Development of the Tropical Reef Fish Fishery: Assessment of Specific Handling Methods for Production of High Quality Chilled Fish

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FINAL REPORT

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PROJECT TITLE:

Development of the tropical reef-fish fishery: assessment of specific handling methods for production of high quality chilled fish.

PROJECT PROPOSER:

This was a joint project between the Queensland Department of Primary Industries and the Northern Territory Department of Primary Industry and Fisheries.

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ii. Non-technical Summary

Practical on-board handling procedures for producing high quality chilled reef fish from tropical waters have been established. Field work was carried out in waters north of Port Douglas, Queensland and in the Timor and Arafura Seas off the Northern Territory. Research concentrated on eight species from four families of reef fish, which commonly compose the commercial catch.

Fish were caught from three different fishing operations: handline in Queensland waters; dropline and trap in Northern Territory waters. The effect of capture method on final fish quality was determined both in the marketplace (Sydney Fish Markets,NSW) and by laboratory analysis using trained sensory assessors and biochemical evaluation. Visual assessment results showed that line fishing maintains a better physical condition than trapping. However, it was found that a trap modification using a nylon mesh-net "trampoline" bottom inside the trap markedly reduced scale loss and physical scarring of the fish. The physical condition of trapped fish was further improved by immediate emptying of the trap into a swim tank of seawater. Severe stress of handlined fish was demonstrated by biochemical analyses which showed extremely low energy reserve levels in most fish immediately post-capture. The physical condition of droplined fish was generally good, but again biochemical analysis indicated high stress levels associated with this method of capture.

Fish from all fishing operations were randomly subjected to one of four on-board handling treatments. The handling procedures included immediate killing of fish by spiking through the hind brain and rapid chilling of the fish using a seawater/freshwater ice slurry. All fish were bled and, post-treatment, gilled, gutted and stored in ice.

Several spiking tools were trialled for efficiency of action and speed of use and one, a wooden-handled 6 inch awl, was selected as most appropriate. Additionally, it was found that spiking to the brain on an angle up through the gills was the more accurate method for many reef fish species.

Ice slurries of different seawater/ice ratios were tested for their chilling efficiency. For tropical fish and slurries made with seawater at tropical temperatures, it was found that a slurry of four parts freshwater ice to one part seawater was the most suitable. Such a slurry has a temperature of 0°C and the consistency of wet concrete, which is beneficial in reducing physical damage to the fish from boat motion. Chilling rates for different sized reef fish at various capture temperatures were determined. Results showed additionally, that it was important to retain fish in the slurry for less than two hours to avoid irreversible whitening of the eye.

Results from comparing handling treatments showed that chilling fish by slurry has greater beneficial effect than spiking alone, especially with respect to rigor extension. Assessment data illustrated that for some species the in-rigor phase of the rigor mortis process was extended to 7 days for fish that were both spiked and slurried. The comparable period for fish from other treatments was less than this, with fish that were not spiked or slurried passing through rigor within 12-18 hours. Overall appearance and colour retention was also enhanced by use of the slurry chilling method, an important marketing point as many tropical reef fish species are brightly coloured. Results of

sensory evaluation of flavour and texture of fish after 7 and 14 days storage in ice showed differences between handling treatments for individual fish species, but the difference was not often statistically significant. Biochemical analysis of the nucleotide breakdown patterns within the fish supported the sensory evaluation results, as there were not large differences in individual nucleotide concentrations after 7 days iced storage.

Overall, research findings indicated that both spiking and the use of ice slurry for immediate post-capture handling of tropical reef fish produced fish of the highest quality.

iii. Background.

The reef fish resource in northern Australia is extensive and well able to supply both local and export markets. In Queensland waters the resource is under strong fishing and tourism pressure, but by adding value to the catch, has potential to provide far greater return to the industry. In Northern Territory waters the reef fishery is essentially underdeveloped and there is strong interest in using this resource within the fishing industry.

Currently, a generally held belief within the reef fishing industry is that product should be frozen to maintain prime quality. However, recent research has shown that tropical reef fish can be landed as top quality fish in chilled form. Higher prices are being achieved for premium quality chilled fish than for frozen product. To attain premium prices, the quality of the product is of paramount importance.

From overseas research, of which a number of studies involved fish taken from tropical waters, it is clear that chilled fish deteriorate at different rates and show different spoilage patterns. Deterioration patterns are species specific and there is often wide variation between fish species from the same family. Hence, handling procedures found to be appropriate for one species are not always directly applicable to other fish species. Specific information on optimal handling methods for tropical reef fish from Australian waters is not yet available.

The method of capture is known to have a large influence on fish quality. Harvest method affects both the physical appearance of the fish and the level of stress to which the fish are subjected. Degree of stress affects the energy reserves within the fish and this has a direct bearing on the duration of rigor mortis phases through which the fish passes.

The method by which the fish dies is important also. It is well known that quick killing reduces the stress the fish goes through at capture and one of the recommended methods for achieving instant death is to spike the fish to destroy the hind brain. Spiking is used extensively to delay loss of quality of fish in Japan and in snapper species from New Zealand waters. However no reports on the effect of spiking tropical fish species have been sighted.

An inherent market advantage of tropical reef fish is their often characteristically bright colour which, however, fades very rapidly on removal of the fish from the water. It is

found that chilling fish in an ice slurry brine retards colour loss. This practice also has the advantage of chilling the fish more rapidly than other methods and can result in prolonging the in-rigor phase of the fish. Such extension of rigor is beneficial as deteriorative changes in the fish flesh do not begin until rigor mortis is complete.

The proposed research will establish the effect of capture method, spiking and chilling fish by ice slurry on tropical reef fish and therefore provide information for attaining premium quality fish.

iv. Need

The current major constraint to premium prices being obtained by fishermen within the reef fishery is lack of available information on how to optimally handle tropical reef species. There is strong and consistent demand for such information from fishermen operating in both Queensland and Northern Territory waters.

v. Objectives

OVERALL OBJECTIVE

To develop catching and handling procedures for tropical reef fish which ensure production of high quality chilled product.

SUB-OBJECTIVES

1. Evaluate the effects of different methods of fish capture on product quality

2. Establish the effect of the spiking technique and short term ice slurry storage on final product quality

3. Determine optimal onboard brine salinities to maximise reef fish colour retention and maintain acceptable salt levels

4. Establish the chilled storage lives of tropical reef species when handlined using optimal conditions determined from the objectives above

5. Through organised workshops and appropriate written material, inform the northern reef fishery of the study findings along with the application and benefits of the technologies

The proposed research, to meet the objectives as described above, would require a period of three years to complete and this was the term for which funding was applied for in the original submission. However, FIRDC only granted funding for the work for a period of one year. Under this condition, the objectives were re-evaluated and research needs prioritised. The determination of chilled storage lives, sub-objective 4, was dropped

entirely due to the impossibility of completion within the time constraint imposed. Sub-objective 3, investigation of optimal salinities and salt uptake rates of reef fish, was restricted to establishing ranges of eye fluid and skin/flesh salinities common among tropical reef fish and determining that this was not radically changed during short term ice slurry brine storage. The other three sub-objectives were considered of primary importance and work concentrated in these areas. Again however, research data collection and analysis was severely limited by the one year time frame.

Given the one year constraint, the objectives were well met and the overall objective was successfully achieved.

vi. Methods

1. Fish capture

Fish were caught in waters north of Port Douglas, Queensland and from the Timor and Arafura Seas in the Northern Territory. Eight species of reef fish from four families were investigated:

Plectropomus leopardus (coral trout)
Pristopomoides multidens (gold band snapper)
Pristopomoides typus (sharp tooth snapper)
Lutjanus sebae (red emperor)
Lutjanus malabaricus (saddletail snapper)
Lethrinus nebulosus (blue lined emperor)
Lethrinus lentjan (red spot emperor)
Lethrinus choerorynchus (lesser spangled emperor)

Fish were caught by handline, dropline and trap in accordance with State fishery regulations and taken from the following depths, handline: to 30m, dropline: 60-100m, trap: 30-60m.

2. Spiking

Instant death of fish was wrought by destroying the hind brain (medulla oblongata) immediately the fish were landed onboard using a 150cm wooden handled awl. Spiking was carried out either by piercing the brain through a point behind the 2nd operculum and following along the lateral line of the fish or by going up on an angle from behind the gill cover, according to fish species.

3. Ice slurries

Ice slurries were made from 4 parts freshwater ice (-10°C - 0°C) to 1 part seawater (26°C - 35°C). The slurry had the consistency of wet concrete and a temperature of 0°C.

4. Handling treatments

Fish were randomly subjected to one of four treatments:

1. Not spiked, held at ambient for 2h
2. Spiked,held at ambient for 2h
3. Not spiked, held in ice slurry for 2h
4. Spiked, held in ice slurry for 2h

Fish were then gilled, gutted and stored in ice for 7d prior to analysis.

5. Rigor measurements

Rigor measurements were conducted essentially according to Iwamoto *et al* (1987)*. Briefly, the fish was placed on a hard surface such that half its body length (from the caudal fin) extended beyond the surface. The degree of tail flaccidness was then measured at selected time intervals. Modification to the method described by Iwamoto, was the development of a "rigometer" for accurate measurement of body stiffness.

*Iwamoto M., Yamanaka H., Watabe S., Hashimoto K. (1987) Journal of Food Science **52**(6) 1514-1517.

6. Analysis

Quality assessment of fish from all treatments was carried out by a trained sensory evaluation panel for parameters of visual appearance and flesh flavour. Additionally, market assessment was made on whole fish landed at the Sydney Fish Markets.

Biochemical analysis of the nucleotide breakdown patterns within the fish was conducted by standard high performance liquid chromatography techniques. Microbiological analysis was not undertaken due to the research priorities changing with the time constraint.

All data obtained was statistically analysed by analysis of variance methods and determination of least significant differences where appropriate.

vii. Results

The purpose of undertaking the research was to provide reef fishermen with practical information for handling reef fish to ensure excellent quality product. This objective was fully realised and included in this report (see attached) is the written information supplied to fishermen as a direct result of this work:

> "Quick killing and chilling can make top fish even better"
> The Queensland Fisherman, March 1991 p28-35
>
> "Recommended handling methods for fresh tropical reef fish"
> Information booklet, September 1991, Department of Primary Industry and Fisheries, Northern Territory Government.

A 2-day workshop entitled "Fish handling and marketing" was held in Darwin, December 1989. The workshop was co-ordinated by the NT Fishing Industry Training Council and attended by a large number of reef fishermen. The first day of the workshop consisted of a series of seminars to get the information across and the second day was devoted to practical hands-on demonstrations of spiking and slurry techniques. The workshop proved very successful and generated even further interest in use of the handling methods.

Additionally, a series of scientific papers are being prepared from the research results for publication within both the fishing industry and the scientific community. The first of these, "The effect of spiking and slurrying on nucleotide degradation of blue lined emperor *Lethrinus nebulosus*" T. Hay and S. Poole (1994), has been prepared to editorial level and is included in this report (see attached).

viii. Benefits

The immediate beneficiary of the research is the fishing sector of the industry as fishermen now have relevant information on which to base fishing operation decisions. However, the benefit of producing premium quality fish passes right through the industry chain to the consumer.

Quantifying the benefit in dollar terms is extremely difficult given the dispersed nature of the reef fishing chain and individual business attitudes within the chain. Fish prices are always market driven and it is known that specific markets will pay a premium price for specific product, for example Japanese restaurants pay more for spiked fish than for fish that are not spiked. It has also been shown that top quality product will earn recognition in the market place and this has been evidenced on the Brisbane market with *Pristopomoides multidens* (marketed locally as king snapper). In 1989, when this species first hit the Brisbane market in reasonable quantity, gilled and gutted chilled fish fetched around $4.50-$6/kg at the retail level. Similar fish now achieve prices as high as $18-$20/kg.

ix. Intellectual Property

Not applicable

x. Further Development

The results from the research undertaken, while providing excellent information for achieving the primary objective, have also demonstrated large gaps in the current knowledge of the full events of rigor as it occurs in tropical reef fish. Greater understanding of the rigor mortis process and the factors which affect it, is crucial to final market quality and distribution of tropical product as deteriorative changes in fish do not occur until rigor is complete.

It is therefore considered that further research into the rigor process of tropical reef fish is of paramount importance.

xi. Staff

One casual technician was employed to assist with the conduction of sensory evaluation panels.

xii. Final Cost

The project was completed on budget and the final Statement of Receipts and Expenditures was submitted by this Department in November 1990.

xiii. Distribution

The content of this report has been conveyed to industry in the Northern Territory and Queensland.
The report is being distributed to:-
FRDC
QDPI library IFIQ
QDPI/FRDC contact- R Pearson
NTDPIF
AUSEAS
CSIRO Division of Fisheries Hobart & Cleveland
QCFO

Quick killing and chilling can make top fish even better

Line and even some net fishermen looking for a marketing edge might find it in quick killing and quick chilling of fish. It's a technique that should improve fishermen's overall returns.

KILLING and chilling reef fish as soon as they're caught improves the quality of the final product.

It's a quality improvement buyers should be able to see with their own eyes, and be prepared to pay a premium for, if you are selling the fish whole. For fillets, the difference is not so easy to recognise, though it should still extend their "shelf life".

The quick kill-and-chill technique traditionally has been used in Japan — where it is called "iki jime" — but is also widely used elsewhere round the world, for fish going to the Japanese market and to local outlets.

New Zealand and Western Australian fishermen have used the technique on snapper. A number of Queensland fishermen are using kill-and-chill on snapper, tropical reef fish and other species, including barramundi.

The usual method is to spike the fish in the brain, cut the gills to make it bleed and then put it into an ice slurry for a couple of hours. After this quick kill and chill, the fish is then lifted out and stored in ice (belly down) in the usual way.

Destroying the fish's brain immediately it's brought aboard stops it struggling and slows chemical changes to the flesh from stress and struggle. Using a spike (such as a spike used for pushing a cord or lace through leather) makes only a small hole and so doesn't open up a large area of flesh to bacteria (compared with, for instance, cutting the fish through the head with a knife).

However all the experts stress that it's not just the quick kill that improves final quality. It is the *combination* of the quick kill and the quick chill.

Putting the fish into an ice slurry is the fastest possible way of reducing the temperature, and so reducing spoilage and natural chemical action in the flesh, without freezing. (You don't want to freeze a whole fish if appearance is important because the freezing of course changes the look of the fish — unless it's deep, deep freezing to around -40°C, outside the range of most on-board freezers.)

Sue Poole, a specialist seafood microbiologist at the International Food Institute in Brisbane, has been studying quick-kill-and-chill techniques with Queensland fish. Here she is explaining some of her work to Peter Spinner, Acting Chairman of the QCFO Line Committee.

More details on the quick-chill-and-kill method are available from Sue Poole at the International Food Institute of Queensland (that's the Department of Primary Industries' former Food Research Branch). The postal address is 19 Hercules Street, Hamilton, Q 4007 and the telephone number (07) 268 8555.

Sue, who is a specialist seafood microbiologist, has been compiling information on the quick-kill-and-chill technique with Queensland (and Northern Territory) fish species. When her present work is finished, probably around the middle of the year, we will publish more details in another article.

She said that the work so far indicates that reactions to the technique

vary between different fish species, and even between individual fish of the same species. Most of her work has involved tropical reef fish.

"It's important to note that the term "reef fish" covers a wide range of species and that, unfortunately, each species tends to behave and/or react quite differently when given the same treatment," she said.

"For example, spiking and slurrying members of the emperor family (*Lethrinus* and *Lutjanus* species) usually results in a very long rigor phase; that is, the time they remain stiff. However for coral trout (*Plectropomus* species) the effect of the same treatment on rigor duration does not seem to be as pronounced but their colour retention is incredibly improved.

"Whatever the exact effect on a particular species, it can be definitely stated that fish treated with spiking and slurry chilling are of far superior quality to those that have not been.

"It must be emphasised that spiking fish by itself is not half as effective as when followed by immersing the fish immediately in an ice slurry. It is the ice slurry treatment, as well as the spiking, that produces the final result."

Fishermen already using the quick-kill-and-chill method have obtained useful information from at least two published articles. One has been written by Kuniji Harada, an advisory officer with the NSW Fish Marketing Authority in Sydney. His article is called "Presenting fish for sale on the Japanese market". The second was written by the New Zealand Fishing Industry Board (NZFIB) and is called "A code of practice for air-freight chilled fish".

Copies of both articles are available from the QCFO State Office in Brisbane. (Contact Research Officer Margaret Delaney on (07) 262 6855 or at PO Box 392, Clayfield, Q 4010.)

The NZFIB article points out that regardless of fishing method the length of time the fish remains on a hook or in a net should be minimised for highest quality. Prolonged soak time in the water, after being caught, will cause the fish to become over stressed, resulting in a reduced shelf life.

After being brought aboard the fish should be carefully handled. Bruising, cuts, scale loss, etc. result in an obvious loss of quality from the point of view of appearance and this becomes accentuated with storage. Do not puncture fish guts as this will release bacteria. Piercing of the skin (apart from spiking) should also be avoided at this stage because this will allow bacteria to attack the flesh.

Chilling of the fish must commence immediately after the fish is landed. The reactions involved in fish spoilage begin immediately after death but they can be minimised by immediate chilling followed by **maintenance of** chilled conditions during all subsequent handling and processing.

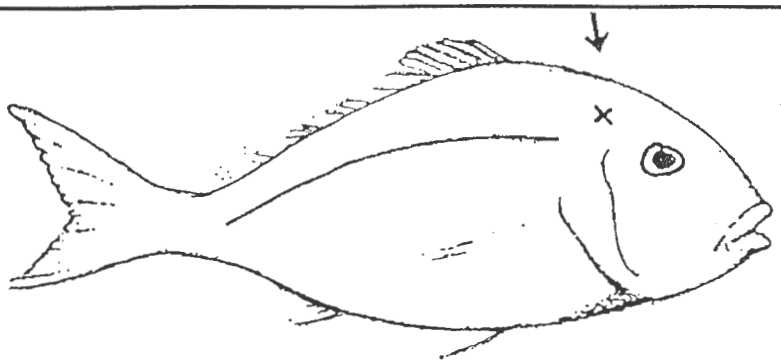
Immediate chilling reduces the risk of gaping fillets; reduces bacterial spoilage because most bacterial growth is reduced in chilled conditions; and slows down spoilage caused by enzymes in the gut and the flesh because enzyme activity is also reduced at lower temperatures.

Ice is the best way to chill fish. Fish flesh freezes at between -2° and -1.5°C. "Chilled" is defined as 0°C, the temperature just above freezing point. (Melting ice has a temperature of 0°C.) Ice is a very efficient means of chilling fish because of its capacity to remove heat from a substance. Ice is easy to use, keeps fish moist and does not require temperature control. Provided ice is made from pure water and it is melting, the temperature of the melt water will be 0°C.

Salt water ice is not recommended for general use. Freshly made salt water ice is very soft, like wet snow. Its temperature is about -3°C. The softness reduces the risk of skin damage to the fish but the coldness causes the gills and eyes to freeze. The eyes turn white, which often causes down grading of the fish in those markets where the appearance of the whole fish is an important attribute.

Fish should be landed as soon as possible after catching. Ideally, fish should be landed within 24 hours but with good chilling and storage techniques, good quality fish can still be landed after three days at sea.

There must always be some ice left covering the fish when it is landed. This will ensure that the fish is either chilled or in the process of being



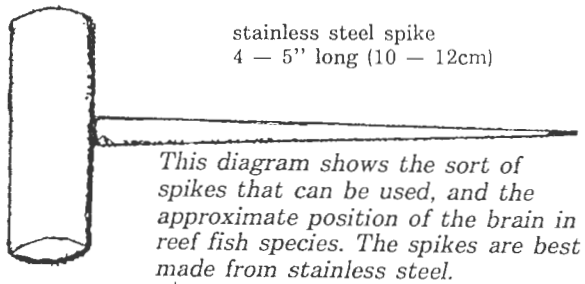
Iki jime technique

x marks point of entry

marks direction

Iki jime Tools

Wooden or stainless steel handle



stainless steel spike 4 — 5" long (10 — 12cm)

This diagram shows the sort of spikes that can be used, and the approximate position of the brain in reef fish species. The spikes are best made from stainless steel.

chilled and *not in the process of warming up*.

Keep the boat clean. Contamination of fish with bacteria from dirty surroundings on board will cause spoilage to be more severe at a later stage. Therefore it is important to:

- clean and disinfect fish holds after every trip;
- always use clean, fresh ice each trip;
- clean and disinfect slurry tanks after every trip; and
- ensure fish cases are properly cleaned by the processor.

The NZFIB article advises that the ice slurry should be used for rapid, effective chilling. An ice slurry is a mixture of sea water and flake or crushed ice made up in tanks on the boat. While there are no strict rules for making up a slurry the following can be used as a guide:— one bucket of sea water to every two buckets of ice (though preferably 3 or 4 buckets of ice).

In rough conditions a thicker slurry is helpful in reducing movement in the tank and so preventing excessive scale loss. Also, when the air tem-

perature is over 20°C, which it is in Queensland on most days of the year, it is better to use three to four buckets of ice.

The temperature of a slurry is about -1°C to -1.5°C. Chilling is rapid because the fish are totally surrounded by chilled water and heat transfer is much faster than in air. While the slurry is in use the correct ratio of ice to sea water must be maintained.

Slurry tanks should be well positioned on board and adequately insulated. Slurry tanks are obviously constructed to fit in with the design and operation of a particular boat. With small boats it is often a matter of fitting them where there is room.

Two or more small tanks are preferable to one large tank because:—

1. They can be cycled.

2. They allow for more precise judgement of the time fish have been in the slurry.

3. They allow for smaller quantities of slurry to be made in times of low catch rates.

Polystyrene, polyurethene or other insulation material should be at least 50 mm thick. An insulated lid will also help to conserve ice.

Fish must be placed in an ice slurry immediately after landing on the boat and must remain in the slurry until thoroughly chilled.

The following table gives a guide to slurry times for different sized fish in Queensland conditions.

Size	Weight	Time to chill to 0°
Small	Up to 1 kg	1 hour
Medium	1 - 3 kg	1 - 2 hrs
Large	3 - 6 kg	2 - 4 hrs

Fish should *not* be left in the ice for longer than the times shown above. (Eye and skin colour might be affected). Even large fish usually are sufficiently chilled after two hours.

After chilling, the fish should be quickly gilled and gutted, and then stored (belly down) in ice.

What's iki jime?

FISH that are spiked for quick killing before chilling are often referred to as "iki jime" fish.

It is a Japanese term. They refer to top-quality fish (usually eaten raw) as "iki" fish. Iki jime is the spiking process that produces iki fish.

Iki jime is pronounced "ick-ee gee-me".

It's not a name most fishermen wrap their tongue round easily. Here we are calling it the "quick-kill-and-chill" method, and the resultant fish as "quick kill-chill fish". That will mean a lot more to most fishermen and buyers than "iki jime", and has the advantage of describing the method at the same time; ie, quick kill, then chill. But, don't forget the step of cutting the gills to bleed the fish between the killing and the chilling.

Spiking fish

Killing fish with a spike can help produce a top quality product. It only works if it is done while the fish is still alive.

The purpose of spiking is to kill the

fish immediately after catching and so prevent the stress conditions which occur if a fish is left to die the normal way. It is important that spiking is carried out in the correct manner so that the brain is pierced. This is only achieved by inserting the spike into the correct position and angle. (See illustration.)

A simple method of determining the angle of entry and the destination of the spike is to cut one fish lengthwise through the head to locate the brain which is a small whitish mass behind the eye. It is then possible to insert the spike from the outside and thus determine the angle required to spike the brain in other fish of the same species.

When spiked correctly the fish will go into a violent convulsion in which all the muscles flex tightly causing the mouth to open, the gills and fins to flare and the body to arch. This should only continue for a few seconds. When the spike is removed the fish will be quite relaxed and floppy.

If this is not the case then the spiking process has not been carried out correctly and should be repeated.

Slurry recipe for success

THIS is Sue Poole's recipe for a chilling slurry.

"An ice slurry made with four parts freshwater ice to one part sea water is the best for our tropical areas.

"It has the consistency of wet concrete and a temperature between -1°C and 0°C.

"It is extremely important that the temperature does not go below -1°C as fish quality will deteriorate at temperatures lower than this. For this reason it is important to *use freshwater ice* rather than seawater ice: seawater ice will take the temperature down lower. It is okay to add seawater to make the slurry but the ice itself should be made from fresh water.

"Fish should remain in the slurry only until they reach 0°C. We have found that for reef fish taken from 26° to 32°C waters, this takes less than two hours, depending on the size of the fish.

"After two hours fish should be removed from the slurry and further processed as required. Fish left in the slurry too long will have irreversibly whitened eyes, as well as leaching of natural skin colour and potential uptake of salt.

"The benefit of using an ice slurry is that it chills the fish so rapidly. This means that the fish is close to or at a temperature of 0°C before the onset of rigor. Because the enzyme action is very slow at this temperature, *the time the fish is in rigor is much extended*.

"For some species, members of the sweetlip family, we found that rigor was not complete for eight days. Because *significant loss of quality* in fish *does not occur until rigor has been completed*, this results in the highest possible quality fish.

"Additionally, the use of ice slurries results in fish that have far better colour than fish that were not put in a slurry, as seen from the photograph of the coral trout on the front of this edition of *The Queensland Fisherman*."

The buyer's point of view

OKAY, now for the crunch question: will the extra trouble involved for fishermen in the quick-kill-and-chill technique mean extra money for their fish?

Probably not, according to one buyer who is handling a growing volume of kill-chill fish.

Eric Cowley, owner of the Southport Fresh Fish Market on the Gold Coast, is a wholesaler and retailer, (and was a net fisherman for 20 years). He buys whole (gilled and gutted) reef fish and barramundi from fishermen who use the kill-chill method. He also buys a wide range of fish handled the conventional way.

Eric is enthusiastic about kill-chill fish but at the same time doesn't want to make a rod for his own back by promising higher prices.

"You can't say a fisherman is going to get a better price per kilo of fish for his product," Eric said recently.

Eric Cowley

"But what it does mean is that buyers are going to take his fish before someone else who isn't using this technique.

"In the long run it's going to mean that buyers will be looking for his fish and he can sell his fish when other fishermen might have trouble getting rid of their product. Fishermen who spike and chill their fish will get a reputation for producing top-quality product and in the long run that's got to mean extra money, not necessarily per kilo of fish but because during the season they will sell more fish more easily and overall be more productive and efficient.

"At the end of the year fishermen who use the quick-kill-and-chill method will make more money than the fishermen who don't use it."

Eric said the difference between kill-chill fish and ordinary fish was very obvious in species of reef fish like coral trout, where colour and general appearance was "miles better". In barramundi the difference was not so obvious on the outside but was easily seen when it came to filleting.

"Anyone who is used to handling fish will spot the difference when they cut the fish," he said. "The colour and the texture of the flesh is definitely better.

"The important thing from a buyer's point of view is that the fish will

maintain its quality for longer. I wouldn't put a time on it, because it can vary with the fish involved, but fish that has been treated this way will certainly give you significant extra 'shelf life'. That's true whatever species it is."

Eric said his kill-chill fish comes from as far north as Yeppoon. It is trucked to Southport in ice overnight. The species involved so far have included red emperor, red-throat emperor, rosy jobfish, parrot, hussar and blackall, plus barramundi in small quantities at the end of the last season and in February.

"I think in years to come the quick-kill-and-chill method is going to become the industry standard for the fishermen who are selling fish whole. It's going to make a big difference in helping local fishermen compete with imported fish. It's going to benefit everyone involved."

For further information contact Eric Cowley at Southport Fresh Fish Markets, 15 Commercial Drive, Southport 4215 or on (075) 32 7479.

How rigor mortis affects quality

RIGOR MORTIS, or rigor, is the process whereby muscle contractions occur shortly after death, causing the muscles to become stiff and inflexible. This process occurs in all animals, including fish.

Immediately after death the muscles are in a relaxed state and can be easily flexed. This is known as the "pre-rigor" state. After approximately one to six hours the muscles enter rigor becoming stiff and will no longer contract under stimulation.

After a further period of time the muscles pass through rigor once more becoming flexible on reaching the post rigor state. For the highest possible quality, **it is important that fish should be as close to 0°C as possible before the onset of rigor.**

The time to reach the post rigor state depends on a number of factors including the species, physical condition of the fish, size, handling while in rigor, degree of exhaustion when caught and the temperature at which the fish is kept.

Some species of fish take longer to reach post rigor depending on their chemical composition. Also if an individual fish is in poor condition due to spawning or poor feeding their energy reserves will be low resulting in a shorter time to reach post rigor. Small fish tend to reach post rigor more rapidly than large fish of the same species.

Handling during rigor can shorten the time that the muscles remain in rigor. It can also cause damage to the flesh if handling is rough resulting in gaping and tearing. The longer a fish struggles while being caught, the more energy it will use up.

The energy reserves are important in the process of rigor mortis and partly determine the time taken to go into rigor. If energy reserves are low the time spent in rigor will be shortened resulting in a reduction in shelf life. (In other words, the more energy the fish has in its muscles when it is brought aboard and spiked, the better.)

The temperature of fish is even more important than energy reserves when entering and passing through rigor. **The lower the temperature during this period then the longer before the post rigor state is reached and hence the longer before any major quality loss begins.**

As well as lengthening the period spent in rigor lower temperatures will lessen the effects of rigor on the texture of the fish flesh. For instance if a fish was left on deck the post rigor state is reached in a matter of hours and fillets taken from these fish are gapy and soft. **Quality is very poor.**

Gaping is generally caused by ineffective chilling on the catching vessel. If fish are not chilled immediately they will go into rigor at a high temperature. The time in rigor will be short and the strength of the muscle contractions which occur at deck temperature will cause tearing of the muscle blocks resulting in gaping.

Fish which are chilled quickly and pass through rigor at about 0°C will be in good condition and the flesh will not suffer any deterioration. With this extension of rigor through rapid chilling the quality of the fish which is landed and packed ashore can be greatly improved.

The immediate killing of fish apparently results in a longer time spent in the "pre-rigor" state; it takes longer for the fish to enter rigor and become stiff.

Enzymes — busy little bodies that ruin fish

ENZYMES — what are they and why do they spoil fish quality?

Enzymes are chemicals that can effect the taste and texture of fish.

Enzymes are necessary in all living creatures to build up and break down tissues. In other words they make and break flesh, skin, etcetera in an endless cycle in living creatures.

Once an animal (such as a fish) dies, enzymes stop building up flesh. However they don't stop breaking down tissue. This affects the flavour and texture of fish.

Extreme heat (in cooking, for instance) destroys enzymes. Normal body heat produces maximum enzyme activity in a piece of fish as a whole. Chilling the whole piece of fish to 0°C reduces enzyme activity.

Though it seems like a contradiction, *part-freezing* a piece of fish can increase spoilage in those parts that aren't frozen.

What happens is that around -1.5°C to -2°C (the temperature produced by seawater ice), water in the fish flesh begins to freeze. The concentration of enzymes *increases* in the parts of the fish not frozen. This increased concentration causes an increase in enzyme activity, and so more loss of quality in those parts of the fish.

If the temperature is lowered further enzyme activity rapidly decreases, and finally virtually ceases altogether around -40°C.

The two main causes of spoilage in fish are enzymes and bacteria.

When fish are partially frozen at -3°C (give or take a degree), the growth of bacteria is prevented. However, as explained, the activity of the enzymes is increased, resulting in more rapid spoilage.

Enzyme spoilage causes changes in the flavour and texture that decrease the eating quality of the cooked flesh: the sweet meaty flavours expected in fresh fish are lost and the texture changes from soft and moist to tough, chewy and stringy.

Quick-kill-and-chill — in summary

HERE is a summary of the "iki jime", or quick-kill-and-chill, fish handling method.

Bring the fish on-board as quickly as possible. This is no problem with reef fish caught on a handline. For a species like barramundi, caught in a net, it means working the net constantly and lifting fish out as soon as possible after it hits the net.

Fish should be spiked as soon as they're lifted from the water. In the case of barramundi (ditto threadfin salmon or other similar species caught in a net), they can be spiked while still in the net beside the boat. Apart from the other benefits, this also makes the fish easier to handle.

Once the fish has been spiked, bleed it by cutting through the top of the gills. (Do this by lifting up the gill cover and inserting the knife blade. The fish will look better than if you make a cut from the throat to the backbone.)

Sink the fish into the ice slurry straight away. Let the fish continue bleeding in the slurry. This will put a lot of blood in the slurry but it is more important to begin to chill the fish immediately.

Most fish should not need to be in the slurry longer than two hours. You will discover the right time with experience. Take the fish from the slurry, and gill and gut them. (If possible do this without breaking the throat, so keeping the head in a straight line with the body and improving appearance.) Then store the fish in ice, belly down (so the belly cavity doesn't fill up with melt water).

RECOMMENDED HANDLING METHODS FOR FRESH TROPICAL REEF FISH

ALF MIKOLAJCZYK

Department of Primary Industry and Fisheries

September 1991

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Recommended Handling Methods For Fresh Tropical Reef Fish

INTRODUCTION

Fresh fish markets demand high quality product. Fish must be as fresh as possible, in premium condition and with optimum skin colour, bright eyes and no scale loss.

In 1988, research undertaken by the Department of Primary Industry and Fisheries and the Queensland International Food Institute established that the storage lives of certain reef fish, when processed and stored on ice correctly, were far longer than originally anticipated (Appendix 1). Subsequent work has led to improved procedures.

To achieve high quality product, follow the procedures for careful handling and rapid chilling which are outlined in this pamphlet.

Although these handling guidelines have been developed for fish caught by either hook or trap methods, adaptation of certain principles can be applied to other methods of fishing, such as trawling or netting.

LANDING YOUR CATCH

1. **Ensure rapid removal of live fish from the fishing gear.**

Fish landed from traps tend to be very lively and should be landed directly into a large bin of fresh seawater, especially when catch rates are high. This process will assist in calming the fish considerably. If fish are landed on the deck, they thrash around violently which causes excess scale loss and bruising. Releasing them directly into contained sea water will minimise damage.

Steel traps should be fitted with a false floor made from nylon prawn net, which will further reduce scale loss and bruising.

It is important not to throw the fish on the deck or use a gaff, both of which will damage the product and accelerate spoilage.

PROCESSING YOUR CATCH

2. **Fish should be bled, or spiked and bled, immediately upon removal from the hooks or fresh seawater bin. Bleeding may be achieved by cutting the gill raker or a main artery. The "Iki-jime" or spiking technique will kill the fish instantly. It is important to spike fish before bleeding. This technique is explained fully in Appendix 2. While fish are being bled they can either be held in plastic bins in a shaded area or immersed in an ice slurry.**

Effective and fast bleeding will reduce discolouration in the flesh, the onset of spoilage and the effects of bruising.

3. **Ideally the slurry should be four parts freshwater ice to one part clean seawater. Slurries must maintain a temperature as close to 0°C as possible, but not bellow -1°C because this causes partial freezing. Partial freezing between -1°C and -6°C accelerates spoilage due to increased enzyme activity (Appendix 3).**

It is important not to add salt to the slurry as this will lower the temperature and cause partial freezing of the fish.

Tests have indicated excessive soak times of fish in an ice slurry will result in bleaching of the skin (especially in red fish), cloudiness of the eyes, and excess salt intake. If bleeding is to be undertaken in an ice slurry it is recommended that fish up to 1kg in weight be processed within 1 hour. Fish larger than 3kg can be left in the slurry for a maximum of 2 hours.

Using a slurry will rapidly reduce the temperature of fish and retard the onset of spoilage. Slurries are more efficient in this regard than straight ice.

Once bled, fish should be gilled and gutted. All gills, gut contents, air bladders and the blood line along the backbone should be removed completely.

Care must be taken to avoid breaking the wings of the fish, spilling the gut contents on cut surfaces and damaging the abdominal wall. Such physical damage, along with remnants of blood in the fish, can accelerate spoilage.

Efficient removal of the blood line can be achieved by using a small hand-held spray gun adapted to a high-pressure spray unit.

Fish should then be made ready for icing by quickly washing with clean sea water to remove excess blood and slime.

After washing, it may be necessary to place fish into a secondary clean ice slurry for a short period to counteract any temperature rise.

PACKING & STORAGE OF FISH

Fish should be "soldier" packed in freshwater ice, with belly facing down - either in bins, in insulated containers or in bulk directly in the hold.

Packing fish in this manner prevents the belly cavity filling with melt-water which can taint the flesh. Care should be taken to ensure fish do not partially freeze. The use of saltwater ice is not recommended as the melting point can be as low as -6°C resulting in partial freezing of the fish.

To chill fish effectively, it is important that the ice should be in contact with as much of the surface of the fish as possible. Avoid stacking fish against each other as this can cause discoloured patches and bleaching on the contact surfaces. Only good clean ice

should be used, preferably flake ice which can be distributed evenly. Ice which has been used for one trip will have a bacterial loading several times higher than the bacterial count on fresh fish, and should not be re-used because it will contaminate the fish. Sufficient ice should be taken on board to cope with maximum catches.

7. **Adequate ice should be placed on the bottom of each bin, around the sides and between each fish. Ice should then be placed over the top of each bin.**

If fish are to be boxed, then correctly designed boxes should be used. Drain holes must be arranged so that melt-water drains down the sides or the ends of the box below, rather than over the fish. Care should be taken when filling and stacking boxes to avoid fish being crushed (Appendix 4).

8. **For bulk ice storage in holds, horizontal partitions should be used at no more than 1 metre intervals to avoid crushing of fish (Appendix 5).**

For bulk storage, extra ice should be used on the floor, against bulkheads and around the sides to absorb heat penetration at these points. Sufficient drainage should be provided.

Whenever chilled air from a refrigeration unit or refrigerated coils is used in conjunction with ice during storage in the hold, care should be taken to ensure that fish are encased completely in ice (Appendix 6). This protects fish from warming up and the drying effects of the refrigeration system. Once again, the operating temperature should be monitored carefully and maintained between -1° and +4°C.

9. **Stored fish should be checked regularly to ensure icing is adequate throughout the entire load and for the duration of the trip. Particular attention should also be given to boxes on the bottom of the load. Additional ice should be added if melting has occurred.**

UNLOADING & FREIGHTING YOUR CATCH

10. **When fish is unloaded for transportation to either the packing establishment or freight terminal, adequate protection is required to minimise temperature rise. Appendix 7 shows the effects on temperature when fish is well and poorly handled.**

Ideally, fish should be loaded directly into a well-insulated, refrigerated transport vehicle. Unloading should be as fast and efficient as possible.

11. **Handling of chilled fish for airfreight must be carried out without delay and ideally in cool premises to minimise possible temperature rise in the product.**

Soldier packing in cartons or containers for transportation is recommended. Regular checks with a thermometer should be carried out to ensure fish are as close to 0°C as possible prior to packaging. It is very difficult to chill fish effectively after they have been packed into containers.

The optimum storage temperature of chilled fish is -1°C to +4°C. The temperature should be maintained at this range at all times.

For distant markets, ice may be added to the boxes of fish. This may be done by placing at least 1kg of ice in a sealed plastic bag which is large enough to be spread over as much of the top of the fish as possible.

If fish is to be airfreighted to market, then airline packaging requirements must be strictly adhered to. These guidelines are available from the airline companies.

Appendix 1

ICE STORAGE LIVES OF REEF FISH CAUGHT IN WATERS OFF THE NORTHERN TERRITORY

SPECIES	SCIENTIFIC NAME	STORAGE LIVES ON ICE (DAYS)
OFFSHORE		
Gold band snapper	*Pristipomoides multidens*	28
Sharp-tooth snapper	*Pristipomoides typus*	25
Red snapper	*Lutjanus erythropterus*	24
Red emperor	*Lutjanus sebae*	29
Rock cod	*Epinephelus spp*	22
INSHORE		
Stripey	*Lutjanus carponotatus*	19
Golden snapper	*Lutjanus johni*	23
Blue-lined emperor	*Lethrinus fraenatus*	21
Moonfish	*Zabidius novemaculatus*	22
Red emperor	*Lutjanus sebae*	28
Rock cod	*Epinephelus spp*	22

The days shown in the above table indicate that the fish were still edible when filleted and cooked i.e. for gold band snapper on day 28. To obtain the storage lives in the above table the fish were processed and stored as follows:

- Fish were landed on deck and bled by cutting the main artery in the throat area without damaging the isthmus.
- After bleeding in bins in a shaded area for 30 minutes, the fish were gilled and gutted. All blood from the backbone was removed.
- Fish were then cleaned thoroughly with fresh sea water and then packed carefully in ice.

NB: It is recommended, despite the long storage lives, that fish be landed in port no later than 6 days after capture to ensure quality product is available for the market.

Appendix 2

IKI-JIME

The purpose of iki-jime or spiking is to kill the fish immediately after capture and so prevent the stress conditions which occur if fish are left to die in the normal way. Spiking the fish can assist in producing a good quality product, but only if it is carried out while the fish is alive.

When implementing the iki-jime technique it is recommended the fish is held by the head with the hand masking the eyes in order to calm the fish.

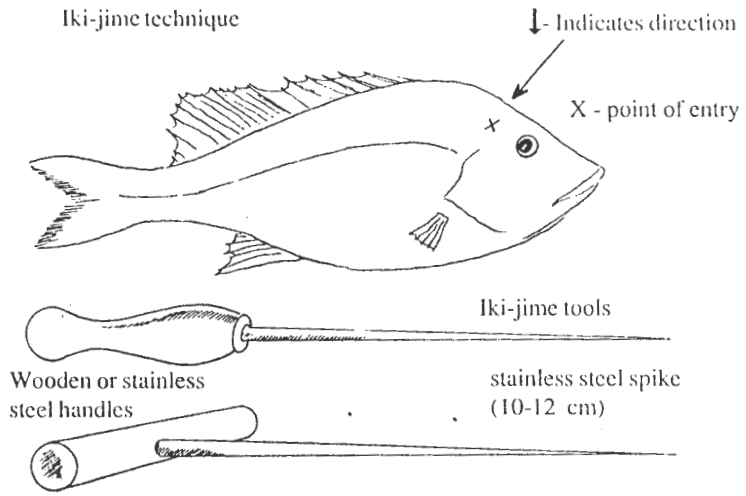
Iki-jime can also prolong the pre-rigor and rigor mortis stages when undertaken in conjunction with rapid chilling. This is important because significant spoilage does not start until the fish is out of rigor.

At low temperatures, rigor can last for a number of days, rather than just hours, as the enzyme activity is reduced while rigor is in progress. The following illustrations demonstrate the correct procedures.

IKI-JIME (SPIKING) TECHNIQUES

1. **Spiking into the brain from the outside.**

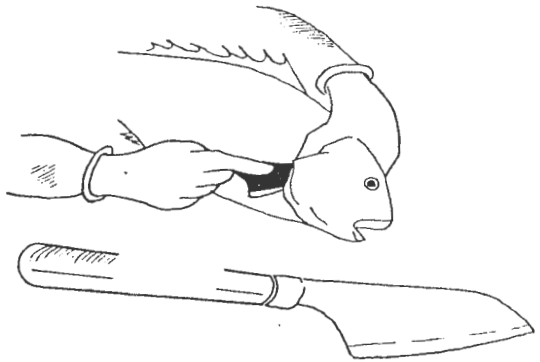
This method is used for most medium sized fin fish. A sharp spike is driven into the brain from the right side or the top of the head for most species. The position of spiking is about 2cm diagonally above and behind the eye, however this must be checked for each fish species. It is important to spike in the right position. The brain can be seen as a small white mass of soft tissue. If spiked correctly the fish will go into violent convulsions for a few seconds, its muscles will tighten, causing the mouth to open, the gills and fins to flare and the body to arch. After removing the spike, the body will relax.



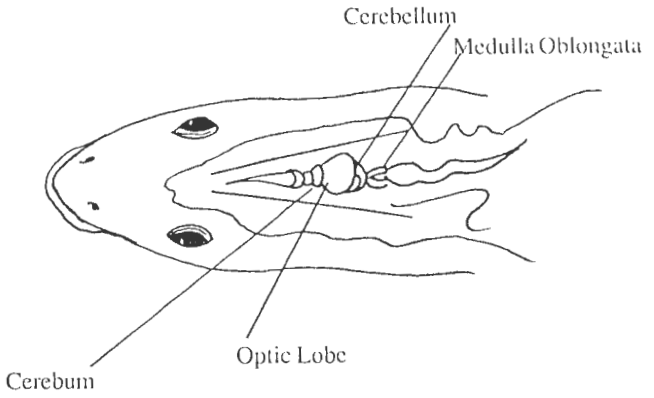
Inserting one of the above tools into the brain at the point indicated on the fish at the direction of the arrow will destroy the hind brain.

2. Spiking into the brain through the underside by opening gill cover.

Smaller species may be spiked into the brain with a sharp knife through the gill opening. The procedure will destroy the brain and also bleed the fish. Care should be taken not to damage the isthmus, i.e. the connecting bridge between the mouth and the body, as this will change the shape of the fish in rigor.



Spiking the brain from under, or over the gill cover.

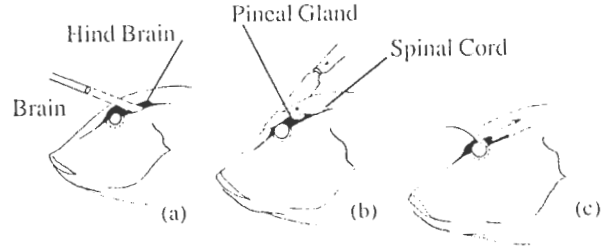


The Medulla Oblongata or Hind Brain is responible for autonomic nervous reactions. Iki-Jime techniques aim to destroy this part of the brain.

3. Exposing the brain and inserting a stiff fibre down the spinal column.

The technique of iki-jime for larger fish such as tuna is carried out by inserting a spike or coring instrument into the brain from the front, through a soft clear patch above the eyes called the pineal window.

Alternatively, a flap of skin is cut from the pineal area to expose the thin membrane or window which covers the brain cavity. A stiff fibre such as monofilament is then passed down the spinal column.



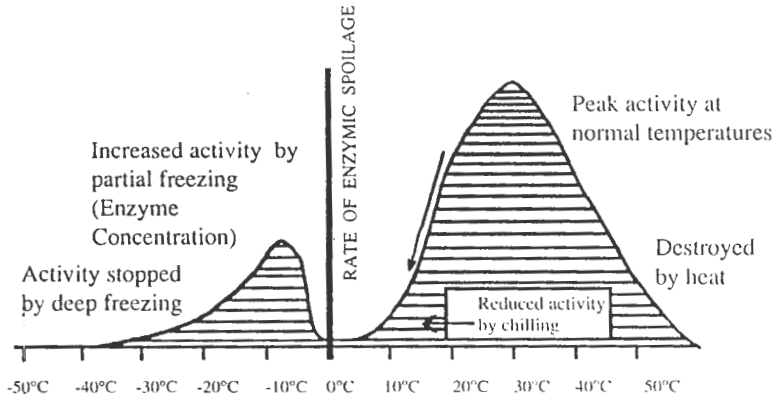
Exposing the spinal column by coring (a) or cutting a flap over the pineal gland (b). Insertion of monofilament line down the spinal column (c).

Appendix 3

WHY PARTIAL FREEZING INCREASES SPOILAGE

The two major causes of spoilage in fresh fish are enzymes and bacteria. When fish are held at -1°C to +4°C bacterial growth is minimised as is enzyme activity. At -2°C to -6°C bacterial growth is still prevented, however the enzyme activity increases thus accelerating spoilage. Enzymic spoilage causes changes in the flavour and texture which result in a decrease of the eating quality of the cooked flesh: the sweet meaty flavours of fresh fish are lost and the texture changes from soft and moist to tough, chewy and stringy.

The rate of enzymic spoilage depends on temperature and enzymic concentration. The change in enzymic activity with temperature is illustrated below.

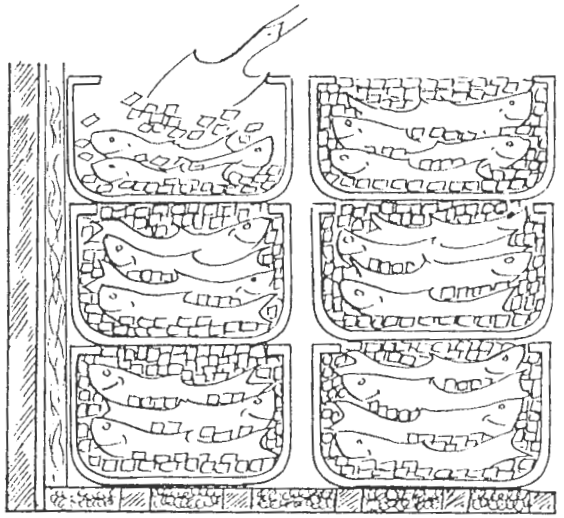


WHAT ARE ENZYMES?

Enzymes are substances which speed up the chemical reactions which cause spoilage and are necessary to build up and break down tissues in all living creatures. After death the building up of tissues no longer occurs but enzymes continue the breakdown process detrimentally affecting the flavour and texture of the flesh.

Appendix 4

ICING OF FRESH FISH IN BINS

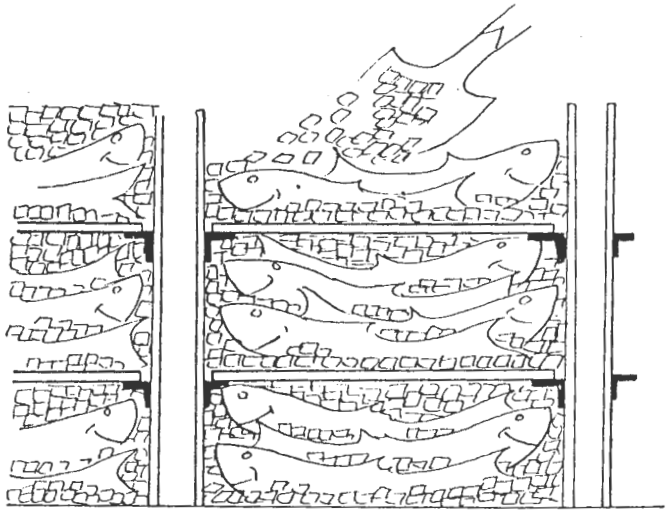


NOTE:

- Walls and floors to be well insulated to maintain chilled conditions.
- Tight stacking of bins will reduce heat transfer to the surface of the bins and reduce ice meltage.
- Bins should not be overfilled to avoid crushing of fish from bins above.
- Ensure ice is liberally distributed throughout and to all surfaces of the fish.
- A good layer of ice should be placed on the bottom and sides of the bins. Ice may also be placed between the ribbing on the floor.

Appendix 5

ICING OF FRESH FISH IN BULK

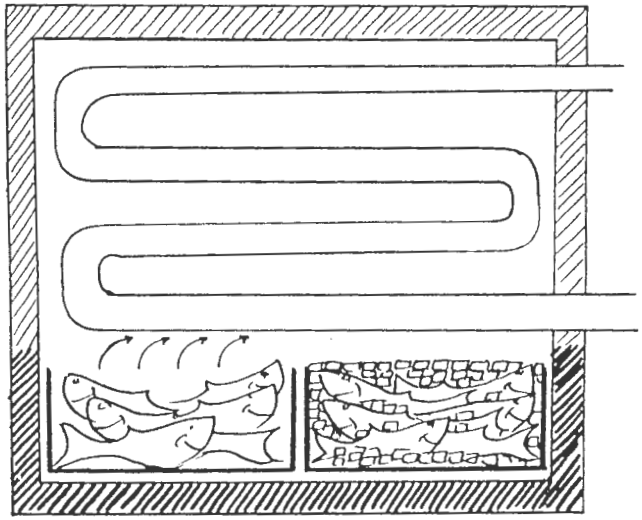


NOTE:

- Walls and floors to be well insulated to ensure chilled conditions are maintained.
- Suitable drainage to be provided for meltwater.
- Ensure ice is liberally distributed to all surfaces of the fish. Care should be taken to ensure good measures of ice are provided on floors and walls of hold.
- Fish are packed on supported shelving to avoid crushing.

Appendix 6

STORAGE OF ICED FRESH FISH IN CHILLED AIR



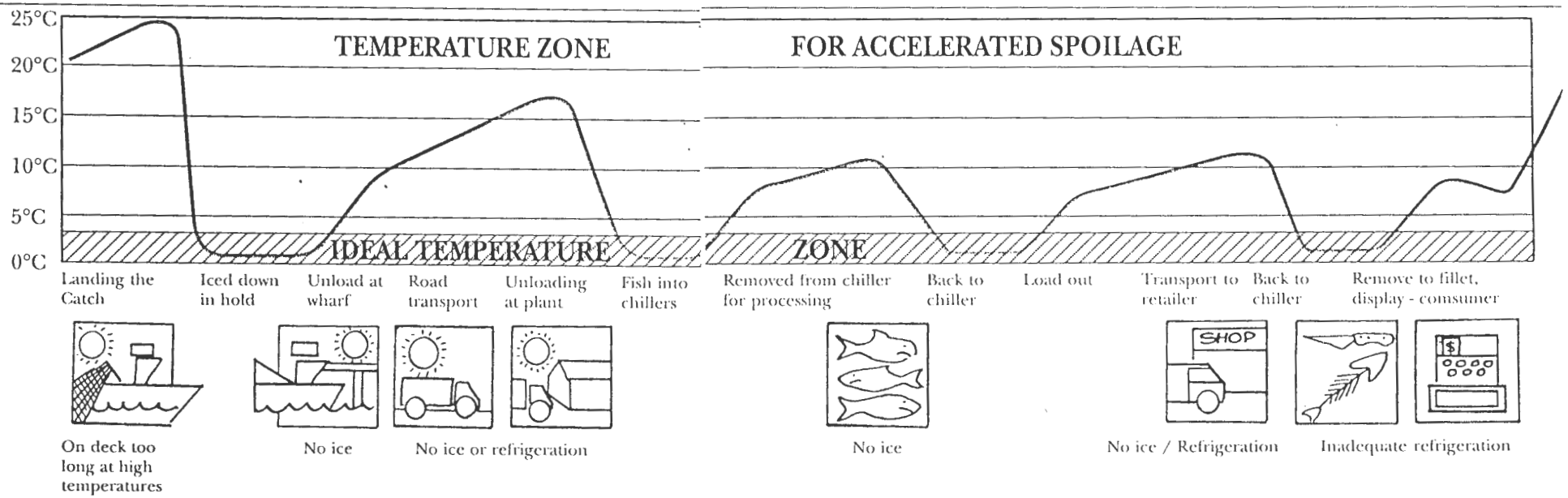
NOTE:

- Cooling coils in a chiller should be located near the roof because cold air is dense and will sink, displacing air warmed by the fish.
- Whenever forced air is used in conjunction with ice care should be taken to ensure fish is totally covered with ice to protect the fish from drying out.
- Extreme care should be taken to ensure fish are not partially frozen below -1°C.

Appendix 7

THE COLD CHAIN

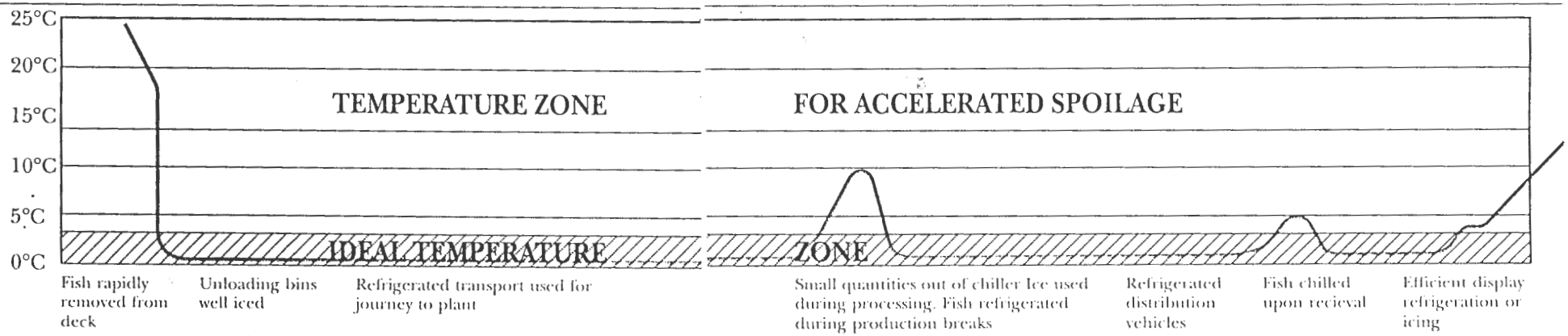
AN INEFFECTIVE COLD CHAIN FOR FRESH SEAFOOD



THE OPTIMUM STORAGE TEMPERATURE SHOULD LOOK

RANGE FOR FRESH SOMETHING LIKE THIS

AN EFFECTIVE COLD CHAIN FOR FRESH SEAFOOD



The Effect of Spiking and Slurrying on Nucleotide Degradation of Blue Lined Emperor *Lethrinus nebulosus*

by

Tracy Hay and Sue Poole

1994

ABSTRACT

Blue lined emperor, *Lethrinus nebulosus*, was trapped in the Timor and Arafura seas off northern Australia in two different seasons, November and May. Fish were subjected to various spiking and ice slurrying treatments and analyzed for their nucleotide breakdown patterns. Results showed that inosine monophosphate (IMP) was the dominant nucleotide present at this time, with only small concentrations of inosine (HxR) and hypoxanthine (Hx) present. K-value was low in all fish from all treatments. Statistical analysis of nucleotide levels did not demonstrate a significant difference between treatments of fish, but there was a highly significant difference ($P<0.01$) in nucleotide concentrations between fish caught in different seasons.

INTRODUCTION

Fisheries resources, like other biological resources, are facing increasing pressures in meeting the needs of the escalating global population. In 1988, global fisheries production peaked at 93 million tonnes (Sorensen *et al.*,1988). During 1990 and 1991, the two most recent years for which the Food and Agriculture Organisation has figures, the world catch began to decline noticeably, indicating that the limit to sustainable landings of wild caught fish were exceeded decades earlier. The decline and in some cases collapse, in fisheries production can be attributed to numerous factors including: over exploitation, introduction of the 200 mile Economic Exclusion Zone (FRDC, 1992), environmental degradation and pollution (Anon, 1994).

World wide demand for high quality fresh seafood is increasing however. Nettleton (1992) commented on the consumer having greater knowledge of the nutritional and health benefits of seafood, particularly from the economically emerging Asian nations (Jeffriess, 1993). This is placing strong pressure on the major seafood suppliers to meet market demand. Global fisheries are unable to maintain the levels of exploitation previously tolerated (Smith, 1994), causing fisheries management authorities to produce policies which increasingly introduce closures to fishing and catch quotas (Anon, 1994).

Management of Australian fisheries is regarded as one of the best in the world and places high emphasis on the need for sustainable fisheries production (Carter, 1994). Waters in the northern half of Australia provide a large, but to a great extent under-exploited, reef fish resource. The north-east waters off the Queensland coast currently support a commercial reef fishery comprising 214 licensed primary vessels with recorded landings of 2313 tonnes and valued at $20.1 million, for the year 1992 (QFMA, 1993). Additionally, Northern Territory offshore reef licences produced a total of 496.7 tonnes of reef fish at a value of $1.4 million (Rees,1992).

However the reef fishery resource is currently fully exploited. Growth of recreational fishing (Gwynne, 1991), increased tourism pressure (Loveday, 1992), declining catch rates, changing market conditions and the increased health and environmental awareness

of Australian society as a whole (Battaglene and Geen, 1992), have all been identified as factors placing pressure on fishermen to adapt their fishing operations within the reef fishery. Improvements in the utilisation of reef fishery resources can be achieved by better catching and handling techniques (Smith, 1994). Value adding in this context is not just extra processing. Rather, it embraces any marketing activity that raises the net value of fisheries production resulting in improved net value of catches. These could include quality improvement, better packaging and use of more appropriate technology (Deeth, 1990). Already changes in Australian market demands have seen fresh fish fetching higher prices than frozen product (Rees, 1992).

A major factor in successfully landing reef fish at local Australian and export markets is the quality of the fish on arrival. Perception of quality is directly related to freshness and freshness is dependant on the phase of rigor-mortis (Harvie, 1982; Harada, 1988). Development and duration of rigor-mortis has been closely linked to depletion of adenosine triphosphate (ATP) (Tomlinson *et al*, 1961; Iwamoto *et al*, 1987).

The onset of rigor and post-mortem biochemical breakdown of fish muscle begins with the hydrolysis of ATP, forming adenosine diphosphate (ADP), then adenosine monophosphate (AMP). AMP is rapidly degraded to inosine monophosphate (IMP) which tends to accumulate initially, as further degradation to HxR and Hx occurs more slowly. A commonly used value, K-value, is the ratio of HxR and Hx to the sum of ATP and related catabolites, expressed as a percentage and is frequently used as a index of fish freshness, as it takes all ATP catabolites into account (Saito *et al.*, 1959; Uchiyama, 1970; Ehira and Uchiyama, 1986; Iwamoto *et al* , 1987; Williams *et al.*, 1991).

The extent to which HxR and Hx accumulate is enzyme specific (Ehira and Uchiyama,1986; Fletcher *et al*, 1986; Murata and Sakaguchi, 1989;) and fish species dependent (Uchiyama *et al*, 1970; Ryder, 1985). The rate at which degradation proceeds is primarily affected by stress levels within the fish at capture. The method of capture and immediate post-harvest handling procedures affect stress levels by elevating metabolic rates and these effects persist for a considerable time (Brett and Groves, 1979; Wells, 1987; Lowe *et al.;* 1993). When post-harvest stress is reduced by killing the fish immediately upon capture, the rate of energy expenditure is minimized and therefore ATP reserves are preserved. The nucleotide degradation process is therefore prolonged. A well known procedure resulting in instant death is spiking the hind brain of the fish (Taniguchi, 1977; Harvie, 1982; Boyd *et al.*, 1984; Harada,1988).

The rate of nucleotide degradation is also affected by temperature of the fish flesh immediately post capture. Nucleotide breakdown is enzyme catalysed and therefore any reduction in fish core temperature will result in a slower reaction. One of the best ways to reduce fish temperature is to immerse the fish in a seawater/ice slurry (Harvie, 1982; Harada, 1988; Price *et al*, 1991), as a liquid medium is more efficient for heat exchange than ice only.

The purpose of this investigation was to examine the effect of spiking and the use of a slurry chilling method on the nucleotide degradation pattern of blue lined emperor *Lethrinus nebulosus*.

MATERIALS AND METHODS

Fishing Operations

Samples of live blue-lined emperor *Lethrinus nebulosus* (0.8-1.2 kg in body weight) were captured in the Timor and Arafura Seas (128^{0}E to 133^{0}E) in waters off the Northern Territory of Australia during November 1989 (n=5 fish per treatment) and May 1991 (n=6 fish per treatment).

Fish were caught in wire traps at a depth up to 30 metres. A "trampoline" of fishing net was stretched tightly above the trap floor to minimise stress and physical damage to the fish during mechanical hauling of the traps. Traps were baited with pilchards and left set for no longer than two hours.

Fish Handling

Immediately on hauling, traps were emptied of their catch into swim tanks filled with fresh seawater. Fish were then randomly subjected to one of four treatments, where all fish were bled by cutting the gill rakers.

Treatment 1 (T1): Not spiked, stored at ambient (29^{0}C) for 2h.

Treatment 2 (T2): Spiked in the medulla oblongata(hind brain), stored at ambient for 2h.

Treatment 3 (T3): Not spiked, stored in a seawater/freshwater-ice slurry (0^{0}C) for 2h.

Treatment 4 (T4): Spiked, stored in a seawater/ice slurry (0^{0}C) for 2h.

All fish were gilled and gutted, soldier packed belly down and stored in ice. At Darwin port, the fish were re-iced and airfreighted to Brisbane. Fish were stored in ice for seven days prior to analysis.

Determination of Nucleotide Degradation

Fish muscle (10g) was homogenised with 50mL of 0.6N perchloric acid for one minute in a Waring blender. The blended material was filtered through Whatman No. 1 filter paper and the acid filtrate quickly neutralised to pH 6.8 with 2N potassium hydroxide. This extract was stored on ice for 30 min, after which the precipitated potassium perchlorate was removed by filtration through sintered glass. The sample was stored frozen until analysis which occurred within 2-3 days.

Separations were achieved on a reverse phase μ-Bondpac C18 stainless steel column (3.9 mm x 30cm, Waters Associates) with a mobile phase of 0.06M K2HPO4 + 0.04m KH2PO4 containing 50mL methanol per litre. The flow rate was 2mL/min. The

absorbance detector was set at 254nm and the response for each of the ATP breakdown products was calibrated by injecting amounts of a standard solution containing 0.166mg/ml of each reference compound. All solutions were passed through a 0.45 m aqueous filter prior to injecting onto the column.

Nucleotide concentrations determined were used to define K-Value by the following equation

$$\text{K value (\%)} = \frac{\text{HxR - Hx}}{\text{ATP + ADP + AMP + IMP +HxR + Hx}}$$

HxR	=	Inosine
Hx	=	Hypoxanthine
ATP	=	Adenosine triphosphate
ADP	=	Adenosine diphosphate
AMP	=	Adenosine monophosphate
IMP	=	Inosine monophosphate

Statistical Analysis

Multifactor analysis of variance was used to analyse the main effects of slurry, spking and trip using type III sums of squares. Initially, variances were compared between groups and dependant variables transformed if variances were found not to be homogeneous. For the dependent variables IMP, Hx and Hx R, the square root transformation was used and for the proportional dependant K value, the arcssin transformation was used.

As all main effects consisted of only two groups, significant differences were interpreted by examining the means. Where significant two or three factor interactions occurred, these were examined by way of a means plot.

RESULTS and DISCUSSION

Concentrations of specific ATP catabolites, IMP, HxR and Hx, are presented in Table 1 along with K-value calculated from all ATP nucleotide breakdown products. Statistical analysis of the data did not demonstrate a significant difference between any of the post-harvest handling treatments. However, such analysis did clearly illustrate a large variability between fish within a treatment, resulting in large standard errors (Table 1). Such variability in nucleotide concentration present in individual fish is commonly found (Boyd *et al*,1984; Bremner *et al*,1988; Poole, unpublished data) and is due to the wild nature of the resource. It is possible that such diversity between individual fish could hide a treatment effect when looking at a single index of assessment.

For all treatments during both trips, the K-values of fish after 7d iced storage were low and below the 20% reference level recommended by Ehira and Uchiyama (1986) for shashimi grade fish. The K-values obtained for *L.nebulosus* are similar to those of other tropical reef fish species caught in the same waters (Williams *et al*, 1991; Poole, unpublished data) and those reported by Bremner *et al* (1988) for reef fish stored for 7d in ice caught off the North West Shelf of Australia. There was no significant difference found between K-values of fish from different treatments.

Concentrations of ATP catabolites (Table 1) show that nucleotide degradation has not proceeded very far in *L.nebulosus* after 7d storage in ice, demonstrated by high levels of IMP being present relative to levels of HxR and Hx. It is well known that IMP concentration is very important with respect to flavour of fish flesh and affects the overall eating quality (Fletcher *et al*, 1986 Murata and Sakaguchi,1988). IMP is found to correlate positively with flavour acceptability (Fletcher *et al*, 1986; Greene and Bernatt-Byrne, 1990) and hence, IMP being the dominant nucleotide present in *L.nebulosus* from all treatments implies that fish were still of excellent quality after 7d storage in ice.

Although statistical analysis did not demonstrate a significant difference in IMP levels between treatments, trends in the data indicated that both spiking and the use of ice slurry did result in a slower degradation of IMP. This is consistent with the findings of Iwamoto *et al* (1987) who found that patterns of nucleotide breakdown of spiked plaice were dependent upon temperature and that degradation of IMP was clearly faster at higher temperatures. It is also in agreement with the knowledge that lowering the temperature of spiked fish results in slower enzymic activity and maintenance of ATP levels (Harada,1988).

In a detailed study of the effects of spiking on fish flesh biochemistry, Boyd *et al* (1984) found a considerable difference in the nucleotide breakdown pattern between spiked and unspiked fish. Their results showed that ATP levels were greater in spiked fish during the immediate post mortem period (up to 8h) compared to unspiked fish and that IMP increased in fish from both treatments, but in spiked fish the increase was delayed and mirrored the changes in ATP. A very important finding of the Boyd *et al* (1984) research was that the differences in nucleotide concentrations between spiked and unspiked fish were indistinguishable after 16.5h. Such spiking effect on nucleotide degradation patterns of *L.nebulosus* was not observed in the investigation carried out here, as fish were first

sampled after 7d storage in ice. This sampling time was chosen so that the research paralleled usual commercial fishing operations in Northern Territory waters as much as possible (Rees,1992). Further research including sampling of fish during the very early post mortem period needs to be undertaken to establish whether spiking delays IMP formation in tropical reef fish.

The concentrations of both HxR and Hx were low in fish from all treatments during both sampling trips (Table 1). This is consistent with IMP being the dominant nucleotide in *L.nebulosus* at 7d iced storage and indicates that biochemical degradation has not proceeded far. Levels of Hx were lower than those of HxR which agrees with Williams *et al* (1991) who found that *L.nebulosus* could be classified as a HxR producer. Statistical analysis demonstrated that there was no significant difference in HxR or Hx levels between fish from any of the treatments.

Nucleotide catabolites were detected at higher levels for all fish samples taken during the November sampling trip as compared to fish caught during May (Table 1.). The dominant ATP catabolites present: IMP, HxR and Hx, were all significantly higher ($P<0.01$) for fish caught during November. It is of note that fish sampling occurred during different seasons and this could bear relevance to physiological condition of the fish. It is suggested that time of season affects the chemical composition of some species of fish (Love, 1975; Botta *et al,* 1987). The difference in chemical composition of fish flesh can be also attributed to stage of sexual maturity and spawning (Shimizu and Wendakoon, 1990). Although the biology of *L.nebulosus* is not fully described, it is known that this species spawns throughout November with no such activity occurring in May (Ramm,pers.comm., Northern Territory Fisheries,1994). It is therefore likely that fish sampled in November and May were of very different physiological states and that this could affect ATP reserves and hence nucleotide breakdown patterns within individual fish.

SUMMARY

The results obtained in this investigation demonstrate the nucleotide degradation pattern of trapped *L.nebulosus* after 7d storage in ice. When fish are handled well post-harvest, IMP is the dominant nucleotide present at this storage time. This fact has a very positive practical implication with respect to quality of fish able to be presented to the marketplace. Additionally, results showed that L.nebulosus is an HxR producer which again is adventitious with respect to fish flavour.

As initial analysis sampling did not occur until after 7d iced storage of fish, the effect of spiking and slurrying on nucleotide degradation was not illustrated by statistical analysis. However trends in the data indicated that both spiking and slurrying did reduce the rate of IMP breakdown. It is likely that effects of spiking and slurrying were observable in the first few hours post-mortem and hence were not recorded in our data.

A large and highly significant difference ($P<0.01$) in nucleotide concentrations between

fish caught in different seasons was observed in this work and it is possible that such difference occurred due to very different physiological states of the fish. Similar research carried out with this hypothesis in mind would clarify this observation.

It is concluded also, that the exact effect of spiking and slurry handling procedures on ATP nucleotides would be illustrated by further research following nucleotide degradation through the very early post mortem stages of fish.

Table 1. Comparison of treatment effect on nucleotide degradation compounds of Blue lined Emperor muscle in relation to time of year

Treatment	Month	IMP	HxR	Hx	K-Value %
		Concentration (μmol g^{-1})			
T1	Nov	15.59 ± 2.32	1.87 ± 0.80	1.41 ± 0.80	15.88 ± 5.06
	May	3.38 ± 0.94	0.57 ± 0.12	0.22 ± 0.14	17.83 ± 5.11
T2	Nov	16.01 ± 3.99	1.69 ± 0.83	1.38 ± 0.79	16.56 ± 7.14
	May	3.55 ± 1.26	0.47 ± 0.32	0.37 ± 0.42	19.08 ± 9.66
T3	Nov	19.76 ± 2.36	1.78 ± 0.54	0.59 ± 0.35	10.61 ± 0.56
	May	4.02 ± 1.51	0.69 ± 0.17	0.53 ± 0.67	23.50 ± 13.65
T4	Nov	14.97 ± 1.30	1.41 ± 0.74	1.05 ± 0.67	13.66 ± 3.15
	May	4.00 ± 0.85	0.50 ± 0.21	0.14 ± 0.05	13.33 ± 2.84

Values are presented as mean and standard deviation

- Treatment 1 (T1): Not spiked, stored at ambient (29°C) for 2h.
- Treatment 2 (T2): Spiked in the medulla oblongata (hind brain), stored at ambient for 2h.
- Treatment 3 (T3): Not spiked, stored in a seawater/freshwater-ice slurry (0°C) for 2h.
- Treatment 4 (T4): Spiked, stored in a seawater/ice slurry (0°C) for 2h.

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