マイワシおよびマサバの死後硬直の進行とATP分解,乳酸蓄積の関係

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Rigor-Mortis Progress of Sardine and Mackerel in Association with ATP Degradation and Lactate Accumulation

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The progress of rigor-mortis was followed together with ATP degradation and lactate accumulation during storage at 0 and 10°C of sardine and mackerel spiked at the brain. Both sardine and mackerel showed rapid progress of rigor-mortis, attaining a full-rigor state at a few hours after death. Although rigor-mortis exhibited a large individual variation, its rate was apparently slower with samples stored at 10°C than with those stored at 0°C for both species. ATP and lactate concentrations in sardine muscle were relatively constant a few hours after death irrespective of the storage temperature, in contrast with rigor-mortis progress. Therefore, many sardine samples exhibited a high rate of rigor-mortis even when they maintained a high concentration of ATP, especially with those stored at 0°C. Mackerel gave rise to a more reasonable relationship between rigor-mortis progress and ATP degradation than did sardine. Correlation of ATP content with that of lactate did not markedly differ from each other between samples stored at 0 and 10°C and was calculated to be around $r=-0.8$ for both sardine and mackerel. It was noted that sardine and mackerel relaxed from a rigor-state at a few hours after spiking when stored at 10°C.

In mammals, the muscle tissue exhibits dramatic metabolic changes after death which differ remarkably from those in a living state. One of these changes is ATP depletion accompanied by rigor-mortis.[1] The ATP concentration is, however, maintained at a certain level for a short period. During this period, creatine phosphate, another high-energy phosphate compound, donates its phosphate group to ADP by enzymatic reaction with creatine kinase to regenerate ATP. It is no doubt that the source of most post-mortem energy under anerobic conditions is glycogen breakdown through glycolysis with simultaneous accumulation of lactate.

The progress of rigor-mortis in association with ATP depletion and lactate accumulation is dependent upon temperature. Locker and Hagyard[2] and Cassens and Newbold[3] reported that beef muscle showed the longer delay phase of rigor-mortis when kept at 15–20°C than at lower temperatures including 0°C. This phenomenon was termed cold shortening and was suggested to be provoked by the release of Ca^{2+} from sarcoplasmic reticulum or mitochondria.[4]

Fish muscle also follows a post-mortem process similar to that of mammals.[5] Recently, Iwamoto *et al.*[6] have found that the ATP degradation rate of spiked plaice was clearly slower at 5–15°C than at 0°C, resulting in retardation of rigor-mortis onset at the former temperatures. This phenomenon was also suggested to be caused by the decrease of Ca^{2+} uptake ability of fish sarcoplasmic reticulum at 0°C.[7]

It is well known that pelagic fishes such as sardine and mackerel have the muscle pH decreasing very quickly after death, easily reaching below 6, in contrast to the bottom fishes.[8–10] However, it seems not easy to follow post-mortem changes in the muscle of pelagic fishes because of their rapid metabolism after death, hence it has been left ambiguous whether they would follow the same rigor-mortis process as that of the bottom fishes.

We have recently had the opportunity to deal

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with live specimens of both sardine and mackerel. The objective of the present study was to follow the rigor-mortis progress of spiked sardine and mackerel specimens during storage at 0 and 10°C in association with ATP degradation and lactate accumulation.

Materials and Methods

Materials

Live specimens of sardine *Sardinops melanosticta* (14.8–21.2 cm body length) and mackerel *Pneumatophorus japonicus japonicus* (20.7–27.5 cm body length) were captured off Shimane Prefecture. They were transported to the Shimane Prefectural Fisheries Experimental Station in a 1 ton seawater tank by supplying O_2 gas and sacrificed by cutting the head (cranial spiking) while alive. It took around 5 h to transport them from the fishing ground to the station. The water temperature was around 20°C during transportation, the same temperature as that of environmental seawater.

Sardine specimens thus treated were divided into two groups each of fifty samples and stored at 0 and 10°C. At 1-h time intervals, five samples were taken out from each 0°C- and 10°C-storage container and subjected to measurements for the rigor index, ATP content, lactate content and pH as described in the following sections. After each series of measurements, these sardine samples were omitted and new samples taken out for the next experiment.

In the case of mackerel, two groups each with seven specimens were stored at 0 and 10°C. At 1- or 2-h time intervals, four samples out of seven were taken out and subjected to measurements of rigor index, ATP content and lactate content, while other three samples were used to measure pH continuously. After each series of measurement, samples were returned to the same containers and used for the next serial experiments.

Analytical Methods

1. Rigor index Rigor-index was measured by the method of Bito *et al.*[11] and used as a parameter of the stage of rigor-mortis.[6]

2. ATP content At the time when the rigor index was measured, 2 g of ordinary dorsal muscle was taken from the same sample and treated with 5 m*l* of 10% perchloric acid. The mixture was homogenized and centrifuged at 3,000 rpm for 3 min. The resulting supernatant was adjusted pH to 6.5–6.8 and the precipitate formed was removed by centrifugation under the same conditions as above. The supernatant thus obtained was analyzed for ATP content by high performance liquid chromatography as reported previously.[6]

3. Lactate content Lactate in the perchloric acid extract was determined by the method of Barker and Summerson.[12]

4. pH Five-grams muscle was homogenized in 2 volume of cold 2 mM iodoacetic acid sodium salt and measured for pH with a Horiba F-8AT pH meter.

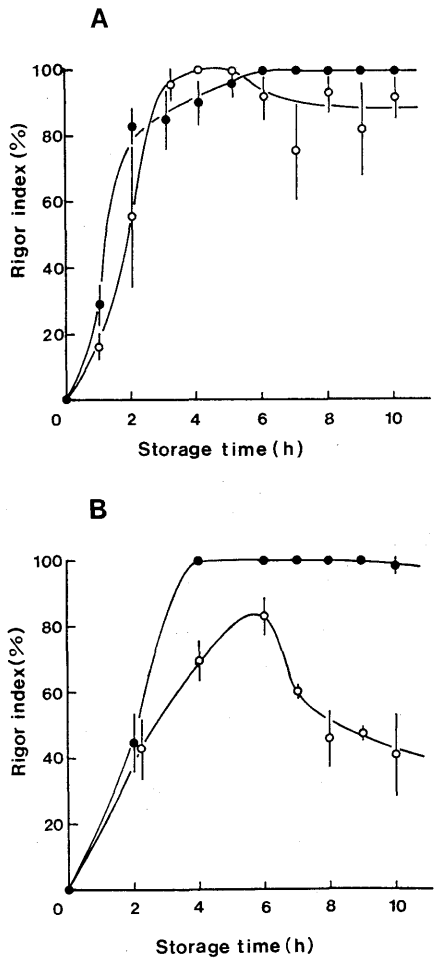

Fig. 1. The progress of rigor-mortis as expressed by the rigor index for sardine (A) and mackerel (B) stored at 0 (●) and 10°C (○). Data are given by mean±standard error (S.E.) from five specimens for sardine and four for mackerel.

Results

Progress of Rigor-Mortis

Both spiked sardine and mackerel showed rapid progress of rigor-mortis as shown in Fig. 1. Although rigor index was in large values of standard error for sardine, especially with samples stored at 10°C, the progress of rigor-mortis was apparently faster at 0°C than at 10°C (Fig. 1A). The rigor index was measured to be around 80% at 0°C at 2 h after spiking, whereas around 60% at 10°C. In the 0°C-storage, the rigor index continued to increase after 2 h and exhibited a full-rigor state (100% rigor index) after 6 h. On the other hand, sardine samples stored at 10°C started decreasing in rigor index after 5 h, suggesting the occurrence of relaxation from rigor.

The progress of rigor-mortis of mackerel was somewhat slower, irrespective of the storage temperature, than that of sardine as shown in Fig. 1B. The storage at 0°C led to the faster rate of rigor-mortis progress again than that at 10°C. It was surprising enough to observe that the rigor index reached a maximum of about 80%, not 100%, at 6 h after spiking, and then decreased rapidly to around 40% after 10 h when marckerel samples were stored at 10°C. Mackerel stored at 0°C were in a full-rigor state after 4 h and exhibited no significant change in rigor index until 10 h after spiking.

ATP Degradation

The ATP content of both sardine and mackerel were determined to be around 6 μmol/g immediately after spiking as shown in Fig. 2.

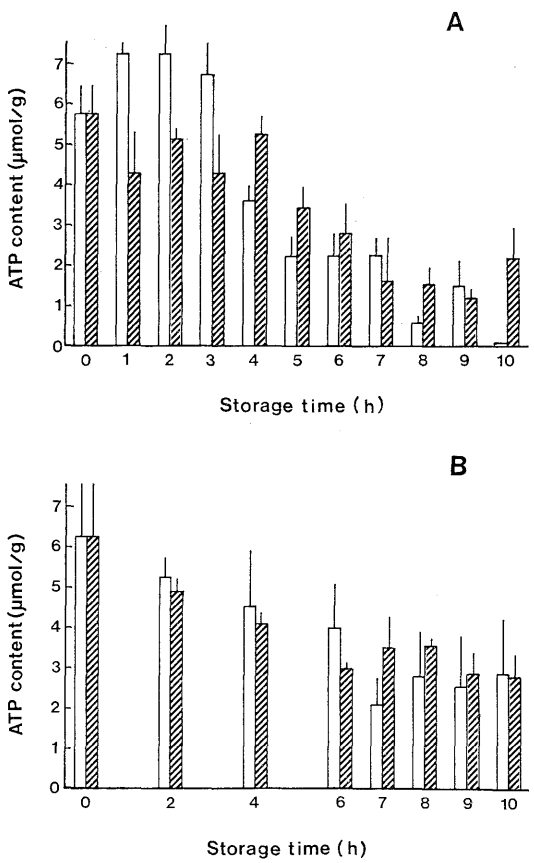

Fig. 2. Changes in ATP content of sardine (A) and mackerel (B) muscles during storage at 0 (□) and 10°C (■). Data are given by mean±S.E. from five specimens for sardine and four for mackerel.

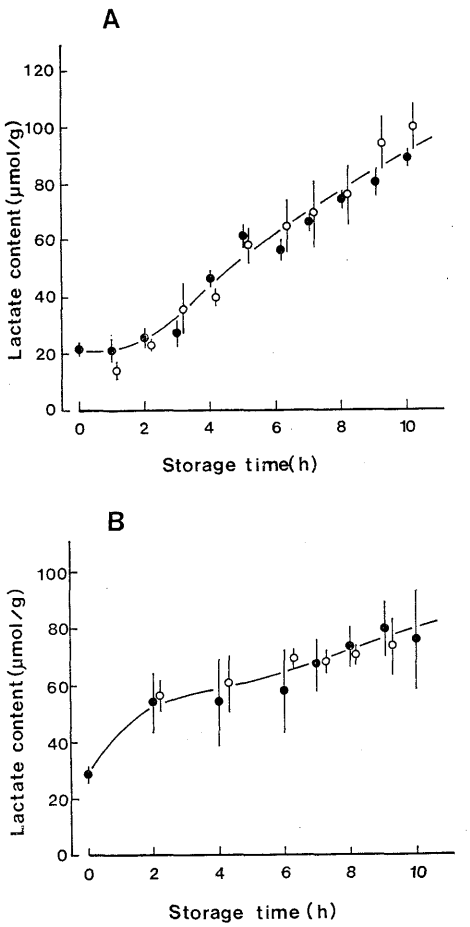

Fig. 3. Changes in lactate content of sardine (A) and mackerel (B) muscles during storage at 0 (●) and 10°C (○). Data are given by mean± S.E. from five specimens for sardine and four for mackerel.

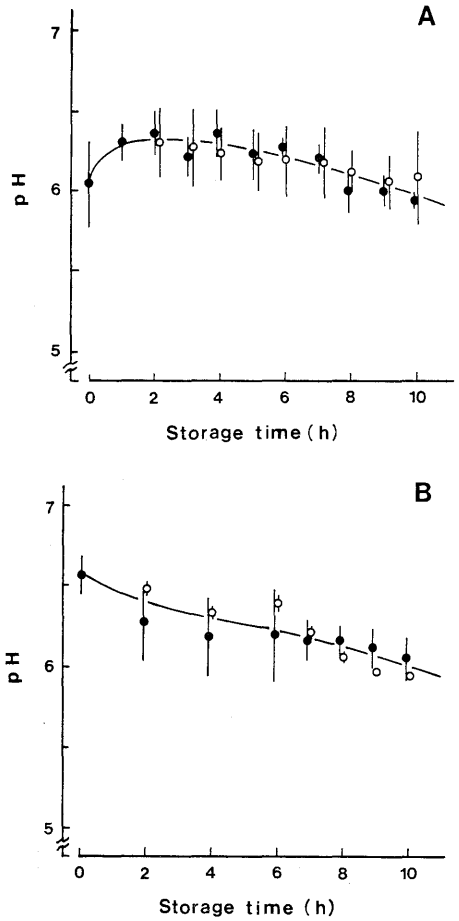

Fig. 4. Changes in the pH of sardine (A) and mackerel (B) muscles during storage at 0 (●) and 10°C (○). Data are given by mean±S.E. from five specimens for sardine and three for mackerel.

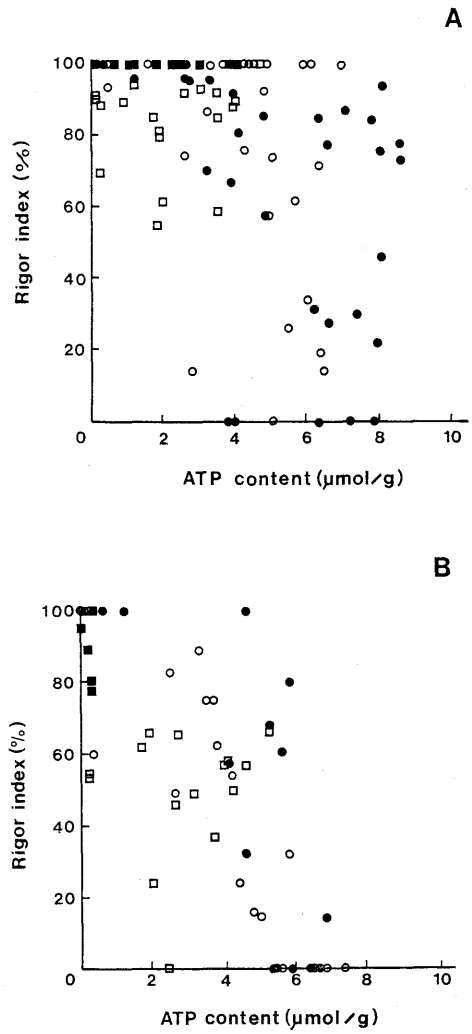

Fig. 5. Relationship between the rigor index and ATP content for sardine (A) and mackerel (B) muscles stored at 0 (●, ■) and 10°C (○, □). Circle and square symbols represent samples before and in, and after full-rigor, respectively; A full-rigor state was attained at 3 h with sardine and 6 h with mackerel, after spiking.

In the case of sardine, the samples stored at 10°C maintained roughly constant ATP concentrations, 4–6 μmol/g, until 4 h, whereas the ATP concentration of those stored at 0°C started decreasing rapidly after 3 h and reached around 2 μmol/g at 5 h after death (Fig. 2A). On the other hand, 10°C-storage resulted in a gradual decrease in the ATP concentration after 5 h up to around 2 μmol/g at 7 h after death.

As for mackerel, there was observed no significant difference in the decay of ATP between samples stored at 0 and 10°C (Fig. 2B). The ATP concentration was around 6 μmol/g at the start of storage and then decreased gradually to around 3 μmol/g after 7 h. However, no obvious retardation of ATP depletion in mackerel muscle occurred within a few hours after death, contrasting well with sardine samples.

Lactate Accumulation

The accumulation rate of lactate did not differ remarkably between samples stored at 0 and 10°C for both sardine and mackerel as shown in Fig. 3. There were, however, some differences observed between these two species in accordance with the ATP degradation profile. Sardine apparently exhibited no accumulation of lactate during 2 h after spiking (Fig. 3A),

while mackerel accumulated a considerable amount of lactate during the same period (Fig. 3B). Another large difference was observed in lactate accumulation between sardines and mackerel after rigor index reached the maximum. Lactate in sardine samples was accumulated at a relatively high rate and its content was determined to be around 80 μmol/g after 8 h (Fig. 3A). On the other hand, lactate accmulation in mackerel muscle was at a slow rate after the rigor index came close to the maximum and was only about 20 μmol/g between 2 and 10 h after spiking, irrespective of storage temperature.

pH Change

Muscle pH tended to decrease during storage for both sardine and mackerel irrespective of storage temperature, although the extent of the decrease was smaller than expected (Fig. 4). In the case of sardine, it has been reported that live specimens have a muscle pH above 7, which decreases rapidly after death.[8] In 3 h after death, some specimens of sardine and mackerel were reported to exhibit the muscle pH below 6.[8,9] In the present study, however, the specimens having the muscle pH below 6 were hardly observed even after 10-h storage at 0 and 10°C. One of the possibilities may exist in the different conditions to sacrifice samples; Spiking was applied in the present study while struggling may be taken place before death in the reported experiments. It remains ambiguous why the muscle pH increased from 6.1 at the start of storage to 6.3–6.4 after a few hours. However, its initial low pH, compared with previously reported values, could be due to the fatigued state of the present sardine samples, since it took 5 h to transport them from the fishing ground to the laboratory.

Relationship between Rigor Index and ATP Content

The relationship between the rigor index and ATP content was examined for both sardine and mackerel. Since sardine and mackerel stored at 10°C relaxed from a rigor state, the relationship between these two factors was distinguished for samples before and after full-rigor as shown in Fig. 5. No good correlation was observed with sardine, especially with samples stored at 0°C. They attained a high rigor index even when the ATP content remained high, 6–8 μmol/g. Sardine stored at 10°C seemed to exhibit a more reasonable relationship. In the case of mackerel, samples stored at 0°C again exhibited high values of rigor index with high content of ATP, compared with those stored at 10°C. Correlations were, however, fairly well for both groups before full-rigor; $r=-0.753$ for samples stored at 0°C and $r=-0.758$ for those stored at 10°C. Most mackerel specimens in full-rigor at 0°C

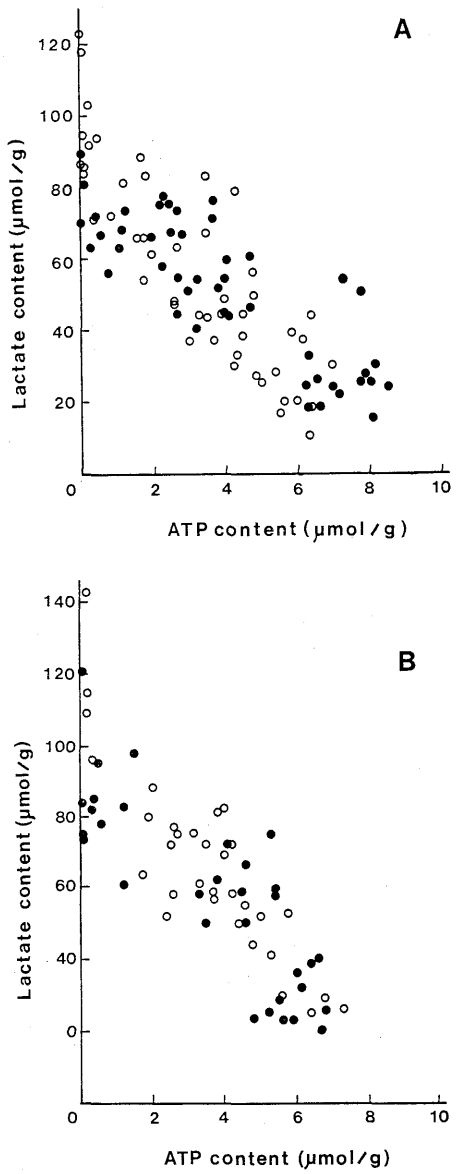

Fig. 6. Relationship between lactate and ATP contents for sardine (A) and mackerel (B) muscles stored at 0 (●) and 10°C (○).

Table 1. Relationship among ATP degradation, lactate accumulation and pH in sardine and mackerel muscles

Species	Correlation	Correlation coefficient
Sardine	ATV vs Lactate	−0.802
	pH vs Lactate	−0.745
Mackerel	ATP vs Lactate	−8.835

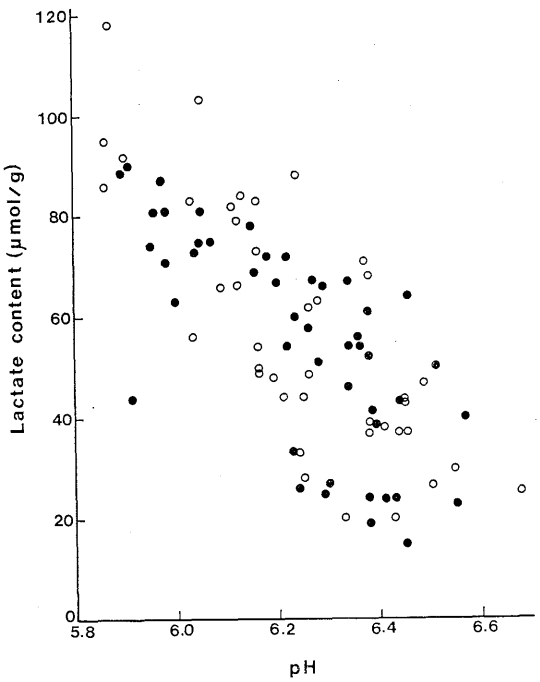

Fig. 7. Relationship between lactate content and pH for sardine muscles stored at 0 (●) and 10°C (○).

consumed ATP completely, thus locating closed square symbols for 16 samples in a small area, an upper left extreme of Fig. 5B.

Relationship between Lactate and ATP Content

In Fig. 6 is shown the relationship between lactate content and ATP. Lactate was gradually accumulated in accordance with ATP degradation in the muscle. Correlation coefficients were calculated from these data and are shown in Table 1. As seen in this table, a fairly good correlation exists between lactate and ATP contents for both sardine and mackerel, although no significant difference was recognized between samples stored at 0 and 10°C and between these two species.

Relationship between Lactate Content and pH

Since the lactate content could not be determined with the same sample as for pH measurement in the case of mackerel, the relationship between them was examined only for sardine. As shown in Fig. 7, there was observed some good correlation, although no significant difference existed between samples stored at 0 and 10°C. The correlation coefficient was calculated to be $r=-0.745$ (Table 1) and rather fairly lower than those reported before, $-0.908\sim-0.941$, for sardine stored at 0°C.[10]

Discussion

Several fish species such as tilapia, red sea bream and plaice have recently been reported to have had their pre-rigor periods shortened when stored at 0°C.[6,13-15] However, some kinds of fish such as cod[16] dolphin fish[17] and grunt[17] have been considered to retard the onset of rigor-mortis at 0°C when compared with the case where the fish was stored at higher temperatures. Therefore, there still remains a controversial relationship between fish rigor-mortis progress and storage temperature. To cope with this discrepancy, the present study was performed using representative pelagic fishes such as sardines and mackerel which exhibit a high activity in swimming, as do dolphin fish and grunt. As is well known, pelagic fishes usually have a very high metabolic rate. Correspondingly, the post-mortem progress rate was found to be extremely high in the present study when compared with bottom fishes[6,13-15] and a full-rigor state was attained after a few hours during 0- and 10°C-storage even by spiking the brain (Fig. 1). Relaxation from rigor was also observed within a short storage period, several hours after death when sample fish were stored at 10°C. However, 10°C-storage apparently resulted in the retardation of rigor-mortis onset for both sardine and mackerel as in the case of red sea bream[14,15] and plaice.[5,6] It should be noted that the present mackerel samples did not reach a full-rigor state. The mechanisms involved have been explained by the extremely poor ability of Ca^{2+} uptake by fish sarcoplasmic reticulum at 0°C.[7] The same mechanisms could be being adopted in the case of sardine and mackerel too.

According to the progress of rigor-mortis, the ATP contents of both sardine and mackerel decreased, although their decreasing profiles were different from each other (Fig. 2). In the case of sardine, the ATP content was almost constant while rigor-mortis proceeded rapidly during the early stage after death. On the other hand, the ATP content in mackerel muscle decreased in accordance with rigor-mortis progress (Fig. 4) and agreed well with plaice,[6] thus exhibiting a better correlation between these two values than with sardine (Fig. 4B). One of the reasons for this phenomenon might be derived from the difficulties in spiking sardine samples while they are quiescent, since post-mortem changes generally accelerate when the fish ceases on struggling.[18,19]

Even after the muscle has attained a full rigor-state, the ATP content still kept decreasing for both pelagic fishes. According to this ATP degradation, lactate was gradually accumulated, and the rate was much higher in sardine than in mackerel (Fig. 3). However, the muscle pH changed to a lesser extent. Therefore, the relationship between the lactate content and the muscle pH for sardine was not so high as expected, especially for samples stored at 0°C (Fig. 6). Abnormal post-mortem changes were also found in a high rate of rigor-mortis progress of sardine samples stored at 0°C while they contained high amount of ATP. Spiked plaice has been reported to attain a full-rigor state when the ATP concentration decreases below 1 μmol/g.[6]

Creatine phosphate is a high-energy phosphate compound and its breakdown is known to precede the decrease of ATP in fish muscle.[5] Although creatine phosphate content was not measured in the present study, it is probable that this compound disappears very quickly after the death of sardine and mackerel.

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