Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes

Camilo Mora[a],*, Maria F. Maya[b]

[a]*Leigh Marine Laboratory, University of Auckland, 349 Goat Island Road, Leigh, New Zealand*
[b]*Seccion de Biología Marina, Universidad del Valle, A.A. 25360, Cali, Colombia*

Abstract

Heat tolerance is commonly determined by exposing organisms to increasing temperatures until they show symptoms of thermal stress or death. Here we carried out an experiment on a blenny species (*Acantemblemaria hancocki*; Pisces: Chaenopsidae) and reviewed the literature to evaluate the extent to which variations in the rate at which temperature is increased in experimental trials affects thermal tolerance of fishes. For the blenny species, we found that thermal tolerance decreases significantly from an intermediate heating rate of ~1 °C/h towards quicker and slower heating rates. In the literature we found very few comparisons of thermal tolerance among heating rates (i.e. eight fish species) and although such comparisons were done over narrow ranges of heating rates, overall they appear to follow the pattern described for the blenny species. We discuss a variety of factors including variations in the levels of acclimation, energy use and body quality among heating rates as the causes for this pattern. However, available data are still limited and further research will be necessary to determine the generality and causes of the pattern we found here. Nevertheless, our results indicate the need for caution in the extrapolation of thermal tolerance data when assessing the tolerance of organisms to environmental phenomena that vary in their rates of warming.

1. Introduction

The experimental determination of the thermal tolerance of species is a common practice in the field of thermal biology. This is motivated not only by our interest in understanding species' thermal physiology but also by our current need to assess the biological effects of different thermal phenomena such as climate change, El Niño, La Niña, overwinters, etc. (Glynn and D'Croz, 1990; Bennett et al., 1997; Addo et al., 2000; Beitinger et al., 2000; Mora and Ospina, 2001, 2002; Kimball et al., 2004; Kimura, 2004). Thermal tolerance is commonly quantified through two different experimental methods. In the "static method", the temperature that causes 50% mortality is determined from a plot of percent mortality at static

temperatures whereas in the "dynamic method", temperature is increased or decreased gradually until a critical point is reached (e.g. equilibrium lost, death). In the dynamic method the critical thermal maximum (CTMax) or minimum is quantified as the mean temperature at which individual fish reach such critical points. The dynamic method has been more broadly used because it is easier to use, requires fewer animals, provide quick data (Lutterschmidt and Hutchison, 1997) and its results are more comparable to natural conditions (Bennett and Judd, 1992; see also Mora and Ospina, 2001).

One problem of using the dynamic method to determine levels of thermal tolerance is the rate at which temperature is changed in the experiments. Rapid rates can produce long lag times between environmental and body temperature, which can overestimate thermal tolerance but may also lead to variations in thermal tolerance in relation to body size (Becker and Genoway, 1979; Lutterschmidt and

*Corresponding author. Fax: +64 9 422 6113.
E-mail address: moracamilo@hotmail.com (C. Mora).

Hutchison, 1997; but see Ospina and Mora, 2004). In contrast, slower heating rates allow fish to acclimatize to new temperatures, which could increase thermal tolerance (Cocking, 1959; Beitinger et al., 2000). However, if heating rates are too slow they could reduce tolerance as temperature could have more time to exert its lethal effects (Cocking, 1959; Beitinger et al., 2000). Finally, in experimental animals, stress hormones are known to increase with time of captivity (Pankhurst and Sharples, 1992); if stress affects thermal tolerance, then one would expect thermal tolerance to reduce at slower heating rates due to the increased captivity time at such heating rates.

Despite the potential incidence of the chosen heating rate on the levels of thermal tolerance this has been poorly studied. As an example, until 2004 the Cambridge Scientific Abstracts Database contained 197 citations referring to ''critical thermal maximum''; of these only five specifically referred to ''heating and/or warming rates''. Out of these five one did not provide any comparison of thermal tolerances among heating rates (Gomez, 1990) and another was based on the rate of temperature penetration in the body of turtles (Webb and Witten, 1973). Our precarious understanding about the effect of the heating rate on thermal tolerance is particularly critical given the broad range of heating rates used so far. Lutterschmidt and Hutchison (1997) reviewing 604 papers on critical thermal maximum reported that among studies heating rates have varied between $10\,°C/min$ to $1\,°C$ per 3.5 days. Beitinger et al. (2000), reviewing 80 publications on thermal tolerance of North American fishes reported heating rates between 0.027 and $60\,°C/h$. Even recommendations for the use of standard rates have been variable (e.g. $0.3\,°C/min$: Becker and Genoway, 1979; $1\,°C/min$: Lutterschmidt and Hutchison, 1997). Here we use different heating rates to quantify the critical thermal maximum of a reef fish species and compiled data from previous studies to search for general patterns on the effect of heating rates on the thermal tolerance of fish.

2. Materials and methods

The blenny fish *Acantemblemaria hancocki* Myers and Reid, 1936 (Pisces, Chaenopsidae) was the focal species of this paper. This is a common species of rocky shores in the Eastern Pacific where it inhabits mainly dead barnacles. Fish of similar size (average total length = 3.1 cm, SD = 0.3) were collected with hand nets and clove oil in Tabogilla Island (Panama) and then transported within 20 min to the laboratory of the Smithsonian Tropical Research Institute in Naos Panama. All fish collections were done during one day and the experiment started the day after. Collected fish were randomly placed into 20 l aquaria (five fish per aquarium) (water temperature in all aquaria was the same as in the field: $24\,°C$). A total of 21 aquaria were deployed for five heating rates ($60\,°C/h$, $1\,°C/h$, $1\,°C/12\,h$, $1\,°C/24\,h$, $1\,°C/48\,h$) and two sets of controls

with ambient temperature. We used three replicate aquaria for each of the treatments and controls.

We estimate any effect of captivity time (and/or its associated stress) by determining the thermal tolerance of fish with a single heating rate of $1\,°C/h$ at different times during the duration of this experiment (days 1, 8 and 25). The data of thermal tolerance with a heating rate of $1\,°C/h$ obtained the day after fish collections was used for the comparison among heating rates but also for this analysis on the effect of captivity time (i.e. day 1 of captivity). Thermal tolerance for the two other captivity times was determined using the two sets of control aquaria 8 and 25 days after the start of the experiment (day 25 was the last day of the experiment). Thermal tolerance was quantified as the mean temperature at which individual fish show erratic swimming. This critical point is widely used as it represents conditions that will ultimately lead to death (Beitinger et al., 2000).

Fish were fed once per day with frozen artemia (food ration of 0.1 ml of artemia per fish). Filtered seawater was supplied constantly to ensure $\sim100\%$ water interchange per day. Aeration was also constant to keep high oxygen concentrations and a continuous mixing of water. All sides of the aquaria, but the frontal side, were covered with Styrofoam to avoid heat lost and changes of temperature in the aquaria through their interface with the surrounding medium. Each aquarium was equipped with an electronic control of temperature (Autonics TZN4S), a sensor of temperature (Autoniz PT100) and a 300 W heater. With this setup water temperature was controlled with $0.1\,°C$ accuracy. These electronic controls were also designed to control water temperature inertia, which can expose fish to undesired thermal effects (here inertia refers to increases in water temperature beyond a desire point when temperature is gradually increased and not to the rate of temperature penetration in the body of fishes). To do so, the electronic controls switched on and off the heaters before reaching the desire temperature so that temperature never exceeded the desire limit. Note that broader inertias are often present at quicker heating rates as temperature cannot be easily stopped at the desire point when it is being quickly increased. The difficulty of keeping constant heating rates throughout an experiment has been an important critique to the dynamic method of quantifying thermal tolerance of fishes (Cocking, 1959) and it is a key assumption in the interpretation of its results (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997).

Upper temperature tolerance data among heating rates and captivity times in *A. hancocki* were presented graphically and compared via ANOVA. Finally, we searched the literature for studies that have specifically analyzed variations in thermal tolerance among heating rates. Our search was based on the indexing databases of Biological Sciences and Biology Digest from Cambridge Scientific Abstracts and from references cited in the papers found.

3. Results

None of the fish held in captivity in control aquaria died or showed symptoms of stress (e.g. unusual behaviors or changes in normal coloration) during the experiment. The effect of captivity stress on fish tolerance was opposite to expected (i.e. tolerance decreasing over time) as thermal tolerance actually increased with increasing captivity time. There were significant differences in thermal tolerance among different lengths of captivity ($F = 4.3$, $p < 0.01$). Fishes held in captivity for 25 days showed higher thermal tolerances than fishes held in captivity for 1 or 8 days (Tukey test, $p < 0.01$) (Fig. 1). However, all the variation in thermal tolerance among captivity lengths was less than 1 °C (Fig. 1).

A. hancocki showed the maximum tolerance at a heating rate of 1 °C/h (i.e. CTMax = 36.6 °C, ± 0.15 SD) and decreased from this point towards slower and faster heating rates [at 60 °C/h CTMax = 35.7 (± 0.6 SD) and at 0.02 °C/h CTMax = 34.3 (± 0.27 SD)] (Fig. 2(A)). Because the data on thermal tolerance showed no homogeneity of variances (Levine's test $F = 27.9$, $p < 0.0001$; Cochran 0.67, $p < 0.0001$) (Fig. 2(A)) we also analyzed variations in thermal tolerance among heating rates with the use of a non-parametric analysis of variance (i.e. Kruskal-Wallis ANOVA). With the use of both parametric and non-parametric tests, thermal tolerance was significantly different among heating rates ($F = 123$, $p < 0.0001$; $H = 75$, $p < 0.0001$): all heating rates but the two slowest ones were significantly different from each other at $p < 0.001$ with the use of a Tukey test with both parametric and ranked data.

Comparisons in thermal tolerance among heating rates were found for eight fish species (Fig. 2). None of these studies determined thermal tolerance over a range of heating rates as broad as the one analyzed in this study. The most recent study on the effect of heating rates, Elliot
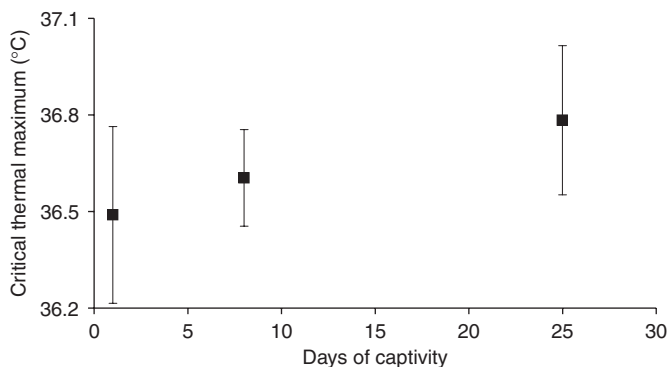

Fig. 1. Effect of captivity time on the thermal tolerance of the blenny fish *Acantemblemaria hancocki*. Thermal tolerance was determined at different times during the duration of the experiment with a unique heating rate of 1 °C/h. Thermal tolerance is expressed as the mean temperature at which fish showed erratic swimming ($\pm$SD). For each captivity time, there were three replicate aquariums with five fish each. Data among aquaria were identical; therefore statistics were calculated combining the data of the 15 fish used for each captivity time.
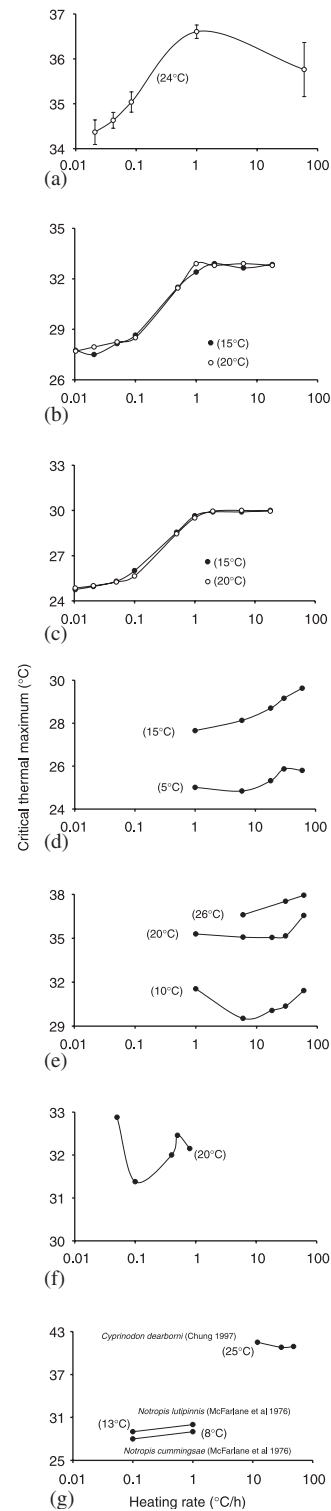

Fig. 2. Relationship between heating rates and thermal tolerance to high temperatures in different fish species. Data is shown as means. Numbers between parentheses indicate the temperature of acclimation in the different studies. In plot A, data among aquaria were identical; therefore statistics were calculated combining the data of the 15 fish used for each heating rate; in this plot vertical bars represent standard deviations of that data. (a) *Acantemblemaria hancocki* (This study), (b) *Salmo salar* (Elliot and Elliot, 1995), (c) *Salmo trutta* (Elliot and Elliot, 1995), (d) *Oncorhynchus kisutch* (Becker and Genoway, 1979), (e) *Lepomis macrochirus* (Cox, 1974; Becker and Genoway, 1979), (f) *Rutilus rutilus* (Cocking 1959) and (g) Other species.

and Elliot (1995) using two *Salmo* species, determined thermal tolerance using slow heating rates and showed trends remarkably similar to ours with those rates (Figs. 2(B) and (C)). Unfortunately, the trend observed with *Salmo* species did not provide information on the side of quicker heating rates (Figs. 2(B) and (C)). Another recent study, Chung (1997) using *Cyprinodon dearborni,* analyzed quick but not slow heating rates and showed a declining trend in thermal tolerance similar to ours on that spectrum of the heating rates (Fig. 2(G)). Older studies commonly used quicker heating rates to determine thermal tolerance and all showed patterns of variation opposite to ours. That is, thermal tolerance increasing with quicker heating rates (Cox, 1974; Becker and Genoway, 1979) (Figs. 2(D)–(F)). An interesting aspect of the comparison between older and more recent studies is that among recent studies temperature of acclimation yielded only minimal variations in thermal tolerance at similar heating rates (Figs. 2(B) and (C)) while such differences were more pronounced among old studies (Figs. 2(D)–(G)).

4. Discussion

Determining the levels of tolerance to temperature of marine organisms is critical to assess the extent to which climate change and other thermal phenomena will affect mortality rates and the abundance and distribution of species. In this regard, out of the available approaches, the dynamic method of determining thermal tolerance in aquatic organisms seems to provide the most reliable data for comparative purposes with environmental conditions (see Bennett and Judd, 1992; Mora and Ospina, 2001). Yet its use will require proper understanding of the factors likely to affect thermal tolerance in experimental trials. In this study, we have shown that the speed of the heating rate is a key factor influencing levels of thermal tolerance in fishes.

The common pattern among recent studies is for thermal tolerance to decrease from an intermediate heating rate (i.e. ~1 °C/h) towards faster and slower rates. Cocking (1959) suggested that heating rates affect not only the time a fish have for acclimation but also the time they are exposed to lethal temperatures. Maximum tolerance gain due to acclimation is then likely to occur at the heating rate at which fish achieve full acclimation (Cocking, 1959). In our results such a rate appears to be 1 °C/h given that at this rate several of the analyzed species showed their maximum tolerance. For ectotherms, full acclimation to high temperatures can be achieved within only few hours (Hutchison, 1976; Chung, 1985, 1995, 2001 Segnini et al., 1993; Lutterschmidt and Hutchison, 1997) suggesting that the increased tolerance at a heating rate of 1 °C/h could indeed obey to the fact that at this rate fish achieve full acclimation to the change in temperature they were exposed to. Reduced levels of tolerance toward faster heating rates could be similarly explained by "inappropriate" acclimation to the quick change in temperature.

Barker et al. (1981) and Hutchison and Murphy (1985) also suggest that rapid changes in temperature can induce heat-shocks effects leading to underestimation of upper thermal limits (Lutterschmidt and Hutchison, 1997).

Cocking's (1959) argued that heating rates slower than the rate for complete acclimation could have harmful effects on fish as they will have longer exposure to the lethal effects of any given temperature. This in turn could explain the reduced thermal tolerance observed in fish exposed to heating rates slower than 1 °C/h. An additional and/or complementary explanation for the reduced tolerance at slow heating rates is the reduction of energetic reserves and/or body deterioration at high temperatures even if temperatures are non-lethal. Elevated temperatures are known to increase metabolism and energetic demands (Hochachka and Somero, 1973). If food supply is kept constant (as in our case) body condition is likely then to deteriorate over time. In fact, we noted that after few days of exposure to slow heating rates fish were more voracious and bonier than fish in control aquaria highlighting their increased food demands and diminished body condition. Hutchison (1976) argued that body condition and energetic reserves are likely to affect thermal tolerance in fishes. This in turn could have reduced tolerance at slow heating rates and/or contributed to the effects of slow heating rates argued by Cocking (1959) and pointed above. Note that stress due to captivity time during long experiments is unlikely to explain the reduced thermal tolerance with slow heating rates since we found that captivity time did not diminish thermal tolerance (Fig. 1).

However, the pattern of decreasing thermal tolerance towards slow and quick heating rates was not without exceptions. Specifically, we found a set of papers reporting increasing thermal tolerance among quick heating rates (Figs. 2(E)–(F)). Interestingly, however, was the fact that all these cases occurred among old studies. Although the mentioned differences may relate to differences in the species used, it may also conform to technical advances in the control of water temperature (e.g. control of water temperature inertia, which is stronger at quicker heating rates, which were commonly used among old studies). Difficulties in maintaining constant heating rates have been previously mentioned as a limitation of old studies (Cocking, 1959). Compared to more recent papers, old studies also showed stronger effects of acclimation on thermal tolerance with the use of similar heating rates. This suggests that methodological differences between these groups of studies may have indeed played an important role in explaining the opposite results shown by old studies about the effects of heating rates on thermal tolerance.

Previous studies have argued the need for standardization of fast heating rates for determining heat tolerance in fishes (e.g. 0.3 °C/min: Becker and Genoway, 1979; 1 °C/min: Lutterschmidt and Hutchison, 1997). Such rates have been argued to be rapid enough to avoid the effects acclimation and slow enough to prevent lags between water and fish body temperature during a trial. However, the

speeds of acclimation are known to vary among species (Chung, 1985, 1995, 2001; Segnini et al., 1993; Lutterschmidt and Hutchison, 1997) and so are the rates of temperature penetration in the body of fishes given variations in the ratio area/volume among species and in the actual body size of species. This suggests that for comparative purposes the recommendation of using standard heating rates could have been premature. If the pattern we describe here holds for other species, for comparative purposes it would be more recommendable to use heating rates at which species show similar responses (e.g. the inflection point at which tolerance declines towards fast and slow heating rates). This will require studies to start reporting thermal tolerance over broad heating rates. Such data would allow more useful extrapolations of thermal tolerance data to thermal phenomena given the possibility of accounting for variations in the rates of warming of different environmental phenomena.

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