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Physiological adaptations in the survival of rotifers (*Brachionus plicatilis*, O. F. Müller) at low temperatures

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Abstract

The biology of the rotifer *Brachionus plicatilis* has been studied extensively in recent years, due to its importance in aquaculture. Today, the culture of several marine fish species relies completely on the daily production of live rotifers.

In the present paper we explore the conditions that facilitate maintaining live rotifers for extensive periods at low temperatures. In addition to its possible contribution in providing reserve stocks for commercial application, these studies may be of ecological importance. They could explain some of the physiological adaptive mechanisms that are involved in the survival of rotifers under adverse environmental conditions.

Experimental results showed relatively high survival rates (82–85%) in rotifers that were cultured at 25 °C and exposed later to −1 °C for 12–14 days. During this period, rotifers were kept without food and their media were not changed. The survival was found to depend on the rotifer culture conditions, prior to exposure to −1 °C. These included the type of food fed to rotifers (yeast or algae), the salinity in which they were cultured, and an essential acclimation period of 2–6 day at 4 °C or 10 °C. The acclimation period was associated with the synthesis of at least one specific protein and accumulation of lipids. Profiles of protein synthesis in rotifers incubated at 10 °C revealed a 94 kD protein, which did not appear in rotifers cultured at 25 or 37 °C. Immunoisolation, using a polyclonal antibody that was prepared against HSP60, revealed that this protein was synthesized in rotifers kept at 10, 25 or 37 °C. However, this antibody did not react with the 94 kD peptide.In addition, rotifers kept at 10 °C accumulated substantial amounts of lipids, including eicosapentaenoic acid (EPA), which is found in the algae fed to them. These results support the hypothesis of specific adaptations to survival at low temperatures during an acclimation period.

Introduction

One of the main obstacles in the large scale production of small mouthed marine fish larvae, is the supply of adequate amounts of live food during the most critical initial stages in their lives. Rotifers of the species *Brachionus plicatilis* O. F. Müller serve traditionally now as the first live food organism offered to the fragile small marine larvae (Hirata, 1980; Lubzens, 1987; Lubzens *et al.* 1989; Hirayama, 1990; Fulks & Main, 1991). Today, the supply of rotifers in marine fish hatcheries relies almost completely on the number of rotifers that can be daily collected ('harvested') from mass cultures. In order not to affect the rotifer standing stock in the hatchery, these numbers depend on the total number and the reproductive rates of the rotifers in culture. Providing rotifers through preserved stocks of resting eggs is one of the strategies that was explored in the past 17 years (Hino & Hirano, 1977; Lubzens *et al.*, 1980; Lubzens, 1981, 1989; Snell & Hoff, 1988; Hagiwara & Hirayama, 1993). Producing rotifer resting eggs that could serve as a reserve stock, or as an immediate source of food, after hatching them is costly. Furthermore, the techniques used today for mass production of rotifers are not fully compatible with mass production of resting eggs. This means that

mass production of rotifer resting eggs is not immediately possible from mass cultured rotifers. There is almost no flexibility for storing surplus live rotifers in the hatchery, for short or longer periods. The need for having access to reserve stocks of rotifers is further alleviated by accidental loss of cultures, periodic and sporadic demands for large quantities and no commercial source for live rotifers.

A few years ago Lubzens *et al.* (1990a) suggested a method for keeping rotifers at 4 °C for days or weeks, at very high densities. However, rotifers at 4 °C must be fed and their medium periodically changed. Preliminary results were reported on high density storage of rotifers at −0.5 and −5.0 °C (Berghahn *et al.*, 1990). In the present paper we explore the possibility of keeping rotifers at −1 °C at very high densities and without supplying them with food or changing their culture medium. We attempt to understand the physiological mechanisms involved in adaptation to very low temperatures, and relate these phenomena to naturally occurring events in field populations.

Materials and methods

Sea water

Natural sea water (salinity 40‰) was obtained and dilutions were performed as described previously (Lubzens *et al.*, 1990a).

Algae

Isochrysis sp. was cultured and harvested as described previously (Lubzens *et al.*, 1990a). *Nannochloropsis* sp. was cultured and treated in a similar way.

Yeast

Caked yeast (*Saccharomyces cerevisiae*), containing 70% water, was purchased from a local bakery and kept at 4 °C for up to 7 days while being used as food for rotifer.

Rotifers

A parthenogenic clone (L-type, size 196 μm × 139 μm, named N.R.E. in our laboratory) was use in the experiments described below. This clone stems from one of the resting that were produced in a laboratory culture. The culture originated from several individuals that were collected at the Dor sea water fish ponds, 40 km south of Haifa, Israel.

Rotifers were generally batch cultured in temperature controlled rooms (25 °C) under constant illumination. They were fed on algae or yeast (depending on the treatment group) for several weeks prior to the experiments.

Experimental design

Rotifers were removed from cultures maintained at 25 °C and were acclimated to 4 or 10 °C before being transferred to −1 °C. During the period of acclimation, which lasted for 0, 2, 4, 6 or 8 d (according to the experiment), rotifers were fed daily and their culture media was changed every 2 days. After the acclimation period and before transferring to −1 °C, the rotifers were sieved and resuspended in sea water at very high densities. The density was determined from counting 0.1 ml triplicate samples.

Ten or 50 ml of the rotifer suspension projected to contain between 20000 to 100000 rotifers were introduced into 50 or 250 ml plastic tissue culture flasks (Costar, CA USA). The flasks were placed with their large surface area facing down, at −1 °C (in the dark) in a water bath, for 12–14 days. The survival of the rotifers was calculated from the density in 3–5 replicate samples (of 0.1 ml) that were taken from each flask (see Lubzens *et al.*, 1990a), at the beginning and end of each experiment.

In these experiments the contribution of the following factors on the survival of rotifers kept at −1 °C were tested: a) type of food (*Isochrysis* or yeast) fed to rotifers; b) the salinity in which they were cultured; c) the time spent at the acclimation temperature of 4 or 10 °C; d) the initial density of the rotifers and e) the volume of the medium in which they were suspended.

The effects of the rate of warming the samples from −1 °C to 25 °C (fast by placing samples in a water bath at 25 °C or slow; by warming at a controlled rate of 1 °C min^{-1}) and the location of the flasks in the water bath (up or down), were also tested in one of the experiments.

Protein synthesis and immuno-isolation

Rotifers that were cultured at 25 °C were later transferred to three different temperatures (10, 25 and 33 °C) for 2 and 24 h. These rotifers were cultured in six 100 ml flasks, at a density of 200 per ml and a total volume of 75 ml, in each flask. During the incubation period they were fed with *Nannochloropsis* sp. *ad lib* and 12.5 μl of ^{35}S methionine (45.2 TBq/mmol;

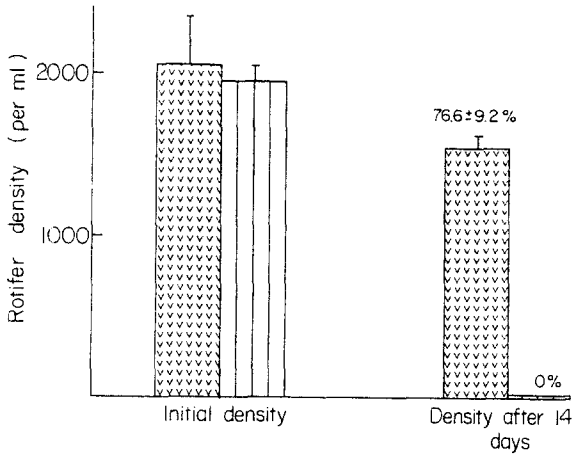

Fig. 1. The effect of food fed to rotifers before cooling to −1 °C. Rotifers were cultured in sea water (salinity 40‰) and fed on either *Isochrysis* or baker's yeast prior to the experiment. They were maintained at 10 °C for 4 d before being transferred to −1°C. During this period, they were fed on their respective type of food. Before exposure to −1 °C, rotifers were suspended in clean 10 ml sea water culture flasks. Each treatment consisted of five replicates. The means of the initial and final densities are shown in the histograms with bars indicating 1 S.D.. The percent of surviving rotifers is shown after 14 d at −1 °C.

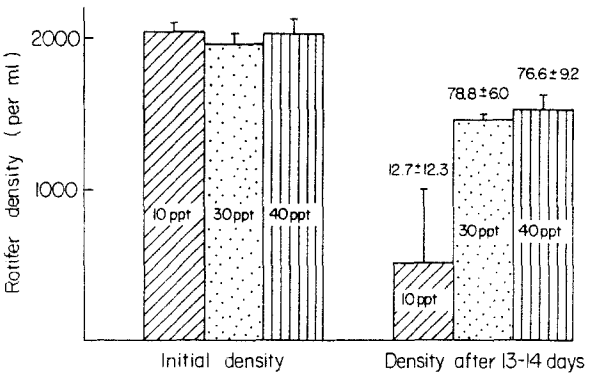

Fig. 2. The effect of salinity on survival at −1 °C. Rotifers were cultured in 10, 30 or 40‰ sea water and fed on *Isochrysis* prior to the experiments. They were incubated at 10 °C for 4 d at the respective salinities, before being placed at −1 °C. Each treatment consisted of five replicate culture flasks, containing rotifers that were suspended in 10 ml of sea water of the respective salinity. The means of the initial and final densities are shown in the histograms with bars indicating 1 S.D.. The percent of surviving rotifers (mean ± S.D.) is shown after 13–14 d.

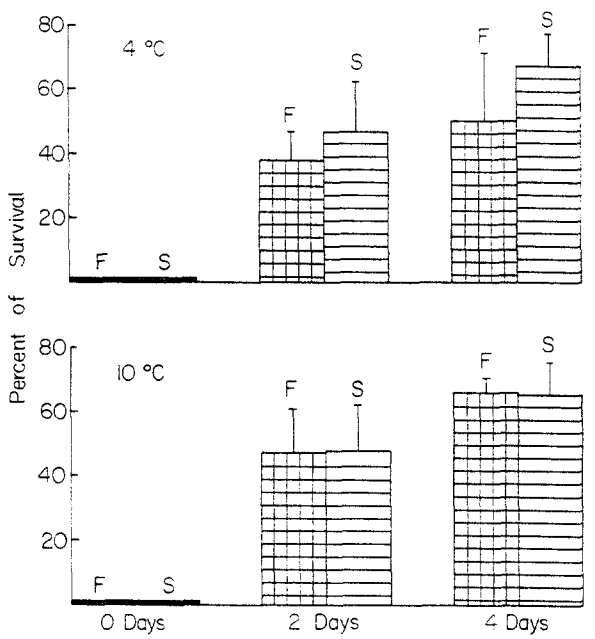

Fig. 3. A. The effect of time (0, 2 or 4 d) spent at 4 or 10 °C on rotifers cultured in 20‰ (A) or 30‰ (B) sea water, before exposing them to −1 °C for 12 d. Rotifers were transferred immediately (F) or warmed slowly (S; 1 ° min^{-1}) from −1 °C to 25 °C. The histograms show the mean of three replicates (except *, where only two survived) and bars indicate 1 S.D.

379.25 MBq ml^{-1}, NEN, Du Pont de Nemours, Germany) was added to each flask. Following the incubation period, the rotifers were collected on a 60 μm mesh sieve, washed in phosphate buffer (0.1 M; pH = 7.5) and homogenized in 2 ml of phosphate buffer, in a Dounce glass homogenizer. Phenylmethylsulfonylfluoride (PMSF) was added (at a final concentration of 0.1 mM) to the buffer solution, prior to homogenization. The homogenate was centrifuged (11 950 *g*, Sorvall, Dupont, USA) for 10 min, and the supernatant was used later for TCA precipitation and immunoisolation.

The incorporation of ^{35}S-methionine into the protein fraction of the homogenate was followed by subjecting triplicate 10 μl from each sample to TCA (trichloroacetic acid) precipitation. Samples were applied on 3MM Whatman square (1 cm) discs and boiled in 10% TCA for 10 min. After cooling, by adding ice to the beaker, discs were washed twice in water, methanol and acetone. The discs were dried with a stream of nitrogen gas, and read in a scintilator counter (Kontron, Packard, USA) after the addition of 4 ml of Opti-Fluor (Packard, USA) to each vial containing one disc.

Specific newly synthesized proteins were isolated from the homogenates by indirect immunoprecipitation using Protein A-Sepharose CL4B beads. Aliquot samples (100–700 μl) containing equal amounts of radiolabel were incubated with 6 μl antisera (SPA-804, prepared in rabbits against HSP60; StressGen, Biotechnologies Corp., Victoria B. C., Canada), at 4 °C

Table 1. The experimental plan testing the effect of the time spent (4,6 or 8 days) at the acclimation temperature (4 or 10°C) of rotifers suspended in 10 or 50 ml, at a density of 2000, 3500 or 5000 ml^{-1}, on their survival. The rotifer flasks were placed in two positions (up or down) in a bath at −1°C for 12–14 days. It was anticipated and later shown that the position in the bath had no effect on survival and the two treatments were therefore combined into one group in the statistical analysis.

Sample volume	Rotifer density	Position	4 °C			10 °C		
			4 days	6 days	8 days	4 days	6 days	8 days
	2000	up	1	1	1	1	1	1
	ml^{-1}	down	1	1	1	1	1	1
10 ml	3500	up	1	1*	1	1*	1	1
	ml^{-1}	down	1	1	1	1	1	1
	5000	up	1			1		
	ml^{-1}	down	1			1		
50 ml	2000	up	1	1				
	ml^{-1}	down	1	1				

* Rotifers in this group died. Since it was possible that the rotifers died immediately after being introduced into the flasks, these groups were excluded from the statistical analysis.

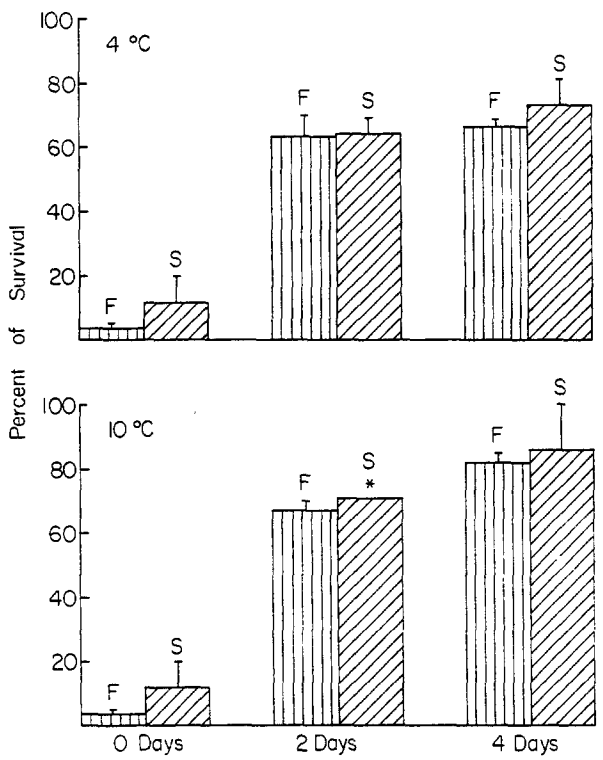

Fig. 3. B.

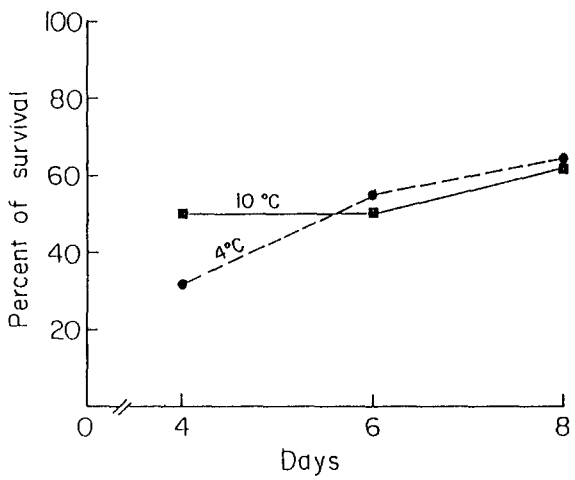

Fig. 4. The interaction between days and temperature on the survival of rotifers exposed to −1 °C.

overnight. Control samples were incubated with non-immunized rabbit serum (Sigma Israel Chemical Co., Israel). The samples were later treated as described in Bidwell *et al.* (1991) and Khayat *et al.* (1994). Total homogenates and immunoisolates were subjected to 3 to 15% SDS-polyacrylamide gradient gel electrophoresis (PAGE), using a mini Protean II electrophoresis cell system (Bio-Rad, Richmond, CA.). The gels were stained with Coomassie blue, dried and autoradiographed by standard procedures. Molecular weight markers were run in each gel and were purchased from Bio-Rad, Richmond, CA.

Lipid analysis

Two experiments were performed to evaluate the amount of lipids in rotifers that were incubated at 10 °C. In *Experiment 1*, rotifers that were cultured at 25 °C and fed with *Nannochloropsis* were suspended at a density of 100 rotifers ml^{-1}, in five flasks each containing 600 ml of sea water at a salinity of 30‰. Three flasks were placed at 10 °C for 12, 24 or 48 h, and two others were incubated at 37 °C for 2 and 12 h. During the incubation period the rotifers were in the dark and fed on algae. Following the incubation period, rotifers were sieved and resuspended in sea water (salinity 30‰) and starved for 2 h at the incubation temperature. A control group was cultured at 25 °C in an identical volume and density and was similarly starved for 2 h.

In *Experiment 2*, rotifers that were cultured at 25 °C were transferred to four flasks and placed at 10 °C for 48 h. Following the incubation period, rotifers from each flask were sieved and resuspended in sea water (salinity 30‰) without food. Two flasks were placed at 10 °C for 4 and 24 h, respectively, and two others were placed at 25 °C for 2 or 4 h, respectively. A control group that was maintained at 25 °C, was also starved for 2 h.

In both experiments, the rotifers were similarly treated after the starvation period which allowed for the absorption of algae that were consumed by the rotifers. Extraction and quantification of total lipids and fatty acids was performed on two replicates of 20000 rotifers (from each flask), according to the methods described by Sukenik *et al.* (1989, 1993). Results were expressed as μg fatty acid (FA) per 10^6 rotifers.

Statistical analysis

The experimental design shown in Fig. 3a & b was unbalanced. However, there was no survival of rotifers transferred directly to −1 °C (time 0). This combination (at salinities of 20 and 30 ‰) was excluded from the statistical analysis. The final analysis pertains to a balanced 2^4 factorial experiment with three replications per cell. An analysis of variance was carried out to show the significance of the main effects and interactions between the four factors. The dependent variable was arcsine p, where $\sqrt{p}$ is the proportion of survived rotifers. The experiment shown in Table 1 was similarly analysed on 30 observations.

General Linear Models procedure including Duncan's multiple range test for variables, was performed using SAS/Stat Users Guide (version 6 SAS Institute Inc., 1989).

Table 2. Statistical analysis of an unbalanced factorial design experiment on 30 observations after the data were transformed by arcsin $\sqrt{p}$.

Source	Degrees of freedom	Pr>F*
Volume	1	0.7710
Days	2	0.047
Volume × Days	1	0.1995
Location	1	0.6739
Volume × Location	1	0.3840
Temperature	1	0.9921
Temperature × Days	2	0.353
Temperature × Location	1	0.9062
Density	2	0.1052
Temperature × Density	2	0.4415
Days × Density	2	0.0543
Days × Location	2	0.1328
Location × Density	2	0.2483

* Probability > F

Results

Rotifers that were cultured in 40‰ salinity sea water and fed yeast did not survive exposure to −1 °C after a 4 day acclimation period at 10 °C (Fig. 1). This was in contrast to similarly treated rotifers that were fed with *Isochrysis* sp. Low survival rates were also observed in rotifers that were cultured in 10‰ salinity sea water (Fig. 2). None of the interactions in the experiment shown in Fig 3A and B were statistically significant ($p \geq 0.10$). Each of the tested effects was found to have a significant contribution to the survival of rotifers kept at −1 °C: salinity ($p = 0.0001$), days at the acclimation temperature ($p < 0.001$) and the acclimation temperature ($p = 0.040$). No differences were found between treatments that were transferred immediately, or warmed slowly (1 °C min^{-1}) to 25 °C ($p > 0.1$). The duration of the acclimation period at 10 °C was critical for the survival of rotifers exposed later to −1 °C (Fig 3A and B). Rotifers that were transferred directly from the temperature in which they were cultured (25 °C) to −1 °C died within 3 days after transfer. An acclimation period of 2 or 4 days, at 4 °C or 10 °C, was essential for survival at −1 °C. In order to determine the best conditions for maintaining rotifers at high densities at −1 °C, five variables were tested simultaneously in one experiment (Table 1). The time spent at the acclimation temperature again contributed significantly to the survival of rotifers (Table 2).

Rotifers required 6 days of acclimation at 4 °C, but only 4 days at 10 °C (Fig. 4). The interaction between all the variables was not statistically significant.

In order to understand the events occurring during the acclimation period, we followed protein synthesis and lipid accumulation in rotifers that were maintained at 10 °C, after being cultured for long periods at 25 °C. Rotifers that were exposed to 10 °C for 24 hr, synthesized a 94 kDa protein that was not found in rotifers cultured at 25 °C or exposed to 33 °C (Fig. 5). This protein was found to be different from HSP60, after subjecting rotifer homogenates to specific immunoisolation, by using a polyclonal antibody (Fig. 6). However, the HSP60 immunoreactive protein was found to be synthesized in rotifers exposed to 33 °C.

Rotifers that were exposed to 10 °C gradually accumulated higher amounts of fatty acids(TFA) than those cultured at 25 °C or those incubated at 37 °C (Table 3). Rotifers that were incubated for as short as 12 h at 10 °C showed almost 3 × higher amounts of total lipids than those kept at 25 or 37 °C. In order to verify that the elevated levels of lipids in 10 °C incubated rotifers were not due to undigested algae in the gut, the rotifers were starved for longer periods in *Experiment 2* (Table 4). Although the amounts of total lipids decreased after 20 h of starvation at 10 °C or 4 h at 25 °C, the rotifers that were incubated for 48 h at 10 °C, contained significantly higher amounts of lipids than those cultured at 25 °C. The fatty acid distribution was also affected by exposing rotifers to 10 °C. Rotifers maintained at 10 °C, showed significantly lower amounts of 16:0 ω7, 18:1 ω7 and 18:1 ω9. The amount of 20: 5 ω3 increased significantly only after 24 h of incubation (Table 4).

Discussion

According to Olsen *et al.* (1993) rate of loss of rotifers was variable but high in starved rotifers placed at temperatures ranging from 0–3 °C. The motility of rotifers was greatly reduced at low temperatures (Berghahn *et al.*, 1990). At −1 °C, only slow ciliary movement was observed and production of eggs ceased almost completely (Lubzens *et al.*, 1990b). This may explain their survival without food during a period of 12–14 days. Survival of rotifers at −1°C was affected by the type of food fed to rotifers before exposing them to low temperatures. Yeast fed rotifers did not survive, while rotifers fed *Isochrysis* sp showed high survival rates. Furthermore, a period of acclimation at a lower temperature was essential for the survival of rotifers. These results support previous preliminary observations, where rotifers not treated in this way (Group 5: Fig. 2, Berghahn *et al.*, 1990) only briefly survived the exposure to low temperature. Algae contain various lipids, mainly long-chain unsaturated fatty acids that are not found in yeast. Although rotifers were found to synthesize lipids *de novo*, the rate of synthesis is slow and most of the lipids that accumulate within the rotifers originate in their food (Lubzens *et al.*, 1985). Rotifers fed with algae accumulate lipids, which are not present in yeast. The lipid content of algae depends on the species (Ben-Amotz *et al.*, 1987) and the conditions in which they are cultured (Sukenik *et al.*, 1989) and affects the lipids that accumulate in rotifers (Lubzens *et al.*, 1989; Whyte & Nagata, 1991).

The required period of acclimation was longer at 4 °C (6 days) than at 10 °C (4 days). The choice of temperatures was rather arbitrary, with 10 °C reflecting a mid-point temperature between −1 °C and 25 °C, which was the routine culture temperature. Choosing 4 °C would obviously be helpful in large-scale operations where cold rooms operating at this temperature are universally available. The results, however, demonstrate that at 4 °C the acclimation period is rather long. Higher levels of survival were found in rotifers cultured at higher salinities, where the freezing point is lower.

Organisms often have to cope with a variety of suboptimal conditions, frequently imposed by climatic changes in the environment. Such conditions are perceived as physiological stresses that an organism has to encounter in order to survive. While it is well known that the functioning of isolated organelles and individual macromolecules can be adversely affected by cold (Franks & Hatley, 1992), more attention was paid to heat injury. This is despite the fact that heat injury takes place at temperatures beyond the normal environmental range, while cold stress is a very real environmental threat. Most temperate ectotherms possess survival mechanism that enable them to cope with seasonal cold conditions in the form of freeze tolerance or freeze avoidance (Franks, 1985). The results in the present paper show that the synthesis of heat shock proteins is triggered by a transient rise in temperature, but not by a fall in temperature. It has also been shown (Cochrane *et al.*, 1991) that heat shock proteins are triggered in rotifers by other stresses, including various toxicants. In bacteria, another set of proteins is synthesized in response to cold shock (Jones *et al.*, 1987). Several of these cold shock proteins were found to function in blocking transcription and translation. In

Table 3. Total fatty acids and fatty acid distribution in rotifers incubated at 10, 25 and 37 °C for various periods of time. Lipid extraction was carried out on rotifers that were starved for 2 hr, at their incubation temperature. General Linear Models Procedure including Duncan's multiple range test was performed on the total fatty acids (TFA) and the fatty acid distribution. Means with the same superscript letter along each line are not significantly different from one another.

Temperature of incubation (°C)	25	10	10	10	37	37	P
Duration of incubation (h)	*	12	24	48	2	12	Pr≥F
Total fatty acids ($\mu g/10^6$ rotifers)	13.9^a	46.2^b	54.8^b	60.5^b	14.5^a	12.1^a	0.0004
Fatty acid distribution (% weight)							
14:0	9.6	10.9	10.8	10.9	10.1	9.3	0.1476
16:0	26.3^a	20.6^c	19.4^c	17.5^d	24.7^d	24.7^d	0.0001
16:1	28.4	33.5	32.8	32.1	29.1	28.0	0.2275
18:0	2.5	2.2	1.7	1.9	3.4	4.7	0.3528
18:1ω9	6.5^a	5.4^{bc}	4.8^c	4.3^{bc}	5.7^a	5.9^{ab}	0.0120
18:1ω7	5.5^a	4.3^{bc}	4.0^{cd}	4.6^d	4.9^{ab}	5.5^{ab}	0.0035
18:2	5.1	6.5	5.8	6.3	8.0	7.5	0.7922
20:4ω6	5.9	5.8	5.5	6.0	4.6	5.3	0.1321
20:5ω3	10.4^{bc}	10.9^b	15.2^a	16.4^a	9.0^c	9.1^c	0.0001

* Control group rotifers that were cultured at 25°.

Table 4. Effect of the duration of starvation at two temperatures (10 and 25°C) on the total fatty acid content and fatty acid distribution of rotifers that were incubated at 10°C for 48 h. General Linear Models Procedure including Duncan's multiple range test was performed on the total fatty acids (TFA) and the fatty acid distribution. Means with the same superscript letter along each line are not significantly different from one another.

Temperature of starvation (°C)	25*	25	25	10	10	P
Duration of starvation (h)	2	2	4	4	20	Pr≥F
Total fatty acids ($\mu g/10^6$ rotifers)	12.7^c	60.9^a	48.8^{ab}	39.7^b	33.1^b	0.0001
Fatty acid distribution (% weight)						
14:0	11.7	13.4	13.0	12.8	12.5	0.04006
16:0	25.8^a	15.8^b	16.7^b	17.0^b	17.2^b	0.0006
16:1	27.0	36.2^a	34.2^a	34.4^a	34.4^a	0.0143
18:0	4.4	1.7	2.0	2.3	2.0	0.0643
18:1ω9	5.8	5.4	5.6	6.1	5.7	0.8659
18:1ω7	2.2	4.9	5.1	5.2	4.5	0.7193
18:2	8.3^a	5.1^b	5.8^b	4.9^b	5.6^b	0.0084
20:4ω6	5.0	3.8	4.0	4.5	4.9	0.4736
20:5ω3	9.9	13.6	13.7	12.8	13.2	0.0666

* Control group that were cultured at 25°, before the starvation period.

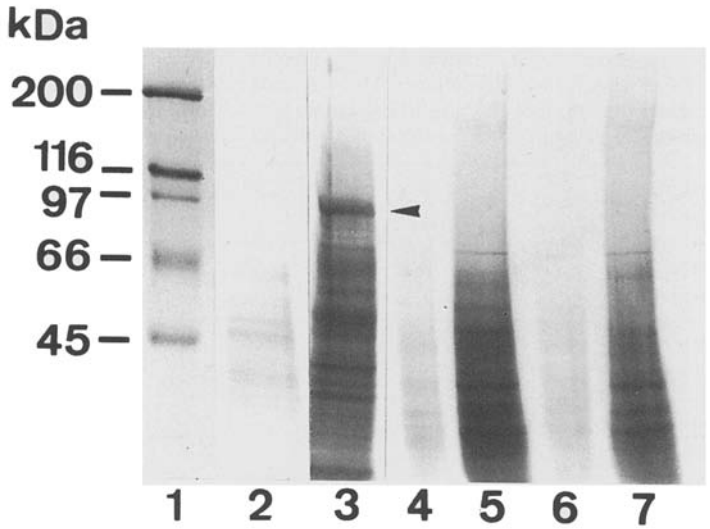

Fig. 5. Electrophoretic comparison between newly synthesized proteins by rotifers incubated with [^{35}S]methionine for 2 or 24 h, at 10 °C (lanes 2 & 3, respectively), 25 °C (lanes 4 & 5, respectively) and 33 °C (lanes 6 & 7, respectively). Equal amounts of labeled proteins were applied to polyacrylamide denatured gradient gels (3–15%), stained with Coomassie blue and exposed to X-ray film. Coomassie blue-stained molecular weight standards are shown in lane 1. The arrow indicates a 94 kD protein synthesized at 10 °C.

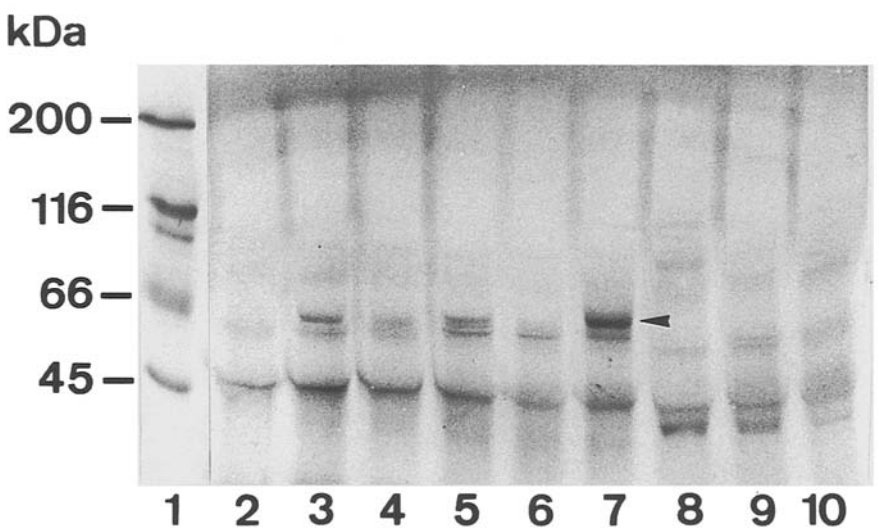

Fig. 6. Electrophoretic comparisons between newly synthesized HSP60 that was immunoisolated from rotifers. Rotifers were incubated with [^{35}S] methionine, at 10 (lanes 2 and 3), 25 (lanes 4 and 5) and 33 °C (lanes 6 and 7), for 2 (lanes 2, 4 and 6) or 24 h (lanes 3, 5 and 7). Equal amounts of labeled proteins were applied to denatured polyacrylamide gradient gels (3–15%), stained with Coomassie blue and exposed to X-ray film. Lanes 8, 9 and 10 are similar to lanes 3, 5 and 7, except that immunoisolation was performed in the presence of non-immunized rabbit serum. Coomassie blue stained molecular weight markers are shown in lane 1. Arrow indicates the position of HSP60 protein.

plants, gene expression was found to change during cold acclimation (Thomashow, 1993). The identity of the 94 kD protein found in rotifers exposed to 10 °C is as yet not known. It may also be associated with changes in the lipid accumulation in rotifers kept at 10 °C.

The total amount and composition of phospholipids in biological membranes was found to change with temperature. An increase in the proportion of unsaturated and long chain fatty acids was found to lower the gel-liquid crystalline phase transition temperature (Tm) of lipids and affect membrane fluidity (Russel, 1990). A decrease in growth temperature resulted in

changes in fatty acid synthesis in micro-organisms (Russel, 1990). In fish, a period of cold acclimation was found to affect the relative proportion of individual phospholipids and the composition and distribution of fatty acids within the phospholipid molecules (Sargent *et al.*, 1989). It was assumed that the cold induced changes were brought about by several mechanisms acting in conjunction, one of them being the increased activities of fatty acid desaturases. In the present paper, the total amount of fatty acids increased significantly during an acclimation period at 10 °C, which was not due to the retention of algae in the gut. Some changes were also noticed in the fatty acid distribution of the accumulated lipids. While a decrease was found in 16:0, 18:1 ω9, 18:1 ω7 and 18:2, an increase was noticed in 16:1 and 20:5 ω3. The origin of 20:5 ω3 may well be in the *Nannochloropsis*, which was used as food for the rotifers. The results suggest that rotifers that were incubated for more than 24 h contain significantly more 20:5 ω3, which is one of the essential fatty acids of marine fish larvae. The possibility of changes in the synthetic pathways of lipids during an acclimation period at 10 °C is currently being investigated.

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