底曳網で漁獲したナミクダヒゲエビの漁獲後のATP関連化合物の濃度変化

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【Research Article】

Post-harvest Change in ATP-related Compounds Concentration in the Tail Muscle of Deep-water Mud Shrimp *Solenocera melantho* Caught by Bottom Trawl

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Abstract

Post-harvest freshness of trawl-caught deep-water mud shrimp *Solenocera melantho* was assessed by measuring the concentration of ATP-related compounds in the tail muscle. In the field study, trawls were towed touching the seabed to catch the shrimps for 15 min, and then the tows were continued off the seabed for 35 and 65 min to prevent more shrimps being caught and to simulate trawling with *S. melantho* in the codend at the towing speed of 2.0 knots. Underwater video camera was connected to record the behavior of the catches inside the codend during towing. *S. melantho* were stored on ice for 5 days after harvesting. *S. melantho* were observed to move toward to the codend, and continued to be stationary without moving after reaching the codend during the trawling. The concentrations of ATP-related compounds during the storage were not different significantly in the tow duration of 35 and 65 min. ATP were found when the catches were retrieved from the codend on the deck of the vessel. Decomposition speed of ATP is high, and AMP and IMP were accumulated during the storage. The onset of HxR was observed after 2-day storage, and Hx was not found during the storage of 5 days.

1. Introduction

The demand for fresh seafood with a guarantee of high quality has increased not only in Japan but also globally. The catching method, catching procedure, and post-harvest treatment greatly impact on fish quality. Changes in quality that occur during storage are influenced by the storage period and conditions but are also affected by the catching methods used.[1] Therefore, it is critical to evaluate capture conditions and subsequent storage conditions to ensure that fresh products are delivered to market.[2]

The shrimp trawl fishery forms one of the major fisheries worldwide, and mostly small-scale fisheries, such as beam trawls and otter trawls are used.[3] Trawling is extremely stressful for animals and can cause severe disturbances to metabolic and respiratory systems. Various factors, such as changes in water temperature, salinity, pressure and light intensity can potentially cause injuries.[4] During trawling, the catches are exposed to numerous stresses in the net that result in mechanical and physiological damage, which ultimately impact on product quality. Tow duration, towing speed, catch composition, and post-catch handling procedures affect the survival rate, and air temperature and duration of air exposure have negative effects on the vitality of shrimp.[5] Fish mortality after harvesting differ according to the catching procedure, and the mortality rate was shown to increase with an increase in the extent of physical damage, which illustrated the negative impact of damage on post-capture survival.[2] Uncontrolled fishery-related factors occur from the net entrance to codend confinement, such as physical contact with the netting and exposure to sediment lifted by the otter boards and footrope during the towing, which are likely to play a significant role in subsequent mortality rates.[6] Shorter tow durations have been shown to result in a higher proportion of alive fish after capture with a trawl.[7] However, the tow duration of bottom trawls differs among regions.[8]

The concentrations of ATP-related compounds are used as chemical indexes of freshness. Fish muscles use chemical energy in the form of ATP and regenerate ATP as it is consumed. However, the muscles lose the ability to regenerate ATP after death and the ATP decomposes as follows[9]:

ATP (adenosine triphosphate)
→ ADP (adenosine diphosphate)
→ AMP (adenosine monophosphate)
→ IMP (inosine monophosphate)
→ HxR (inosine)
→ Hx (hypoxanthine)

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The degradation behavior of ATP-related compounds during the generation of energy in live fishes may differ according to the species and habitat temperature.[10] *K*-values, defined as the ratio of HxR plus Hx for total ATP-related compounds, can be calculated from the concentration of ATP and its breakdown products,[11] and fluctuate and differ among fishing methods and fish species. *K*-values of trawl-caught fish from prolonged tow durations can vary widely.[8] The staying period of fishes, i.e., from the time they enter the net during towing until they are hauled up on to the fishing boat, is expected to affect the deterioration of freshness. *K*-values of fish caught by trawl and transported to the fish market were found to be positively correlated with the number of days that the fishing boat was at sea.[12] The post-catch condition of freshness affects the degree of increase in *K*-values during transportation and in marketing.[13] However, there are some cases that freshness is hard to be evaluated by *K*-value, because the difference in composition of ATP, ADP, AMP, and IMP is not reflected in *K*-value.[14, 15] For this reason, the concentration of each of ATP-related compounds is important to evaluate the freshness.

Deep-water mud shrimp (*Solenocera melantho*, hereafter referred to as "mud shrimp") are widely distributed in the Indo-West Pacific and serve as an important species in commercial fisheries in Japan.[16, 17] The main aim of the present study was to examine the post-harvest freshness of trawl-caught mud shrimps during the storage of 5 days on ice by assessing the concentration of ATP-related compounds in their tail muscles.

2. Materials and methods

The trawl used in the present study was made of polyethylene netting, had a total length of 23.5 m, and was equipped with wing canvas kites made of polyester cloth (size: 1.0×1.0 m, thickness: 1 mm) at both wings which function is to open the trawl mouth horizontally. The mesh sizes of the body net and codend were 37.9 mm and 20.2 mm, respectively. The attack angle between the canvas kites and the towing direction was 22° and the distance between both canvas kites was 7.2 m at a towing speed of 2.0 knots (approximately 1.03 m s^{-1}).[18]

The research operations for trawling were conducted by Nansei-maru, a research vessel of Kagoshima University, in the middle of Kagoshima Bay at depth of 220–223 m on 4th October and 22nd November 2013. Trawling included three kinds of experimental tow described as follow, because tow duration is approximately 40 min to 1 h in commercial trawl which main target are shrimps.[19, 20]

Trawls were towed touching the seabed, shrimps were caught, and then the tows were continued off the seabed to prevent more shrimps being caught, which simulated bottom trawling with the mud shrimps in the codend. The implementation of a standard towing time of 15 min is advisable to reduce the frequency of unnecessarily large catches.[21] Excessive catch sizes reduce the feasibly of effectively sorting the catches.[22, 23] Therefore, we used a tow duration of 15 min to catch the mud shrimps in the present study.

Three kinds of experimental tow were used; In the first operation, the net was towed on the seabed for 15 min with a warp length of 900 m (defined as the "control"), in which the trawl was towed while touching the seabed and shrimp and fish were caught. In the second operation, the net was towed on the seabed for 15 min (warp length of 900 m), then hauled up and towed off the seabed for 65 min with a reduced warp length of 400 m to prevent any shrimp from being caught and to simulate bottom trawling with the shrimps inside the codend (defined as "control + 65 min"). In the third operation, the net was towed on the seabed for 15 min, then hauled up and towed off the seabed for 35 min with a reduced warp length of 400 m (defined as "control + 35 min").

Towing speed was fixed at 2.0 knots but was reduced to less than 2.0 knots during the hauling-up process to reduce the warp length. During the operations, the sea conditions were calm, i.e., no waves or wind, and almost no current in Kagoshima Bay which is a semi-closed deep-water. Therefore, tow duration and towing speed were precisely controlled. Depth and temperature sensors (DST milli-F, Star-Oddi) were connected to the middle of the footrope to measure the depth of the footrope and seawater temperature at 1 s intervals during towing. Effective tow durations were calculated from the trawl depth profiles derived from the depth and temperature sensor measurements.[22]

Underwater video camera equipped with two LED lights (Trawl camera, JT electric Co., Ltd.) was connected to the front and on the upper part of the codend to record the behavior of the catches inside the codend during towing (**Fig. 1**).

After towing, the net was hauled up onto the vessel deck, but the codend was directly transferred from the sea to a container with seawater and ice to prevent the catches from being ex-

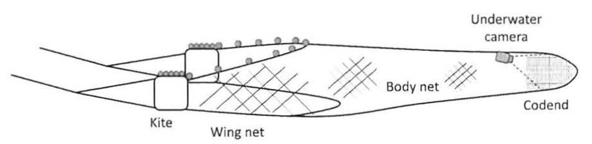

Fig. 1 Schematic drawings of trawl used in the experiments. Underwater video camera was connected to the front and on the upper part of the codend to record the behavior of the catches inside the codend.

posed to air, because air-exposure during sorting is very stressful.[4] All mud shrimps were still alive when the catches were taken from the codend, and 10 individuals were immediately and randomly selected from the container for the extraction of ATP-related compounds onboard the research vessel. To minimize stress, selected alive mud shrimps were immediately sacrificed by rapidly twisting their bodies before the extraction of ATP-related compounds. The remaining alive mud shrimps were transferred into a styrene foam box and stored on ice in the laboratory. 10 Individuals were taken from the styrene foam box for the extraction of ATP-related compounds at the storage of 1, 3, and 5 days in October experiments, and at the storage of 1 and 2 days in November experiments which complement data missing in October experiments.

ATP-related compounds were extracted from the tail muscles of mud shrimps using the methods stipulated by Hu *et al.*[24] The procedure of extracting ATP-related compounds is as follows: The body temperature of the mud shrimps and temperature in the styrene foam box were measured using a stick thermometer (TT-508, Tanita Co., Ltd.). The tail muscle samples of 1 g which internal organs and shells of tail muscle had been removed[25] were smashed using a glass rod in 10 volumes of 5% perchloric acid (PCA). The PCA used for the extraction of ATP and its derivatives was neutralized with KOH and the pH of the mixture was adjusted to 2.0–3.5. The volume of the solution was increased to 20 mL, and the supernatant was obtained by filtering the mixture through a membrane with 0.45 μm pore size to remove the small floating aggregates. The filtrate was further neutralized by adding 20 mM phosphate buffer (pH 7.5) for storage of the sample.

The sample solutions of extracted ATP-related compounds were kept frozen at -80°C at a freezer. The concentrations of the ATP-related compounds of prepared sample solutions were determined within 1 month using high-performance liquid chromatography (HPLC).

3. Results

The depth of the net and seawater temperature during towing in October and November experiments are shown in **Fig. 2-1** and **Fig. 2-2**, respectively. The depth of the "control" operation was revealed to be 221 m, which was same as the water depth of the operation site. The changing depth of the footrope in October experiments was about 180 m at the beginning of the towing above the seabed, and the depth decreased with towing time to a constant in the second and third operations. The sensor measurements estimated that the trawl was towed on the seabed for 15 min in the first operation, control + 65 min in the second operation, and con-

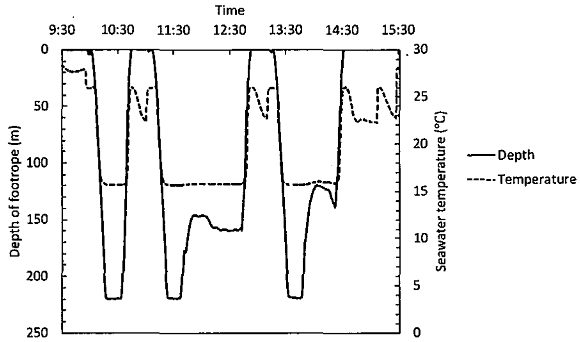

Fig. 2-1 Changes in the depth of the footrope and the seawater temperature during towing the net in October experiments.

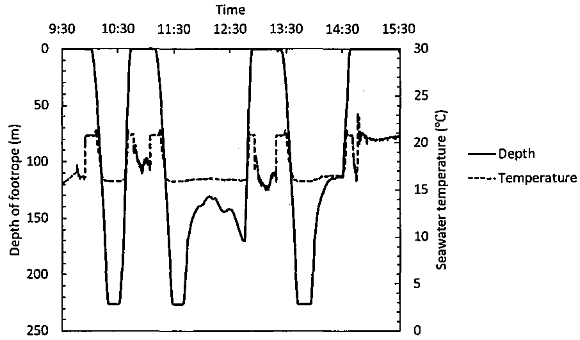

Fig. 2-2 Changes in the depth of the footrope and the seawater temperature during towing the net in November experiments.

trol + 35 min in the third operation. Seawater temperature ranged between 15.7 and 16.1°C during towing touching and off the seabed. The changing depth of footrope in November experiments was almost same as that in October experiments. Change of seawater temperature during towing in November experiments was from 15.9 to 16.5°C. Thus, the influence of changing seawater temperature on the freshness of mud shrimps was considered to be negligible in the present study.

The catch compositions for each operation are shown in **Table 1**. The total catch weight of the first, second, and third operations varied between 12.63 and 27.67 kg. The weight of mud shrimps was 0.23–0.93 kg, and accounted for 1.7–7.4% of the total catch weight.

Images inside the codend recorded with the underwater camera during towing are shown in **Fig. 3**. The mud shrimps were observed to move toward to the codend while trawl was towed touching the seabed. Various kinds of fish were seen swimming inside the codend or escaping from the field of view for the camera, however, all shrimps could not swim and continued to be stationary without moving after reaching the codend.

All mud shrimps showed strong signs of life with tail-flipping and moving their limbs immediately after being retrieved from the codend on the deck of the vessel. The

Table 1 Catch species and weight for each experimental tow.

Species	Catch weight (kg)					
	Oct.			Nov.		
	Cont.	+35min	+65min	Cont.	+35min	+65min
Caelorinchus jordani	3.70	2.10	3.60	0.80	0.90	0.50
Hoplostethus crassispinus	3.40	0.31	2.40	2.60	0.92	0.50
Conger myriaster	1.90	0.90	0.82	0.40	0.30	0.60
Malakichthys griseus	0.42	0.32	0.46	-	0.04	-
Cubiceps aquamiceps	0.28	0.00	0.27	-	0.10	-
Physiculus chigodarana	0.10	0.20	0	-	0.04	0.14
Hoplobrotula armata	-	0.17	0.37	0.30	-	-
Lepidotrigla hime	-	-	-	-	0.02	-
Solenocera melantho	0.82	0.93	0.83	0.30	0.42	0.23
Other shrimps	16.10	7.70	13.40	13.50	12.40	10.10
Ibacuc ciliatus	-	-	-	0.19	0.10	0.38
Holothuroidea sp.	0.95	-	1.01	-	-	-
Asteroidea	-	-	-	-	0.2	0.26
	27.67	12.63	23.16	18.09	15.44	12.71

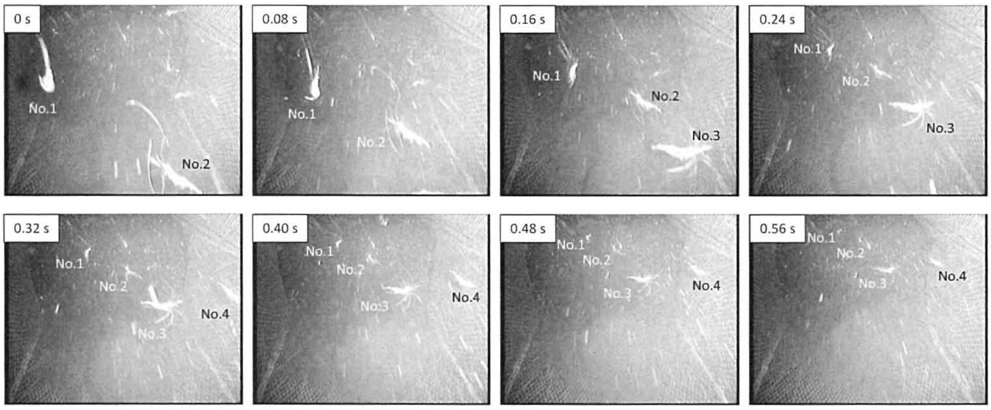

Fig. 3 Captured images by intervals of 0.08 s from video clips recorded with underwater camera. The shrimps were moved toward to the codend during towing touching the seabed. The body of the shrimps reflect the LED light mounted to the underwater camera.

mean body weight of mud shrimps used for ATP-related compound analysis was 21.30 (S.D. $\pm$6.81) g, and the mean body weight of mud shrimps did not differ significantly between experimental operations (Steel's test, $P>0.05$). Both the body temperature of the mud shrimps and the temperature in the storage styrene foam boxes with ice were maintained from -0.2 to -0.1°C during the storage. The concentrations of ATP-related compounds in the tail muscles of mud shrimps are shown in **Fig. 4-1–4-5**.

ATP-related compounds, except HxR and Hx, were detected in the tail muscles of sampled mud shrimps immediately after being retrieved from the codend. Significant differences in concentrations of ATP, ADP, AMP, and IMP were not found with tow durations of control, control + 35 min, and control + 65 min during the storage (Steel's test, $P>0.05$) (**Fig. 4-1**). The ATP disappeared, the concentrations of ADP decreased, and the concentrations of AMP and IMP increased in all operations after 1 day of storage (**Fig. 4-2**). The onset of HxR was observed after 2-day storage with tow durations of control and control + 35 min operations (**Fig. 4-3**). HxR were found in all samples after 5 days of storage, however significant differences were not detected among the operations among three kinds of operations (Steel's test, $P>0.05$) (**Fig. 4-5**). The concentrations of AMP increased in the all samples from the three kinds of operations from immediately after harvesting to 1 day of storage. The AMP concentrations were commonly observed to be the highest among the concentrations of ATP-related compounds during the storage.

4. Discussion

1) Evaluation of shrimp freshness

It is important to assess the effects of fishing operation conditions on the freshness of the catches to improve procedures, storage, and transport protocols and thus produce and

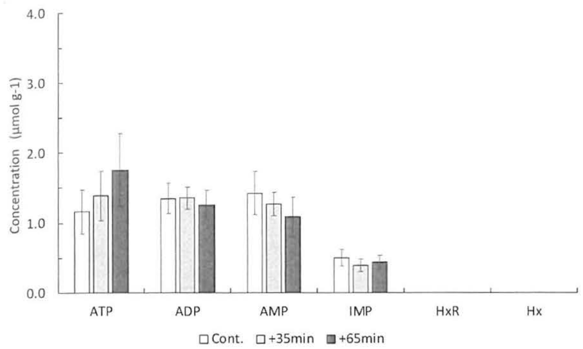

Fig. 4-1 Concentrations of ATP-related compounds in the tail muscles of trawl-caught deep-water mud shrimp immediately after retrieving from the codend in October and November experiments.

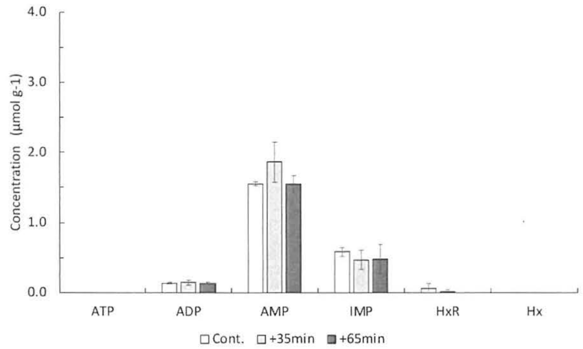

Fig. 4-3 Concentrations of ATP-related compounds in the tail muscles of trawl-caught deep-water mud shrimp after 2 days of storage in November experiments.

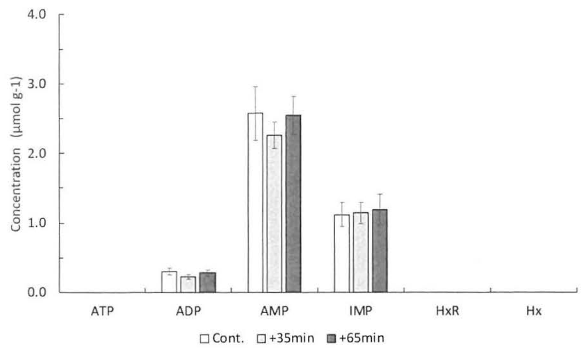

Fig. 4-2 Concentrations of ATP-related compounds in the tail muscles of trawl-caught deep-water mud shrimp after 1 day of storage in October and November experiments.

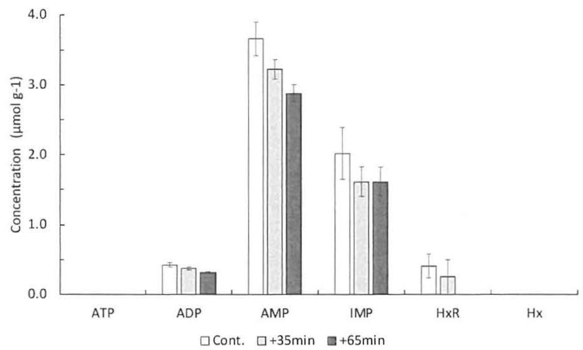

Fig. 4-4 Concentrations of ATP-related compounds in the tail muscles of trawl-caught deep-water mud shrimp after 3 days of storage in October experiments.

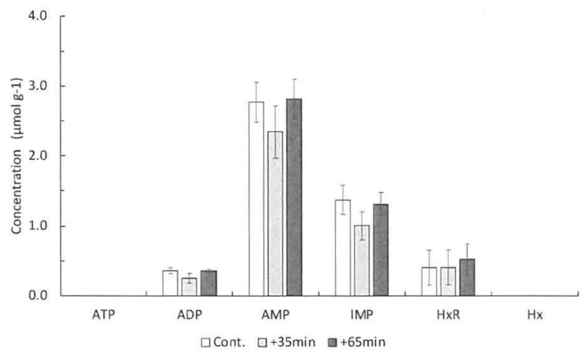

Fig. 4-5 Concentrations of ATP-related compounds in the tail muscles of trawl-caught deep-water mud shrimp after 5 days of storage in October experiments.

supply fresh seafood. In the present study, we examined the change in the freshness of the mud shrimps caught by trawl; immediately after being retrieved from the codend and over 5 days of storage on ice.

In previous studies, the freshness of trawl-caught fish from prolonged tow durations varied widely from nearly 0% to an extremely high level of K-value, because tow duration is defined as the period that the trawl is towed and continually touching the seabed.[8] Thus, the duration from when the fish enter the codend until hauling up is not necessarily the same as the tow duration of a trawl, for example, if the fish enter the codend just before the net is hauled up, the duration that the fish spent at the codend would be shorter than the tow duration. Therefore, the estimated freshness might be lower than the real value. This was an issue that we were not able to control, because we could not correctly identify the point at which each sampled shrimp entered the net and reached the codend, and thus the durations that the individuals were exposed to the stress of towing were unknown. In efforts to minimize such a problem, the effective tow duration was precisely controlled with a period of the trawl touching the seabed during towing in order to catch the mud shrimps and then followed by towing of the trawl above the seabed to simulate trawling with the mud shrimps inside the codend.

Seafood freshness is determined by means of sensory and chemical analysis and microbial population evaluation.[26] In the present study, the freshness of mud shrimps was assessed by measuring nucleotide breakdown products.[27] Fish muscles use chemical energy in the form of ATP and regenerate ATP as it is consumed. However, muscles lose the ability to regenerate ATP after death[9] and most of the enzymes involved in the breakdown of ATP to IMP are autolytic. The degradation of IMP to HxR and then to Hx is mainly due to spoilage by enzymatic activity.[28]

The main ATP-related compounds in the muscles of trawl-caught Norway lobsters were ATP and AMP, and low concentrations of IMP and HxR were found immediately after harvesting.[29] ATP concentrations have been shown to decrease in the muscle tissue of trawl-caught Norway lobster during storage, while AMP concentrations increase to become the main ATP-related compounds.[30, 31] The main ATP-related compounds found in trawled Norway lobster was AMP, while tow duration (15, 60, and 150 min) did not affect the levels of ATP, ADP, and AMP.[31] In the present study, extraction of ATP-related compounds in the tail muscles of mud shrimps revealed that the ATP were found immediately after harvesting, and the contents of ATP disappeared and the concentration of ADP were low throughout the 5-day storage, whereas AMP levels were high; reflecting a rapid conversion of ATP into AMP. In addition, HxR was detected 2 days after storage and Hx was not found during the 5-day storage. Decomposition speed of ATP is high after harvesting, and AMP and IMP were accumulated during the storage on ice.[32, 33] Thus, our findings indicated that the decomposition speed of AMP and IMP was low in the tail muscle tissue of mud shrimps. The content of IMP contributes to taste of fish meat, especially in raw fish (*Sashimi*), because IMP is a constituent of the *Umami*-flavor, and whereas Hx accumulation is associated with a progressive loss of flavor and finally leads to a bitter taste.[34]

Changes in K-values, which serve as an index for estimating freshness, in response to tow duration revealed that more fish had higher K-values with longer tow durations. K-values vary greatly according to operation conditions, the fishing area, and fish species. Towing speed, seawater temperature, and total catch size are expected to be positively correlated with K-values.[8, 12, 13] Longer tow durations have been shown to result in larger catches but higher mortality rates.[35] The ratio of damaged shrimps has been shown to increase with longer tow durations.[31] However, in the present study, all post-harvest mud shrimps were still alive and the increase in the trawl period from 35 to 65 min did not cause a change in the concentrations of ATP-related compounds in the tail muscle.

The effect of trawling on animals is a result of a combination of different stressors that are applied almost simultaneously, for example, increased exercise due to the animals trying to escape from the net,[36] physical damage due to abrasion between animals and objects in the codend of the net, and changes in water pressure during towing occur simultaneously during trawl operations.[27] Norway lobsters respond to threatening situations by producing tail-flips which involves a series of rapid swimming movements. The normal towing speeds of commercial trawls range between 2 and 3 knots and exceed the average escape-swimming speed of Norway lobsters. Therefore, a high proportion of Norway lobsters that are directly in front of the net are likely to be caught irrespective of their initial orientation.[36] In the present study, the behaviors of mud shrimps inside the net were recorded using the underwater camera. The mud shrimps were carried toward the codend without swimming against the flow, and after reaching the codend, the mud shrimps remained stationary. It is probable that the cramped conditions within the codend may have restricted the movement of mud shrimps, and tow duration of the present study did not affect the concentration of ATP-related compound in the tail muscle.

In the present study, the tow duration for catching the mud shrimps was 15 min, however tow duration touching the seabed is approximately 40 min to 1 h in commercial trawl which main target are shrimps.[19, 20] Therefore, catch size is expected to be larger in commercial operations than in the present study. The codend circumference as well as mesh size play important roles in the catch selectivity. The codend forms a typical bulge during the towing, which expands to maximum diameter as the catch accumulates. A narrow codend expands to form a smaller bulge and it takes less time to achieve the maximum diameter.[37] The drag pressure against the catch is the predominant component of the codend drag.[38] In addition, the amount of trash fish increases as the mesh size of the codend decreases, which effects the freshness of fish inside the codend. The water pressure against the fish and shrimp during the towing is expected to differ according to the total catch size inside the codend. The number of fishes in the codend is expected to affect the duration of agony before death and the degree of freshness maintained in the catch.[8, 12, 13] It is important to consider mesh size of codend to avoid catching non-target small fish for resource management, but also to improve the quality of the catches.

2) Further development for fresh shrimp

The demand for the supply of live animals has increased in Asia and in other markets for purposes of displaying live fish in aquariums and tanks, e.g., in seafood restaurants, supermarkets, and retail outlets, and trawl-caught animals are increasingly being used to supply the live market.[39, 40] It is important to investigate the effects of trawling operation conditions on the catch freshness to improve transportation and storage protocols. In the present study, the low levels of ATP detected immediately after being retrieved from the codend indicated that even the mud shrimps exposed to a short tow duration (i.e., 15 min) were already exhausted. However, ATP remained in the tail muscle tissue of trawl-caught mud shrimp which suggests that this species has a high capacity to recover from an extreme physiological and stressed state.[31] The transport of live animals serves to exploit higher market prices in the fishing industry. Specifically, the demand for fresh shrimp, especially live shrimp, has recently increased. The transport and storage of live crustaceans has been made possible through technological advances.[41] In the present study, post-harvest mud shrimps were kept on ice. It is necessary to examine the effect of tow duration on the live shrimps after harvesting to further develop techniques to improve the quality and freshness of the catches.

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【研究論文】

底曳網で漁獲したナミクダヒゲエビの漁獲後のATP関連化合物の濃度変化

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和文要旨

底曳網で漁獲したナミクダヒゲエビを漁獲直後から5日間氷蔵して，筋肉中に含まれるATP関連化合物の濃度変化を調べた。曳網速度は2.0ノットで一定，着底曳網15分間でエビ類を採集した後（コントロール），35分間，65分間の離底曳網を行った。コッドエンド前端に取り付けた水中カメラの映像からナミクダヒゲエビはコッドエンド内で静止し続けているのが観察された。コントロール，離底曳網35分，65分ではATP関連化合物濃度に有意差はみられず，氷蔵1日後にはATPは消失し，2日後からHxRが検出され始め，Hxは検出されなかった。

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