to induce the copepod to dormancy are 130~150‰. The revival rate was 100% (a) and 90% (b) in both sexes when the dormant animals were transferred back to normal sea water just after the experiment was terminated.

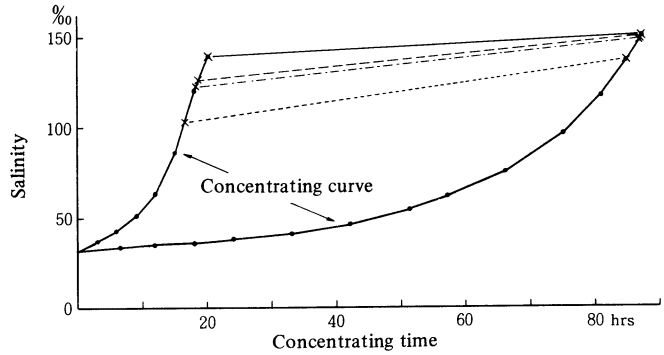

Fig. 2. Effects of concentrating seawater on the occurrence of dormancy in the animals. (at different concentrating rates).
——— : ♀) Salinity at which all of the individuals become
– – – – : ♂) dormant.
------ : ♀) Upper end of salinities in which swimming or
---------- : ♂) crawling individuals are seen.

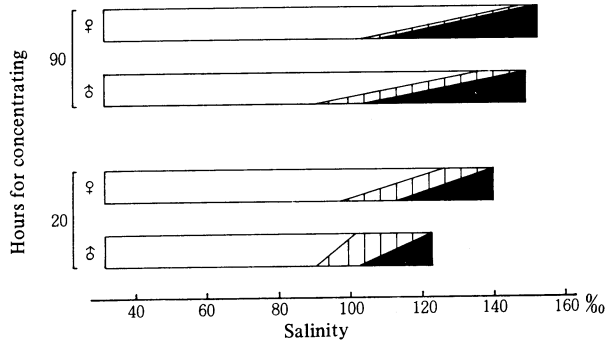

Fig. 3. Changes of the activity of the animals at two different rates of concentrating seawater. (schematic illustration).
Open space : Swimming or crawling.
Striped space : Laying with moving appendages.
Solid space : Laying motionless.

To observe the effect of storing temperature on the survival during the dormancy caused by high salinity, a lot of dormant animals were prepared by concentrating the sea water containing normally active animals up to a salinity of 180‰ in about 100 hours. They were subdivided into three lots and respectively stored at temperatures −3~3°C, 1~7°C and 20~26°C for 45 days, during which period their revival rates were examined at definite intervals. As shown in Fig. 4, the highest revival (i.e., survival) rate was obtained when the animals were stored at 1~7°C: for example, 60% of the dormant animals revived after 30 days. The survival rate decreased rapidly after two weeks when stored at −3~3°C. Most of the individuals died within one week when stored at 20~26°C.

In order to know the combined effect of temperature and salinity on the occur-

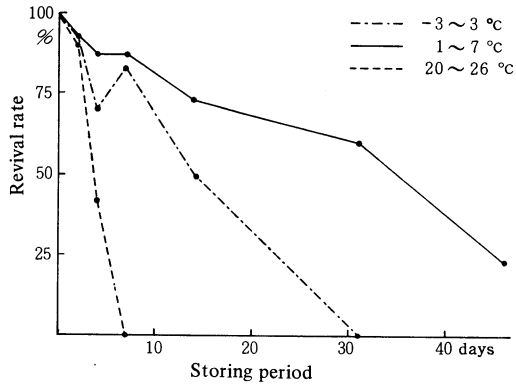

Fig. 4. Changes of the revival rate of the salt-dependent dormant individuals stored at different temperatures.

rence of dormancy, an experiment was made in which animals cultured at 18°C were exposed to various temperatures and salinities and their motility was observed. In the course of concentrating the sea-water medium from a salinity of 32.5‰ up to 150‰ in 20 hours, samples were taken out four times: once in the beginning, twice in the midst and once at the end. Each sample obtained at salinities of 33, 67, 99 and 150‰ was kept at temperatures of 0, 2.5, 5, 7, 9, or 25°C for 24 hours. Though the results were not sufficiently precise as shown in Table 4, it appeared that dormancy occurs at higher temperatures with increasing salinities of the solutions in which the animals were placed.

Table 4. Relationship between salinity and temperature on the occurrence of dormancy in the animals

Temperature (°C)		0	2.5	5	7	9	25
Salinity (‰) 33	female	–	±	+	+	+	+
	male	–	–	+	+	+	+
67	female	–	–	+	+	+	+
	male	–	–	±	+	+	+
99	female	–	–	–	±	±	±
	male	–	–	–	–	–	±
150	female	–	–	–	–	–	–
	male	–	–	–	–	–	–

\+ : Active individuals only (swimming or crawling).
– : Inactive individuals only (laying motionless).
± : Both active and inactive individuals.

DISCUSSION

"Dormancy" in general means a temporary interruption of activity or growth in a living organism. It is substantially classified into two phenomenons: one is the loss of activity induced by extreme inadequacy of the environmental conditions, and the other, being called "diapause" is a state that naturally occurs at a certain period in the normal course of development or growth. The dormancy dealt with in this paper is obviously the former one. Such a dormant state has been so far observed in the adults of *Tigriopus* by some other researchers. ISSEL[9] reported that as soon as the density

of sea water in rock pools is raised to a certain level, the copepod *Tigriopus fulvus* falls in a state of apparent death, from which it can awake even after a very long time and regain normal activity when the water is sufficiently diluted. Afterward, FRASER[10] experimentally confirmed this phenomenon in the same species.

Now it has become clear that in the adults of *Tigriopus japonicus* the dormancy occurs at very high salinities as in *T. fulvus*. When the animals cultured in sea water of a salinity of about 34‰ were directly transferred into the media of a graded series of salinities ranging from 0‰ to 181‰ they fell into dormancy at salinities above 100‰ although in these high salinities they gradually died during dormancy. According to RANADE[11] the maximum immersion period, after which no recovery took place, was 30 hours at salinity 180‰ in *T. fulvus*. In our experiments on *T. japonicus*, all the individuals died within 24 hours at a salinity of 181‰. This species may therefore be considered as being more sensible to high salinities than *T. fulvus*. When the sea water containing the copepod was concentrated slowly by evaporation, not all of the animals fell into dormancy until the salinity went up to 130~150‰. The animals made dormant at these salinities could survive after a long period of storage at comparatively low temperatures ranging 1~7°C. In this case the rate of evaporation was not an important factor for their survival as it took 20 to 100 hours for the salinity of the medium to attain the final level of about 150‰.

Temperature conditions should be considered as another important factor inducing dormancy in the adults of *T. japonicus*. TAKEDA[4] reported that the rearing temperature affects the resistance of this species to high temperatures and that the animals reared at high temperatures can tolerate higher temperatures. In the present experiments the animals cultured at 25°C and 20~15°C fell into a dormant state at 8~4°C and 2~−2°C respectively. This result possibly indicates that the temperature causing dormancy of this copepod changes with the rearing temperature and that a rapid temperature decline of some 20°C from their rearing temperature causes dormancy.

It was shown by RANADE[11] that temperature tolerance in *T. fulvus* goes up with increasing salinities. MATUTANI[8] also reported that heat resistance in *T. japonicus* increases as the animal is adapted to concentrated sea water. From the experiments on the combined effect of temperature and salinity in causing dormancy, the animals fell into dormancy at higher temperatures as the environmental salinity became higher. Females were less inclined to fall into a dormant state than males throughout the present experiments. This agrees with the tendency of females of better enduring significant changes in either temperature or salinity than males as shown by IGARASHI[5] and MATUTANI[6].

Regarding the possibility of storing this copepod in large quantities so as to serve as a live food for larval fish at any desired time, our experimental results suggest that a successful method may be developed by storing adult animals in a dormant state. In view of a higher survival rate and greater practicability, a method of inducing dormancy

at a temperature of about 20°C below the culturing temperature and successively storing the dormant animals at that temperature seems to be superior.

SUMMARY

Experiments have been done on the salinities and temperatures causing dormancy in the adults of *Tigriopus japonicus*. The results are as follows;

(1) The animals cultured in sea-water media (salinity, 32.5~34‰) at 25°C and 20~15°C wholly lie dormant in 8~4°C and 2~−2°C, respectively.

(2) These dormant animals of both sexes show a revival rate of 80% after being stored at 0°C for a month.

(3) When the sea water containing the animals is gradually concentrated by evaporation at 23~26°C, all the individuals fall into dormancy at salinities of 130~150‰. At these salinities the dormant animals survive for more than one month at 1~7°C.

(4) The animals fall into dormancy at higher temperatures as the environmental salinity is higher.

(5) Females are slightly more resistant to falling into dormancy than males, either at lower temperatures or at raised salinities.

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Tigriopus japonicus（ハルパクチコイダ橈脚類）成体の休眠生態について

笠原正五郎・秋山敏男

筆者らは，餌料生物としての利用における *T. japonicus* の培養，ならびに保存に関する基礎的研究を行なっているが，ここでは，後者に関連して行なった本種成体の休眠生態に関する二三の観察結果について報告する。

(1) 25℃で培養したものは4～8℃で，15～20℃で培養したものは −2～2℃でそれぞれ全個体が休眠状態となり，1日後に元の水温に戻すとすべてが蘇生した。また，16℃で培養し0℃で休眠した個体をそのまま0℃で保存した場合，1か月後に80%（雌雄ともに），2か月後に55%（雌），20%（雄）の蘇生率を示した。

(2) 飼育海水の濃縮による高塩分条件下では，濃縮速度（但し塩分約150‰へ20～100時間の範囲）と特に関係なく，いずれの場合も塩分130～150‰（水温23～26℃）程で全個体が休眠状態となり，それらを1～7℃に置いた場合，1か月後に60%（雌雄混在）の蘇生率が得られた。

(3) 休眠生起の条件としての塩分と温度の関係については断片的な検討にとどまったが，塩分濃度が高くなるにつれ，いわゆる冷休止帯よりさらに高い水温で休眠状態になることが認められた。

(4) 以上の実験を通じ，概して雌は雄より休眠状態に陥り難い一方，休眠からの蘇生率は若干良好であった。