Studies on the physiological response of the wrasse, *Notolabrus tetricus*, greenback flounder, *Rhombosolea tapirina*, and horseshoe leatherjacket, *Meuschenia hippocrepis* to water temperature changes during holding and transport

Professor G.N.R. Forteath
J.D. Findlay
A. Steffans

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FISHERIES
RESEARCH &
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Project 92/125.15

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Section I: Blue Throated Wrasse

Materials and Methods

Approach 1

Principal Investigator: Professor Nigel Forteath
Address: University of Tasmania, Department of Aquaculture
P.O. Box 1214, Launceston Tas 7250
Telephone: 03 63 243802 Fax: 03 63 243804

Non-Technical Summary

The need to understand the water temperature at which fish can be comatised for live transport is obvious. Likewise it is essential to discover the water temperature at which the majority of fish will survive for extended periods. It may be necessary to transport fish to Asia, Japan or the USA. These journeys may necessitate the live product having to endure at least 24hrs in transport boxes.

In 1995, work commenced at the University of Tasmania to study the temperature at which the blue throated wrasse, *Notolabrus tetricus*, horseshoe leatherjacket, *Meuschenia hippocrepis* and greenback flounder, *Rhombosolea tapirina* became comatosed and temperature at which survival was at least 80%.

Two approaches were decided upon. The first was to obtain information using a dynamic cooling method, whereby, the water temperature in which the fish were held was lowered by a set number of degrees per hour. This method would allow us to determine the Incipient Lethal Temperature i.e. the water temperature below which the fish would not survive. However, it was also important to find out if those fish which apparently withstood the treatment recovered when returned to the holding tank temperature.

The second approach entailed removing fish from their holding tank and placing them directly into seawater at a given temperature (static approach), holding them at this temperature for a set period and then returning them to the original tank for recovery. It was hoped that coma would be induced prior to the lethal temperature. This was not necessary the case. Indeed this approach highlighted the fact that while metabolic rate was greatly reduced death often resulted before come induction.

During the experiments mortalities were recorded. However, it is also important to continue the experiment once fish are returned to their original holding tank temperature since mortalities may continue for some time after apparent recovery.

Section I experiments on wrasse demonstrated that this species is not able to tolerate water temperatures below 6.9°C for any length of time without mortalities increasing. However, the initial temperature at which the fish are held will raise or lower this critical cold temperature. Thus although comatosed or temperatured shocked, death will ensue after a short-time period below 6.9°C or higher depending on the acclimation temperature.

Section II experiments highlight the very narrow temperature range between survival and death for wrasse. The work also points out that survival during coma or temperatures which reduce metabolic rate is limited and the fish must be kept at warmer temperatures for long-term (12hr or 24hr) transport.

Section III compares the work carried out on wrasse with the greenback flounder. The latter shows a much greater tolerance to low water temperatures and is an excellent species for live fish transport. It is apparent that although flounder can be held at very low temperatures, coma temperatures appear to vary widely between individuals and are difficult to define. However once again the data point out that there is a very fine line between comatose temperature and the death-point, while transport temperature is warmer than comatising temperature.

Unfortunately, limited data are available at present on the horseshoe leatherjacket (Section IV).

Background

Interest in the live fish trade has increased steadily over the past five years. Species previously unexploited by fishermen are being targeted. Unfortunately no information is readily available on temperature tolerance of these animals and coma induction using cold water is preferable to using drugs to anaethetise them during transport.

Three teams of researchers have been investigating suitable transport methods. One in Queensland, studying coral trout and barramundi for the most part and two in Tasmania concentrating on morwong, blue throated wrasse, flounder and leatherjackets.

During 1995, the research on the blue throated wrasse, *Notolabrus tetricus*, was focused on determining holding tank requirements and examining the lower incipient lethal and critical thermal minimum water temperatures. At the same time horseshoe leatherjackets, *Meuschenia hippocrepis*, were captured and studied to try to ascertain their lower incipient lethal and critical thermal minimum water temperatures. We believe that meaningful work on the transport of live fish requires a determination of water temperatures which comatose specimens and subsequently permit at least 80% recovery during transit. Thus during 1995, the following studies commenced:

- thermal minimum for wrasse;
- thermal minimum for horseshoe leatherjackets; and
- tank design.

To achieve our goals, the National Sea Food Centre awarded us a grant of $29,000 over the one year period. However, work of this nature is likely to take researchers longer than one year to obtain meaningful results. Furthermore, we wished to compare the behaviour of the wrasse and leatherjacket with the greenback flounder. A large grant was awarded by FRDC to Dr Purser of the Aquaculture Department, to investigate the aquaculture potential of flounder. We considered it important to determine the thermal minimum for this species since it appeared likely to us that live export was an important value adding market for the species. Permission was obtained from FRDC to include this species in our work. The results reported here cover data obtained in 1995, 1996 and 1997. It is hoped that these data will increase the success rate of live fish reaching market and permit the live fish industry to design a robust and meaningful protocol.

The author wishes to acknowledge the work undertaken by Mr. Alby Steffans who submitted his research as a thesis for which he was awarded the degree of Master of Applied Science. The results reported on the Blue Throated Wrasse (Section I) are produced here with the full permission of Mr. Steffans. The author of this report was a supervisor for Mr. Steffans and his research was tailored into the project. Section II are results of research by Mr. James Findlay who is undertaking a PhD degree under the author's supervision. These results are likewise provided with the student's permission.

Need

Serious losses occur during the transport of live fish. Furthermore transport in water is costly due to its weight, while reluctance by airlines to carry seawater adds to difficulties. The use of drugs to anaethetise the fish is regretable and irresponsible. Thus protocols developed using cold water to comatise the stock is preferable. The latter requires quality refrigeration equipment and information on suitable fish holding water temperatures before, during transport, and on return to holding tanks. The need for such data is of paramount importance for each species of fish.

Objectives

To develop suitable protocols for cold water comatisation of the blue throated wrasse, greenback flounder and horseshoe leatherjacket.

To determine suitable water temperature for long-term transport of wrasse, flounder and leatherjackets.

Research Methods and Results

Section I: Blue Throated Wrasse

Materials and Methods

Approach 1

Specimens were collected by angling over reefs at the mouth of the Tamar Estuary. The fish were held in a 1000 L tank on board the fishing vessel and transported in the tank back to base. The latter initially included only two 4000 L Rathbun tanks, a biological filter, U.V. filter and a 200 L reservoir. However, shock loading of non-purged fish prevented efficient water quality control and subsequent holding of captured specimens was undertaken in a large fish trap placed in the Tamar. Fish were held in this trap for a minimum period of 24hrs before transferring them to shore based holding systems.

Experiments were undertaken in four 'reefer' containers (refrigeration/freezer shipping containers 6.5m x 2.4m x 2.4m). Both acclimation of the fish and experiments on the dynamic reduction (Critical Thermal Minimum) were carried out in 3 x 380 L circular tanks set-up as recirculating systems. Calculations for the biofilter size and appropriate stocking densities are set out in papers by Forteath (1990) and Hart and O'Sullivan (eds) (1993). Maintenance of the tanks was carried out daily and regular monitoring of water temperatures, dissolved oxygen, total ammonia, pH, nitrite and salinity were undertaken. Photoperiod was held as closely as possible to natural Light/Dark lengths according to season. Likewise holding temperatures were set to correspond to season. Thus during summer, light schedules were 16L/8D and water temperature held at 20°C. In the winter, fish were held at 10L/12 D and 10°C and during Autumn the animals were held at 12L/12D and 15°C.

Two experimental approaches were adopted:

- Determination of the Critical Thermal Minimum (CT Min) using a dynamic approach i.e. reducing water temperature slowly over a period of time; and
- Incipient Thermal Temperature (ILT) using a static TL_{50} procedure. Fish were transferred from a holding temperature (20, 15, 10°C) directly into a lower temperature.

C.T. Min Methodology

Following acclimatation to one of the test temperatures (10°C, 15°C or 20°C) the following procedure were undertaken:

i) water temperature was reduced by $1\,^{\circ}\mathrm{C}\,h^{-1}$ until all the test fish died - final endpoint;

(ii) temperature and dissolved oxygen were recorded every 30 minutes;

(iii) mortalities were recorded throughout the experiments;

(iv) length, weight and sex of all specimens were recorded; and

(v) water quality parameters logged.

C.T. Min Results

The C.T. Min at 10°C acclimation is calculated to be 4°C . The slope of the regression shown in Figure 1 predicts an increase in cumulative percent mortality of 27.4% for every 1°C fall in temperature below 6.3°C.

At an acclimation temperature of 15°C, the CT Min is 4.33°C while the slope of the regression (Figure 2) predicts an increase in cumulative percent mortality of 23.6% for every 1°C drop in temperature below 7.2°C.

Fish acclimatised at 20°C temperature had a mean CT Min of 6.78°C and the regression (Figure 3) predicts an increase in cumulative present mortality of 20.3% for every degree in water temperature below 10.1°C.

Static TL_{50}

Figure 4 shows mortalities over a 24 hour period when wrasse were first acclimated to 10°C. At 5°C (S.D. 0.85) it appears that about 50% of the fish die. Furthermore, for every one degree fall in temperature below 5.86°C of the static water, it is predicted that mortalities will increase by 43.43%. From Figure 4, it also appears that the incipient lethal temperature is about 5.86°C.

Fish acclimated at 15°C have TL_{50} of 6°C (S.D. 0.84) with mortality increasing to 53.7% for every 1°C fall is static water temperature below 6.9°C. The 1.L.T is about 6.9°C.

Figure 6 shows a TL_{50} of approximately 8.3°C when fish are acclimated at 20°C (S.D. = 1.2). Mortality up to 24 hours is shown as 36% for every one degree fall in water temperature. The temperature above which death is no longer a function of temperature is 9.54°C.

Median lethal exposure times were calculated for wrasse abruptly transferred for 24L into static temperatures of 5°C, 4.5°C, 4°C and 3°C respectively. (Over a 24 hr period, no mortalities were recorded at 6°C). The data were plotted an long-linear paper as shown in Figure 7. Death is predicted to be instantaneous at 0.01 h at a temperature of -1.7°C.

Figures 8 and 9 show similar log-linear plots for median lethal exposure times of fish acclimated at 15°C and 20°C and exposed to static water temperatures of 6°C, 5.5°C, 4°C and 3°C in the case of 15°C acclimation and 8°C, 7.5°C and 7°C, for fish acclimatised at 20°C.

Development of thermal profiles

The data presented above permit possible thermal profiles to be developed for *N. tetricus*. These are shown in Figures 10 - 12.

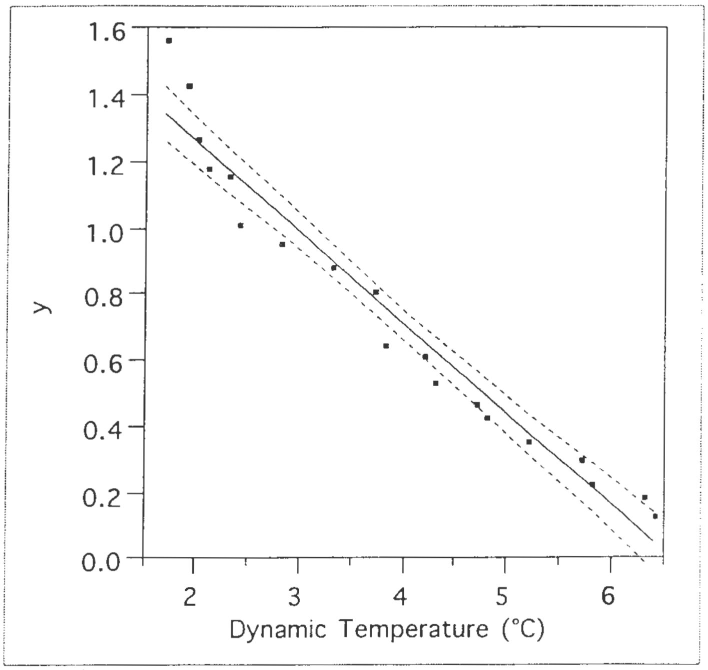


Figure 1 y (y = asin√ cumulative mortality, with 95% confidence belts) versus dynamic water temperature (°C) in blue-throat wrasse, *Notolabrus tetricus,* acclimated to 10°C. Mortality derived from 6 groups (3 x 9; 3 x 10) of fish.

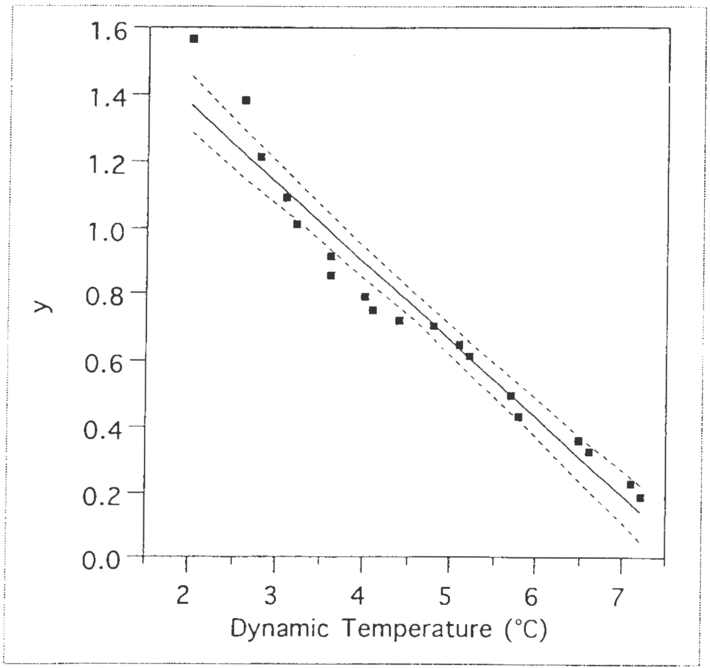


Figure 2 y (y = asin√ cumulative mortality, with 95% confidence belts) versus dynamic water temperature (°C) in blue-throat wrasse, *Notolabrus tetricus,* acclimated to 15°C. Mortality derived from 6 groups (3 x 9; 3 x 10) of fish.

Comparisons between static and dynamic survival temperatures

It is interesting to make comparisons between the results of these static and dynamic experiments. At the acclimation temperature of 10°C, the mean dynamic CT Min value of 3.64°C was significantly different to the mean static TL_{50} value of 5°C ($P<0.05$). Likewise, at 15°C acclimation, the mean dynamic CTMin value of 4.33°C was significantly different to the static TL_{50} mean of 6°C ($P<0.05$). At 20°C the mean dynamic CT Min value of 6.78°C also was significantly different to the TL_{50} valve of 8.3°C ($P<0.05$).

Discussion

The results presented above demonstrate that wrasse may show very different mortalities at given temperatures depending on acclimation or lack thereof. These findings are important for the live wrasse trade since fishermen will have to make a decision on the type of equipment they employ to prepare the fish for transportation. However, it must be clearly stated that acclimation in fish is a long-term process and the methodology used in the dynamic trials (1°C h^{-1}) cannot be considered an acclimation regime *sensu strictu* but neither can it be denied that a long and thorough acclimation period would be relevant to industry. The reported study was designed to provide data applicable to applied methods for preparing fish for live transport.

This study certainly suggests there is an advantage in the dynamic approach i.e. slowly lowering water temperatures over a time period. Wrasse appeared to survive colder temperatures than those subjected to static thermal shock. The static method appears to poorly prepare the fish for the subsequent thermal shock. One reason for the different tolerances found may be stress. The increased handling during the static cooling of the animals may have increased mortalities. However in Section II we found the dynamic approach caused erratic behaviour in the fish, sometimes leading to death.

The results likewise show the importance of holding fish for transport at low temperatures prior to induction of coma. Fish held at 20°C quickly die at temperatures below 6°C whereas those held at 10°C may tolerate 4°C. It is reasonable to suggest that fish comatosed by low temperature would consume less oxygen and produce less metabolic waste than fish held at warmer temperatures. Water quality is always more difficult to maintain at higher temperatures particularly because oxygen is reduced and there is likely to be more unionised amonia present. Thus it is important to lower water temperature during transport.

This study does not give an indication of mortalities which may follow sudden temperature changes which my or may not induce coma. It is quite conceivable that since coma induction is very close to death point, recovery after 2hrs at, for example, the Incipient Thermal Temperature by a large percentage of the fish was short lived and mortalities may increase over time as a result of the thermal shock. This is an important consideration. In order to ascertain survival more comprehensively further static and dynamic trials have been undertaken.

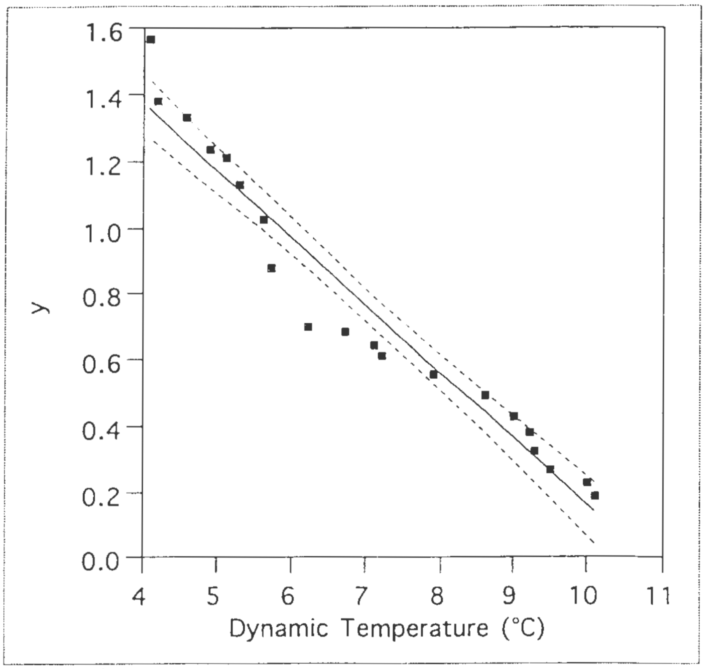


Figure 3 y (y = asin√ cumulative mortality, with 95% confidence belts) versus dynamic water temperature (°C) in blue-throat wrasse, *Notolabrus tetricus,* acclimated to 20°C. Mortality derived from 6 groups (3 x 9; 3 x 10) of fish.

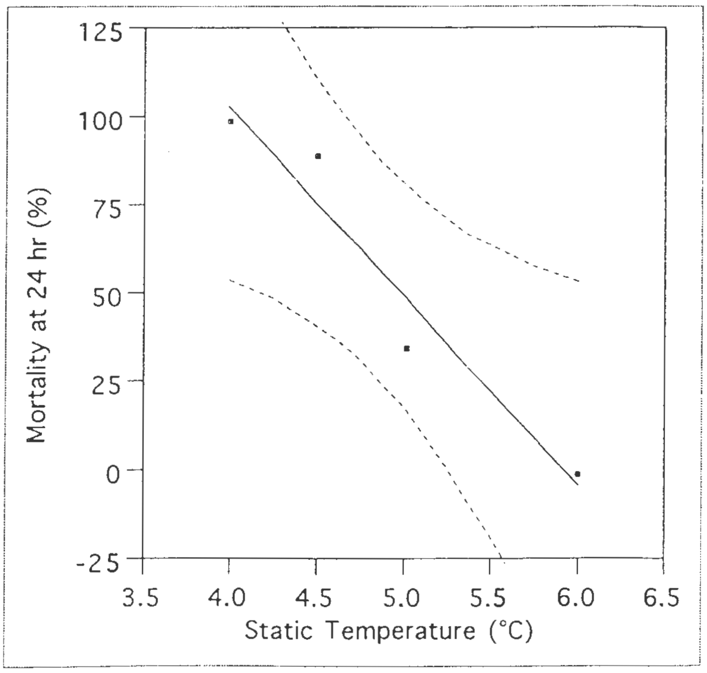


Figure 4 Mortality at 24 hours ($\bar{x}$% with 95% confidence belts) versus static water temperature (°C) in blue-throat wrasse, *Notolabrus tetricus,* acclimated to 10°C. Graphic interpolation of yields a mean static TL50 of approximately 5°C. Mortality values were derived from three groups of 20 fish and one group of thirty fish.

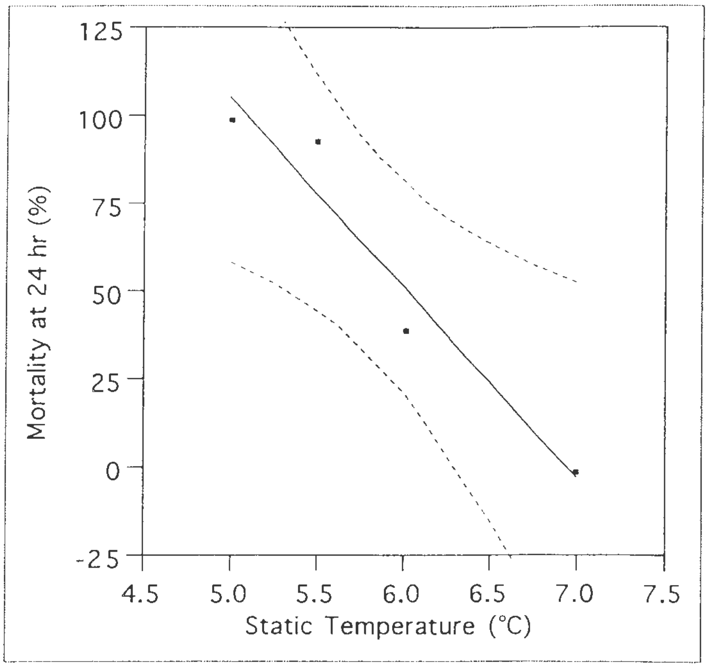


Figure 5 Mortality at 24 hours ($\bar{x}$% with 95% confidence belts) versus static water temperature (°C) in blue-throat wrasse, *Notolabrus tetricus,* acclimated to 15°C. Graphic interpolation of yields a mean static TL50 of approximately 6°C. Mortality values were derived from three groups of 20 fish and one group of thirty fish.

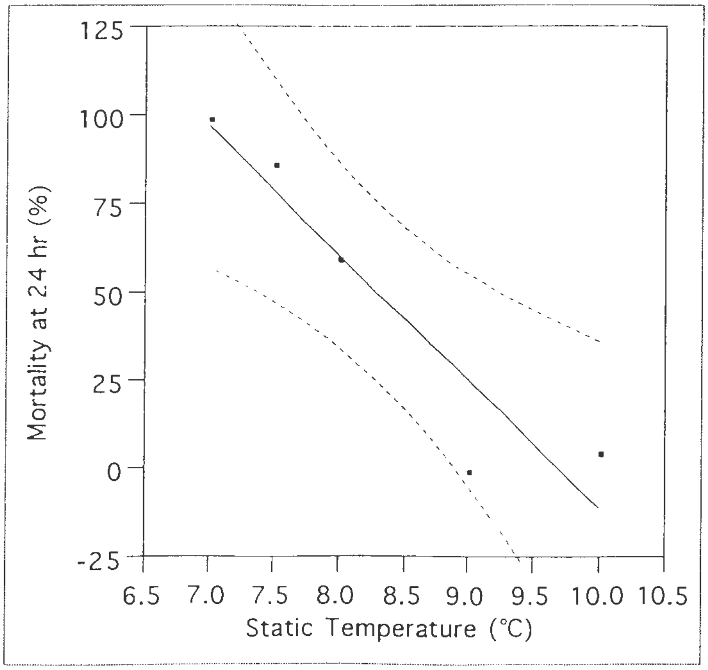


Figure 6 Mortality at 24 hours ($\bar{x}$% with 95% confidence belts) versus static water temperature (°C) in blue-throat wrasse, *Notolabrus tetricus,* acclimated to 20°C. Graphic interpolation of yields a mean static TL50 of 7.07°C. Mortality values were derived from four groups of 20 fish and one group of thirty fish.

Section II: Blue Throated Wrasse

Introduction

Wrasse were brought back to the University campus and held in recirculation systems for a minimum period of two weeks prior to experimentation. The fish were fed squid and water quality constantly monitored in the holding facility. Water temperature was maintained at 15°C.

It was important to construct a series of temperature controlled tanks which would permit either a dynamic reduction in temperature from 15°C to 0°C or a series of static systems which would maintain water temperature at given temperatures. These units were designed and set-up close to the fish holding facilities. The systems were fine tuned to give a very accurate temperature.

The experiments were designed to ascertain whether the live fish industry would benefit from establishing systems which gradually cooled the water or used a system consisting of tanks holding water at a given temperature or temperatures. It is recognised that lowering the water temperature 1°C per day is uneconomic but a more rapid approach is feasible.

Materials and Methods

A refrigerated system was etablished which permitted seawater to be lowered by either 1.5°C or 2.5°C per hour from a standard holding temperature of 15°C. Furthermore, the system also enabled the water temperature to be raised by 0.5°C or 1°C per hour until 15°C was reached.

A second refrigerated system was set-up which ensured a series of static seawater temperatures were maintained ranging from 0.5°C to 15°C.

Each dynamic experiment consisted of three tanks into which 1.3kg of wrasse were placed. The wrasse were starved for 48hrs prior to experimentation.

Mortalities were recorded during the experiments and for 24hrs after the water temperature, and hence fish, were returned to 15°C. It was considered that all mortalities up to the 24hr time period at 15°C were caused by thermal shock.

In the static experiments, wrasse were transferred from 15°C straight into the test water temperature in which they were held for either 2hrs, 12hrs, or 24hrs, prior to being returned to 15°C. Mortalities were recorded throughout the experiments and for 24hrs after fish were returned to seawater at 15°C. Three tanks were used for each test temperature and 1.3kg of wrasse were placed in each tank.

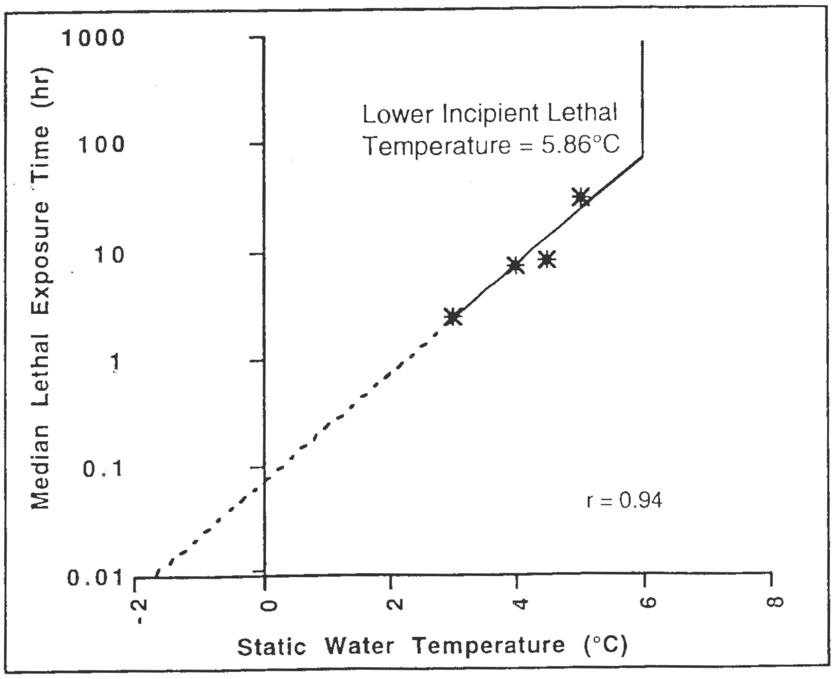


Figure 7 Median lethal exposure temperature (hours) versus static water temperature in four groups of blue-throat wrasse, *Notolabrus tetricus*, (3 x 20; 1 x 30) acclimated to 10°C.

Results

Figure 13 shows the results of the dynamic trials. It can be seen from the figure that at 5°C survival is 0% i.e. by lowering the water temperature from 15°C to 5°C at 1.5°C per hour all fish died. Conversely, lowering the temperature to 6.9°C did not cause mortalities. Interestingly, mortalities did not change when temperatures were reduced more rapidly (Figure 13). The wrasse were sometimes comatised at this temperature.

Figure 14 shows results of the static hold experiments for wrasse held at temperatures within the range 4.4°C to 8°C for 2 hours, within the range 6°C to 10°C for 12hrs, and within the range 6.5 to 10.3 for 24hrs. Percentage survival 24hrs after the fish were returned to 15°C is shown on the Y-axis.

Discussion

Section I explored the Critical Thermal Minimum for wrasse: acclimation at 15°C resulted in the CT Min being predicted to be 4.33°C. In the dynamic experiments reported in Section II the death end-point is 5°C. The CT Min work in Section I also predicts 23.6% cumulative percentage mortality for every 1°C fall in temperature below 7.2°C. Thus these experiments indicate considerable variability between survival below 7°C. Furthermore, the importance of following survival over an extended period of time once the original acclimation temperature is reached (15°C) cannot be ignored. In Section II we have stressed the need to count mortalities for 24hrs after subjecting the fish to thermal shock once fish are returned to the acclimation temperature. In the experiments carried out in Section I this was not done and mortalities were scored during treatment and the initial return to the acclimation temperature only. This approach may have masked the number of mortalities resulting from the treatment. Mortalities in the second of group of experiments were found to increase in spite of the fish being returned to the acclimation temperature. Given this scenario 5°C is probably a lethal temperature for the blue throated wrasse.

Section II static experiments are extremely important since they demonstrate that temperature variations of as little as 0.5°C may have a marked effect on fish survival. It can be seen (Fig. 14) that wrasse held at 5.59°C had a greater survival (2hr immersion) than those held at 5.5°C (2hr immersion). This clearly demostrates the critical nature of temperature reduction for wrasse. A very small temperature difference may have a marked effect on subsequent survival. Furthermore, these results would not have been possible without the procurement and design of a sensitive water temperature control apparatus. Experiments carried out in Section I were not undertaken using this apparatus and relied on a model to ascertain CT Min. We believe that the results in Section II highlight the need for a sensitive refrigeration system to ensure an accurate comatising temperature for wrasse. Comatising these fish is difficult.

The results of the static hold experiment (Fig. 14) suggest a protocol for comatising or at least greatly reducing their metabolic rate and subsequent transport of wrasse. Two hour emersion at 6.9°C will comatise the fish but subsequent survival is only 80%. However, an initial water temperature of between 6.9 - 7.3°C followed by transport in water at 9.5°C may result in most of the wrasse surviving. Of course some specimens may increase their metabolism but clearly this is more preferable than 100% mortality which may well result if the fish are held at less than 9.5°C for 24hrs. Similarly for a 12hr journey the fish, after initial cold shock, can be transported safely at 9°C.

When using the dynamic approach, it was found that at temperatures just prior to coma inducement, the wrasse behaved extremely erratically, often damaging themselves on the sides of the tank. This did not occur in the static experiments. The latter approach was subsequently abandoned because of the behaviour of the fish. These observations suggest that wrasse do not necessarily become comatosed just above the death-point. Coma is not easy to induce. The erratic behaviour also points out that a dynamic approach may be more stressful on the fish that the static.

Section III: The Greenback Flounder, *Rhombosolea tapirina*

Introduction

The Greenback flounder has been bred in captivity for several years now. Market research suggests that this species will be readily accepted in the live fish trade and such value adding is likely to increase the economic viability of the species. Unlike the blue throated wrasse and horseshoe leatherjacket, there is increasing likelihood that the greenback founder will become a farmed species in the next two or three years thereby ensuring ready supplies of this fish for live export.

Materials and Methods

All fish used in the trials were reared in captivity and on-grown in Rathbun tanks on campus. Feeding of specimens was terminated 48hrs prior to each trial and 10 fish were used in each of three trial tanks.

The same sensitive refrigeration apparatus used in both the dynamic and static experiments in Section II was used for the work and the methodology is set out in Section II.

Results

Figure 15 shows percentage survival of greenback flounder following dynamic and static trials. Mortalities were recorded for 24hrs after fish were returned to 15°C (acclimation temperature).

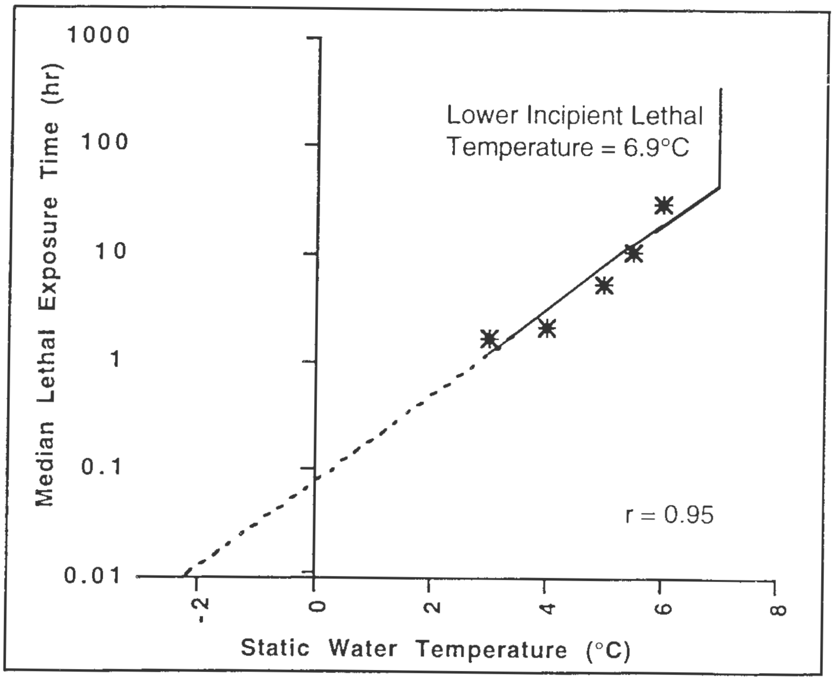


Figure 8 Median lethal exposure temperature (hours) versus static water temperature in five groups of blue-throat wrasse, *Notolabrus tetricus,* (4 x 20; 1 x 30) acclimated to 15°C.

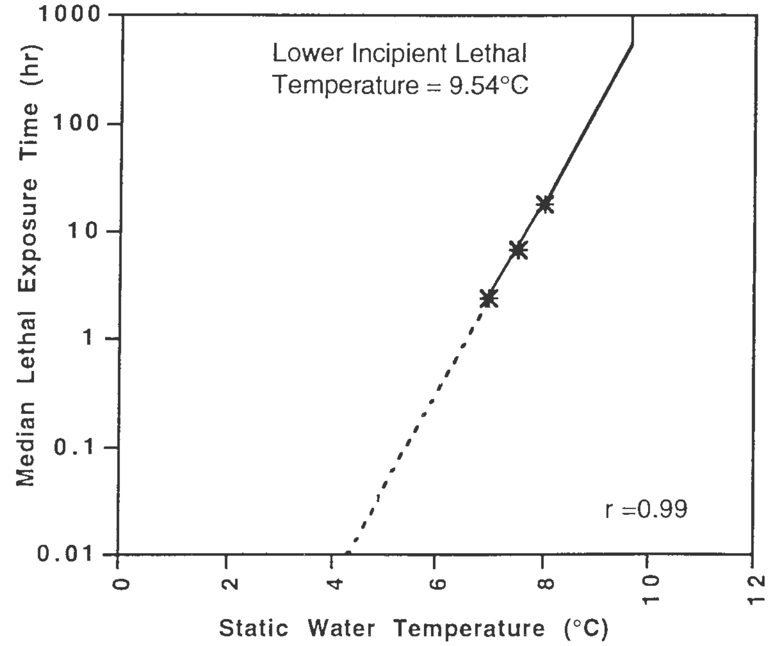


Figure 9 Median lethal exposure temperature (hours) versus static water temperature in four groups of blue-throated wrasse, *Notolabrus tetricus,* (3 x 20; 1 x 30) acclimated to 20°C.

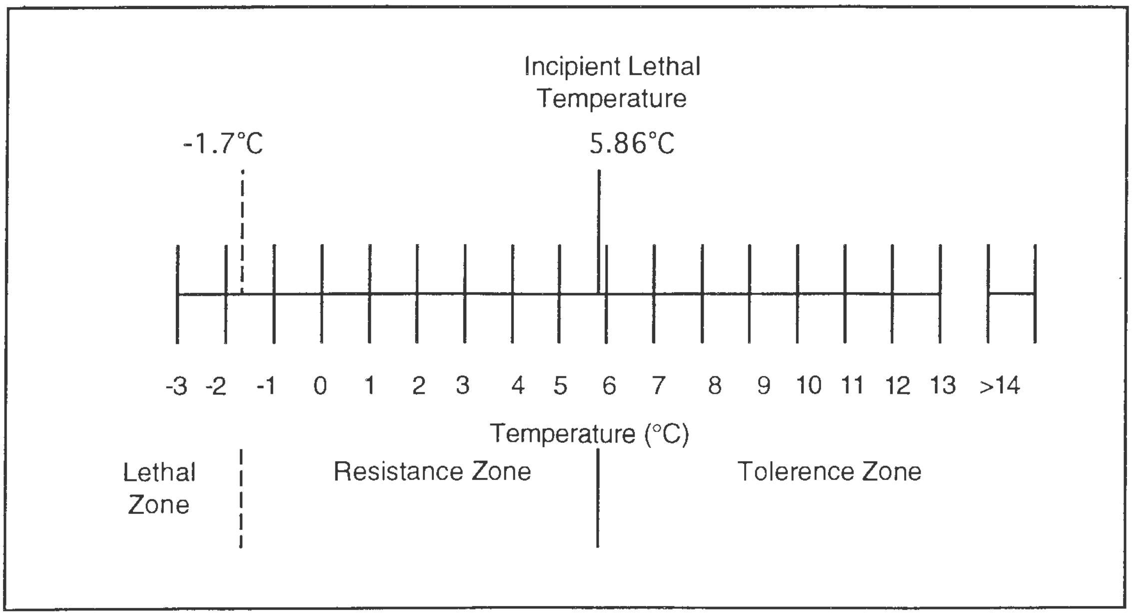


Figure 10 Static thermal profile for Blue-throat Wrasse, *Notolabrus tetricus* , acclimated to 10°C and subjected to various low temperatures for 24 hours. Lethal Zone = conditions intolerable for any period of time; death due to cold a certainty. Resistance Zone = fish may survive for limited periods; exposure time interval for 50% mortality = $0.0714 \times 10^{0.4993x}$. Tolerance Zone = death no longer a function of temperature; survival time infinite. Dotted lines dividing Lethal and Resistance Zones indicate approximate transition temperature. See text for details.

Figure 14 gives comparative data between flounder, wrasse and horseshoe leatherjacket which were subjected to static seawater temperature changes before being returned to 15°C.

It can be seen from the data in Figure 15 that the percentage of greenback flounder which survive a dynamic temperature change from 15°C to 1°C and then returned to 15°C is remarkably high (80%). Indeed, mortalities were zero until water temperature fell below 1.5°C. However, between 1.5°C and 1.4°C there was a sudden increase but between 1.4°C and 1°C mortalities were only 10%.

In the static experiments, flounder were able to tolerate 2hr immersion in water at 0°C with 50% survival when returned to 15°C. However, survival was zero if held at 0.9°C for 12 or 24hrs. Nevertheless, it should be noted that flounder survived 12h immersion in water at 3°C and 24hr immersion at 3.6°C. Nevertheless, even at these very low temperatures flounder were not necessarily comatosed.

Discussion

The results shown in Figures 14 and 15 clearly indicate that the greenback flounder is an excellent species for the live fish trade. Unlike wrasse, this fish species is able to tolerate extremely low seawater temperatures, offering much greater flexibility in choosing the correct temperature for comatisation than in wrasse. Furthermore, a cold initial water temperature is likely to permit a greater survival rate since during extended transit water temperatures will rise in containers.

An increase in water temperature during transportation is an obvious problem with species which cannot be held for significant periods at low temperatures. As pointed out in Section II wrasse cannot be held at low temperatures for long: this is in contrast to the greenback flounder. Even at 4.5°C flounder have close to 100% survival after 24hrs at this temperature. The need to increase water temperature after reducing the metabolic rate of wrasse makes this species far more difficult to transport live than flounder.

Figures 14 and 15 once again highlight the narrow temperature difference between survival and death in fish subjected to low temperatures. This is most clearly seen in Figure 15 (Dynamic) where mortalities suddenly increased between 1.5°C and 1.48°C. A similar finding is referred to in Section II for wrasse. These are important data for industry and should not be ignored. In Section I the model predicted mortalities but the find differences between a survival temperature and death was masked. We are continuing the research and hope to present meaningful protocols for coma induction and subsequent water holding temperatures during transport. Nevertheless, it is apparent that the fine line between coma inducement and death will necessitate investment in well designed and accurate refrigeration equipment, particularly for species such as wrasse which are far less tolerant of low temperatures than the green-back flounder. Furthermore the static approach may be the prefered approach. In Section I the dynamic system seemed to work best but subsequent mortalities, (not recorded) may have been high.

Section IV: Horseshoe Leatherjacket, *Meuschenia hippocrepis*

All specimens of horseshoe leatherjackets were captured by hooks or traps at the mouth of the Tamar estuary. Unfortunately, a major oil slick occurred at the sampling site in 1995 which resulted in this fish species leaving the effected reef and further samples were difficult to obtain. As a result little data has been obtained on this species to-date.

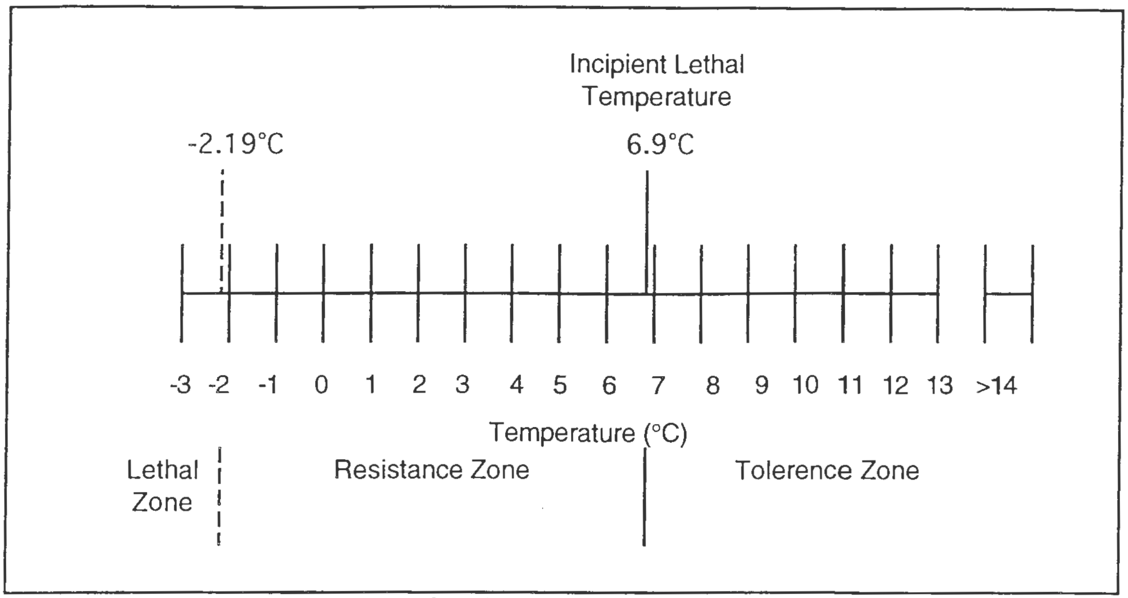


Figure 11 Static thermal profile for Blue-throat Wrasse, *Notolabrus tetricus* , acclimated to 15°C and subjected to various low temperatures for 24 hours. Lethal Zone = conditions intolerable for any period of time; death due to cold a certainty. Resistance Zone = fish may survive for limited periods; exposure time interval for 50% mortality = $0.0748 \times 10^{0.3981x}$. Tolerance Zone = death no longer a function of temperature; survival time infinite. Dotted lines dividing Lethal and Resistance Zones indicate approximate transition temperature. See text for details.

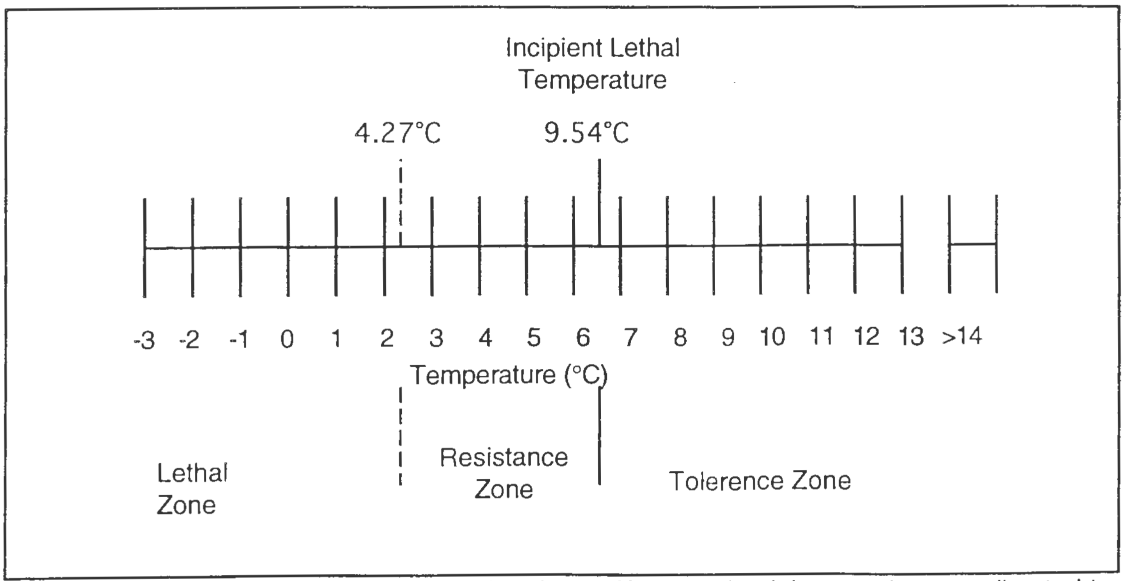


Figure 12 Static thermal profile for Blue-throat Wrasse, *Notolabrus tetricus* , acclimated to 20°C and subjected to various low temperatures for 24 hours. Lethal Zone = conditions intolerable for any period of time; death due to cold a certainty. Resistance Zone = fish may survive for limited periods; exposure time interval for 50% mortality = $1.905 \times 10^{0.8718x}$. Tolerance Zone = death no longer a function of temperature; survival time infinite. Dotted lines dividing Lethal and Resistance Zones indicate approximate transition temperature. See text for details.

The same apparatus used for water temperature control in the wrasse research was used in experiments on *M. hippocrepis*, and all fish were maintained in a temperature controlled holding tank and fed daily prior to experimentation. Water quality was measured each day.

Materials and Methods

Only static hold experiments were carried out. The materials and methodologies were similar to those reported in Section II.

Results

Figure 14 shows percentage survival of the horseshoe leatherjackets held for 2hrs, within the temperature range 5 to 7°C.

Discussion

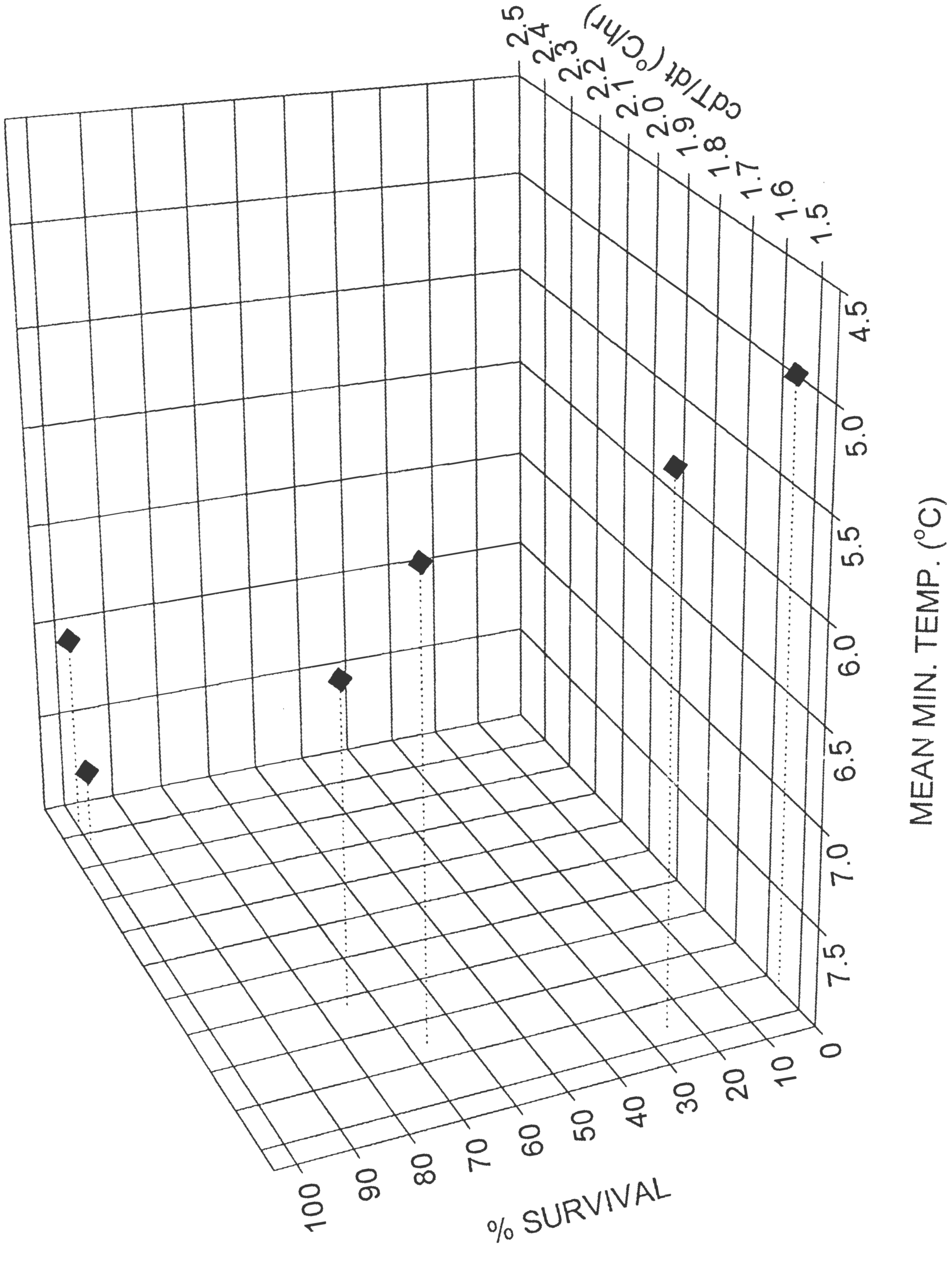
While it is unfortunate that further data on the horseshoe leatherjacket are not available at present, Figure 14 does tend suggest that this species is less tolerant of cold water than either wrasse or flounder. Even at 7°C for 2hrs survival has fallen to 80%. As seen in Figure 14 wrasse survival at 6°C for 2hrs was 100% although it fell rapidly below this temperature. It is possible that significant mortalities will not occur until temperature is reduced to 4°C or so but the narrow range between death and survival found in both the wrasse and flounder cannot be ignored. More research is required on this species.

Conclusions

- Experiments to determine the Critical Thermal Minimum and lower Incipient Lethal Temperature for a given species require accurate refrigeration units.
- It is not economically feasible to acclimate fish to the ILT since acclimation should be undertaken at a rate of about 1°C per day.
- There appears to be no advantage in using a dynamic approach to comatising fish for live export.
- Static cooling is successful so long as the operator recognises:
    - i) coma is difficult to induce;
    - ii) there exists a very fine temperature range between death-point and coma induction;
    - iii) the coma induction temperature is not sustainable in transit for several hours; however, coma can be maintained using slightly elevated temperature;
    - iv) it is easier to reduce metabolic rate than induce coma;
    - v) transport water will be warmer than the coma inducing water temperature;
    - vi) different temperate fish species have very different tolerances to cold water;
    - vii) percentage survival can not be ascertained until 24hrs after fish are returned to initial acclimation temperature.
- wrasse subjected to 5°C will die;

- wrasse can be comatised between 6.9 - 7.3°C for a short period by must be transfered to at least 8.5°C for transit times of up to 12hrs and 9°C for 24hrs;
- wrasse and horseshoe leatherjackets will behave extremely erratically if water temperature is lowered slowly, often resulting in death;
- flounder can tolerate temperatures as low as 2°C for 2hrs and 3°C for up to 12 hrs. For 24hrs transit 3.5°C is safe;
- very low temperatures for flounder did not always induce coma but certainly reduced activity; and
- 75% of horseshoe leatherjackets may survive water temperatures of 5°C for 2hrs.

Figure 13. Percentage survival of blue throated wrasse at different cold temperatures. Initial and final holding temperature was 15°C and temperature reduction (dynamic) is shown on the right-hand side of the figure (°C/hr). Mortalities were recorded for 24 hours after each experiment.



WRASSE DYNAMIC SUMMARY

Figure 14. Combined static percentage survival data for wrasse, flounder and horseshoe leatherjackets. Initial and final holding temperature was 15°C. Mortalities were recorded for 24 hours after each experiment.

COMBINED STATIC HOLD DATA FOR FLOUNDER, WRASSE AND HORSESHOE LEATHERJACKETS

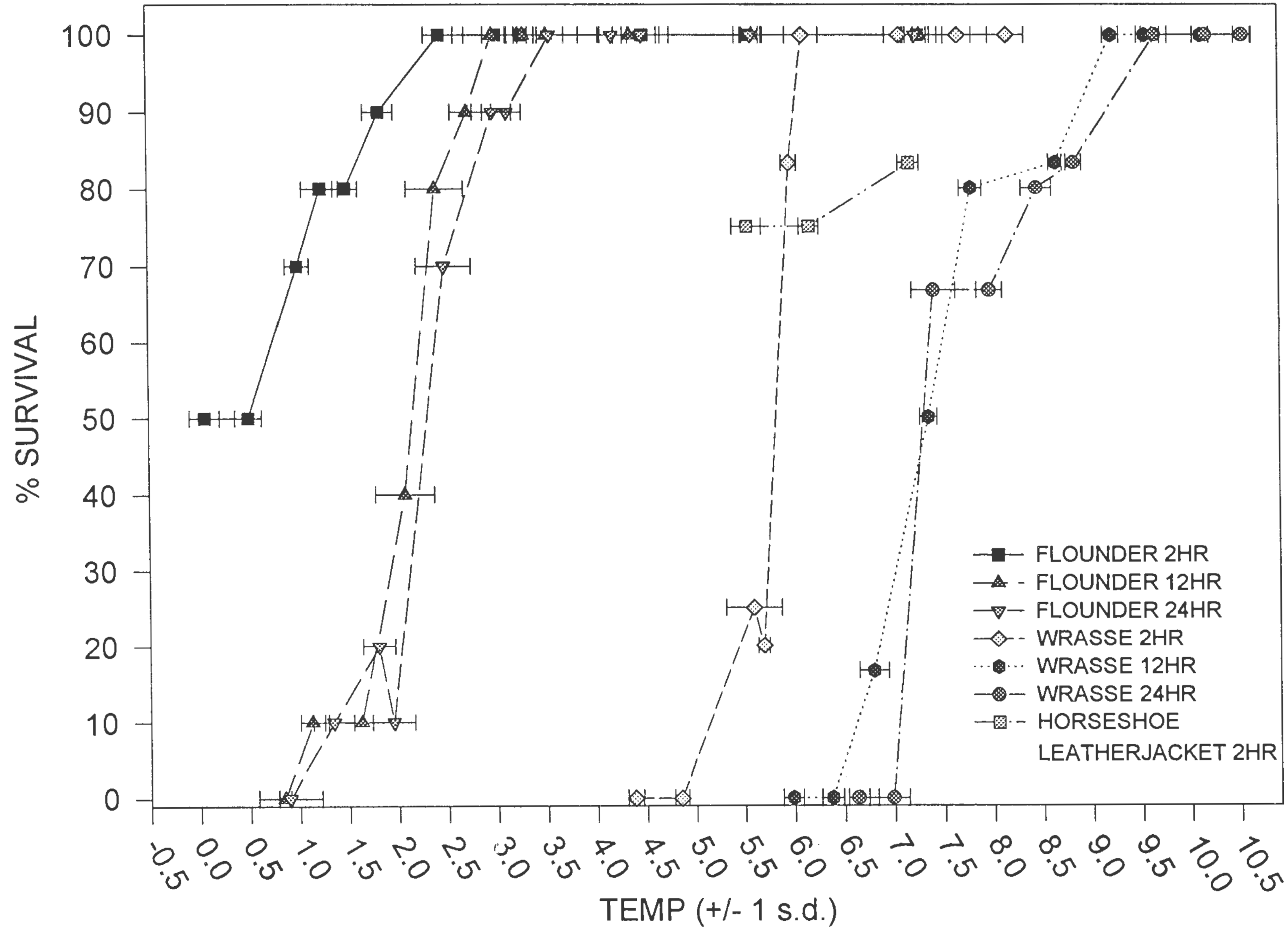


Figure 15. A comparison between static and dynamic water temperature changes on flounder survival at various cold temperatures. Initial and final holding temperature was 15°C. Mortalities were recorded for 24 hours after each experiment.

COMPARISON OF STATIC VS DYNAMIC TEMPERATURE CHANGE FOR FLOUNDER