The Journal of Experimental Biology 216, 3450-3460

doi:10.1242/jeb.088856

RESEARCH ARTICLE

Precision of archerfish C-starts is fully temperature compensated

Philipp Krupczynski and Stefan Schuster*

Department of Animal Physiology, University of Bayreuth, D-95440 Bayreuth, Germany

*Author for correspondence (stefan.schuster@uni-bayreuth.de)

SUMMARY

Hunting archerfish precisely adapt their predictive C-starts to the initial movement of dislodged prey so that turn angle and initial speed are matched to the place and time of the later point of catch. The high accuracy and the known target point of the starts allow a sensitive straightforward assay of how temperature affects the underlying circuits. Furthermore, archerfish face rapid temperature fluctuations in their mangrove biotopes that could compromise performance. Here, we show that after a brief acclimation period the function of the C-starts was fully maintained over a range of operating temperatures: (i) full responsiveness was maintained at all temperatures, (ii) at all temperatures the fish selected accurate turns and were able to do so over the full angular range, (iii) at all temperatures speed attained immediately after the end of the C-start was matched – with equal accuracy – to 'virtual speed', i.e. the ratio of remaining distance to the future landing point and remaining time. While precision was fully temperature compensated, C-start latency was not and increased by about 4 ms per 1°C cooling. Also, kinematic aspects of the C-start were only partly temperature compensated. Above 26°C, the duration of the two major phases of the C-start were temperature compensated. At lower temperatures, however, durations increased similar to latency. Given the accessibility of the underlying networks, the archerfish predictive start should be an excellent model to assay the degree of plasticity and functional stability of C-start motor patterns.

Key words: M-cell network, acclimation, motor circuit, functional stability, circuit homeostasis.

Received 26 March 2013; Accepted 16 May 2013

INTRODUCTION

A fundamental integrative property of poikilothermic animals is that they manage to breathe, escape and maintain other vital functions even when changes in temperature drastically change cellular and molecular properties within the underlying circuits. An excellent example of a vital motor response that should not be compromised by temperature effects is the so-called C-start shown by most escaping teleost fish (e.g. Eaton et al., 1977; Eaton et al., 2001). This characteristic pattern allows fish to achieve the highest attainable acceleration by first bending their body into the shape of a letter C and then, with all fins erected, pushing off as much water as possible in as little time as possible (Weihs, 1973; Webb, 1975). Evidently, the fish must be able to keep the level of acceleration, short latency and directionality adaptive even when a change in temperature affects the elements of the underlying circuitry. In connection with the robust and vital behaviour they mediate, the accessibility of the underlying circuits (Furshpan and Furukawa, 1962; Faber and Korn, 1978) has made the C-start networks of fish one of the major models and sources of insight in neuroethology (e.g. Faber et al., 1989; Zottoli and Faber, 2000; Eaton et al., 2001; Korn and Faber, 2005). C-starts are driven by a surprisingly small set of a few hundred reticulospinal neurons in the hindbrain of the fish. Among these a set of six identified cells, which can be recognized from one fish to the next, play a major role in enabling the very fastest starts. When these are killed, C-starts are still possible, but no longer at the very top performance level (Liu and Fetcho, 1999; Kimmel et al., 1980). Among these, the pair of Mauthner (M-) cells is particularly interesting: in the intact system the M-cells are the largest, fastest conducting and first neurons to respond to a startle stimulus. A single spike in one of the two cells releases the C-start pattern and determines whether the body will bend towards the left or right side (e.g. Zottoli, 1977; Eaton et al., 1981).

Taking full advantage of this experimentally amenable system, two previous studies (Preuss and Faber, 2003; Szabo et al., 2008) succeeded in analysing both acute and acclimation effects of temperature on escape probability and directionality, and in relating these to cellular and synaptic changes at the level of the M-cell and associated circuitry. They thereby not only provided the first evidence for compensation effects in the vertebrate central nervous system but also linked them to properties of an identified neuron. Acute cooling increased behavioural responsiveness and decreased directional selectivity and this could directly be explained by changes in the dendritic cable properties of the M-cell and by changes in the balance between excitatory and inhibitory inputs into the cell (Preuss and Faber, 2003). A particularly interesting finding was that acclimation allowed the fish to compensate for the acute effects of cooling, but not of warming up: a drastic increase in escape probability and decrease in directionality upon warming was not compensated for. Again, the finding could be explained at the level of the M-cell as a lack of compensation for the increased excitatory synaptic inputs (Szabo et al., 2008). Environmental temperature affects the M-cell system in many ways and can even elicit M-cell spiking (Sillar and Robertson, 2009).

The accessibility of the underlying networks makes C-starts highly attractive for studying how well function is balanced against environmental perturbations. However, C-starts seem to lack one major ingredient of a good model: to accurately measure the effects

of temperature changes on accuracy, one should know exactly what the intended output (i.e. turn angle, speed after C-start) would be in the given situation. If this is not known it is not possible to detect small deviations from the 'desired' pattern. Evidently, this is at odds with the very nature of an escape: all that should be known *a priori* – both to a predator and to the experimenter – is that the C-start should probably be directed away from the source. This means that the intended direction of an escape can only be known with an ~180 deg accuracy, making it impossible to detect small deviations between desired and actual direction in a given situation. Furthermore, although the escape should be fast, no study has yet identified exactly what speed the escape should initially have in a given situation. Most importantly, for an escape, variability in angle and speed in response to the same situation would seem to be an advantage rather than being detrimental. This degree of inherent and required variability would seem to prevent the experimenter from knowing precisely what the animal should do in a given test situation and to then precisely quantify the errors brought about by changes in temperature.

The situation is completely different with a newly discovered type of C-start shown by hunting archerfish (Fig. 1). Briefly, these fish dislodge aerial prey items with a shot of water fired from their mouth (e.g. Smith, 1936; Lüling, 1963). To increase the chance of actually catching the dislodged prey despite the presence of competitors, shooting comes packaged with a C-start motor pattern, the 'predictive start': after a brief view of the initial motion of the falling prey, the fish can initiate a C-start that turns them right towards the later landing point and pushes them off with a matched speed so that the fish would arrive at the right spot in just the right time (e.g. Schuster, 2012). With its aim well known for a given combination of initial values of prey motion, the archerfish 'predictive' C-start would appear to be perfectly suited to study how well C-starts can be buffered against even slight changes in internal operating parameters that would affect function. The high accuracy attainable by studying archerfish C-starts could thus disclose an inherent buffering capability of C-start circuitry that very likely is also present in escapes but masked by our ignorance of the 'randomizing' additions the fish presumably adds to make its course unpredictable. Most importantly, the methodological advantages of using archerfish predictive C-starts come together with tough ecological demands for temperature-compensated functionality at all operating temperatures: their complex mangrove biotopes force the fish to cover considerable distances in their search for hunting grounds. The fish consequently often face dramatic and rapid changes in light conditions, salinity and temperature over the course of a few hours (S.S., unpublished observations). Shooting without a predictive start or with one that is not well aimed would usually mean losing the food to the more numerous competitors. Moreover, because the rapid starts are performed right below the water surface, they are conspicuous to the many aquatic and aerial predators around. So, it would probably be better for the fish to not start at all than launch an inaccurately aimed predictive C-start that misses the later landing point.

Using the methodological advantages of the archerfish predictive starts and the high constraints on precision, we explored the degree to which C-start manoeuvres can be functionally buffered against changes in temperature. Specifically, we analysed whether the fish can keep up the constant high release probability, fine-tuned selection of speed and angle, short latency and speed of the C-start motor pattern throughout the temperature range at which they hunt in the wild.

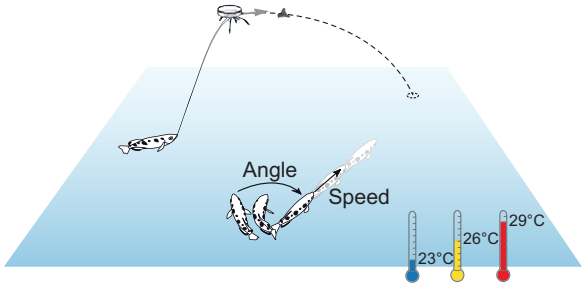

Fig. 1. The archerfish predictive start as a sensitive assay of temperature compensation in C-starts. Shortly after aerial prey is dislodged by a shot, archerfish can initiate a C-start, their so-called predictive start. The kinematics of this start are selected based on information sampled during the initial motion of the falling prey, from which the fish derive height, direction and speed and predict where prey is going to land. By adjusting their C-start accordingly, the fish rotate by just the correct angle and start with an appropriate speed that is matched to distance and timing. Because the required speed and aim at the end of the C-start is known for any combination, even small deviations of actual performance can be sensitively detected. In the tests reported here, a change in average alignment or an increase in scatter of the alignment below 1 deg could have been detected. Moreover, the fine-tuned archerfish C-starts provide an intrinsic control for unchanged motivation: the fish will dislodge prey only when they are in the mood to hunt and to catch prey. Thus, they provide the cues that release predictive starts only when they are motivated. The inset introduces colours used in all subsequent figures for the three main experimental temperatures 23, 26 and 29°C.

MATERIALS AND METHODS

Animals

Experiments were performed on a group of five archerfish, *Toxotes chatareus* Hamilton 1822. The body lengths of the fish (from snout to caudal peduncle) ranged from 7.6 to 10.9 cm (9.1±0.7 cm, mean ± s.d.). Fish were kept and all experiments were carried out in a large tank (1.0×1.0×0.6 m) filled to a height of 35 cm with brackish water (conductivity 3.5–3.7 mS cm^{-1}). A light regime of 12 h:12 h was maintained and experiments were started no earlier than 5 h after light onset. Because all group members responded to dislodged prey, their responses were pooled.

Experimental setup and acclimation

The basic design is illustrated in Fig. 1. One dead fly (*Calliphora* sp.) at a time was wetted and stuck on the lower side of a transparent acrylic circular platform (50 mm in diameter), mounted 30 cm above the water surface in the centre of the tank. Dislodged flies fell ballistically (see Rossel et al., 2002). Experiments started at a temperature of 26°C. After 15 days of testing, the temperature was changed to 23°C, followed by 29°C and then – as a final control for stability of the general performance – an additional phase of 26°C. Each phase started with 7–10 days of acclimation in which the fish were kept in training. After this time, experiments were run for 3–4 weeks. To ensure comparable motivation, the number of trials run per day differed at the different temperatures: 30 trials per day at 26°C, 20 trials per day at 23°C and 35 trials per day at 29°C. Accordingly, the fish were tested for 15 days at 26°C, 27 days at 23°C, 21 days at 29°C and then another 15 days at 26°C. By performing the experiments in a temperature-stabilized room, whose temperature was accurately set 2°C below the tank's intended temperature, a 300 W heater sufficed to set temperatures with an accuracy of ≤0.1°C.

Recording

We used digital high-speed video recording at 500 frames s^{-1} (HotShot 1280M, NAC Deutschland, Stuttgart, Germany; lens Sigma 20 mm f/1.8 EX DG). The camera viewed the water surface from a height of 1.5 m at a right angle. Contrast was optimized by diffusely illuminating the tank from below by shining four halogen spotlights (200 W each) onto a diffusor plate (mounted below the tank to cover its full area). Additionally, two halogen spotlights (400 W each) were directed upwards onto a sheet that covered 2.0×2.0 m at a height of 1.8 m above the water surface. The resulting Michelson contrast between the falling fly (reflected light 31.3 cd m^{-2}) and its background (reflected light 422.4 cd m^{-2}) was 0.86 in all experiments. Because target and fish were at different distances, we needed to correct for the resulting metric distortions. To achieve this, we projected the falling prey onto the water surface, taking into account the known distortion in perspective that applied for the fly's current height level. From the way flies fell, the height level that we needed for this calculation could simply be derived from the time of falling, a procedure also used previously (e.g. Rossel et al., 2002; Schlegel and Schuster, 2008).

Data analysis

The recordings were processed using ImageJ (developed at the National Institutes of Health) and custom-written software. We evaluated latency, aspects of the C-start kinematics, and the accuracy of the fish's rapid predictive turn and its take-off speed. Responsiveness was 100% at all temperatures, i.e. in all situations in which one group member fired at the prey and dislodged it, at least one group member showed a C-start that was finished before prey impact. To ensure that the C-starts were selected solely on the basis of information on prey motion but not on the basis of the responses of the other fish, we analysed only the C-start of the fish that responded first, as described previously (e.g. Rossel et al., 2002; Wöhl and Schuster, 2007; Schlegel and Schuster, 2008). To ensure that accuracy was not due to additional mechanosensory input from the splashing impact of prey, all analyses (including the take-off part that immediately followed the C-start) exclusively relate to C-starts after which the fish took off before the prey's impact. Because obstacles strongly influence the fish's initial turn (S. Wöhl and S.S., unpublished), we excluded cases in which another fish blocked the direct path to the later impact point. In order to easily measure latency and to critically assay turning accuracy, we required the starts to turn the fish by at least 10 deg. In a number of trials the dislodged fly simply fell vertically – these trials were not included in the present analysis because 'predicting' the later landing point would have been trivial in such cases. In addition, cases were excluded in which the fish could simply follow the prey's motion; a minimum angle of 10 deg was required between the fish's path to the point of impact and the fly's horizontal trajectory. Latency was derived from the number of frames between the onset of prey movement and the initiation of the C-start. Duration of the C-start and its two stages were similarly obtained. Stage 1 was defined as the time the fish needed from initiating the turn until its body was maximally bent, and stage 2 as the time from maximum bend until the end of the turn leading to the subsequent take-off phase. Accuracy of the turn was assessed as follows: the error e of the aim of the C-start was taken at the end of stage 2 as the minimum distance a line in the initial direction had from the fly's later landing point. As in earlier papers (e.g. Rossel et al., 2002), this distance was considered negative if the line intersected the projected path of the fly before the impact point; otherwise, it was taken as positive. The following analysis gave the accuracy of the speed adjustment: immediately at the end of stage 2, speed values were taken from the change in position of the fish's centre of mass (see Wöhl and Schuster, 2006) in four consecutive 10 ms intervals. Unless stated otherwise, the mean of these four speed values is reported.

Statistical analysis

Unless stated otherwise all tests were run using SigmaPlot (version 11.0, Systat Software Inc. 2008) and performed two-tailed with an alpha level of P=0.05. In *post hoc* tests, the level of significance was examined using sequential step-down Bonferroni corrections. Normality of data was assayed using Shapiro–Wilk tests and additionally confirmed with Q–Q plots coupled with histograms. For parametric data, equal variance was checked using Levene tests. As non-parametric tests (for latency and C-turn properties), we used Mann–Whitney U-tests and Kruskal–Wallis one-way ANOVA on ranks, *post hoc* tested with Dunn's method. Parametric tests (for error in aim and take-off speed) consisted of one-sample and two-sample t-tests and one-way ANOVA, *post hoc* tested with the Holm–Sidak method, and repeated measures ANOVA. Correlations were tested either using Pearson correlation (parametric) or Spearman rank correlation (non-parametric).

Tests on how temperature affected the relationship between take-off speed and 'virtual speed' were run in R (version 2.10.1, R Development Core Team, 2009) using multivariate linear models. To compare whether 'virtual speed' (i.e. remaining distance per remaining time) or distance by itself better describes the variability in take-off speed, we used two different multivariate linear models with either 'virtual speed' or distance as predictor. Model selection was based on the method described previously (Rödel et al., 2004; Burnham and Anderson, 1998; Wagenmakers and Farrell, 2004) using the second-order Akaike's information criterion (AICc). In summary, the most suitable model is the one with the smaller Δ_i (=AICc–minAICc) and the larger Akaike weights w_i. Additionally, R was used to test equal variance of non-parametric data using rank-based modified Brown–Forsythe tests.

Stability

Table 1 reports all aspects of the C-starts both for the initial phase at 26°C and in the final control phase at the same temperature. None of the aspects showed any significant changes (see Results for an

Table 1. Comparison of initial and final measurements at 26°C demonstrates stability of overall conditions

Variable	Initial	Final	Statistics
Latency (ms)	73.9±14.6 (91)	72.4±15.6 (104)	U-test P=0.28
Size of turn (deg)	43.6±24.5 (91)	41.7±24.6 (104)	U-test P=0.50
Total turn duration (ms)	49.8±17.8 (91)	49.6±16.2 (104)	U-test P=0.95
Stage 1 duration (ms)	19.7±5.2 (91)	20.5±6.4 (104)	U-test P=0.36
Stage 2 duration (ms)	30.1±14.8 (91)	29.1±11.3 (104)	U-test P=0.96
Error e (mm)	−2.70±1.59 (91)	0.05±2.09 (104)	t-test P=0.31
Take-off speed (m s^{-1})	1.095±0.287 (89)	1.130±0.296 (101)	t-test P=0.41

Initial and final values are shown as means ± s.d. with N in parentheses.

Table 2. Latency, accuracy and duration of the C-start remained constant after the brief acclimation period

Temperature	Latency	Error e	Turn duration
23°C (*N*=119)	r_s=0.038, *P*=0.69	*r*=0.148, *P*=0.11	r_s=0.115, *P*=0.21
26°C (*N*=195)	r_s=−0.068, *P*=0.34	*r*=0.053, *P*=0.49	r_s=−0.025, *P*=0.73
29°C (*N*=115)	r_s=0.134, *P*=0.15	*r*=−0.080, *P*=0.40	r_s=−0.056, *P*=0.56

Data are the correlation of latency, accuracy and duration with time.

explanation of the meaning of the respective quantities). Hence, the general properties of the C-starts had not changed over the course of the present experiments, as would have been possible, in principle, by changes in social structure within the group or growth of the fish during the study period. Values subsequently reported for 26°C relate to pooled data from the first and final phase at 26°C.

RESULTS

A 100% release probability is maintained over the full range of temperatures

Because the fish had to actively dislodge prey, the experiments provided an automatic intrinsic control that the fish were motivated to hunt and to capture prey. Fish fired and successfully dislodged prey at all temperatures examined. Most surprisingly, however, whenever a target was dislodged, its initial motion always sufficed to release a predictive start of at least one of the fish that brought the fish on course before the prey had landed. A remarkable 100% release rate of predictive starts held at all temperatures: of 119 cases at 23°C, 119 released a predictive C-start. Similarly, each of 115 presentations at 29°C released a C-start and so did all 195 presentations at 26°C. The success rate of the first responder was also constant (75.2%, 79.5% and 72.6% at 23, 26 and 29°C, respectively). Furthermore, both the number of fish that managed to start quickly enough as well as the probability that the shooter was among them were remarkably unaffected by temperature: an average of three (2.98, 3.05 and 2.86 fish at 23, 26 and 29°C, respectively) of the five fish managed to be on their way at least 40 ms before prey impact. The probability that the fish that had actually fired the shot was among them was 87.4%, 87.2% and 87.8% at 23, 26 and 29°C, respectively. This lack of failure strongly contrasts with findings on the temperature effect on release probability in escape C-starts (e.g. Szabo et al., 2008).

Acclimation is fast

An acclimation phase of about 1 week at a novel temperature was thus sufficient to ensure constant responsiveness of the fish. Surprisingly, the same held true for all parameters we examined in this study. Table 2 reports this for three major parameters that will be described in detail below: two (latency and duration of the C-start) that are not fully temperature compensated and one (alignment after C-start) that is perfectly temperature compensated. All three parameters showed no tendency to change during the testing period that followed the 1 week acclimation. The brief acclimation time at the novel temperature generally left the fish fully acclimated in all aspects of their predictive C-starts that we analysed in this study. The only aspect whose stability we were unable to test was the precision in judging distance and timing, and setting take-off speed accordingly. To assay this capability requires a large dataset of starts assembled over an extended time, thus restricting the temporal resolution at which a change could have been detected. Nevertheless, it would have had to occur rapidly, and be completed in the ~4 week testing phase. However, we were able to follow the course of changes in each of the other properties that we will describe throughout this paper and none of them showed a significant trend over time.

Latency is not temperature compensated

While responsiveness remained constant at all temperatures, latency, i.e. the time between the onset of prey movement and onset of the C-start, increased with decreasing temperature (Fig. 2; Kruskal-Wallis: *H*=189.75, d.f.=2, *P*<0.001, Dunn's test: all pairwise *P*<0.05). Moreover, there was no indication that further acclimation would reduce this shift in latency (Table 2). In the range from 29 to 23°C, median latency increased about 4 ms per 1°C cooling. Besides its effect on median latency, cooling also affected the shape of the latency distributions: whereas the distribution was sharply focused at 29°C, it became slightly broader at lower temperatures (rank-based Brown–Forsythe: *P*=0.004). In addition, the

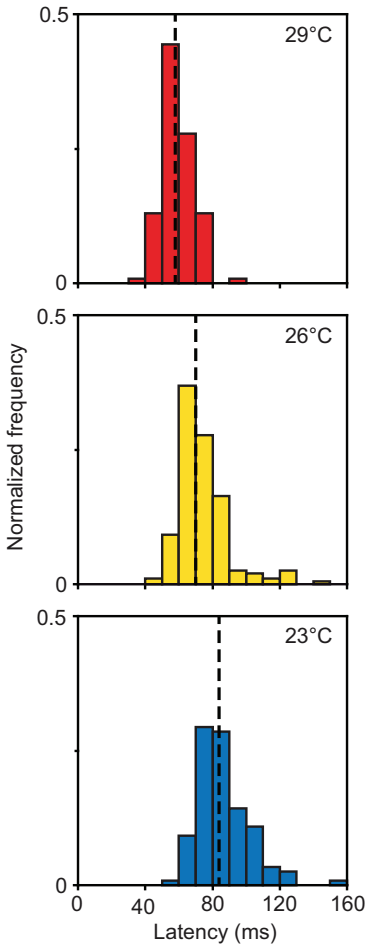

Fig. 2. Latency is not temperature compensated. Fish took less than 1 week to acclimate to the experimental temperature, after which time latency values were constant. Median latency (dashed lines) increased by about 12 ms with a 3°C cooling (Kruskal–Wallis: *H*=189.75, d.f.=2, *P*<0.001, Dunn's test: all pairwise *P*<0.05). Cooling also broadened the latency distributions, an effect that cannot be attributed to a changed internal motivation of the fish. Histograms are based on *N*=119, 195 and 115 responses at 23, 26 and 29°C, respectively, and normalized so that each total frequency equals 1. Binning starts at zero with bin widths of 10 ms.

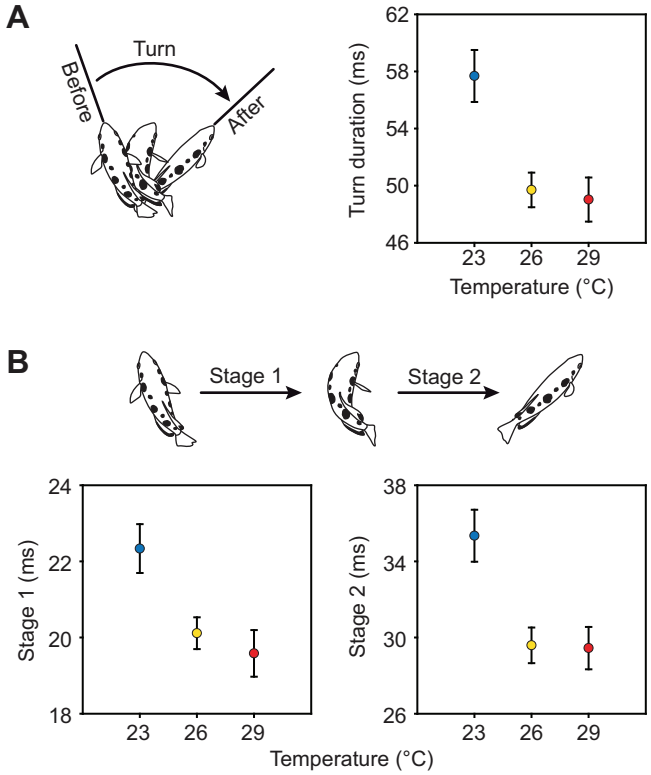

Fig. 3. Kinematic aspects of the C-starts are only partly temperature compensated. (A) Cooling below 26°C increased total duration of the predictive C-starts. (B) Splitting the turn into its two distinct stages, stage 1 (bending into a C-shape) and stage 2 (return flip), showed that the two stages are similarly affected by temperature. In A and B, durations are reported as means ± s.e.m. *N*=119, 195 and 115 C-starts analysed for 23, 26 and 29°C, respectively. Kinematic features were stable after a 1 week acclimation period to the experimental temperature. All durations were significantly larger at 23°C than at the two higher temperatures (Kruskal–Wallis: all tests $P<0.001$, Dunn's tests: $P<0.05$) but did not differ significantly between 26 and 29°C (Dunn's tests: $P>0.05$).

distributions appeared to be more skewed at lower temperatures, an impression that is compatible with calculations of higher moments of the distribution: skewness increased from 0.5 at 29°C to 1.4 at 23°C and kurtosis increased from 0.6 to 3.7.

Kinematics of the C-start manoeuvre change with temperature

After the brief acclimation period the temporal aspects of the C-start manoeuvre were also stable, with no indication of any further changes during testing. The kinematics were only partially temperature compensated (Fig. 3). The total duration of the C-start manoeuvre was significantly longer at 23°C than at both 26 and 29°C, but not significantly different between the two higher temperatures (Fig. 3A; Kruskal–Wallis: $H=17.60$, d.f.=2, $P<0.001$, Dunn's test: $P>0.05$ for 26 *versus* 29°C, $P<0.05$ for 23°C *versus* 26 and 29°C). The same pattern also held for the two phases, stage 1 and stage 2, of the C-start manoeuvre (Fig. 3B). The duration of the initial bending phase (stage 1) was significantly longer at 23°C but constant at 26 and 29°C (Kruskal–Wallis: $H=12.32$, d.f.=2, $P=0.002$, Dunn's test: $P<0.05$ for 23°C *versus* 26 and 29°C). Similarly, the subsequent rapid straightening phase (stage 2) was also significantly longer at 23°C but constant at the two higher temperatures (Kruskal–Wallis: $H=14.96$, $P<0.001$, d.f.=2, Dunn's test: $P<0.05$ for 23°C *versus* 26 and 29°C). The increased total duration of the C-start at the lower temperature seemed to be accompanied by a change in the shape of the distribution of C-start duration. At 23°C, the distribution of total time was about 10 ms broader than at the higher temperatures (rank-based Brown–Forsythe: $P=0.02$), whereas no difference in the shape of the distributions was evident at 26 and 29°C. Cooling from 26 to 23°C thus increased the total duration of the manoeuvre from 46 to 56 ms (or about 3 ms °C^{-1}) and slightly broadened the distribution of turn duration. Acclimation to higher temperatures above 26°C seemed to leave C-start kinematics unchanged.

The duration of the two stages has previously been shown to be quite variable and related – among other factors – to the degree of turning (Wöhl and Schuster, 2007). It is therefore important to stress that turn sizes were equally distributed at the three temperatures (Kruskal–Wallis: $H=0.03$, d.f.=2, $P>0.99$; see Fig. 4B) so that the observed differences in acclimated turn duration are due to temperature and not to the fish making systematically smaller turns at higher temperatures.

Precision of post-start alignment is unaffected by temperature

Already, the first tests after the brief acclimation period showed that the accuracy of the predictive starts was not at all affected by temperature. To account for the trial-to-trial variability in the way the target moved relative to the responding fish, we defined the error e as illustrated in Fig. 4A. Zero error means that the C-start leaves the fish perfectly aligned to the later point of impact, an error of −10 mm means that strictly following the initial alignment would lead the fish 10 mm past the landing point, in a direction towards the starting point of the prey. The error would be +10 mm if it was directed away from the point at which prey motion started. Histograms in Fig. 4A show that at all temperatures the errors were symmetrically distributed and always had a mean of zero (one-sample *t*-test: in all cases $P>0.05$). As reported in Table 2, this must already have been established at the end of the brief acclimation period, as we found no trend in error e over time. Moreover, not only did the average start remain precisely aligned towards the later landing point but also the scatter around this aim was not affected by temperature: the standard deviations of the distributions obtained at different temperatures were not significantly different (Levene: $P=0.95$). Hence, both mean aim as well as scatter around that aim were fully temperature compensated after the brief acclimation period.

With changes in temperature of only 3°C, it is natural to ask whether the expected changes in the aim were not simply too small to be detectable. Given the high accuracy of the initial alignment (evident in Fig. 4A), which translates to a zero mean error angle with a standard deviation of no more than 4.3 deg, a typical standard error of the mean in our experiments was less than 0.4 deg. Hence, we should have been able to detect even slight effects of incomplete temperature compensation, with a resolution of 1 deg. It is also important to stress that the criteria described in Materials and methods ensured that we included only the most demanding starts and excluded cases in which precision would have been trivial to accomplish. Yet, the temperature compensation could have been achieved by restricting the angular range at which C-starts were launched. We therefore analysed the correlation of actual total turn size during the C-start manoeuvre with the amount of turning that would be required to be aligned to the later landing point. Would the fish be able to select an appropriate turning angle only in a restricted angular range? Fig. 4B shows that this is clearly not the case: C-starts came from a broad angular range at all temperatures and were equally accurate no matter what the required size of the

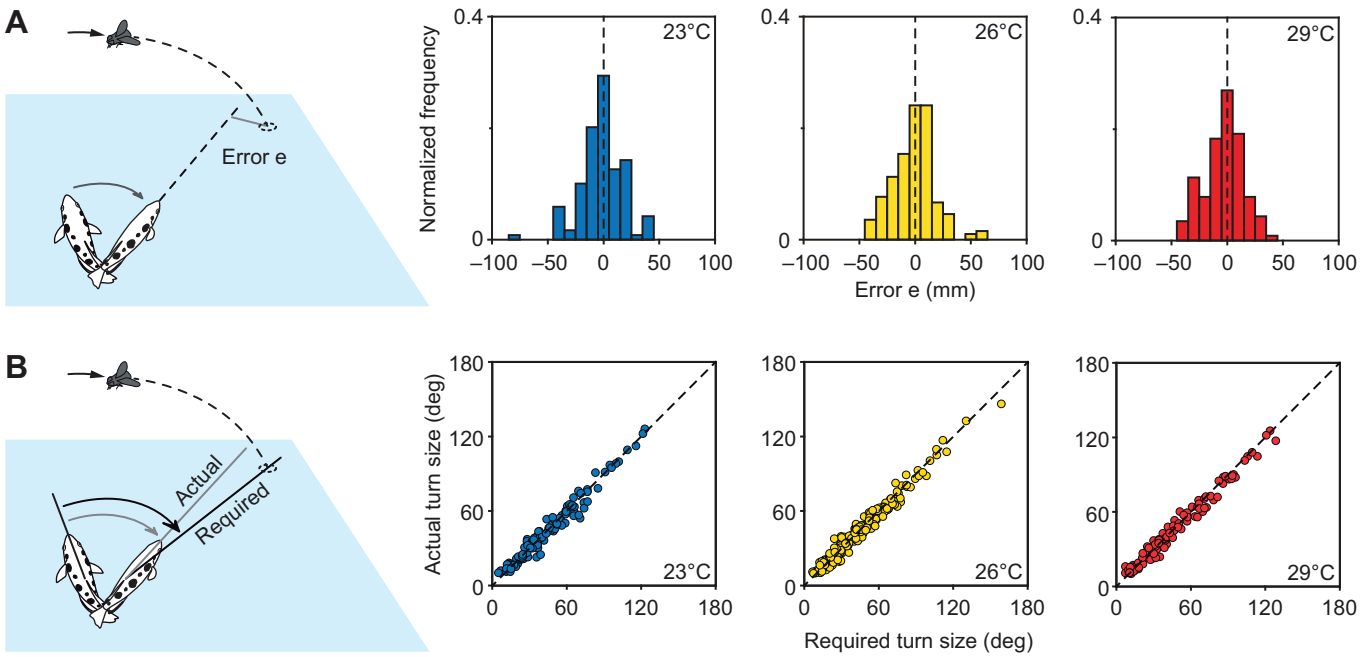

Fig. 4. The precision of aim after C-start is completely temperature compensated over the full angular range. (A) Histograms showing the distribution of errors in the initial alignment of the fish at the end of their C-starts. The accuracy of alignment was assayed by examining how close continued motion in this direction would bring the fish to the later impact point (error e). Note that target direction varied from trial to trial relative to position and orientation of the responding fish. Errors were not affected by temperature (ANOVA: $F_{2,426}$=0.374, P=0.69) and aim after C-start was right towards the future landing point at all temperatures (mean error e not significantly different from zero; one-sample t-tests: in all cases P>0.05). Also, the scatter around the perfect average alignment was not affected by temperature: standard deviations of the respective error distributions were not significantly different (Levene: P=0.95). Histograms are based on N=119, 195 and 115 C-starts at 23, 26 and 29°C, respectively, and are normalized so that each total frequency equals 1. Centre bin from −5 to +5 mm with bin widths of 10 mm. Zero error is indicated by dashed lines. (B) Plots of actual *versus* required aim after C-start shows that the turns were – at all temperatures – accurately set over the full range of required turning angles. The correlation was highly significant (P<0.001) at all temperatures and regression lines are not significantly different from those expected if actual turn size equalled the required turn size (dashed lines).

turn was (R^2=0.97, 0.97, 0.98 for a temperature of 23, 26 and 29°C, respectively).

The ability to compute post-start speed is unaffected by temperature

In addition to aligning the fish to where the prey is going to land, the C-start pushes the fish off with a speed that appears to be matched to distance and remaining time so that the fish will arrive shortly after the impact of its prey and at minimum travel cost (Wöhl and Schuster, 2006). Would the fish's ability to judge distance and timing be compromised after a change in temperature? This should in principle be detectable from an analysis of how the fish set their post-C-start take-off speed.

To establish such an approach we first examined whether the fish actually had acquired constant speed when stage 2 of their C-starts had ended and also whether they initially kept that speed (Fig. 5). To address this we took – for all starts at the three major temperatures – speed levels in four successive intervals of 10 ms duration that followed the end of stage 2. Fig. 5 reports the changes in speed between these intervals. Mean speed change between successive intervals was zero at all temperatures. Hence, take-off speed was attained in the first 10 ms subsequent to stage 2 and remained constant for the first 40 ms after take-off. It is thus safe to say that at all temperatures fish took off at a constant speed (whose absolute level, however, could be different from start to start so as to be matched to the novel conditions) and had attained this speed level immediately after the C-start (Fig. 5; one-sample t-test: in all cases P>0.05; repeated measures ANOVA: in all cases P>0.05).

To judge the fish's ability to infer distance and timing at the different temperatures and to set its take-off speed accordingly, we needed to find a range of speed levels that would be equally attainable at all temperatures. Cooling shifted the distribution of post-C-start take-off speed towards lower values (Fig. 6; ANOVA: $F_{2,417}$=13.20, P<0.001, Holm–Sidak test: all pairwise P<0.05) but even at 23°C the fish were able to reach top speed levels of over 15 fish lengths s^{-1}. Because the fish themselves commanded target motion it was crucial to check whether shooting power was similar at the three temperatures. For instance, a decline in shooting power at the lower temperature would result in a smaller mean distance travelled by the falling prey and this, in turn, would cause a shift in take-off speed towards smaller values. Direct measurement of the actual distances the dislodged prey items travelled showed that they were the same at all temperatures (Kruskal–Wallis: H=0.87, d.f.=2, P=0.65), thus showing that shooting power was unchanged. Moreover, the range of distances that the responding fish actually had to cover was not different at the three temperatures (Kruskal–Wallis: H=0.87, d.f.=2, P=0.65) and hence it can safely be concluded that the distribution of speed (Fig. 6) was also not confounded by the change in the mean distance the fish needed to cover, pointing to basic limitations of the fast-start motor system in the cold.

To analyse temperature compensation in the fish's ability to judge distance and time from the adjustment in take-off speed, we needed to select a range of speed levels that the fish could actually attain equally well at all temperatures. Based on Fig. 6 we selected the range up to 15 fish lengths s^{-1} (on average 1.37 m s^{-1}) as one that

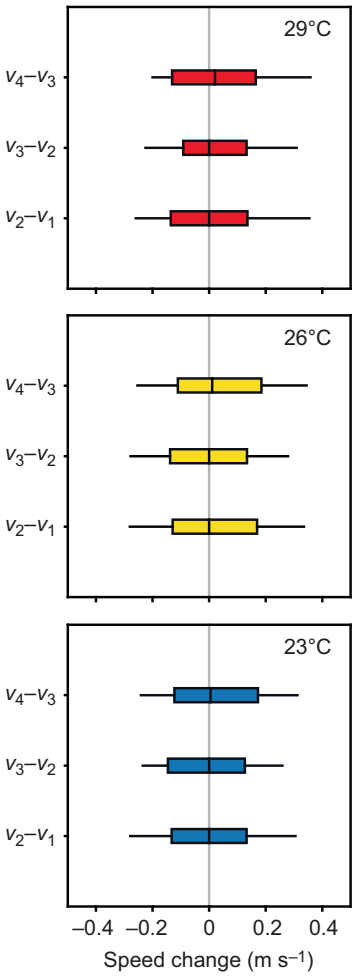

Fig. 5. Evidence that the C-starts resulted in stable initial take-off speed levels at all temperatures. Take-off speed, attained at the end of the C-start, was constant during the first 40 ms of the subsequent translation. For each response, speed values v_1, v_2, v_3 and v_4 were taken from the changes in position during the first, second, third and fourth 10 ms interval, respectively, of the fish's initial translatory motion. Boxplots show the changes in speed between the first two (v_2–v_1) and successive intervals. At all temperatures, mean changes were always not significantly different from zero (one-sample *t*-tests: in all cases $P>0.05$) and there was no significant difference among the intervals (repeated measures ANOVA: all tests $P>0.05$). N=117, 190 and 113 responses at 23, 26 and 29°C, respectively. Boundaries (whiskers) of boxplots show 25th/75th (10th/90th) percentiles, lines mark median. Grey lines show speed change of 0 m s^{-1}.

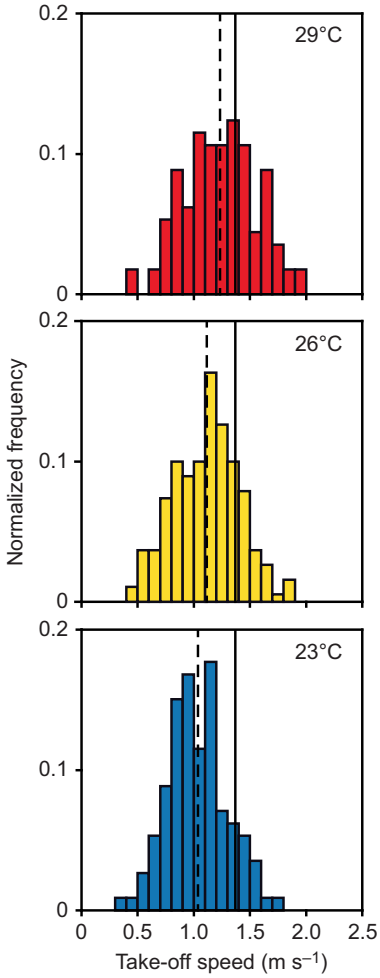

Fig. 6. The range of attainable speed depends on temperature. The distribution of initial speed values of starting fish was temperature dependent (ANOVA: $F_{2,417}$=13.20, $P<0.001$, Holm–Sidak test: all pairwise $P<0.05$). This must be accounted for when take-off speed is used to assay how temperature affects the fish's ability to judge distance and time. Speed levels from 0 to 15 lengths s^{-1} (solid line) are attainable at all temperatures. Histograms are based on N=117, 190 and 113 responses at 23, 26 and 29°C, respectively, and normalized so that each total frequency equals 1. Binning starts at zero with bin widths of 0.1 m s^{-1}. Mean speed is indicated by dashed lines.

would allow us to find out from the adjustment in take-off speed how well the fish had judged the situation.

We first asked whether take-off speed would better be predicted by the distance the fish had to cover to the point of prey impact or to this distance divided by the remaining time until impact (i.e. the 'virtual speed'). The analysis reported in Table 3 clearly shows that at all temperatures take-off speed was better predicted by virtual speed and not by distance alone. Fig. 7 therefore plots take-off speed *versus* virtual speed to assay the fish's judgement of distance and timing at the three experimental temperatures. Actual and virtual speed correlated equally well at all temperatures and no significant differences in slope (P=0.69) or intercept (P=0.06) of the regression lines could be detected (multivariate linear model: R^2=0.574, $F_{3,151}$=67.89; $P<0.001$). This suggests that the fish had perfectly temperature compensated their ability to set take-off speed (within the accessible range). This is also evident from a more direct way of measuring precision by analysing the distribution of the differences between actual speed and virtual speed. All distributions (not shown) had zero means and standard deviations (around 0.18 m s^{-1}) did not differ between temperatures (Levene: P=0.87).

The findings have two implications: first, in the cold, deficits in the motor system become apparent and maximum take-off speed declines; second, the 'computational' aspect of the problem – judging distance and timing, and setting take-off speed accordingly – is completely temperature compensated.

Compensation in an extended range

Measurements made outside our standard temperature range illustrate how important the intrinsic motivational control was: both at 21°C (39 predictive starts in 39 trials) and at 32°C (19 predictive

Table 3. Linear models show that 'virtual speed' describes the actual take-off speed attained at the end of the C-starts better than distance

Temperature	Model	k	AICc	Δ_i	w_i
Temperature as fixed factor	**Virtual speed**	**5**	**–133.640**	**0**	**1.000**
	Distance	5	−88.908	43.732	3.190×10^{-10}
23°C	**Virtual speed**	**3**	**–23.291**	**0**	**0.912**
	Distance	3	−18.609	4.682	0.088
26°C	**Virtual speed**	**3**	**–74.109**	**0**	**1.000**
	Distance	3	−51.190	22.920	1.055×10^{-5}
29°C	**Virtual speed**	**3**	**–26.803**	**0**	**1.000**
	Distance	3	−10.889	15.914	3.501×10^{-4}

k, number of parameters in model; AICc, corrected Akaike information criterion; Δ_i, AICc–minAICc; w_i, Akaike weights.
The best fitting model in each case is shown in bold.

starts in 19 trials), responsiveness was 100%. That is, when the fish set their prey into motion they would also always produce C-starts. Furthermore, at both temperatures, the starts were accurately aimed, the mean errors were not different from zero (one-sample t-tests: in both cases $P>0.05$) and the standard deviations (0.02 m) were the same as those in the error distributions at all other temperatures (Levene: $P=0.999$). However, the motivation to hunt and to actually dislodge prey was largely affected by temperature. This is evident, for instance, from the mean number of dislodgements we were able to obtain within approximately 1 h of experimentation: 10 at 21°C, 20 at 23°C, 30 at 26°C, 35 at 29°C and fewer than 20 at 32°C.

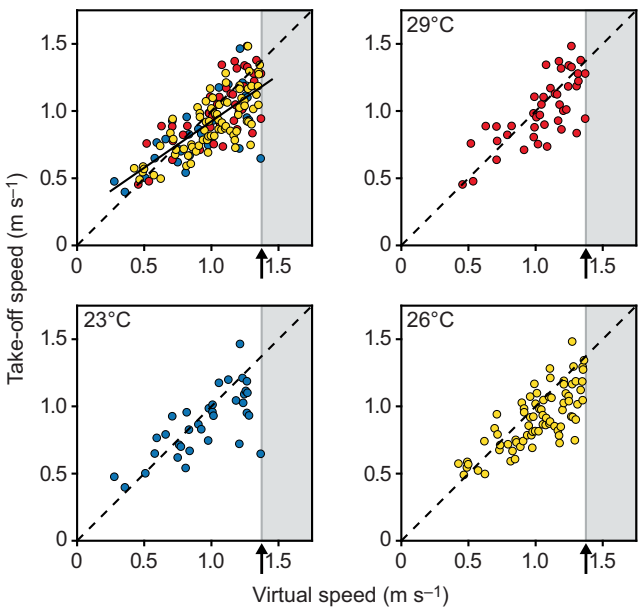

Fig. 7. Precision of setting initial speed is temperature compensated. At all temperatures the initial speed (take-off speed), attained immediately after the C-start, correlated best with 'virtual speed', i.e. the level of constant speed that would allow the fish to arrive simultaneously with the falling prey. To account for the decline in maximum speed with decreasing temperature, we compared the accuracy in setting initial speed only in the speed range that was accessible for the fish at all three temperatures. Based on the findings of Fig. 6, this was taken as 15 fish lengths s^{-1} (arrow). N=35, 77 and 43 C-starts at 23, 26 and 29°C, respectively. Overall correlation is indicated in the top left plot (includes all data and regression line). Multivariate linear model: $R^2=0.574$, $F_{3,151}=67.89$; $P<0.001$; no difference in slope ($P=0.69$) and intercept ($P=0.06$). The three other plots show take-off speed *versus* required virtual speed for the three experimental temperatures (dashed lines indicate where speed was equal to virtual speed).

DISCUSSION

The fine-tuned archerfish predictive starts provide a sensitive assay of how well C-start manoeuvres can be buffered against temperature-induced changes in performance. In archerfish, the C-start must be matched to time and position of the point at which its ballistically falling prey will later impact and the effects of incomplete temperature compensation could readily and sensitively be detected. Moreover, the starts automatically offer an intrinsic control for matched motivation at different temperatures: because the fish first have to dislodge their prey, they elicit predictive C-starts only when they are actually motivated to hunt.

Functional stability of fast starts

Our findings, obtained after a brief acclimation period, suggest that the functionality of the predictive C-starts is completely buffered against changes in environmental temperature. Whenever the fish are motivated to hunt, the release probability of their accurately aimed predictive starts is 100% throughout the full range of temperatures at which we observed hunting in the wild (about 23–30°C; S.S., unpublished data). Both the accuracy of the initial aim at the end of the C-start and the ability to set take-off speed according to estimated distance and timing appear to be completely temperature compensated. Note that we could have detected changes in either average alignment after the C-starts or the scatter around this average alignment with an accuracy of better than 1 deg. Moreover, the acclimation to a novel temperature must have been rapid, given that all properties were stable after our brief acclimation period of about 1 week. Our findings therefore point to a remarkable capacity of C-start networks to be 'functionally stable', i.e. to ensure precise functionality under changed operating conditions.

In goldfish, escape C-start acclimation allowed the animal to compensate only for the acute effects of cooling but not of warming up. An increase in escape probability and a decrease in directionality upon warming remained uncompensated even after 4 weeks (Szabo et al., 2008). Nevertheless, it is still possible that we presently largely underrate the degree of functional stability of escape C-starts. Stability of function may be masked by three major experimental difficulties with escapes. (i) Efficient escapes necessarily involve a random element that should not easily be detected by any observer. But without independent evidence on the acute levels of this random element only rather crude assays of functionality are possible. (ii) This implies that large changes in temperature are needed to see effects on directionality and these changes may not always be in the normal operating range of the animal. (iii) Escape C-starts share a further major difficulty with other assays of maximum locomotor capabilities – the problem of controlling motivation (e.g. Losos et al., 2002). While it seems

natural to assume that the motivation to perform a life-saving start is always constant, this may not actually be true when alternatives exist: 'freezing' or simply suppressing the C-start could be alternative manoeuvres that might actually be better at lower temperatures. In the cold, doing nothing could be better than a C-start that is launched too late and that has insufficient power to move the fish rapidly enough out of the zone of danger.

Other C-start patterns of hunting fish would also allow both motivation to be controlled and accuracy to be assayed. Directed C-starts have been described, for instance, in fruit-catching Middle America machaca *Brycon guatemalensis* (Krupczynski and Schuster, 2008; Schuster, 2012). These stream-living fish respond to the falling motion of figs. The interesting part of their response is the way their starts accommodate the later amount of drift. The C-starts of *Brycon* rotate the fish in a direction that seems to automatically accommodate the amount of drift the fish will later face. We therefore hope that fast starts of these and other fish may provide useful systems to sensitively explore the more general capacity of the C-start network design to ensure functional stability after changes in temperature.

Speed of acclimation

Because several weeks of acclimation were required in other fish (Webb, 1978; Sidell et al., 1973; Johnson and Bennett, 1995; Johnson et al., 1996; Szabo et al., 2008) and because incomplete compensation has been reported even after 8 weeks of acclimation (Webb, 1978), we had expected the acclimation of our fish to still be incomplete when tests started. We thus had hoped to detect at least small systematic changes during the first weeks. Surprisingly, we found no such changes (Table 2), even in those variables (latency and C-start duration) that were not temperature compensated. Thus, the acclimation appears to be accomplished in less than a week at the new temperature.

Rapid acclimation would seem to be a clear advantage for archerfish in the wild. In their natural mangrove habitats the interaction of tidal water movement with freshwater inflow causes temperature fluctuations at a given location. In addition, the irregular changes in water depth force the fish to move out from a good hunting ground to other suitable spots. Because these may differ in temperature from the previous one (for example because water depth or shading vary), archerfish probably have to regularly cope with changes in temperature. Yet, within the range of temperatures in which they hunt, their C-starts still need to be precise: incorrectly aimed starts mean overshooting prey impact and losing food while risking being spotted by predators. Hence, the ecological context would seem to strongly favour mechanisms that ensure rapid functionality after naturally occurring changes in operating temperature.

The situation in archerfish could be reminiscent of the extremely rapid acclimation found in marine intertidal invertebrates, for which the term 'immediate compensation' was suggested (e.g. Hazel and Prosser, 1974). In a brief interval following an acute massive temperature change, physiological properties of these animals are often unchanged, which frequently is described as the properties having effective Q_{10} values close to unity. How would archerfish C-starts be affected by acute temperature changes? Would it be possible to discover 'immediate compensation' at least in the computations that underlie setting of C-start angle and take-off speed? Unfortunately, approaching the effects of acute changes on the behavioural performance is tricky: to critically and sensitively assay accuracy of the archerfish predictive start sampling of a few days is typically necessary. This is to ensure that starting conditions were really comparably challenging (see criteria introduced in Materials and methods). Based on the strong acute effects of temperature changes on all fish fast starts analysed so far (Webb, 1978; Beddow et al., 1995; Preuss and Faber, 2003; Szabo et al., 2008), we think that an 'immediate compensation' of archerfish C-starts is not very likely. However, studies on acute effects are required to disclose the actual speed of acclimation and to dissect the involvement of various components (i.e. computations and motor power) of the archerfish C-start circuitry. At present it can only be said that the overall acclimation in the archerfish C-starts is remarkably fast – when compared with the several weeks needed in other fish (see above).

Incomplete compensation of latency and C-start duration

While the C-start precision was temperature compensated, the latency of the C-start was not. Mean latency and variability of latency were increased at lower temperature (Fig. 2) and these effects did not acclimate any further. It may be tempting to speculate that latency increased to maintain function, i.e. to allow sampling of more information and thus to ensure maintained precision after cooling. While this holds true at a decreased visual contrast of prey motion relative to its background (Schlegel and Schuster, 2008) and at low light levels (P.K. and S.S., unpublished), it probably does not explain the changes in latency. First, latency changed with absolute temperature but not with changes from the initial temperature. Second, the amount of change was similar for latency and for the contraction phase (Stage 1), a relationship that we failed to see when latency was increased to maintain precision (Schlegel and Schuster, 2008) (P.K. and S.S., unpublished). Many factors – from visual transduction to the first contraction that makes the C-start visible – contribute to response latency and all of them are temperature dependent (see Lenz et al., 2005). So the most remarkable aspect of C-start latency appears to be that it showed so little variability and varied so consistently with temperature.

Which factors are most important in mediating functional stability?

Fig. 8 shows that producing a fine-tuned C-start based on initial target motion is an integrative process with components that are affected by temperature in different ways: it requires the representation of information on the initial movement of prey

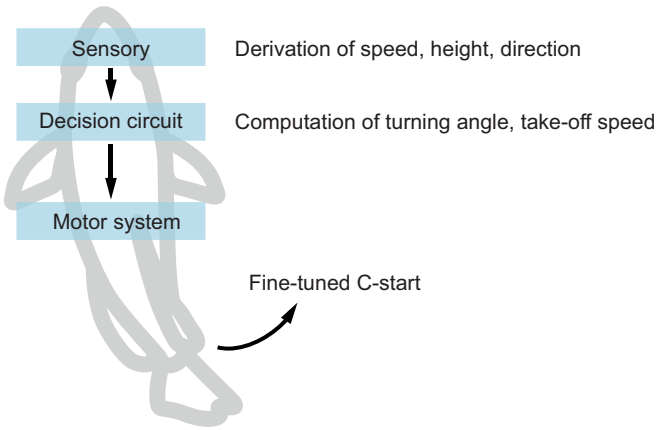

Fig. 8. Substrates that contribute to temperature compensation of the predictive start.

(Rossel et al., 2002; Schlegel and Schuster, 2008), 'computations' to derive the required angle of C-start turning and required post-C-start take-off speed, and the capability of the body trunk muscles to work against resistance and fulfil what the circuits have computed should be done. Clearly, both the visual functions and the 'computational' aspects seem to be completely temperature compensated (Figs 4, 7) and compensation is achieved within no more than a week (Table 2).

What would be interesting to explore further is the acclimation in archerfish trunk muscle. A huge body of literature (for reviews, see Bennett, 1985; Bennett, 1990; Rome, 1990) suggests that contraction speed does acclimate slowly – over the course of weeks – after acute changes in temperature. After cooling, an increase in the number of motor units can make up for the decline in contraction speed. Also, slow adjustments of the activity of myofibrillar ATPase activity – a major determinant of contraction speed – have been described (e.g. Bennett, 1990; Rome, 1990; Johnson and Bennett, 1995; Johnson et al., 1996). Therefore, what seems remarkable in the motor system of archerfish is that C-start kinematics were stable after only 1 week of acclimation. If acclimation in muscle occurred as in other fish, it should still be ongoing and detectable from changes in stage 1 or stage 2 duration during the testing phase, which was not the case. If acclimation was much slower than in other fish then it would still have been ongoing during the final experiments at 26°C that followed after a total of 5 weeks at 29°C. However, neither was a change in turn duration seen during the final tests at 26°C (r=−0.087, P=0.38) nor was turn duration different from that obtained in the first phase to which our fish had been acclimated for at least 1 year (Table 1). So either the archerfish muscles do not acclimate at all or they acclimate in less than about a week. The first option seems unlikely given that, 1 week after a 6°C change in temperature (from 23 to 29°C, see Materials and methods), the duration of the C-start (and of both its major phases) was stable and was equal to that at 26°C.

An alternative explanation, the use of thermally insensitive elastic recoil mechanisms (e.g. Anderson and Deban, 2010) seems to be at odds with our findings in Fig. 3. The 'loading' phase would correspond to stage 1 of the C-start and is affected by temperature in very much the same way as the subsequent straightening phase. Also, some sort of 'buffering' of the internal operating temperature of the trunk muscles is at odds with the shift in take-off speed in the cold (Fig. 6). Our findings thus raise the possibility that archerfish trunk musculature accommodates rapidly.

Accessibility of archerfish Mauthner-associated network

Our findings suggest that the archerfish C-starts may help to bring a property of C-start circuitry into focus that remains hidden in C-start escapes: an exquisite stability of function against changes in operating conditions. The behavioural accessibility of the archerfish predictive C-starts comes packaged with neurophysiological accessibility of the Mauthner-associated hindbrain networks. Recent findings (P. Machnik, unpublished) show that archerfish do have Mauthner cells that are as easy to record from *in vivo* as they are in goldfish. The effects of temperature on the archerfish M-cells appear to parallel the findings in goldfish (Preuss and Faber, 2003; Szabo et al., 2008) and many properties of the archerfish M-cell are temperature sensitive. The accessibility of the archerfish C-start circuit may therefore facilitate our future understanding of the neuronal part played in functional stability, i.e. how function is stabilized when so many cellular and synaptic properties are strongly affected even by small changes in temperature.

ACKNOWLEDGEMENTS

We thank Antje Halwas and Karl-Heinz Pöhner for technical support and Stefan Gross for statistical advice. Peter Machnik, Gerrit Begemann and two anonymous reviewers greatly helped us with highly stimulating comments and suggestions on the manuscript.

AUTHOR CONTRIBUTIONS

S.S. and P.K. conceived the project, P.K. conducted the experiments, and S.S. and P.K. analysed the data and wrote the paper.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SCHU1470/2, 7 and 8).

REFERENCES

Anderson, C. V. and Deban, S. M. (2010). Ballistic tongue projection in chameleons maintains high performance at low temperature. *Proc. Natl. Acad. Sci. USA* **107**, 5495-5499.

Beddow, T. A., Leeuwen, J. and Johnston, I. A. (1995). Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. Exp. Biol.* **198**, 203-208.

Bennett, A. F. (1985). Temperature and muscle. *J. Exp. Biol.* **115**, 333-344.

Bennett, A. F. (1990). Thermal dependence of locomotor capacity. *Am. J. Physiol.* **259**, R253-R258.

Burnham, K. P. and Anderson, D. R. (1998). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. New York, NY: Springer-Verlag.

Eaton, R. C., Bombardieri, R. A. and Meyer, D. L. (1977). The Mauthner-initiated startle response in teleost fish. *J. Exp. Biol.* **66**, 65-81.

Eaton, R. C., Lavender, W. A. and Wieland, C. M. (1981). Identification of Mauthner-initiated response patterns in goldfish: evidence from simultaneous cinematography and electrophysiology. *J. Comp. Physiol. A* **144**, 521-531.

Eaton, R. C., Lee, R. K. K. and Foreman, M. B. (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. *Prog. Neurobiol.* **63**, 467-485.

Faber, D. S. and Korn, H. (1978). Electrophysiology of the Mauthner cell: basic properties, synaptic mechanisms, and associated networks. In *Neurobiology of the Mauthner Cell* (ed. D. S. Faber and H. Korn), pp. 47-131. New York, NY: Raven Press.

Faber, D. S., Fetcho, J. R. and Korn, H. (1989). Neuronal networks underlying the escape response in goldfish. General implications for motor control. *Ann. N. Y. Acad. Sci.* **563**, 11-33.

Furshpan, E. J. and Furukawa, T. (1962). Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *J. Neurophysiol.* **25**, 732-771.

Hazel, J. R. and Prosser, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620-677.

Johnson, T. P. and Bennett, A. F. (1995). The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *J. Exp. Biol.* **198**, 2165-2175.

Johnson, T. P., Bennett, A. F. and McLister, J. D. (1996). Thermal dependence and acclimation of fast start locomotion and its physiological basis in rainbow trout (*Oncorhynchus mykiss*). *Physiol. Zool.* **69**, 276-292.

Kimmel, C. B., Eaton, R. C. and Powell, S. L. (1980). Decreased fast-start performance of zebrafish lacking Mauthner neurons. *J. Comp. Physiol.* **140**, 343-350.

Korn, H. and Faber, D. S. (2005). The Mauthner cell half a century later: a neurobiological model for decision-making? *Neuron* **47**, 13-28.

Krupczynski, P. and Schuster, S. (2008). Fruit-catching fish tune their fast starts to compensate for drift. *Curr. Biol.* **18**, 1961-1965.

Lenz, P. H., Hower, A. E. and Hartline, D. K. (2005). Temperature compensation in the escape response of a marine copepod, *Calanus finmarchicus* (Crustacea). *Biol. Bull.* **209**, 75-85.

Liu, K. S. and Fetcho, J. R. (1999). Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish. *Neuron* **23**, 325-335.

Losos, J. B., Creer, D. A. and Schulte, J. A. (2002). Cautionary comments on the measurement of maximum locomotor capabilities. *J. Zool. (Lond.)* **258**, 57-61.

Lüling, K. H. (1963). The archerfish. *Sci. Am.* **209**, 100-108.

Preuss, T. and Faber, D. S. (2003). Central cellular mechanisms underlying temperature-dependent changes in the goldfish startle-escape behavior. *J. Neurosci.* **23**, 5617-5626.

R Development Core Team (2009). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/

Rödel, H. G., Bora, A., Kaetzke, P., Khaschei, M., Hutzelmeyer, H. and von Holst, D. (2004). Over-winter survival in subadult European rabbits: weather effects, density dependence, and the impact of individual characteristics. *Oecologia* **140**, 566-576.

Rome, L. C. (1990). Influence of temperature on muscle recruitment and muscle function in vivo. *Am. J. Physiol.* **259**, R210-R222.

Rossel, S., Corlija, J. and Schuster, S. (2002). Predicting three-dimensional target motion: how archer fish determine where to catch their dislodged prey. *J. Exp. Biol.* **205**, 3321-3326.

Schlegel, T. and Schuster, S. (2008). Small circuits for large tasks: high-speed decision-making in archerfish. *Science* **319**, 104-106.
Schuster, S. (2012). Fast-starts in hunting fish: decision-making in small networks of identified neurons. *Curr. Opin. Neurobiol.* **22**, 279-284.
Sidell, B. D., Wilson, F. R., Hazel, J. and Prosser, C. L. (1973). Time course of thermal acclimation in goldfish. *J. Comp. Physiol.* **84**, 119-127.
Sillar, K. T. and Robertson, R. M. (2009). Thermal activation of escape swimming in post-hatching *Xenopus laevis* frog larvae. *J. Exp. Biol.* **212**, 2356-2364.
Smith, H. M. (1936). The archer fish. *Nat. Hist.* **38**, 2-11.
Szabo, T. M., Brookings, T., Preuss, T. and Faber, D. S. (2008). Effects of temperature acclimation on a central neural circuit and its behavioral output. *J. Neurophysiol.* **100**, 2997-3008.
Wagenmakers, E.-J. and Farrell, S. (2004). AIC model selection using Akaike weights. *Psychon. Bull. Rev.* **11**, 192-196.
Webb, P. W. (1975). Acceleration performance of rainbow trout, *Salmo gairdneri*, and green sunfish, *Lepomis cyanellus*. *J. Exp. Biol.* **63**, 451-465.
Webb, P. W. (1978). Effects of temperature on fast-start performance of rainbow trout, *Salmo gaidneri*. *J. Fish Res. Board Can.* **35**, 1417-1422.
Weihs, D. (1973). The mechanism of rapid starting of slender fish. *Biorheology* **10**, 343-350.
Wöhl, S. and Schuster, S. (2006). Hunting archer fish match their take-off speed to distance from the future point of catch. *J. Exp. Biol.* **209**, 141-151.
Wöhl, S. and Schuster, S. (2007). The predictive start of hunting archer fish: a flexible and precise motor pattern performed with the kinematics of an escape C-start. *J. Exp. Biol.* **210**, 311-324.
Zottoli, S. J. (1977). Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish. *J. Exp. Biol.* **66**, 243-254.
Zottoli, S. J. and Faber, D. S. (2000). The Mauthner cell: what has it taught us? *Neuroscientist* **6**, 25-37.