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RESEARCH ARTICLE

Heat- and humidity-induced plastic changes in body lipids and starvation resistance in the tropical fly *Zaprionus indianus* during wet and dry seasons

T. N. Girish[1], B. E. Pradeep[1] and Ravi Parkash[2,*]

ABSTRACT

Insects in tropical wet or dry seasons are likely to cope with starvation stress through plastic changes (developmental as well as adult acclimation) in energy metabolites. Control and experimental groups of *Zaprionus indianus* flies were reared under wet or dry conditions, but adults were acclimated at different thermal or humidity conditions. Adult flies of the control group were acclimated at 27°C and low (50%) or high (60%) relative humidity (RH). For experimental groups, adult flies were acclimated at 32°C for 1 to 6 days and under low (40%) or high (70%) RH. For humidity acclimation, adult flies were acclimated at 27°C but under low (40%) or high (70%) RH for 1 to 6 days. Plastic changes in experimental groups as compared with the control group (developmental as well as adult acclimation) revealed significant accumulation of body lipids owing to thermal or humidity acclimation of wet season flies, but low humidity acclimation did not change the level of body lipids in dry season flies. Starvation resistance and body lipids were higher in the males of dry season flies but in the females of wet season flies. Adults acclimated under different thermal or humidity conditions exhibited changes in the rate of utilization of body lipids, carbohydrates and proteins. Adult acclimation of wet or dry season flies revealed plastic changes in mean daily fecundity; and a reduction in fecundity under starvation. Thus, thermal or humidity acclimation of adults revealed plastic changes in energy metabolites to support starvation resistance of wet or dry season flies.

KEY WORDS: Tropical drosophilid, Developmental acclimation, Adult acclimation, Heat acclimation, Humidity acclimation

INTRODUCTION

In the wild habitats of diverse insect taxa, incidence of food shortage is associated with seasonal changes in abiotic conditions (Tauber et al., 1986; Danks, 2004). Several studies have shown heritable variation in starvation resistance in diverse insect taxa (see Rion and Kawecki, 2007). Genetic variation for starvation resistance has been examined in different *Drosophila* species (Matzkin et al., 2009a), in geographical populations of *D. melanogaster* (Hoffmann and Parsons, 1991; Parkash and Munjal, 1999; Robinson et al., 2000; Hoffmann et al., 2001) and on the basis of laboratory selection experiments (Chippindale et al., 1996; Hoffmann et al., 2005). In contrast, stress-induced plastic changes in starvation resistance have been investigated mainly in *D. melanogaster* (1) on the basis of adult flies acclimated to different stressors (Bubliy et al., 2012) and regarding (2) the developmental acclimation effects of constant versus summer-simulated conditions (Hoffmann et al., 2005), (3) the developmental acclimation effects of different humidity levels (Parkash et al., 2014b) and (4) the lack of geographical differences in plastic responses for starvation resistance of *D. leontia* (Aggarwal, 2014). Developmental acclimation under summer-specific thermal conditions revealed higher starvation resistance compared with acclimation under winter conditions (Hoffmann et al., 2005). Further, *D. melanogaster* flies reared in high humidity evidenced an increase in resistance to heat as well as to starvation (Parkash et al., 2014b). Adult acclimation of *D. melanogaster* flies reared under standard growth conditions showed a lack of cross-tolerance between starvation and resistance to heat or cold (Bubliy et al., 2012). However, these previous studies did not consider combined acclimation effects resulting from developmental as well as adult acclimation. Such combined plastic effects for starvation resistance are likely to reflect acclimatization of flies in wild habitats.

In ectothermic organisms, seasonally varying thermal conditions induce plastic responses for morphological and life history traits (Bochdanovits and de Jong, 2003; de Jong, 2005, 2010; Behrman et al., 2015). Both thermal and humidity conditions vary significantly in subtropical regions. In the tropics, wet or dry seasons differ approximately two-fold in relative humidity, but thermal changes are limited. Seasonal phenotypic plasticity in tropical climates has been demonstrated for wing pattern polyphenism in the African butterfly *Bicyclus anynana* (Roskam and Brakefield, 1999) and for drought resistance in the mosquito *Anopheles gambiae* from Africa (Wagoner et al., 2014) and in *Drosophila leontia* (Parkash and Ranga, 2014). In tropical insect taxa, wet or dry conditions are likely to elicit seasonal phenotypic plasticity of starvation resistance, which has received little attention in the literature thus far. However, a single study has investigated seasonal phenotypic plasticity of starvation resistance in a sub-tropical African butterfly, *Bicyclus anynana* (Pijpe et al., 2007). In *B. anynana*, there are wet or dry seasonal morphs that vary in body coloration as well as in reproductive behaviour, i.e. they have two extended generations per year, and show higher fecundity in the wet season morph than in the dry season morph. *Bicyclus anynana* from that study showed greater starvation resistance in the dry season morph (living under the cooler, autumn and winter temperature of 18°C) than in the wet season morph (spring and summer temperature of 27°C). Seasonal plastic differences in starvation resistance of *B. anynana* revealed associated changes in resting metabolic rate: adult butterflies acclimated at 27°C had higher resting metabolic rates (CO_2 production) than butterflies acclimated at 18°C. However, Pijpe et al. (2007) did not investigate the plastic effects of humidity acclimation or plastic changes in the energy

[1]Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam 515134, India. [2]Department of Genetics, Maharshi Dayanand University, Rohtak 124001, India.

*Author for correspondence (rpgenetics@gmail.com)

T.N.G., 0000-0002-5048-5283; B.E.P., 0000-0001-8022-8168; R.P., 0000-0001-9880-3941

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metabolites supporting starvation resistance in *B. anynana*. To the best of our knowledge, seasonal plasticity of starvation resistance has not been investigated in drosophilids living under warm dry or warm wet seasons in tropical regions.

In insects as well as ectothermic vertebrates (lizards and fishes), changes in the levels of carbohydrates, lipids and proteins are associated with thermal conditions (Sheridan, 1994; Chapman, 1998; Hochachka and Somero, 2002; Shen et al., 2005). For example, diapause involves accumulation of body lipids in insects (Arrese and Soulages, 2010). Further, analysis of basal levels of metabolic pools of 12 *Drosophila* species has revealed greater starvation resistance as well as higher levels of body lipids in *D. mojavensis* and *D. arizonae*, which are adapted to the hot environment of US deserts (Matzkin et al., 2009a,b). The modest increase in starvation resistance of *D. melanogaster* flies grown under warmer temperature also supports the association of starvation resistance with higher temperature (Hoffmann et al., 2005). Therefore, plastic changes in starvation resistance as well as body lipids owing to heat acclimation may be expected, but supporting empirical data are lacking. In diverse insect taxa, effects of thermal hardening and/or acclimation on drought resistance have been investigated in tse-tse flies, beetles and *Drosophila* species, but these studies did not consider starvation resistance (see Chown et al., 2011). Further, plastic changes in starvation resistance and body lipids in response to heat or humidity acclimation in tropical drosophilids have also received less attention.

Physiological mechanisms for stressor-induced plastic changes involve accumulation and utilization of various energy metabolites. In diverse insect taxa, single or multiple bouts of cold, desiccation or starvation stress in adults have revealed utilization of energy metabolites such as carbohydrates, body lipids and proteins (Benoit et al., 2010; Teets et al., 2011, 2012; Rosendale et al., 2017). These studies have shown stressor-specific utilization of carbohydrates under cold or desiccation stress, or body lipids under starvation. A longer duration of starvation (36 weeks) reduced dehydration tolerance as well as incurred significant energetic costs in the American dog tick, *Dermacentor variabilis* (Rosendale et al., 2017). Thus, energy metabolism plays an important role in the ability of diverse insect taxa to survive stressful conditions that they encounter during their lifetime. For example, accumulation and utilization of carbohydrates are associated with cold or drought hardening and/or acclimation of *D. melanogaster* (Kostal et al., 2011), *Belgica antarctica* (Benoit et al., 2007; Teets et al., 2011, 2012) and *D. immigrans* (Tamang et al., 2017), and with heat hardening in *Zaprionus indianus* (Kalra et al., 2017). For starvation, utilization of body lipids is well known in diverse insect taxa such as drosophilids (Marron et al., 2003), larvae of the migratory locust (Hill and Goldsworthy, 1970), the African fruit beetle (Auerswald and Gäde, 2000), the American dog tick (Rosendale et al., 2017) and many other insects (Arrese and Soulages, 2010). There is evidence of accumulation of body lipids in laboratory selected starvation resistant strains of *D. melanogaster* (Chippindale et al., 1996; Hoffmann et al., 2005). If cold hardening or acclimation can cause changes in the levels of energy metabolites in ectotherms living in cold environments, we may expect heat-induced changes in energy metabolites of tropical insect taxa.

In the present study, we assessed the combined effects of developmental and adult acclimation on starvation-resistance-related traits in *Zaprionus indianus* Gupta 1970 flies reared under dry or wet conditions. Further, we tested plastic changes owing to adult acclimation under three sets of thermal or humidity conditions. The adult flies of the control group were acclimated at 27°C and at low (50%) or high (60%) relative humidity (RH) conditions, which match with ambient conditions of the flies at the start of the dry or wet season because these thermal or humidity conditions change subsequently during the late period of wet or dry seasons. Accordingly, for experimental groups of flies, we assessed the effects of thermal acclimation at 32°C but under low (40%) or high (70%) RH conditions. In another experimental group of flies, we assessed the effects of humidity acclimation at low (40%) or high (70%) RH but at 27°C. We investigated plastic changes in the levels of body lipids in adult flies of each season acclimated at 32°C for 1 to 6 days, or acclimated to low or high humidity for 1 to 6 days (40% or 70% RH). Thus, different groups of flies (control and experimental groups) were reared under identical thermal or humidity conditions but were subjected to different adult acclimation conditions. For wet or dry season flies, we compared rates of utilization of energy metabolites under different durations of starvation stress (12, 24, 36 or 48 h). Finally, we assessed possible costs of starvation stress on mean daily fecundity of adult flies acclimated to wet or dry seasonal conditions. Thus, we tested plastic changes in starvation resistance of *Z. indianus* resulting from different adult acclimation conditions of wet or dry seasons.

MATERIALS AND METHODS

Collection and cultures

Zaprionus indianus individuals were collected during two seasons, the dry season (March–April: T_{avg}=29±2.3°C, RH=38±3.4%) and the wet season (June–July: T_{avg}=26±2.2°C, RH=72±4%), from the tropical south Indian locality Puttaparthi (14.17°N, 77.81°E). For this locality, meteorological data on seasonal changes in T_{avg} and percent RH for the last 10 years were obtained from www.worldweatheronline.com (see Fig. 1A,B). In Puttaparthi, the light:dark cycle is 12 h:12 h during the dry season and 13 h:11 h during the wet season. Thus, there are no significant seasonal changes in the photoperiod. For the two seasons, wild-collected *Drosophila* flies (using the bait-trap method) were used to assess the percent relative abundance of *Z. indianus* (Fig. 1C). The relative abundance was calculated as the number of wild-caught *Z. indianus* individuals divided by the number of all the different drosophilids collected during either the dry or the wet season (2 months each) from six locations in the university town of Puttaparthi. The relative abundance of dry season flies is likely to be skewed because of the slightly lower number of *Drosophila* species during the dry season. Further, collection records of the last 3 years from the similar sites were used to find repeatability of relative abundance of *Z. indianus* during the dry or wet seasons.

Approximately 400 wild-caught flies of *Z. indianus* from each season (wet or dry) were used to initiate cultures at low density (approximately 60 pairs per bottle). The flies were reared on the standard cornmeal–agar–yeast medium following Markow and O'Grady (2006) in 200 ml wide-mouth bottles which were covered with muslin gauze so as to maintain low or high relative humidity conditions for different life-cycle stages of *Z. indianus*. Therefore, the dry and wet season flies were tested during the respective seasons. Relative humidity was maintained with Metrex humidity chambers (www.metrexinstruments.com; MEC-30) which were set at low or high humidity. The relative humidity level inside culture bottles was monitored through sensors of digital hygrometers for cultures kept at low or high humidity levels. We investigated F_2 flies (both sexes) of *Z. indianus* to assess plastic changes in starvation related traits owing to different adult acclimation conditions. Further, for each season, both male and female adult *Z. indianus* were acclimated to heat or humidity conditions to examine plastic changes in starvation

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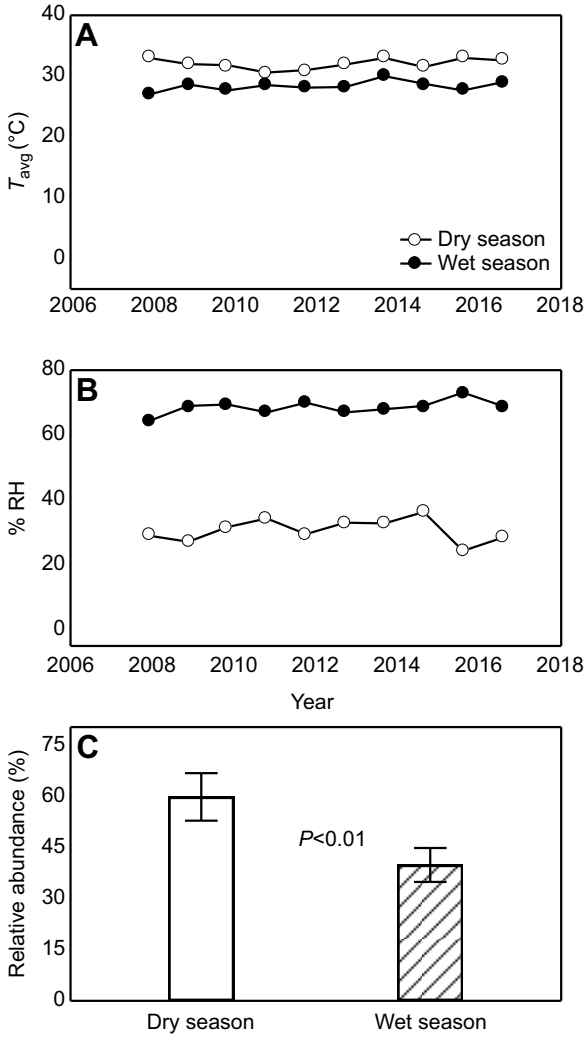

Fig. 1. Wet or dry seasonal changes in climatic variables and relative abundance of *Zaprionus indianus*. (A,B) Data on seasonal changes in average temperature (T_{avg}; A) and percent relative humidity (% RH; B) over the last 10 years for the south Indian locality (Puttaparthi, 14.17°N) of the origin of *Z. indianus*. (C) Percent relative abundance of *Z. indianus* during the dry or wet season (based on the past 3 years of collections through the bait-trap method; $n \approx 900$ for each season); data are shown as means±s.d.

resistance and energy metabolites owing to adult acclimation conditions. The physiological age of the flies was kept similar for control as well as experimental groups of flies for analysis of plastic changes in body lipids and other metabolites of *Z. indianus*.

Experimental set-up

Seasonally varying wild-caught flies were reared under wet or dry conditions, i.e. dry season flies at 27±1°C and 40±2% RH, and wet season flies at 27±1°C and 70±2% RH. These groups of flies were subjected to different adult acclimation conditions which flies encounter during early or late periods of wet or dry seasons. Thermal or humidity conditions change subsequently during the later part of dry or wet seasons. The ambient humidity level decreases from 50% RH to 40% RH during the dry season whereas humidity increases from 60% RH to 70% RH during the wet season. Thus, flies reared under wet or dry conditions were subjected to different adult acclimation treatments: (1) the control group was acclimated at 27°C and low (50%) or high (60%) RH conditions; (2) one experimental group was acclimated at 32°C (i.e. mean temperature during the late season) for 1, 2, 4 or 6 days and the RH was either low (40%) for the dry season flies or high (80%) for the wet season flies; and (3) another experimental group was subjected to humidity acclimation at 40% RH (for dry season flies) or 70% RH (for wet season flies) for 1, 2, 4 or 6 days but at 27°C. For experiments on different durations of thermal or humidity acclimation (1 to 6 days), we examined age-related changes in unacclimated as well as acclimated flies of 1, 2, 4 or 6 days. Such experiments are expected to reveal age-related changes in the level of body lipids (see Fairbanks and Burch, 1970). In order to determine adult acclimation effects, control as well as experimental groups of flies (both sexes) were used to analyze plastic changes in starvation resistance and energy metabolites (body lipids, carbohydrates and proteins). For assessment of plastic changes in body lipids owing to different durations (1, 2, 4 or 6 days) of heat or humidity acclimation of adult flies, age-related changes were analysed in control and experimental groups of flies. Plastic effects were tested in three replicates of 20 flies for the control (non-acclimated) group as well as for different acclimation groups of wet or dry season flies. The utilization of energy metabolites (body lipids, carbohydrates and proteins) was analyzed under different durations of starvation stress (12, 24, 36 or 48 h) in the vials containing non-nutritive agar medium. Finally, plastic changes in mean daily fecundity were analyzed in the control group of flies at 27°C as well as in adult flies acclimated to thermal (32°C) or humidity (40 or 70% RH) conditions. Mean daily fecundity was also assessed in flies of control and experimental groups subjected to 24 h starvation stress.

Starvation resistance

Starvation resistance was measured in three replicates of 20 flies (both sexes) after developmental and adult acclimation to wet or dry conditions. Starvation assays were carried out at 27°C with flies of similar physiological age (6 days) for control as well as experimental groups of flies. Further, starvation resistance was also assessed in flies after adult acclimation to thermal or humidity conditions. For each vial, 10 adult flies of each sex and each acclimation treatment were used for analysis. The experimental set-up for estimation of starvation resistance involved a lower vial and an upper inverted vial of the same size (10×3 cm). The lower vial contained a sponge impregnated with 4 ml of distilled water and 1 mg sodium benzoate (anti-bacterial agent). Flies were kept in the upper vial with non-nutritive agar medium at the base and were covered with muslin gauze. The set of these two vials was wrapped with adhesive tape at the junction and kept in the humidity chamber (www.metrexinstruments.com; MEC-30) maintained at 90% RH. The numbers of dead flies were recorded twice a day (07:00 and 19:00 h) for the first day and subsequently three times a day (07:00, 15:00 and 23:00 h) until all flies had died from starvation. Such data were used for starvation survival curves based on Kaplan–Meier analysis. The log-rank test was used to compare sex-specific differences for each treatment group.

Assessment of body-size-related traits

To estimate season-specific changes in body-size-related traits (wet and dry mass, body water content and hydration level), three replicates of 20 flies were used. Individual flies were weighed on a Sartorius microbalance (model CPA26P, 0.001 mg precision; http://www.sartorious.com) and then reweighed after drying for 48 h at 60°C. Total body water content was estimated as the difference in mass before and after drying at 60°C. Hydration level was estimated as the ratio of body water content to dry mass (Gibbs et al., 1997).

Body lipids estimation

Body lipid content was estimated in individual flies of three replicates of 20 flies (control as well as acclimated) from each season, sex and acclimation treatment. Individual flies were dried at 60°C for 48 h, and dry mass was obtained by weighing on a Sartorius microbalance (model CPA26P; 0.001 mg precision). Body lipids were extracted by placing individual flies in 2 ml centrifuge tubes (http://www.tarsons.in) containing 1.5 ml of diethyl ether. These tubes were subjected to 200 rpm shaking at 37°C for 24 h; the solvent was then replaced and the process was repeated for 24 h. Finally, the solvent was removed and individuals were again dried at 60°C for 48 h and reweighed. Body lipid content was calculated per individual by subtracting the lipid-free dry mass from the initial dry mass per fly. The body lipid content was normalized to respective dry mass following Robinson et al. (2000).

Carbohydrate estimation

We followed Marron et al. (2003) for quantifying carbohydrates in three replicates of 20 flies of each season, sex and acclimation treatment. Twenty flies were homogenized in 4 ml double-distilled water and the homogenates were centrifuged at 8106 ***g*** for 5 min. Thereafter, 100 µl of supernatant from each sample was aliquoted for further analysis. Carbohydrate content in each sample was converted to glucose using 10 µl of amyloglucosidase (8 mg ml^{-1}; Sigma-Aldrich, www.sigmaaldrich.com) and samples were incubated overnight. Finally, the glucose amount was quantified after adding 1 ml of Infinity glucose reagent (Thermo-Fisher Scientific, www.thermofisher.com) to each tube. The absorbance was spectrophotometrically recorded at 340 nm within 1 h to quantify glucose levels. Solutions with known glucose concentrations were used to make standard curves.

Protein estimation

Protein levels were measured using the Bradford method (Bradford, 1976). Protein content was estimated in each of the three replicates of 20 flies of each season and sex. Twenty flies were homogenized in 1 ml of lysis buffer and the homogenate (in 1.5 ml Eppendorf tube) was subjected to centrifugation at 9600 ***g*** for 10 min at 4°C in a Fresco 21 centrifuge (Thermo-Fisher Scientific). Further, 100 µl of sample was mixed with 900 µl of Bradford reagent (Sigma-Aldrich). Finally, the absorbance was quantified spectrophotometrically at 595 nm and the amount of protein was estimated in reference to a standard curve based on bovine serum albumin.

Change in energy metabolites

Rate of change in accumulation (+) as well as utilization (−) of each energy metabolite was calculated as a function of different durations (1 to 6 days) for thermal or humidity acclimation for each season and sex. The rate of change in each energy metabolite was calculated in three replicates of 20 flies. To determine the rate of accumulation of body lipids, flies were acclimated to thermal (at 32°C) or humidity (at 40% or 70% RH, respectively) conditions for 1, 2, 4 or 6 days. The rate of utilization of body lipids or carbohydrates or proteins under starvation stress for 12, 24, 36 or 48 h was measured in flies of different treatment groups.

Mean daily fecundity

Mean daily fecundity was measured in control as well as experimental groups of flies acclimated to different heat or humidity conditions. The physiological age of the flies was kept constant. Mean daily fecundity was assessed at 27°C under low or high humidity for control as well as acclimated flies from dry or wet season treatments, respectively. Mean daily fecundity was analyzed in adult flies of each season acclimated to thermal (at 32°C) or humidity (at 40% or 70%) conditions for 4 days. Further, mean daily fecundity was also estimated in flies of different treatment groups subjected to 24 h starvation stress. We estimated fecundity in three replicates of 10 flies (using one virgin female and one virgin male per vial). The flies were transferred to fresh food vials every day, and the number of eggs laid by each female after 24 h was recorded daily for 10 days, and data were represented as mean±s.e. daily fecundity. For these experiments, live yeast was not provided in the food medium.

Treatment and analysis of data

Data on plastic changes in body size, starvation resistance and energy metabolites in flies reared under wet or dry conditions are shown in Table 1. Seasonal differences in body-size-related traits, starvation resistance and energy metabolites (body lipids, carbohydrates and proteins) were compared with Welch's two-

Table 1. Seasonal differences in body mass, hydration level, starvation resistance, body lipids, carbohydrates and proteins of male and female *Zaprionus indianus* (control group) reared under dry or wet conditions (developmental acclimation) followed by acclimation of adults at 27°C and under low (50%) or high (60%) relative humidity (RH)

Trait	Sex	Dry season	Wet season	Fold	Welch's test
Wet mass (mg fly^{-1})	M	1.94±0.030	1.76±0.024	1.10	56.92***
	F	2.18±0.028	1.96±0.026	1.11	69.57***
Dry mass (mg fly^{-1})	M	0.590±0.03	0.585±0.02	1.01	1.94 n.s.
	F	0.680±0.02	0.676±0.03	1.01	2.15 n.s.
Water content (mg fly^{-1})	M	1.35±0.04	1.18±0.03	1.14	40.55***
	F	1.50±0.05	1.28±0.05	1.17	39.11***
Hydration WC/DM	M	2.29±0.03	2.02±0.04	1.13	64.41***
	F	2.21±0.05	1.89±0.03	1.17	67.56***
Starvation resistance (h)	M	100±2.24	120±2.41	1.20	63.24***
	F	80±2.18	150±2.35	1.88	221.36***
Body lipids (µg mg^{-1} fly^{-1})	M	130±2.48	150±3.12	1.15	55.01***
	F	100±2.16	180±3.58	1.80	211.18***
Carbohydrates (µg mg^{-1} fly^{-1})	M	200±4.60	210±3.54	1.05	19.32***
	F	266±3.90	250±3.10	1.06	38.17***
Proteins (µg mg^{-1} fly^{-1})	M	205±2.30	180±3.89	1.14	65.99***
	F	250±3.45	226±4.86	1.11	46.37***

Data are means±s.e. ***P<0.001; n.s., non-significant. WC, water content; DM, dry mass.
For each season and sex, each trait was analyzed in three replicates of 20 flies (6 days old). Levels of significance with Welch's two-tailed test are shown.

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tailed test and in terms of fold difference (Table 1). Starvation survival was recorded for male and female flies (three replicates of 20 flies) of *Z. indianus* reared under wet or dry conditions. Starvation survival curves were generated using Kaplan–Meier analysis with MEDCALC statistical software (www.medcalc.org) and statistical significance was tested with the log-rank test (Fig. 2). Plastic effects of acclimation treatment and sex were calculated on the basis of two-way ANOVA (Table 2). The data on starvation resistance and body lipids were used to calculate absolute acclimation capacity (AAC; i.e. trait values of acclimated flies–control flies) and relative acclimation capacity (RAC; i.e. AAC divided by the control value of unacclimated flies) as suggested by Kellett et al. (2005). For these two measures, we represent data in the form of bar diagrams (Fig. 3). Seasonal plastic changes in body lipids in the control group of flies and after different durations of thermal acclimation are illustrated in Fig. 4. Tukey's test was used to determine the significance level of differences between treatments. Data on the rate of metabolite change as a function of

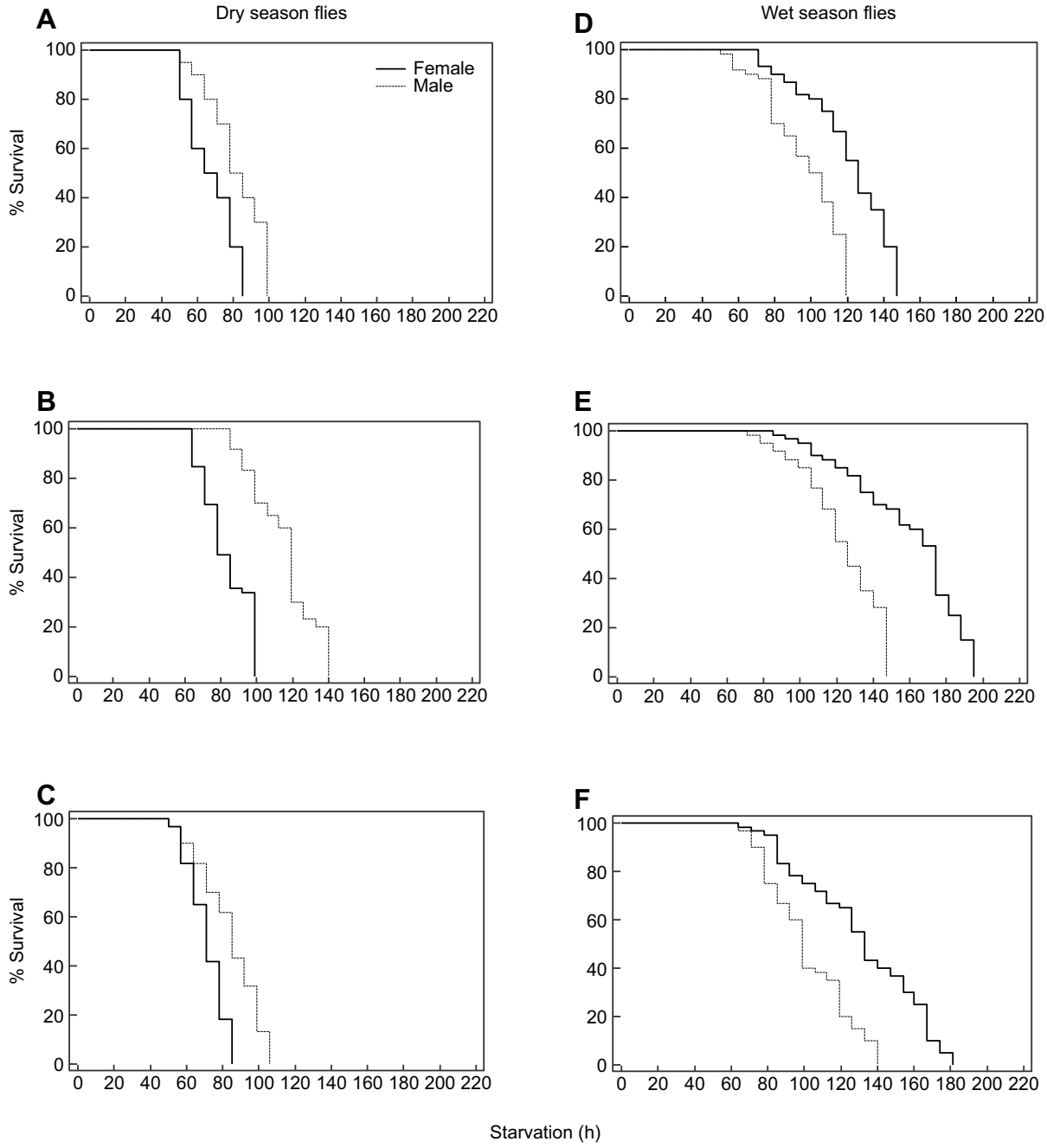

Fig. 2. Plastic changes in starvation resistance (based on Kaplan–Meier analysis) of *Zaprionus indianus* owing to adult acclimation under different thermal or humidity conditions. (A,D) Adult flies of the control group were acclimated at 27°C and low (50%) or high (60%) RH for 6 days. (B,E) Adult flies of one experimental group were thermally acclimated at 32°C for 6 days and at low (40%) or high (70%) RH. (C,F) Humidity acclimation of another experimental group of flies at low (40%) or high (70%) RH and at 27°C. Sexual dimorphism for starvation survival hours differs across wet or dry conditions (based on the log-rank test, $P<0.01$). Data are means±s.e. of three replicates of 20 flies for each treatment.

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Table 2. Results of two-way ANOVA testing the effects of adult acclimation to thermal or humidity conditions on starvation resistance and body lipids in male and female *Z. indianus* reared under wet or dry conditions

	Dry season flies					Wet season flies			
	Humidity acclimation			Thermal acclimation		Humidity acclimation		Thermal acclimation	
	d.f.	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Starvation resistance									
Sex	1	16,524	51,867***	30,250	43,694***	46,240	47,457***	57,760	68,262***
Acclimation	1	1	2.0 n.s.	30,250	43,694***	7840	8046***	46,240	54,647***
Sex×Acclimation	1	0	1.0 n.s.	2250	3250***	160	164***	1440	1702***
Error	156	0		1.0		1.0		1.0	
Body lipids									
Sex	1	0.033408	6153.1***	0.104142	111,351***	0.033640	1173.5***	0.030250	1055.2***
Acclimation	1	0.000020	3.6 n.s.	0.095942	102,584***	0.237160	8273.0***	0.123210	4298.0***
Sex×Acclimation	1	0.000001	0.2 n.s.	0.017598	18,816***	0.004840	168.8***	0.003610	125.9***
Error	156	10^{-6}		10^{-6}		3×10^{-5}		3×10^{-5}	

***P<0.001; n.s., non-significant.

different durations (1, 2, 4 or 6 days) of thermal or humidity acclimation were analyzed through regression analysis for calculation of regression slope values (Tables 3 and 4). Seasonal differences in slope values were compared with a Student's *t*-test. Finally, accumulation of body lipids owing to thermal or humidity acclimation is schematically represented in Fig. 5. Statistica 7 and MEDCALC were used for statistical calculations as well as illustrations.

RESULTS

Season-specific plastic changes in the control group of flies

For the control group, data on body hydration level, starvation resistance and energy metabolites of male and female flies of *Z. indianus* reared under dry or wet conditions followed by adult acclimation are given in Table 1. The hydration level of *Z. indianus* flies showed seasonal differences, i.e. the level of hydration was ∼15±2% higher in dry season flies than in wet season flies. For each

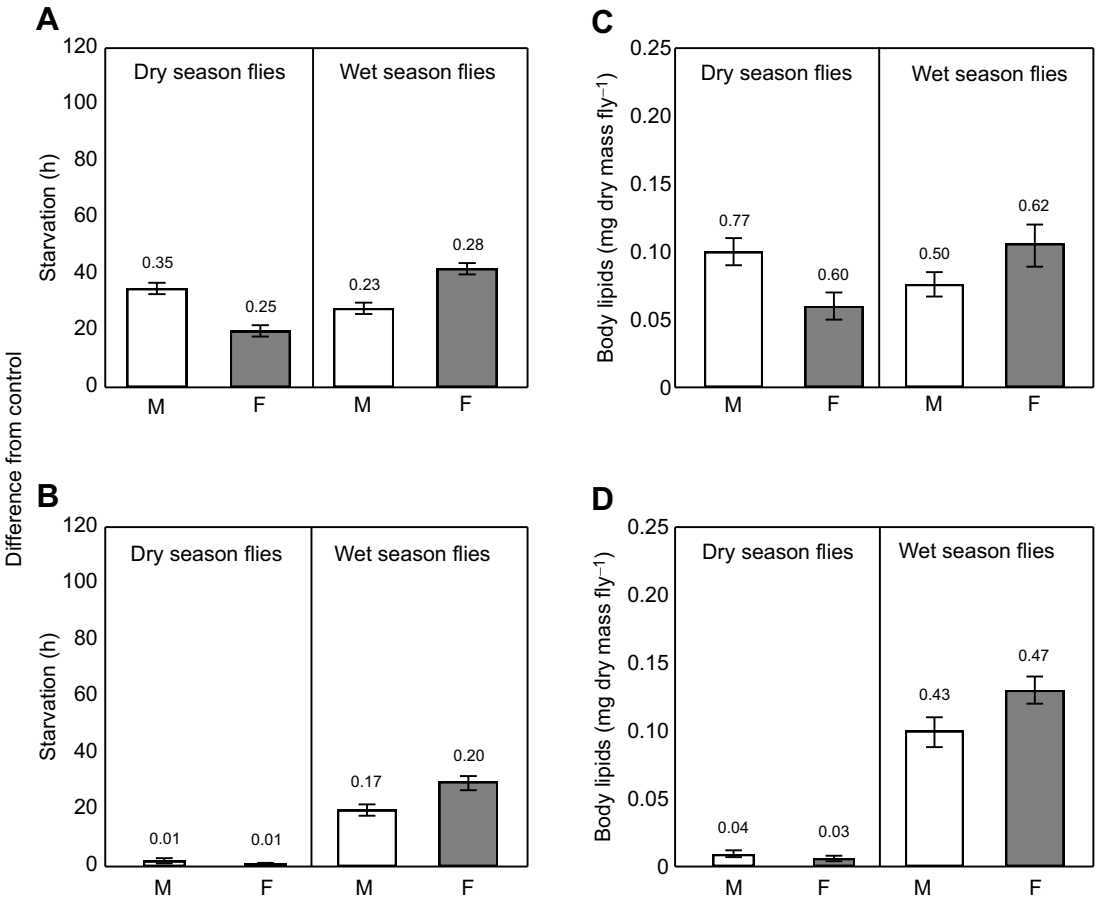

Fig. 3. Seasonal and sex-specific adult acclimation effects in *Zaprionus indianus*. Comparison of the increase in starvation resistance and body lipids [absolute acclimation capacity (AAC): acclimated–control] of dry or wet season flies owing to thermal acclimation at 32°C (A,C) or humidity acclimation at 40% or 70% RH (B,D) for 6 days. For each trait and sex, values differ significantly across seasons (P<0.01). For each acclimation treatment, bars represent AAC whereas values on the top of each bar refer to relative acclimation capacity (RAC). Data are means±s.e. of three replicates of 20 flies for each treatment.

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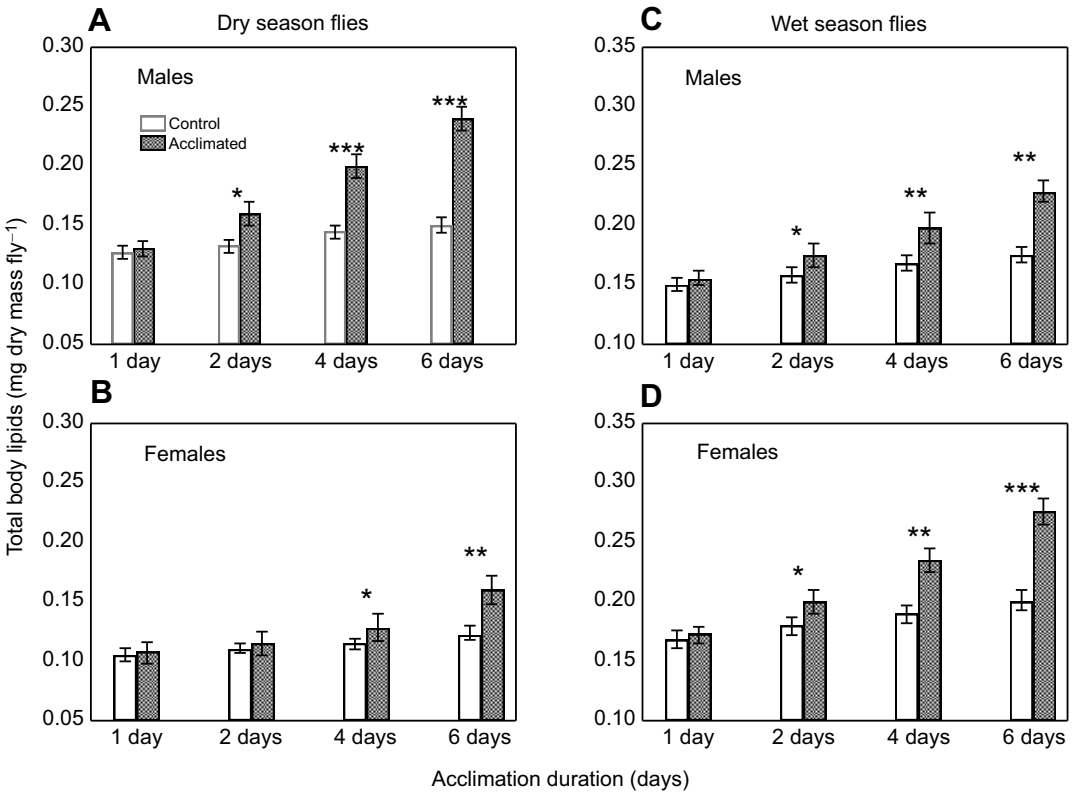

Fig. 4. Heat-induced plastic changes in the levels of body lipids in *Zaprionus indianus* of dry or wet seasons. Plastic changes in body lipids owing to thermal acclimation at 32°C under low (40%) or high (70%) RH conditions in male (A,C) and female *Z. indianus* (B,D). Age-related changes have been shown for control flies as well as adults acclimated for different durations (1, 2, 4 or 6 days) at 32°C. Data are means±s.e. of three replicates of 20 flies for each treatment. Statistical difference between each pair of control and acclimated flies was tested with Student's *t*-test (**P*<0.05; ***P*<0.01;****P*<0.001).

season, wet and dry mass were higher in female flies than male flies of both seasons. Dry season flies had ∼10% more wet mass than wet season flies. Dry mass did not vary across seasons despite sex-specific differences for each season (Table 1). *Zaprionus indianus* dry season flies showed ∼15% more body water than wet season flies. Further, seasonal differences in starvation resistance and body lipids showed sexual dimorphism, i.e. higher starvation resistance and body lipids in male dry season flies and in female wet season flies (Table 1). In contrast, protein levels were ∼12% higher in dry season flies than in wet season flies. Finally, there was an ∼5% difference in the level of carbohydrates between dry and wet season flies (Table 1). Except dry mass, all of the traits showed significant seasonal differences (*t*-test, $P<0.001$; Table 1). Thus, combined effects of developmental and adult acclimation of flies from the control group revealed significant seasonal differences in body hydration, starvation resistance and energy metabolites of *Z. indianus* from a tropical locality. In the present work, we did not investigate genetic differences between seasonal populations through common garden experiments. However, the observed phenotypic differences are likely to include genetic differences between seasonal populations of *Z. indianus*.

Combined plastic changes owing to developmental and adult acclimation effects on starvation resistance

Starvation survival following developmental acclimation (dry or wet conditions) and then adult acclimation at 32°C for 6 days under low or high humidity is shown in Fig. 2. Combined acclimation effects revealed seasonal as well as sex-specific

Table 3. Rate of accumulation (+) of body lipids of adult flies owing to thermal (32°C) or humidity (40% or 70% RH) acclimation (1, 2, 4 or 6 days) and rate of utilization (–) under starvation stress (12, 24, 36 or 48 h) in male and female *Z. indianus* reared under dry or wet conditions

	Dry season			Wet season		
	Male	Female	$t_{1,58}$	Male	Female	$t_{1,58}$
Rate of accumulation (μg h^{-1})[a]						
Thermal acclimation	+0.67±0.02	+0.55±0.02	37.47***	+0.60±0.01	+0.75±0.03	43.75***
Humidity acclimation	+0.03±0.01	+0.03±0.01	n.s.	+0.62±0.03	+0.83±0.02	53.86***
Rate of utilization (μg h^{-1})[a]						
Thermal acclimation	−1.16±0.01	−1.10±0.02	10.59***	−0.83±0.02	−0.90±0.02	21.86***
Humidity acclimations	−1.00±0.02	−0.94±0.02	17.14***	−0.74±0.01	−0.80±0.03	18.01***
Control	−0.87±0.01	−0.81±0.02	19.76***	−0.68±0.02	−0.73±0.02	15.61***

[a]Regression slope values represent the rate of accumulation or utilization of body lipids.
***$P<0.001$; n.s., non-significant.
For each season, sex-specific differences were compared by Student's *t*-test.

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Table 4. Rates (regression slope values) of utilization of carbohydrates and proteins under starvation stress (12, 24, 36 or 48 h) in control and low humidity (40% RH) or thermal (32°C) acclimated dry season *Z. indianus* (both sexes)

		Rate of utilization (μg h^{-1}) of dry season flies		
	Sex	Control	Humidity acclimation	Thermal acclimation
Carbohydrates	M	−1.66±0.04[a]	−1.71±0.02[b]	−2.06±0.05[c]
	F	−1.52±0.03[a]	−1.58±0.04[b]	−1.80±0.03[c]
Proteins	M	−1.40±0.03[a]	−1.47±0.02[b]	−1.66±0.02[c]
	F	−1.34±0.04[a]	−1.42±0.03[b]	−1.60±0.02[c]

Different letters indicate significant differences based on Tukey's test.

differences in starvation resistance of *Z. indianus* (Fig. 2A,D). Male dry season flies showed greater starvation resistance than female dry season flies. However, for the wet season, starvation resistance was higher in females than in males, and overall, wet season flies were more starvation tolerant than dry season flies (Fig. 2A,D). Dry season flies evidenced lower plastic responses in starvation resistance owing to thermal acclimation at 32°C than wet season flies (Fig. 2B,E). In contrast, combined effects owing to developmental acclimation followed by humidity acclimation of adults revealed no change in starvation resistance of either male or female dry season flies (Fig. 2C) but a significant increase in starvation resistance of wet season flies (Fig. 2F). Thus, adult acclimation effects on starvation resistance involve thermal effects for dry season flies but both thermal and humidity effects for wet season flies of *Z. indianus*. Further, plastic changes owing to humidity or thermal acclimation have shown significant effects owing to sex, acclimation and their interaction, except for plastic responses for humidity acclimation of dry season flies (ANOVA; Table 2).

Plastic changes in starvation resistance and body lipids owing to adult acclimation capacity

Data on acclimation capacity (AAC and RAC) for starvation resistance and body lipids of *Z. indianus* adult flies acclimated to thermal or humidity conditions are shown in Fig. 3. The physiological age of the flies was kept constant (6 days) for control as well as experimental groups of flies, which were assayed simultaneously. For starvation resistance, plastic responses of dry or wet season flies owing to thermal acclimation are shown in Fig. 3A whereas effects of humidity acclimation are shown in Fig. 3B. For dry season flies, adult thermal acclimation effects are significant (RAC=0.35 for males, 0.25 for females, $P<0.01$), but there was no effect of low humidity acclimation (RAC=0.01, $P>0.05$). Starvation resistance of wet season flies showed similar responses with thermal or humidity acclimation (Fig. 3A,B).

Plastic changes in body lipids owing to thermal or humidity acclimation (for 6 days) are consistent with adult acclimation effects on starvation resistance of *Z. indianus* (Fig. 3A,B versus C,D). For dry season flies, the level of body lipids significantly increased after thermal acclimation of flies at 32°C for 6 days. However, there were no changes in the level of body lipids after low humidity acclimation (Fig. 3C,D). For wet season flies, heat- or humidity-triggered plastic changes were quite significant (RAC=0.50 to 0.62, $P<0.01$). Thus, thermal and low versus high humidity acclimation of adult flies supports plastic changes in starvation resistance through increases in the levels of body lipids.

Thermal or humidity-induced accumulation of body lipids

Plastic changes in body lipids in the control group of flies of different ages (1, 2, 4 or 6 days) and in experimental groups after different durations (1, 2, 4 or 6 days) of thermal acclimation of newly eclosed adult *Z. indianus* are shown in Fig. 4. Age-related changes (1, 2, 4 or 6 days) in body lipids in the experimental groups of flies are also shown in Fig. 4. Unacclimated flies of different ages exhibited a modest increase (10 to 20%) in total body lipids. In the case of thermally acclimated flies (1 to 6 days), a significant increase in the level of body lipids was due to thermal effects while effects of age-related changes were quite low. For dry season flies, thermal acclimation duration had a significant effect on the accumulation level of body lipids of male flies compared with females (Fig. 4A,B). In contrast, both male and female wet season flies showed increases in body lipids owing to thermal acclimation (Fig. 4C,D). Thus, different durations of thermal acclimation responses resulted in an increase in the level of body lipids, and such seasonal as well as sex-specific differences are shown in Fig. 4.

The level of body lipids did not change in response to low humidity acclimation in male or female dry season flies (Fig. 4A,B). On the contrary, humidity acclimation revealed significant plastic changes in body lipids of both sexes. However, plastic changes in body lipids do involve interaction effects owing to thermal or humidity acclimation conditions. For dry season flies, thermal acclimation of adult flies may include major effects owing to thermal acclimation at 32°C and little effect owing to low humidity. In contrast, for wet season flies, it is difficult to partition the adult acclimation effects owing to thermal or humidity acclimation.

Plastic changes in the rates of accumulation or utilization of body lipids

Based on the data of plastic changes in body lipids as a function of different durations of thermal or humidity acclimation, we assessed

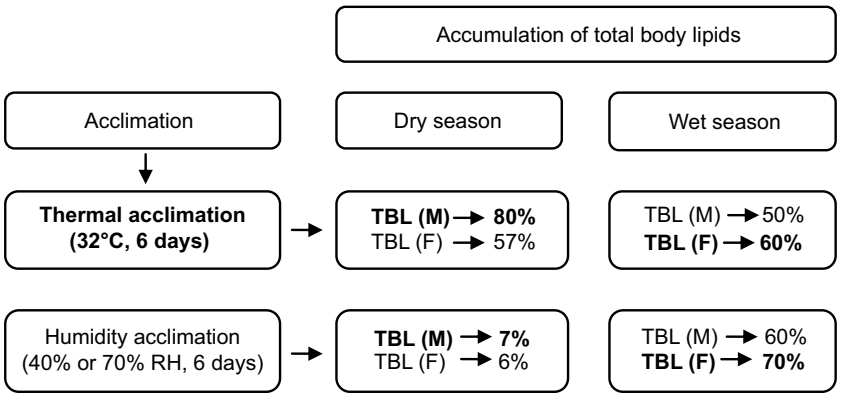

Fig. 5. Sexual dimorphism for accumulation of body lipids owing to thermal or humidity acclimation. Schematic representation of plastic changes in total body lipids (TBL; %) in dry or wet season male (M) and female (F) *Z. indianus* owing to either heat acclimation at 32°C for 6 days or low versus high humidity acclimation for 6 days. Thermal acclimation effects are higher for dry season males whereas low humidity showed little effect. For wet season flies, both thermal and humidity acclimation effects are significant (shown in bold).

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whether rates of accumulation varied across seasons as well as sexes of *Z. indianus* flies; data on regression slope values are given in Table 3. In dry season flies, low humidity acclimation durations did not trigger accumulation of body lipids, but thermal acclimation revealed significant accumulation effects (Table 3). The lower rate of lipid accumulation in females (+0.55 µg h^{-1}) than males (+0.67 µg h^{-1}) is consistent with sexual differences in starvation resistance (Table 3). In contrast, for wet season flies, both abiotic factors (ecologically relevant thermal as well as humidity acclimation) resulted in a higher rate of lipid accumulation in females owing to humidity as well as thermal acclimation as compared with males (Table 3).

We found contrasting levels of differences in the rates of utilization of body lipids under starvation stress in flies of dry versus wet seasons (Table 3). In dry season flies, rates of utilization of body lipids under starvation stress were significantly higher than in wet season flies (P<0.001; Table 3). For flies of both seasons, rates of utilization were higher in thermal or low humidity acclimated flies than in flies of the control group (P<0.001; Table 3). Sex-specific differences were also significant for the rates of accumulation or utilization of body lipids for flies of both seasons.

A schematic representation of percent change in accumulation of body lipids after 6 days of thermal or humidity acclimation of adult *Z. indianus* reared under dry or wet conditions is shown in Fig. 5. A comparison of heat or humidity acclimation (6 days) revealed sex-specific as well as seasonal differences in the accumulated levels of body lipids. Low humidity (40% RH) acclimation showed no effect on body lipids of either male or female dry season flies (Fig. 5).

Utilization of carbohydrates and proteins in dry or wet season flies

Dry season flies utilized carbohydrates and proteins under starvation stress. Data on the rates of utilization of carbohydrates and proteins in dry season flies (control and acclimated groups) are shown in Table 4. For both carbohydrates and proteins, the rates of utilization were 20% higher in thermally acclimated flies (32°C) compared with unacclimated flies (P<0.001; Table 4). However, the rates of utilization after low humidity acclimation revealed less of an effect (~4 to 6%). Sex-specific differences were significant for rates of utilization of carbohydrates and proteins in control as well as acclimated flies. However, we observed non-utilization of carbohydrates and proteins by control and acclimated wet season flies. In contrast, there is evidence of utilization of carbohydrates and proteins under starvation in xeric and mesic species of drosophilids (Marron et al., 2003). Therefore, our results of non-utilization of carbohydrates in wet season flies could be biased because of our inability to detect minor changes in the carbohydrate level.

Plastic changes in fecundity owing to thermal or humidity acclimation of adult flies

We assessed changes in the mean daily fecundity of adult flies acclimated to thermal or humidity conditions as compared with controls (Fig. 6). Mean daily fecundity was higher for wet season than for dry season flies (P<0.01; Fig. 6A,C). Thermal acclimation at 32°C for 4 days increased the level of daily fecundity of flies. In contrast, mean daily fecundity was approximately 20% higher in female flies acclimated to high humidity conditions. In flies subjected to 24 h of starvation stress, a reduction in mean daily fecundity was observed in control as well as acclimated groups of flies (Fig. 6B,D). Starved flies of different groups of acclimated or unacclimated (control group) dry season females revealed lower mean daily fecundity as compared with wet season females. Thus, thermal or humidity acclimation of adult females showed significant differences in mean daily fecundity of *Z. indianus*.

DISCUSSION

In the tropical drosophilid *Z. indianus* (reared under wet or dry conditions), we investigated plastic changes in starvation-related traits owing to adult acclimation under different conditions of heat or humidity. Dry or wet seasonal phenotypes include developmental as well as adult acclimation effects besides genetic differences in the progeny of seasonal wild-caught flies. However, the latter aspect was not investigated in the present study. We observed significant seasonal as well as sex-specific differences in starvation resistance and in the rates of accumulation of body lipids (but not of carbohydrates and proteins) as a function of different durations of heat or humidity acclimation. Dry season flies utilized body lipids, but also 20% to 30% of carbohydrates and proteins. Such plastic changes in energy metabolites differed across season as well as sexes. Our results show that plastic changes in the level of body lipids support increased starvation resistance. We observed plastic changes in mean daily fecundity in response to heat acclimation of *Z. indianus*. Starved flies showed a reduction in mean daily fecundity in the control group of flies as well in those flies acclimated to heat or humidity conditions. Thus, heat- or humidity-induced plastic responses confer the ability to cope with starvation stress in *Z. indianus* of wet or dry seasons.

Adult acclimation effects

An assessment of RAC has shown that plastic changes in heat resistance are constrained by their basal level in eight *Drosophila* species (Kellett et al., 2005). However, another study revealed geographical differences in the RAC of heat resistance in populations of *D. melanogaster* (Sgrò et al., 2010). Plastic responses owing to cold or heat hardening and/or acclimation are able to mitigate deleterious effects of these stressors. Previous studies have shown that after developmental acclimation, there is a modest increase in the starvation survival of *D. buzzatii* reared under constant or fluctuating thermal conditions (Sarup and Loeschcke, 2010) and in *D. melanogaster* reared under constant versus summer conditions (Hoffmann et al., 2005). Further, basal levels of starvation resistance vary across different *Drosophila* species (Matzkin et al., 2009a) and plastic responses for starvation resistance could also vary across species. In the present work, we found significantly higher levels of starvation resistance and body lipids of *Z. indianus* as a consequence of the combined effects of developmental and adult acclimation (Fig. 3). Plastic responses evidenced sex-specific differences for the flies of each season, i.e. higher RAC values for starvation and body lipids in dry season males and wet season females. For starvation resistance and body lipids, thermal acclimation (32°C, 6 days) of adult flies of both seasons revealed a significant increase. In contrast, adult acclimation of dry season flies to low humidity did not evidence plastic changes in starvation resistance and body lipids, but high humidity acclimation of adult wet season flies resulted in increased starvation resistance and body lipids.

Plastic changes in body lipids owing to heat acclimation of adult flies

During the lifetime of an organism, stressors cause perturbations in the levels of energy metabolites and survival depends upon maintenance of energetic homeostasis (Chapman, 1998; Hochachka and Somero, 2002; Grönke et al., 2005; Hildebrandt

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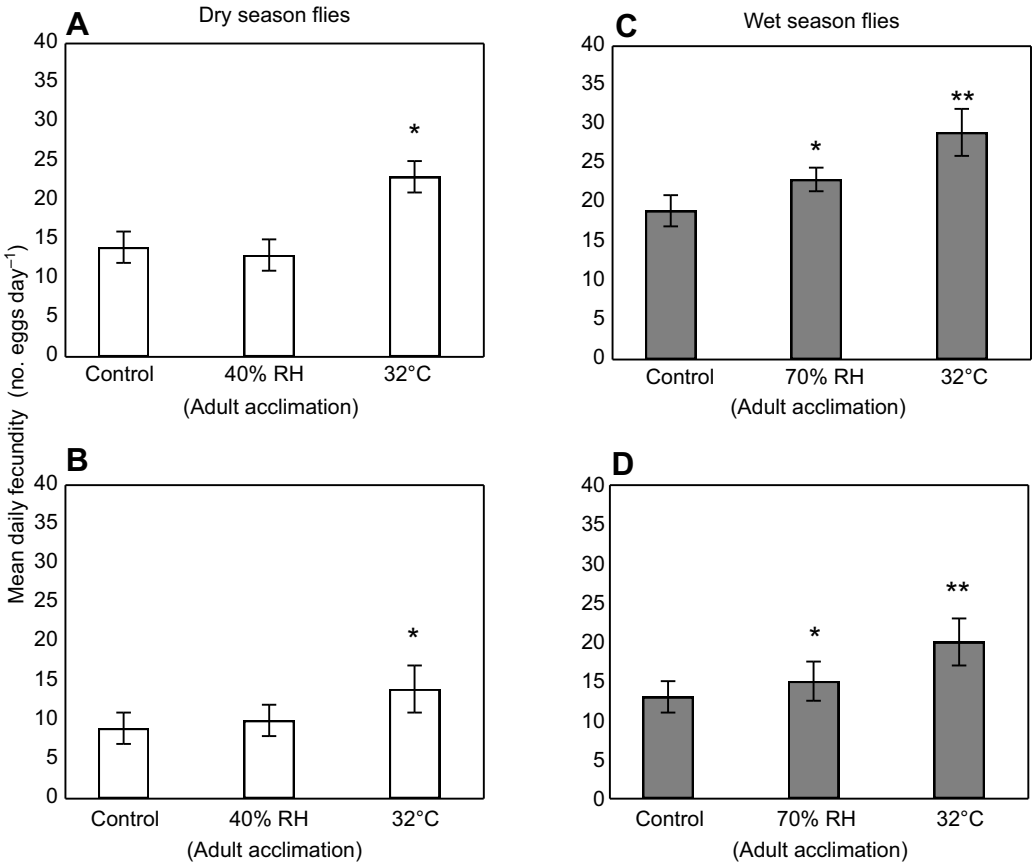

Fig. 6. Plastic changes in mean daily fecundity due to heat or humidity acclimation. Mean daily fecundity of *Z. indianus* flies reared under dry (50%) or wet (60%) RH conditions at 27°C (control group) and for experimental groups (adult flies subjected to thermal acclimation at 32°C or low versus high humidity for 4 days). The physiological age of flies was kept similar (4 days) for each treatment group. Flies of these treatment groups subjected to 24 h starvation showed a reduction in daily mean fecundity (B,D). Data are means±s.e. of three replicates of 10 flies for each treatment. Statistical significant differences between control and acclimated flies were analyzed with Student's *t*-test (*P<0.05; **P<0.01).

et al., 2011). For drosophilids facing periodic starvation, adult flies are likely to depend on the stored energetic fuels (Arrese and Soulages, 2010). During starvation, utilization of body lipids is ubiquitous but causal factors for lipid accumulation require empirical evidence (Rion and Kawecki, 2007). Thermal acclimation (32°C, 6 days) of adult flies of both wet and dry seasons resulted in a significant increase in the levels of body lipids. Such plastic responses for accumulation of body lipids can meet the requirements of lipid utilization under starvation stress and could enable flies to maintain energetic homeostasis.

Effects of humidity acclimation of adults on accumulation of body lipids

Previous studies on starvation clines in several drosophilids from India have shown increased starvation resistance of southern populations living in warm and humid habitats (Parkash and Munjal, 1999; Parkash and Aggarwal, 2012). Genetic effects on starvation resistance are associated with a humidity gradient on the Indian subcontinent, i.e. they are negatively correlated with latitude (Parkash and Aggarwal, 2012). Therefore, to obtain empirical evidence, we tested plastic responses in starvation resistance as well as body lipids after humidity acclimation. For dry season flies, low humidity acclimation did not alter the level of body lipids in either sex. In contrast, for wet season flies, high humidity acclimation revealed a linear increase in the level of body lipids as a function of different acclimation durations (1 to 6 days). Thus, high humidity level affected the accumulation of body lipids in *Z. indianus*. Further, seasonal as well as sex-specific differences in the rates of accumulation of body lipids are consistent with plastic changes in the starvation survival of *Z. indianus*. For example, higher rates of accumulation of body lipids in wet season females and in dry season males are consistent with observed plastic changes in starvation survival. Further, lower starvation resistance of dry season flies can be explained on the basis of accumulation of body lipids due to thermal but not low humidity acclimation.

Seasonal plasticity in the utilization of energetic metabolites

Plastic changes in resistance to multiple stressors (cold, heat or desiccation) have received greater attention in the literature than starvation resistance (Chown and Nicolson, 2004; Overgaard et al., 2008; Angilletta, 2009; Parkash et al., 2014a). Previous studies have shown that lipids are the preferred sources of energy utilization under starvation whereas carbohydrates are metabolized as a result of desiccation in *Drosophila* species (Rion and Kawecki, 2007; Hoffmann, 2010). However, there are reports on the use of a mix of energy metabolites under desiccation and/or starvation in mesic and desert *Drosophila* species (Marron et al., 2003), *D. melanogaster* (Djawdan et al., 1998), the mosquito *Culex pipiens* (Benoit et al., 2010), *Belgica antarctica* (Teets et al., 2011) and the American dog tick (Rosendale et al., 2017). It may be argued that desiccation- or starvation-specific utilization of energy metabolites seems to vary

across insect taxa that differ in their ecological adaptations (Marron et al., 2003). Beyond interspecific comparisons, intraspecific analysis (temporal or spatial) of energetic consequences of desiccation or starvation stress has received little attention.

In the present work, we tested utilization of carbohydrates and proteins under starvation in flies acclimated to dry conditions. The rationale is that dry season *Z. indianus* flies seem more constrained under starvation compared with wet season flies. In dry season flies, lipid storage is lower and only thermal acclimation (not the low humidity acclimation) could increase the lipid levels. Therefore, utilization of carbohydrates and proteins under starvation is consistent with the survival strategy of dry season flies. Thus, seasonal phenotypic plasticity of starvation resistance in *Z. indianus* could be constrained by a shift in the use of energetic resources.

Sexual dimorphism in starvation resistance and body lipids

Several *Drosophila* species (reared under standard laboratory conditions) have shown sex-specific differences in starvation resistance, i.e. females with larger body size show longer starvation resistance than smaller males (Matzkin et al., 2009a). In *Z. indianus* reared under wet or dry humidity conditions, there are significant sex-specific differences in starvation resistance. In dry season flies, males have higher starvation resistance and body lipid levels than females. However, wet season female flies showed significantly higher starvation resistance and body lipids as compared with males. These observations reflect that abiotic factors are likely to induce sex-specific plastic changes in body lipids to support starvation resistance of *Z. indianus*. Such sex-specific differences in the levels of energy metabolites are also associated with desiccation resistance in *D. hydei*, i.e. a higher desiccation resistance in males of *D. hydei* is associated with a greater amount of trehalose as compared with females (Kalra and Parkash, 2014). Thus, plastic changes in the level of energy reserves are associated with sexual dimorphism of starvation resistance in *Z. indianus*.

Changes in food medium owing to low or high humidity conditions

We raised *Z. indianus* under dry or wet conditions, which is likely to affect the laboratory food medium. Feeding behaviour of *Drosophila* larvae varies genetically owing to rover or sitter variants of the foraging gene (Kaun et al., 2008), and owing to solid or semi-solid food under dry or wet humidity conditions, respectively (Shen, 2012a,b). In *D. melanogaster*, the larval feeding response (measured on the basis of contractions of mouth hooks) is higher on liquid glucose–agar medium as compared with solid glucose–agar medium (Shen, 2012a,b). A previous study has investigated food responses of *D. melanogaster* larvae (e.g. Kaun et al., 2008) but such analyses have not been made for food acquisition by *Z. indianus* larvae and adults. It is likely that laboratory food medium condition (drier or semi-solid) can impact feeding rates of *Z. indianus* larvae and adults. Plastic changes owing to developmental acclimation (under dry versus wet relative humidity) have revealed seasonal as well as sex-specific differences in the acquisition of three energy metabolites: increased levels of proteins and carbohydrates have been shown in flies reared under dry season (low humidity) conditions whereas higher levels of body lipids are evident in wet season flies (Table 1). Our data on plastic changes owing to developmental acclimation in starvation resistance and three energy metabolites (body lipids, carbohydrates and proteins) seem to involve disproportionate levels of nutrient reserves from wet or dry food medium as well as possible plastic changes in the feeding behaviour of *Z. indianus*.

Plastic changes in mean daily fecundity owing to adult acclimation

Several studies support trade-offs between stress resistance and life history traits (see Nylin and Gotthard, 1998; Hoffmann, 2010). Laboratory strains selected for resistance to cold, heat, desiccation or starvation revealed a reduction in mean daily fecundity and other life history traits in *D. melanogaster* (Hoffmann, 2010). Drosophilids exposed to multiple stressors in the wild may favour an energetic homeostasis to cope with harsh climates (Bochdanovits and de Jong, 2003; Angilletta, 2009). Accordingly, plastic responses owing to acclimation are likely to mitigate change in reproductive output under ecologically relevant climatic conditions. A comparison of control and experimental groups of flies subjected to thermal acclimation at 32°C revealed plastic changes in fecundity of *Z. indianus*. Thermal or high humidity acclimation of adult female flies significantly increased mean daily fecundity. Such plastic responses could be species specific owing to the ability of *Z. indianus* to adapt to drier conditions in the tropics. These observations support the higher relative abundance (60%) of *Z. indianus* during the dry season as compared with 35% during the wet season. Further, starvation (24 h) led to a reduction in mean daily fecundity of *Z. indianus*, but plastic responses were observed in both control as well as acclimated flies. Thus, plastic changes in mean daily fecundity vary in flies acclimated to thermal or humidity conditions.

Conclusions

During their lifetime, insects are able to cope with seasonal changes in temperature and humidity through phenotypic plasticity of morphological and physiological traits. Tropical drosophilids from southern India are resistant to heat and starvation, as evidenced by genetic clines, but previous studies did not consider plastic changes in starvation resistance. In *Z. indianus* flies reared under wet or dry conditions, we observed significant seasonal as well as sex-specific differences in starvation survival, i.e. dry season males and wet season females showed higher lipid levels as well as starvation resistance. Thermal or humidity acclimation of adults elicited accumulation of body lipids for both sexes of wet season flies whereas only thermal acclimation increased starvation-related traits in dry season flies. Rates of accumulation as well as utilization of body lipids also revealed seasonal differences. Thus, seasonal phenotypic plasticity of starvation in *Z. indianus* involves increased storage and lower rates of utilization in wet season flies. Finally, thermal or humidity acclimation of virgin flies resulted in increased levels of fecundity in *Z. indianus*. However, thermal or humidity acclimation of wet or dry season flies resulted in a reduction in mean daily fecundity under starvation (24 h). Therefore, plastic changes of starvation resistance and body lipids confer adaptive changes for better survival of *Z. indianus* living under dry or wet climatic conditions in the tropics. Thus, seasonal phenotypic plasticity for starvation resistance in *Z. indianus* seems critical for ecological success as an invasive species in the tropics.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.P.; Methodology: T.N.G.; Software: T.N.G., B.E.P.; Formal analysis: T.N.G., R.P.; Resources: B.E.P.; Data curation: T.N.G.; Writing - original

draft: R.P.; Writing - review & editing: R.P.; Visualization: B.E.P.; Supervision: R.P.; Project administration: B.E.P.

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