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Water temperature explains part of the variation in basal plasma cortisol level, within and between- fish species

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Abstract :

Within the thermal tolerance range of fish, metabolism is known to escalate with warming. Rapid thermic changes also trigger a series of physiological responses, including activation of the stress axis, producing cortisol. Fish have adapted to their environment by producing low level of plasmatic cortisol when unstressed (basal), so that thriving in their natural temperature should not impact their basal cortisol levels. Yet, surprisingly little is known on how temperature affects cortisol within and between fish species. Here, we conducted a phylogenetic meta-analysis to (1) test whether temperature can explain the differences in basal cortisol between species and (2) evaluate the role of temperature on differences in cortisol levels between individuals of a same species. To do so, we retrieved basal plasma cortisol data from 126 studies, investigating 33 marine and freshwater fish species, and correlated it to water temperature. Intra-species variability in basal plasma cortisol levels was further investigated in two species, the European sea bass Dicentrarchus labrax and the Nile tilapia Oreochromis niloticus. Factors such as life stage, sex and weight were also considered in the analyses. Overall, our phylogenetic analysis revealed a clear positive correlation between basal cortisol level and the temperature at which the fish live. The role of temperature has also been confirmed within D. labrax, while it failed to be significant in O. niloticus. In this paper, influence of habitat, life stage, sex and weight on basal plasma cortisol levels are also discussed. Since some abiotic parameters were not included in the analysis, our study is a call to encourage scientists to systematically report other key factors such as dissolved oxygen or salinity to fully depict the temperature-cortisol relationship in fishes.

Keywords : Baseline cortisol, European Sea Bass, Meta-analysis, Metabolism, Nile Tilapia, Stress

1 | INTRODUCTION

Cortisol is the major corticosteroid in fishes and has demonstrated to play a central role in their capacities to cope with environmental challenges. Cortisol production involves the hypothalamo–pituitary–interrenal axis (HPI) (Mommsen *et al.*, 1999). Briefly, the production and release of cortisol occurs in response to stress in interrenal cells, located in the teleost head kidney, and is mainly controlled by the adrenocorticotropic hormone (ACTH), which is released by the pituitary gland into the blood (Sumpter, 1997). Cortisol in the blood mobilizes energy and activates physiological mechanisms to allow fish to cope with stressors (Sadoul & Vijayan, 2016), affecting many biological processes, including immunity, appetite, growth or reproduction (Tort, 2011; Sadoul & Vijayan, 2016; Schreck & Tort, 2016).

Cortisol levels are known to rise in response to acute changes of many biotic and abiotic factors (Castanheira *et al.*, 2017; Sadoul *et al.*, 2017; Alfonso *et al.*, 2020a; Bessa *et al.*, 2021), including temperature (Alfonso *et al.*, 2021; Goikoetxea *et al.*, 2021). Post-stress cortisol levels, extensively studied, have demonstrated the existence of intra-specific variability, probably as a consequence of differences in sensitivity to a same level of ACTH, rather than differences in the interpretation of a same stressor (Pottinger & Carrick, 1999; Khan *et al.*, 2016; Samaras & Pavlidis, 2018). Yet, this variability in post-stress cortisol has been observed to relate to differences in genotype and/or the life history (Fevolden *et al.*, 1999; Pottinger & Carrick, 1999; Barton, 2002; Chatziplis *et al.*, 2020; Bordin & Freire, 2021). The significant heritability in post-stress cortisol levels (Vandeputte *et al.*, 2016) combined to the proven possibility to select on this phenotype clearly demonstrate the important role of the genotype in this variability (Pottinger & Carrick, 1999). Life history has also been demonstrated to modulate the way fish respond to stress events later in life (Auperin & Geslin, 2008; Robinson *et al.*, 2019).

Pre-stress cortisol levels, also called basal (or resting or baseline) cortisol levels, are overall less studied, probably because of the difficulty to obtain standardized measures. While intra-species variability in basal cortisol levels undoubtedly exists, little is known on the drivers of this variability, even if some evidences suggest some environmental and genetics drivers (e.g., temperature, coping styles, HPI sensitivity) (Samaras & Pavlidis, 2018; Tudorache *et al.*, 2018; Alfonso *et al.*, 2020b, 2021). Inter-species variability in the basal levels of cortisol are also clear, with some species displaying very low levels, around 2 ng/mL for domestic rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), and some like the European seabass *Dicentrarchus labrax* L., showing basal levels 40-50 times higher (Barton, 2002; Fanouraki *et al.*, 2011; Samaras *et al.*, 2016, 2018). Whether this is phylogenetically driven or an adaptive response to a specific environment is unknown.

As ectotherms, fish body temperature is driven by water temperature. Hence, living in warm water has inherent effects on fish physiology, metabolism and behaviour (Clarke & Johnston, 1999; Somero, 2004). Within the thermal tolerance range of species, metabolism is known to exponentially increase with temperature (Somero, 2004; Schulte *et al.*, 2011), though the increase of metabolic rate tends to level off or to be reduced when reaching the upper thermal limits of individuals (Claireaux & Lagardère, 1999). In parallel, rapid thermic changes are well known to trigger a series of physiological responses including activation of the stress axis, producing cortisol. In a recent review, we reported that temperature increase could affect fish stress physiology in multiple ways (Alfonso *et al.*, 2021). Chronic temperature increase can, for instance, lead to enhanced metabolic reactions, including those producing cortisol, but can also be perceived as chronic stress when temperature impairs homeostasis (Schulte *et al.*, 2011; Alfonso *et al.*, 2021). These changes in cortisol are concomitant to other changes on the stress regulation of HPI axis, and could be responsible for physiological adaptations (Goikoetxea *et al.*, 2021). For instance, in brook trout *Salvelinus fontinalis* (Mitchill 1814), it has been demonstrated that when fish is facing its upper thermal limit, plasmatic cortisol increases concomitantly with glucose as well as heat shock protein 70 in gills (Chadwick *et al.*, 2015;

Chadwick & McCormick, 2017). These physiological adaptations help to face the increase in temperature but at the cost of a reduced growth (Chadwick & McCormick, 2017; Alfonso *et al.*, 2021). Individuals well adapted to their water temperature are, however, supposedly unstressed, and should display low levels of basal cortisol. It is nevertheless unknown whether these levels (in unstressed individuals) are also driven by temperature within and between fish species, as for metabolism (Somero, 2004; Schulte *et al.*, 2011), i.e. increased basal cortisol production caused by increased metabolism. Due to the central role of the HPI axis in coping abilities, understanding the link between basal cortisol level and temperature in fishes is of primary importance for reaching sustainable growth of the aquaculture sector and fisheries industries in the context of global warming (Goikoetxea *et al.*, 2021; McKenzie *et al.*, 2021).

Here, we conducted a phylogenetic meta-analysis to (1) test whether temperature can explain the differences in basal cortisol between species and (2) evaluate the role of temperature on differences in basal cortisol levels between individuals of a same species. To do so, we retrieved basal plasma cortisol data and water temperature associated from 126 studies, investigating 33 marine and freshwater fish species. To further investigate the intra-species variability in basal plasmatic cortisol levels linked to temperature, we made a specific focus on two well studied species from marine and freshwater: the *D. labrax* and the Nile tilapia *Oreochromis niloticus* L.. Other factors were considered in the analyses, such as habitat (freshwater vs. marine), life stage, sex or weight

2 | MATERIALS AND METHODS

2.1 | Literature search

Three different searches were conducted using all databases in Web of Science (Clarivate Analytics, PA, USA). In the first search, we focused on the basal cortisol level of the 250 most produced fish species according to statistics from FishStatJ over the period 2010-2019 (https://www.fao.org/fishery/en/statistics/software/fishstatj/en; consulted July 2021). The species list was then reduced to keep only scientific names. The genus was fully spelt out when only the first letter was provided. Groups of species were removed and crossings between species were separated in two different species. The resulting list of 161 species (**Text S1**) was used for a search in web of knowledge the 18th august 2021 performed with only one additional keyword: “basal cortisol” (using “AND” as connector). In this search, only 'basal' was used as a keyword for getting non-stressed cortisol values, while ‘baseline’ or ‘resting’ could also have been considered but this would have greatly broadened the search scope. The search resulted in a total of 291 publications (thereafter called the “all fishes” list).

To further investigate the pattern at the within-species level, we performed the literature search with an enlarged term (i.e., “cortisol" rather than “basal cortisol”) on two distinct species, *D. labrax* and *O. niloticus*. Keywords for these searches were:

- (“*Dicentrarchus labrax*” OR “European seabass” OR “European sea bass”) AND “cortisol”

- (“Nile tilapia” OR “*Oreochromis niloticus*”) AND “cortisol”

Searches were performed on the 12th July 2021 and the 6th October 2021 and resulted in 321 and 386 publications respectively (thereafter called the “only sea bass” and “only Nile tilapia” list respectively).

2.2 | Data management and PRISMA diagram

The three lists of publications were reviewed by a single co-author to reduce technical variability in results interpretations (SA for the “all species” list; CH for the “only sea bass” list; EB for the “only Nile tilapia” list). Each publication was first reviewed based on the abstract and all irrelevant studies were removed. After this initial trimming, the lists included 260, 81 and 147 studies, respectively (PRISMA Checklist; see **Figure S1**). For all lists, we then only kept studies for which mean basal cortisol was measured in the plasma and for which the temperature and the total number of individuals tested were provided. In addition, when two or more conditions were tested, we chose to keep the condition referred by the authors as the "Control" conditions (i.e., unstressed individuals) for extracting the basal cortisol measures. When authors did not clearly state the unstressed condition, we considered that unstressed individuals were reared in an environment that did not suddenly change within the last week and supported their basic needs (e.g., correct oxygenation, correct density, good feeding, no contamination). When some environmental and/or husbandry data (e.g., dissolved oxygen concentration, stocking density) were not provided in the paper, we considered that they were complying to the basic needs of the species, and fish were therefore classified as "unstressed individuals". We ensured that water temperatures were all within the thermal range of the studied species, following ranges provided in Dahlke *et al.* (2020). This screening led to a total of 126, 42 and 63 suitable studies for "all fishes", "only sea bass" and "only Nile tilapia" lists, resulting in datasets containing 398, 78 and 75 cortisol values respectively and associated temperature (**Supplementary material**). Note that four values above 1000 ng/ml were removed from the "only Nile tilapia" dataset, as they were considered as technical outliers (probable conversion error from authors which did not respond to our solicitations). These steps are illustrated in the PRISMA diagram (**Figure S1**). All collected data and R code are available in the **Supplementary material**.

2.3 | Life history traits and phylogeny

For each study, we collected (when available), the information on the sex of the tested-individuals (only males, only females or a mix of both), their life stage (adult or juvenile), and their mean body mass. A column was also added to the dataset to categorize species based on whether they live rather in fresh- or marine-water. For euryhaline fishes, this information was retrieved from the paper.

For the “all fishes” dataset, we created the phylogeny using the *rotl* package (Michonneau *et al.*, 2016), which provides an interface to the 'Open Tree of Life' (Hinchliff *et al.*, 2015), where all our species were available. The tree was pruned using the R package *picante* 1.6-2 (Kembel *et al.*, 2010). Branch length was obtained from the 'Open Tree of Life' providing ultrametric trees. The phylogenetic tree of the taxa included in the study is provided in **Figure S1**.

2.4 | Explaining basal cortisol production pattern according to temperature

The “all fishes” dataset was analysed in two ways. First, by considering the mean of basal cortisol at the mean temperature values of each species, across all studies. This allowed to test whether cortisol overall increases with the average temperature the species are usually reared at (between species analysis). Second, by using all raw data, with the aim of testing the effect of temperature within species (see 2.4.2). Results of this second analyses were confirmed by focusing on two species *D. labrax* “only sea bass” and *O. niloticus* “only Nile tilapia”.

2.4.1 | Between species

We fitted a linear model explaining the average log transformed cortisol values using average temperature values for each species with the MCMCglmm function (Markov Chain Monte Carlo generalized linear mixed model) (Hadfield, 2010). We set the number of iterations to 1,000,000, used an initial burn in value of 30,000 and a thinning interval of 100. We defined priors necessary for the Bayesian modelling with inverse-Wishart distribution for the variance structure using parameter settings for non-informative priors (expected variance, $V = 1$; degree of belief, $\nu = 0.02$). If the posterior mean was different from 0 (i.e., if the 95% credible intervals (CI) of the estimates of intercept and slope do not span zero), then the tested fixed effect was considered to have a significant effect on basal cortisol level.

2.4.2 | Within species

The same approach was conducted but using log-transformed basal cortisol values of each raw contained in the "all fishes" dataset. The observations were weighted by the inverse of the sample size. We first ran a model that included all fixed effects (temperature, habitat, weight, sex and life stage) as well as all random effects (phylogeny, studies and cortisol measurement methods). Note that the same inverse-Wishart distribution was used for defining non-informative priors (expected variance, $V = 1$; degree of belief, $\nu = 0.02$) for random factors. We then removed one fixed effect at a time and classified all models based on their Deviance Information Criterion (DIC) using the MuMIn package (Barton, 2019). Once the best model was found, we acted similarly for the random effects (**Table S1**). The mean weight of fish was not available in all studies (95 studies were lost, i.e., 75 %), and when added to the full model, it was not significant, so that we kept the main model with four fixed effects and three random factors, that was the most parsimonious regarding the DIC (**Table S1**). For the "only sea bass" dataset, all studies were conducted in marine water, and the sex was available only in 16 observations. We thus only conserved temperature, weight and life stage in the model. Regarding the "only Nile tilapia" dataset, all fish were tested in freshwater, and the sex was available in only 58 observations (out of 79). In addition, when performing model comparison, adding the sex as fixed effect in the model resulted in the worst model regarding DIC. Sex was thus discarded and only the temperature, weight and life stage were conserved in the model. The trace and distribution of all variables were checked visually, as well as the autocorrelation between iterations. Each model was run at least five times to check for the consistency of the results (including parameter estimates and DIC). To illustrate these effects, we used the outputs of the MCMCglmm models and plotted them with the ggplot2 package (Wickham *et al.*, 2022).

2.5 | Controlling for heterogeneity

We used MCMCglmm to partition the total heterogeneity I^2 among different sources: variation explained by study identity, cortisol measurement method, species relatedness (phylogeny) and by residual variation (Nakagawa & Santos, 2012). We calculated the degree of phylogenetic signal using the phylogenetic heritability index, H^2, which is the variance attributable to phylogeny in relation to the total variance linked to species expected in the data (Nakagawa & Santos, 2012). H^2 is equivalent to Pagel's lambda coefficient (Pagel, 1999), where values close to 1 are associated with strong phylogenetic signals as opposed to values close to 0. All data and R code are available in the Data.

3 | RESULTS

3.1 | Variation of basal cortisol level in plasma between fish species

Basal cortisol levels varied between the 33 fish species analysed. Lowest values were found for the Meagre *Argyrosomus regius* (Asso 1801) with a mean of 1.31 ± 0.53 ng/mL at an average temperature of 19.5 °C (**Figure 1, Table S2**). On the opposite, the highest basal plasma cortisol values were found for the Wuchang bream *Megalobrama amblycephala* (Yih 1955) with a mean of 332.41 ± 38.31 ng/mL at an average temperature of 26.6 °C (**Figure 1, Table S2**). The log-transformed mean plasma cortisol values for each species increased as a function of the mean water temperature (Posterior Mean: 0.13; 95% CI: [0.06, 0.20]; $p<0.001$; **Figure 2)**.

3.2 | Variation of basal cortisol level in plasma within fish species

All Fishes

Adding "species" as a random factor, did not improve the model in comparison to the one with the phylogenetic signal, but it was kept to calculate the H^2 (**Table S3**). The phylogenetic heritability index accounted for 20% (**Table S3**) of the total variance of the data, which was relatively weak. The residual variance was only 4%, indicating that most of the variance in the data was accounted for by the model. Regarding fixed effects, only temperature had a significant and positive effect on the basal cortisol level (**Figure 3a**, **Table 1**). This indicates that log-transformed basal cortisol level increased steadily with temperature within species, independently of their phylogenetic relatedness, the study and the cortisol measurement method. The sex, development stage and habitat had no effect on the basal plasma cortisol level (**Table S1**).

Dicentrarchus labrax

When considering *D. labrax*, there was an overall positive effect of both the weight and the temperature at which the fish were reared ($p<0.05$ for both; **Table 1**). Thus, fish reared at higher temperatures display higher level of plasma cortisol and larger individuals produced more cortisol relative to smaller ones (**Figure 3b**). It is worth noting that the best model (DIC= 163.85) did not include life stage.

Oreochromis niloticus

The best model was the one with weight only (DIC = 73.7), but since we were interested in the effect of temperature, we conserved it in the model (DIC = 76.8, **Table 1**). There was no significant effect of temperature on basal plasma cortisol level in *O. niloticus* ($p=0.11$) but only a significant effect of weight on basal cortisol level ($p<0.05$), so that larger individuals produced more cortisol relative to smaller ones (**Figure 3c**).

4 | DISCUSSION

By analysing the dataset including all 33 species, we detected that water temperature - within the species thermal tolerance range - explains a significant part of the variations in basal plasma cortisol level, within and between fish species. This pattern was less clear when focusing on the two case study species, in which the plasma cortisol level was found to significantly increase with temperature in *D. labrax*, while it did not in *O. niloticus*. Altogether our results confirm that, in unstressed individuals, the basal cortisol level follows the temperature effect, both within and between fish species. Weight was the only factor that influenced some of our results, with heavier fish showing higher basal plasma cortisol concentration within the two fish species focused on. It is worth mentioning that in the present study, some abiotic (e.g., salinity, dissolved oxygen concentration, pH) and husbandry (e.g., stocking density, life history) factors, that may have affected basal cortisol levels, were most of the time, not reported in original articles. Hence, they were not included in the formal analysis, which represent a caveat, further discussed below.

In this study, we also provide the mean (± SE) of the basal plasma cortisol level for 33 species of interest for both aquaculture and conservation purposes, as well as the variations expected due to temperature in each species (**Table S2**). This could serve as reference for researchers to check how consistent their measured values are with the literature. It is nevertheless worth mentioning that, although the plasma cortisol overall increases in fishes as function of the water temperature, some species display deviation from expected cortisol level based on temperature data. For instance, *A. regius* and Turbot *Scophthalmus maximus* L. displayed cortisol values well below the linear regression. At the opposite, values above the linear regression were observed for some species including the Caspian trout *Salmo caspius* (Kessler 1877), *D. labrax* and *M. amblycephala* (**Figure 2**). Whether this has ecological consequences remains to be described, but since cortisol influences energy allocations, even in a resting state (Sadoul & Vijayan, 2016), this might translate into differences in overall metabolism. Yet, inter-species differences in sensitivity to cortisol (e.g. through differences in corticoid receptors MR and GR quantity and affinity to cortisol) might modulate these ecological consequences. For instance, differences between species in the relative expression of MR and GR2 were previously reported (Sadoul *et al.*, 2018) with probable consequences on the amplitude of the response to cortisol.

Other environmental factors, probably related to the ecological niche of the species are likely to contribute to the residual variability in basal cortisol levels. We were able to test only the effect of marine vs. freshwater which did not capture any of the variability. As far as we know, no formal study ever investigated cortisol differences between marine and freshwater fish species. Fanouraki *et al.* (2011) briefly compared cortisol release rates between seven marine fish species and two freshwater fish species from selected studies, with a tendency for higher cortisol production in the latter compared to the former. Within euryhaline species, clear conclusions are difficult to draw because of cofounding factors. Indeed, changes in salinity encountered in the wild are often correlated to a change in multiple other water parameters (pH, oxygen concentration, etc..) but also often to specific life history events, such as migration or reproduction (Björnsson *et al.*, 2011; Baker *et al.*, 2013; Baker & Vynne, 2014; Vargas-Chacoff *et al.*, 2018). For instance, Vargas-Chacoff *et al.* (2018) investigated how elevated temperatures impact osmoregulation capacities and stress response of the anadromous Atlantic salmon *Salmo salar* L. depending on water (fresh vs. marine). Overall, plasma cortisol levels of *S. salar* were higher in Sea water than in freshwater, for a temperature ranging from 14-24 °C. In Gilthead seabream *Sparus aurata* L. there was a clear interaction between temperature and salinity on plasmatic cortisol levels. Indeed, when acclimated to 12 and 19 °C, cortisol production followed a "U" shape depending on salinity, while plasmatic cortisol levels were stable at 26 °C (Vargas-Chacoff *et al.*, 2020). Salinity (classified here as a categorical ordinal variable), should be better depicted in the future studies, to better highlight its effect on cortisol-temperature relationship. Also, in the papers reviewed, data regarding environmental parameters such as oxygen concentration, ammonia, CO_2 concentration, photoperiod or pH, as well as details regarding husbandry procedures, were (partially or totally) missing in most instances. It is worth mentioning that those factors are important for fish physiology, including cortisol release (especially when reaching extreme values) (Pickering & Pottinger, 1987; Kwong *et al.*, 2014; Sadoul *et al.*, 2017; Alfonso *et al.*, 2020a; Gesto *et al.*, 2020) but due to the lack of data, this prevented their use as explaining factors in the models used in the present study. One might wonder what would be the effects of these factors on the outcomes of the full model. The aim of making a formal meta-analysis is also to provide a sufficient number of studies and species to overcome any potential effects of confounding factors. Here, the residual variance of the random factors was very low (4%), meaning that most of the variance was explained by the chosen model. Hence, tiny effects related to husbandry procedures are negligible.

Factors related to the individual state, life stage and sex failed to significantly explain part of the basal cortisol variability between species. These are surprising results because basal cortisol levels and the response to stress were found to be both affected by life stage in fishes' literature. For instance, the whole-body cortisol remains stable at rest from 1 to 5 weeks post hatching while the cortisol level in response to stress increases over time in yellow Perch *Perca Flavescens* (Mitchill 1814) (Jentoft *et al.*, 2002). In Sockeye Salmon *Oncorhynchus nerka* (Walbaum 1792), the life stage (e.g., outward migration to marine waters, return migration to freshwater systems) has been proved to also affect the physiological features of individuals including circulating cortisol level (Baker & Vynne, 2014). A remarkable example on the interaction between life stages and basal cortisol has recently been provided by studying cortisol accumulated over years in the opercula of the yelloweye rockfish *Sebastes ruberrimus* (Cramer 1895) (Charapata *et al.*, 2022). By monitoring cortisol levels in females aged from 1 to 90 years, the authors showed that cortisol concentration was overall stable from 1-7 years, and suddenly increased between 10-11 years, which corresponds to the sexual maturity period (Mean ± SE, 11±1 years). At a smaller scale, cortisol concentration was also shown to vary daily or with seasonality in some fish species, also rending difficult the obtention of a clear pattern when analysing all fish species together (Pickering & Pottinger, 1983; Planas *et al.*, 1990; Fatira *et al.*, 2014). Also, the life history, especially early life, of individual (e.g. stress due to cortisol exposure, tank cleaning or water temperature variation) could affect the basal cortisol level in response to further stress events later in life (juvenile, adult stage) (Varsamos *et al.*, 2006; Auperin & Geslin, 2008; Robinson *et al.*, 2019). So, a potential effect of life stage on basal plasma cortisol level in our study could be masked by up- and down variations of cortisol level during fish life and/or differences in life histories that are mostly unknown in the individuals analysed in our meta-analysis (Baker *et al.*, 2013).

Sex is generally said to interact with basal cortisol levels in fishes but literature is sometimes contradictory on the direction of such relationship depending on species, context and/or life periods. For instance, in unstressed conditions, male and female zebrafish *Danio rerio* (Hamilton 1822) display similar basal cortisol levels while when exposed to unpredictable chronic stress, the cortisol level did increase in male but not in females, resulting in difference between sexes under stress (Rambo *et al.*, 2017). In the *O. nerka*, female display greater basal level of cortisol during the spawning period (Kubokawa *et al.*, 2001). In juveniles' *D. labrax* undergoing sex differentiation, no differences in basal cortisol were found between males and females (Goikoetxea *et al.*, 2022). Finally, it is worth mentioning that the sexual maturity is also an important factor affecting cortisol production within sex, e.g. mature female overall display a relatively higher concentration of plasma cortisol than non-mature females (Pickering & Christie, 1981). This could lead to difference in basal cortisol level. Therefore, sex-induced differences on cortisol level are likely species and context dependent in fishes (e.g., reproduction/spawning period, territorial defence, foraging, stress), explaining the absence of a general trend in our study.

The increase of basal circulating cortisol level as a function of temperature within fish species has been partially confirmed in our study when focusing on a marine species, *D. labrax*. This relationship was however not significant in the freshwater species we investigated, *O. niloticus*. This absence of link between temperature and cortisol in *O. niloticus*, need to be further investigated in order to verify (1) if this is a repeatable result for this specific species and others; (2) if this can be explained by the small temperature range investigated in this tropical fish, mostly between 20-30° C; (3) if this is the consequence of unaccounted variation due to unknown parameters maybe varying between studies (e.g., water quality, life history, handling procedures), or (4) simply the result of a sample size too small.

In our meta-analyses, we carefully removed cortisol values of stressed individuals to avoid biasing the outputs. As briefly discussed above, some information related to water parameters (e.g. nitrite/nitrate concentrations, pH, salinity) were however not always present in the papers reviewed, and deteriorated water could mask correlations between temperature and cortisol, by inducing stress in fishes (Pickering & Pottinger, 1987; Alfonso *et al.*, 2020a). This also concerns a range of experimental conditions known to affect cortisol levels, such as stocking density or blood sampling procedure since that information is sometimes lacking (e.g., time after handling, time of the day). The paucity of information on the effect of other abiotic parameters on cortisol level is a call for systematically report them in the studies. In addition, gathering data on more stenothermic fish (e.g., Artic/Antarctic species), would also help better understanding how temperature affect cortisol, because they -in some ways- display sharp specific adaptive physiological features to cope with extreme temperatures (Seebacher *et al.*, 2005; Hudson *et al.*, 2008).

Weight of individuals was found to affect basal plasma cortisol level in both *D. labrax* and *O. niloticus*, with larger fish displaying higher basal cortisol levels than smaller ones. Previous research investigated the relationship between cortisol and weight in studies performed in the South American catfish *Rhamdia quelen* (Quoy & Gaimard 1824) (Barcellos *et al.*, 2012; Koakoski *et al.*, 2012). The authors concluded that the weight did not directly affect basal plasma cortisol level, as well as the dynamic of the stress response, but it was rather the age of fish that did affect the cortisol release upon a stress event (Barcellos *et al.*, 2012; Charapata *et al.*, 2022). Younger fish respond faster to stress than older fish. This divergent response to stress according to age was hypothesized to be due to variations in the timing of maturation of the HPI axis or variations in the perception of stressor (Koakoski *et al.*, 2012). In our meta-analysis, it was impossible to test the effect of age on basal plasma cortisol level ‘per se’, since this data was not always mentioned in the studies reviewed, but the life stage did not affect the basal cortisol level in plasma, as discussed above. This is nevertheless consistent with the results of the studies carried out in *R. quelen*, where no effect of age was found on the basal cortisol levels (Barcellos *et al.*, 2012; Koakoski *et al.*, 2012). Furthermore, habituation to environment and/or stress is known to decrease the cortisol level, including cortisol release in fish (Barton *et al.*, 1986; Houslay *et al.*, 2019; Kenter *et al.*, 2021). We thus expected that larger fish (i.e., older and experienced fish regarding stress) would be less affected by environmental factors and respond less to stress than smaller (younger) fish. For instance, in *S. salar* even the dynamic of stress is similar, smaller fish (0.5 kg) appeared to display greater basal cortisol level compared to larger ones (3 kg) but no statistics were performed by the authors to confirm this trend (Hemre & Krogdahl, 1996). Results of our meta-analysis contradict the hypothesis that larger fish would display lower cortisol levels than smaller fish due to habituation to stress and environmental variations. Further research is needed to clarify all the biotic and abiotic factors that influence cortisol release in fishes.

5 | CONCLUSION

Overall, our study demonstrates that, in unstressed individuals, the basal cortisol levels are following temperature effects within and between fish species. At the intra-specific level, it is worth mentioning that this has been demonstrated in *D. labrax* but not in *O. niloticus*. Cortisol values should therefore always be compared between studies after accounting for this effect. Whether these differences have consequences on the known downstream physiological responses to cortisol is nevertheless unknown. Finally, further studies should include other environmental (e.g., salinity, dissolved oxygen concentration, pH), husbandry (e.g., stocking density, life history) or life (feeding type, habitat) factors to better underpin the role of temperature, as well as its interaction with other factors in basal cortisol production.

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CONTRIBUTION

S.A conceptualized the experiment and methodology, performed the literature review, wrote the original draft of the manuscript, and reviewed and edited the manuscript. C.H performed the literature review and reviewed and edited the manuscript. E.B performed the literature review and reviewed and edited the manuscript. B.S and B.G acquired the funding, conceptualized the experiment and methodology, supervised the literature review, analysed the data, and reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

All the data that support the findings are available in supplementary material.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Supporting material

Text S1. List of the 161 species used in the study.

S1 PRISMA Checklist. PRISMA flow diagram describing literature search, selection, and analyses. The orange rectangles narrow the numbers of studies per dataset prior to the analysis steps (purple rectangles) performed on the basal cortisol level within each study. Numbers are provided per dataset: red for “all fishes”; blue for “only sea bass”; and green for “only Nile tilapia”; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses (TIF)

Fig. S1. Phylogenetic tree of the 33 fish species used in the phylogenetic meta-analysis computed using the R package *rotl*. All data and R code supporting the figure are available in **S1 data**.

Table S1. Comparison of the models including final fixed factors selected (temperature, habitat, life stage and sex for “all fishes”; and temperature and weight for both “only sea bass” and “only Nile tilapia”. These models were compared based on their DIC using the MuMIn package (Barton, 2019), which also provides the weight of each model. DIC, Deviance Information Criterion. (XLSX)

Table S2. Number of values (n), basal cortisol level (ng/mL; mean ± SE) and temperature (°C; mean ± SE) of the 33 species investigated in the meta-analyses. (XLSX)

Table S3. Estimates of heterogeneity I2 (%) from the normalized-mean meta-analysis and the CV meta-analysis for the best model with all random factors for the three different datasets “All fishes”, “European sea bass” and “Nile tilapia”. The MLPMA allowed partitioning I2 among varying levels. Posterior mean and upper and lower 95% CIs for each estimate of I2 are provided. H2 is phylogenetic heritability, the proportion of variability in the data attributable to the phylogenetic component of MLPMA calculated following Nakagawa and Santos, 2012. CI, credible interval; CV, coefficient of variation; MLPMA, multilevel phylogenetic meta-analysis. (XLSX)

S1 Data. All data for the meta-analysis as well as the R code to run the different models. (ZIP)

REFERENCES

Alfonso, S., Sadoul, B., Cousin, X., & Bégout, M.-L. (2020a). Spatial Distribution and

Activity Patterns as Welfare Indicators in Response to Water Quality Changes in European Sea Bass, Dicentrarchus Labrax. *Applied Animal Behaviour Science*, 104974.

Alfonso, S., Zupa, W., Manfrin, A., Fiocchi, E., Spedicato, M. T., Lembo, G., & Carbonara, P. (2020b). Stress Coping Styles: Is the Basal Level of Stress Physiological Indicators Linked to Behaviour of Sea Bream? *Applied Animal Behaviour Science*, *231*, 105085.

Alfonso, S., Gesto, M., & Sadoul, B. (2021). Temperature Increase and Its Effects on Fish Stress Physiology in the Context of Global Warming. *Journal of Fish Biology*, *98*, 1496–1508.

Auperin, B., & Geslin, M. (2008). Plasma Cortisol Response to Stress in Juvenile Rainbow Trout Is Influenced by Their Life History during Early Development and by Egg Cortisol Content. *General and Comparative Endocrinology*, *158*, 234–239.

Baker, M. R., & Vynne, C. H. (2014). Cortisol Profiles in Sockeye Salmon: Sample Bias and Baseline Values at Migration, Maturation, Spawning, and Senescence. *Fisheries Research*, *154*, 38–43.

Baker, M. R., Gobush, K. S., & Vynne, C. H. (2013). Review of Factors Influencing Stress Hormones in Fish and Wildlife. *Journal for Nature Conservation*, *21*, 309–318.

Barcellos, L. J. G., Kreutz, L. C., Koakoski, G., Oliveira, T. A., da Rosa, J. G. S., & Fagundes, M. (2012). Fish Age, Instead of Weight and Size, as a Determining Factor for Time Course Differences in Cortisol Response to Stress. *Physiology and Behavior*, *107*, 397–400.

Barton, B. A. (2002). Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. *Integrative and Comparative Biology*, *42*, 517–525.

Barton, B., Schreck, C., & Barton, L. (1986). Effects of Chronic Cortisol Administration and Daily Acute Stress on Growth, Physiological Conditions, and Stress Responses in Juvenile Rainbow Trout. *Diseases of Aquatic Organisms*, *2*, 173–185.

Barton, K. (2019). Package ‘ MuMIn ’. *R cran*.

Bessa, E., Sadoul, B., Mckenzie, D. J., & Geffroy, B. (2021). Group Size, Temperature and Body Size Modulate the Effects of Social Hierarchy on Basal Cortisol Levels in Fishes. *Hormones and Behavior*, *136*, 105077.

Björnsson, B. T., Stefansson, S. O., & McCormick, S. D. (2011). Environmental Endocrinology of Salmon Smoltification. *General and Comparative Endocrinology*, *170*, 290–298.

Bordin, D., & Freire, C. A. (2021). Remarkable Variability in Stress Responses among Subtropical Coastal Marine Teleosts. *Marine Biology*, *168*, 1–17.

Castanheira, M. F., Conceição, L. E. C., Millot, S., Rey, S., Bégout, M. L., Damsgård, B., … Martins, C. I. M. (2017). Coping Styles in Farmed Fish: Consequences for Aquaculture. *Reviews in Aquaculture*. 2017, 23–41, doi:10.1111/raq.12100.

Chadwick, J. G., & McCormick, S. D. (2017). Upper Thermal Limits of Growth in Brook Trout and Their Relationship to Stress Physiology. *Journal of Experimental Biology*, *220*, 3976–3987.

Chadwick, J. G., Nislow, K. H., & McCormick, S. D. (2015). Thermal Onset of Cellular and Endocrine Stress Responses Correspond to Ecological Limits in Brook Trout, an Iconic Cold-Water Fish. *Conservation Physiology*, *3*, 1–12.

Charapata, P., Oxman, D., McNeel, K., Keith, A., Mansouri, F., & Trumble, S. (2022). Lifetime Hormone Profiles for a Long-Lived Teleost: Opercula Reveal Novel Estimates of Age-Specific Reproductive Parameters and Stress Trends in Yelloweye Rockfish (Sebastes Ruberrimus) . *Canadian Journal of Fisheries and Aquatic Sciences*, *1728*, 1712–1728.

Chatziplis, D., Oikonomlou, S., Loukovitis, D., Tsiokos, D., Samaras, A., Dimitroglou, A., … Pavlidis, M. (2020). QTL for Stress and Disease Resistance in European Sea Bass, Dicentrarhus Labrax L. *Animals*, *10*, 1668.

Claireaux, G., & Lagardère, J. P. (1999). Influence of Temperature, Oxygen and Salinity on the Metabolism of the European Sea Bass. *Journal of Sea Research*, *42*, 157–168.

Clarke, A., & Johnston, N. M. (1999). Scaling of Metabolic Rate with Body Mass and Temperature in Teleost Fish. *Journal of Animal Ecology*, *68*, 893–905.

Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H. O. (2020). Thermal Bottlenecks in the Life Cycle Define Climate Vulnerability of Fish. *Science*, *369*, 65–70.

Fanouraki, E., Mylonas, C. C., Papandroulakis, N., & Pavlidis, M. (2011). Species Specificity in the Magnitude and Duration of the Acute Stress Response in Mediterranean Marine Fish in Culture. *General and Comparative Endocrinology*, *173*, 313–322.

Fatira, E., Papandroulakis, N., & Pavlidis, M. (2014). Diel Changes in Plasma Cortisol and Effects of Size and Stress Duration on the Cortisol Response in European Sea Bass (Dicentrarchus Labrax). *Fish Physiology and Biochemistry*, *40*, 911–919.

Fevolden, S. E., Røed, K. H., Fjalestad, K. T., & Stien, J. (1999). Poststress Levels of Lysozyme and Cortisol in Adult Rainbow Trout: Heritabilities and Genetic Correlations. *Journal of Fish Biology*, *54*, 900–910.

Gesto, M., Zupa, W., Alfonso, S., Spedicato, M. T., Lembo, G., & Carbonara, P. (2020). Using Acoustic Telemetry to Assess Behavioral Responses to Acute Hypoxia and

Ammonia Exposure in Farmed Rainbow Trout of Different Competitive Ability. *Applied Animal Behaviour Science*, *230*, 105084.

Goikoetxea, A., Servili, A., Houdelet, C., Mouchel, O., Hermet, S., Clota, F., … Benjamin, B. (2022). Natural Cortisol Production Is Not Linked to the Sexual Fate of European Sea Bass. *Fish Physiology and Biochemistry*, *48*, 1117–1135.

Goikoetxea, A., Sadoul, B., Blondeau-Bidet, E., Aerts, J., Blanc, M. O., Parrinello, H., … Geffroy, B. (2021). Genetic Pathways Underpinning Hormonal Stress Responses in Fish Exposed to Short- and Long-Term Warm Ocean Temperatures. *Ecological Indicators*, *120*, 106937.

Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software*, *33*, 1–22.

Hemre, G. I., & Krogdahl, Å. (1996). Effect of Handling and Fish Size on Secondary Changes in Carbohydrate Metabolism in Atlantic Salmon, Salmo Salar L. *Aquaculture Nutrition*, *2*, 249–252.

Hinchliff, C. E., Smith, S. A., Allman, J. F., Burleigh, J. G., Chaudhary, R., Coghill, L. M., … Cranston, K. A. (2015). Synthesis of Phylogeny and Taxonomy into a Comprehensive Tree of Life. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 12764–12769.

Houslay, T. M., Earley, R. L., Young, A. J., & Wilson, A. J. (2019). Habituation and Individual Variation in the Endocrine Stress Response in the Trinidadian Guppy (Poecilia Reticulata). *General and Comparative Endocrinology*, *270*, 113–122.

Hudson, H. A., Brauer, P. R., Scofield, M. A., & Petzel, D. H. (2008). Effects of Warm Acclimation on Serum Osmolality, Cortisol and Hematocrit Levels in the Antarctic Fish, Trematomus Bernacchii. *Polar Biology*, *31*, 991–997.

Jentoft, S., Held, J. A., Malison, J. A., & Barry, T. P. (2002). Ontogeny of the Cortisol Stress Response in Yellow Perch (Perca Flavescens). *Fish Physiology and Biochemistry*, *26*, 371–378.

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., … Webb, C. O. (2010). Picante: R Tools for Integrating Phylogenies and Ecology. *Bioinformatics*, *26*, 1463–1464.

Kenter, L. W., Breton, T. S., & Berlinsky, D. L. (2021). Comparing Stress Responses of F1 and Domesticated Striped Bass (Morone Saxatilis) Following a Repeated Acute Stressor. *Aquaculture Research*, *52*, 4786–4798.

Khan, U. W., Øverli, Ø., Hinkle, P. M., Pasha, F. A., Johansen, I. B., Berget, I., … Vage, D. I. (2016). A Novel Role for Pigment Genes in the Stress Response in Rainbow Trout (Oncorhynchus Mykiss). *Scientific Reports*, *6*, 28969.

Koakoski, G., Oliveira, T. A., da Rosa, J. G. S., Fagundes, M., Kreutz, L. C., & Barcellos, L. J. G. (2012). Divergent Time Course of Cortisol Response to Stress in Fish of Different Ages. *Physiology and Behavior*, *106*, 129–132.

Kubokawa, K., Yoshioka, M., & Iwata, M. (2001). Sex-Specific Cortisol and Sex Steroids Responses in Stressed Sockeye Salmon during Spawning Period. *Zoological Science*, *18*, 947–954.

Kwong, R. W. M., Kumai, Y., & Perry, S. F. (2014). The Physiology of Fish at Low PH: The Zebrafish as a Model System. *Journal of Experimental Biology*, *217*, 651–662.

McKenzie, D. J., Geffroy, B., & Farrell, A. P. (2021). Effects of Global Warming on Fishes and Fisheries. *Journal of Fish Biology*, *98*, 1489–1492.

Michonneau, F., Brown, J. W., & Winter, D. J. (2016). Rotl: An R Package to Interact with the Open Tree of Life Data. *Methods in Ecology and Evolution*, *7*, 1476–1481.

Mommsen, T., Vijayan, M., & Moon, T. (1999). Cortisol in Teleosts: Dynamics, Mechanism of Action, and Metabolic Regulation. *Reviews in Fish Biology and Fisheries*, *9*, 211–268.

Nakagawa, S., & Santos, E. S. A. (2012). Methodological Issues and Advances in Biological Meta-Analysis. *Evolutionary Ecology*, *26*, 1253–1274.

Pagel, M. (1999). Inferring the Historical Patterns of Biological Evolution. *Nature*, *401*, 877–884.

Pickering, A. D., & Christie, P. (1981). Changes in the Concentrations of Plasma Cortisol and Thyroxine during Sexual Maturation of the Hatchery-Reared Brown Trout, Salmo Trutta L. *General and Comparative Endocrinology*, *44*, 487–496.

Pickering, A. D., & Pottinger, T. G. (1983). Seasonal and Diel Changes in Plasma Cortisol Levels of the Brown Trout, Salmo Trutta L. *General and Comparative Endocrinology*, *49*, 232–239.

Pickering, A. D., & Pottinger, T. G. (1987). Poor Water Quality Suppresses the Cortisol Response of Salmonid Fish to Handling and Confinement. *Journal of Fish Biology*, *30*, 363–374.

Planas, J., Gutierrez, J., Fernandez, J., Carrillo, M., & Canals, P. (1990). Annual and Daily Variations of Plasma Cortisol in Sea Bass, *Dicentrarchus Labrax*L. *Aquaculture*, *91*, 171–178.

Pottinger, T. G., & Carrick, T. R. (1999). Modification of the Plasma Cortisol Response to Stress in Rainbow Trout by Selective Breeding. *General and Comparative*

Endocrinology, *116*, 122–132.

Rambo, C. L., Mocelin, R., Marcon, M., Villanova, D., Koakoski, G., de Abreu, M. S., … Bonan, C. D. (2017). Gender Differences in Aggression and Cortisol Levels in Zebrafish Subjected to Unpredictable Chronic Stress. *Physiology and Behavior*, *171*, 50–54.

Robinson, N. A., Johnsen, H., Moghadam, H., Andersen, Ø., & Tveiten, H. (2019). Early Developmental Stress Affects Subsequent Gene Expression Response to an Acute Stress in Atlantic Salmon: An Approach for Creating Robust Fish for Aquaculture? *G3: Genes, Genomes, Genetics*, *9*, 1597–1611.

Sadoul, B., Alfonso, S., Bessa, E., Bouchareb, A., Blondeau-Bidet, E., Clair, P., … Geffroy, B. (2018). Enhanced Brain Expression of Genes Related to Cell Proliferation and Neural Differentiation Is Associated with Cortisol Receptor Expression in Fishes. *General and Comparative Endocrinology*, *267*, 76–81.

Sadoul, B., Friggens, N. C., Valotaire, C., Labbé, L., Colson, V., Prunet, P., & Leguen, I. (2017). Physiological and Behavioral Flexibility to an Acute CO 2 Challenge, within and between Genotypes in Rainbow Trout. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*, *209*, 25–33.

Sadoul, B., & Vijayan, M. M. (2016). Stress and Growth. In *Fish Physiology* pp. 167–205 Elsevier Inc.

Samaras, A., Santo, C. E., Papandroulakis, N., Mitrizakis, N., Pavlidis, M., Höglund, E., … Gorissen, M. (2018). Allostatic Load and Stress Physiology in European Seabass (Dicentrarchus Labrax L.) and Gilthead Seabream (Sparus Aurata L.). *Frontiers in Endocrinology*, *9*, 1–13.

Samaras, A., & Pavlidis, M. (2018). Regulation of Divergent Cortisol Responsiveness in

European Sea Bass, Dicentrarchus Labrax L. *PLoS ONE*, *13*, 1–13.

Samaras, A., Papandroulakis, N., Costari, M., & Pavlidis, M. (2016). Stress and Metabolic Indicators in a Relatively High (European Sea Bass, Dicentrarchus Labrax) and a Low (Meagre, Argyrosomus Regius) Cortisol Responsive Species, in Different Water Temperatures. *Aquaculture Research*, *47*, 3501–3515.

Schreck, C. B., & Tort, L. (2016). The Concept of Stress in Fish. In *Fish physiology* pp. 1–34 Elsevier.

Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology*, *51*, 691–702.

Seebacher, F., Davison, W., Lowe, C. J., & Franklin, C. E. (2005). A Falsification of the Thermal Specialization Paradigm: Compensation for Elevated Temperatures in Antarctic Fishes. *Biology Letters*, *1*, 151–154.

Somero, G. N. (2004). Adaptation of Enzymes to Temperature: Searching for Basic 'Strategies'. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, *139*, 321–333.

Sumpter, J. P. (1997). The Endocrinology of Stress. *Fish stress and health in aquaculture*, *819*, 95–118.

Tort, L. (2011). Stress and Immune Modulation in Fish. *Developmental and Comparative Immunology*, *35*, 1366–1375.

Tudorache, C., Slabbekoorn, H., Robbers, Y., Hin, E., Meijer, J. H., Spaink, H. P., & Schaaf, M. J. M. (2018). Biological Clock Function Is Linked to Proactive and Reactive Personality Types. *BMC Biology*, *16*, 1–13.

Vandeputte, M., Porte, J. D., Auperin, B., Dupont-Nivet, M., Vergnet, A., Valotaire, C., … Chatain, B. (2016). Quantitative Genetic Variation for Post-Stress Cortisol and Swimming Performance in Growth-Selected and Control Populations of European Sea Bass (Dicentrarchus Labrax). *Aquaculture*, *455*, 1–7.

Vargas-Chacoff, L., Arjona, F. J., Ruiz-Jarabo, I., García-Lopez, A., Flik, G., & Mancera, J. M. (2020). Water Temperature Affects Osmoregulatory Responses in Gilthead Sea Bream (Sparus Aurata L.). *Journal of Thermal Biology*, *88*, 102526.

Vargas-Chacoff, L., Regish, A. M., Weinstock, A., & McCormick, S. D. (2018). Effects of Elevated Temperature on Osmoregulation and Stress Responses in Atlantic Salmon Salmo Salar Smolts in Fresh Water and Seawater. *Journal of Fish Biology*, *93*, 550–559.

Varsamos, S., Flik, G., Pepin, J. F., Bonga, S. E. W., & Breuil, G. (2006). Husbandry Stress during Early Life Stages Affects the Stress Response and Health Status of Juvenile Sea Bass, Dicentrarchus Labrax. *Fish & shellfish immunology*, *20*, 83–96.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., … Dunnington, D. (2022). Package ‘Ggplot2’ Create Elegant Data Visualisations Using the Grammar of Graphics.

Tables:

Table 1. Outputs of the MCMCglmm models Model1a, 1b and 1c for the “all fishes”, “only sea bass” (*Dicentrarchus labrax*) and “only Nile tilapia” (*Oreochromis niloticus*) datasets respectively. Estimates with 95% CIs not spanning zero are considered statistically significant (significance codes: ***: <0.001 ;**:0.01; *: 0.05).

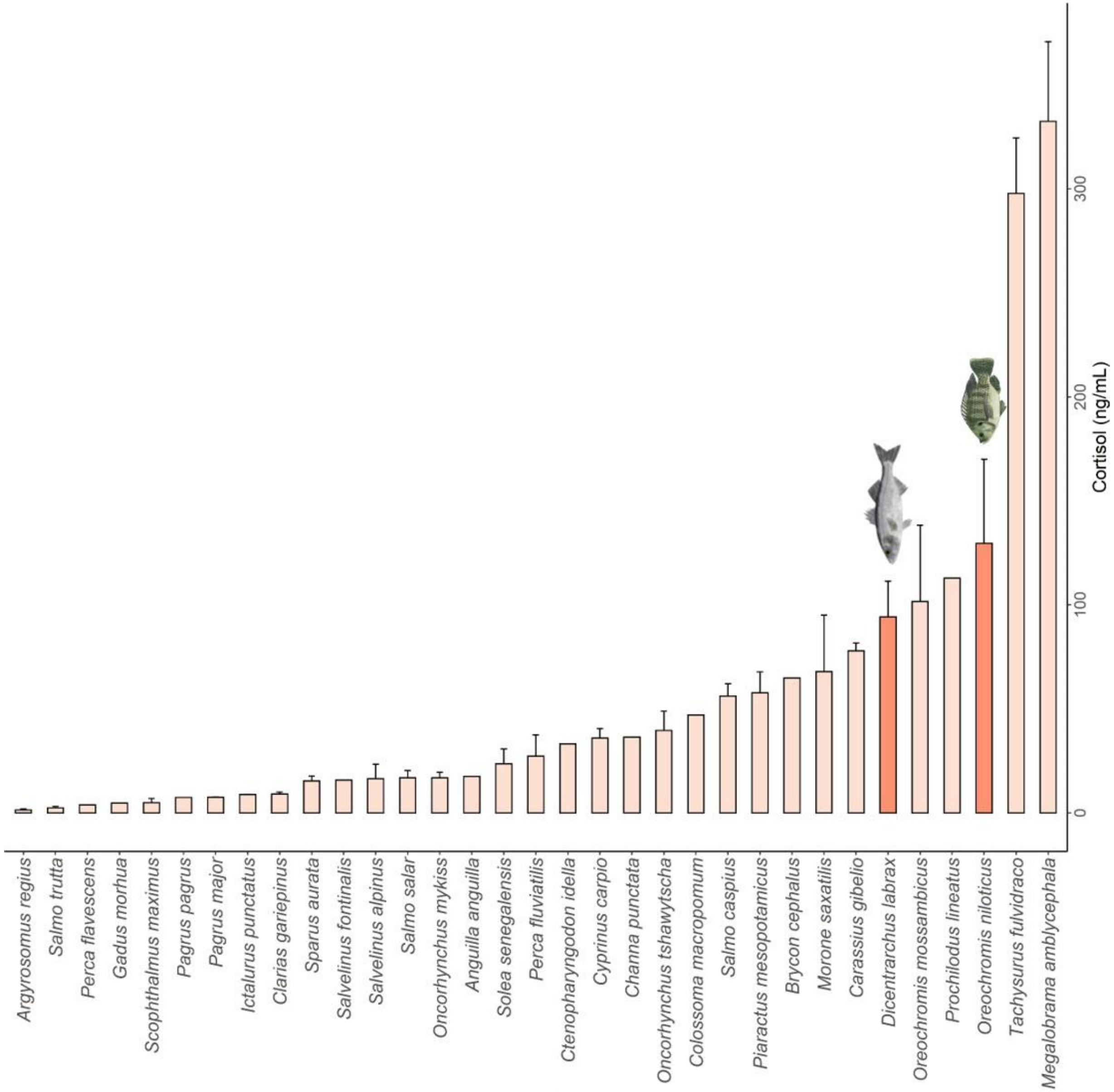
	Fixed effects	Random	**Posterior Mean**	**l-95% CI**	**u-95% CI**	**pMCMC**
All Fishes	*Intercept*	model 1a	1.47630	-1.07452	4.22035	0.258
	Temperature	model 1a	0.08029	0.04500	0.11430	<0.001***
	Environment : Freshwater vs Marine	model 1a	0.07021	-0.56046	0.70388	0.824
	Life Stage : Adult vs Juvenile	model 1a	-0.42311	-0.94782	0.09581	0.107
	Sex : Female vs Male	model 1a	0.86648	-0.27801	2.05127	0.140
	Sex : Female vs Mixed	model 1a	0.61157	-0.31673	1.53280	0.197
Only Sea Bass	*Intercept*	model 1b	2.7486975	1.6068333	3.8533550	<0.01**
	Temperature	model 1b	0.0384466	0.0017255	0.0736820	<0.05*
	Weight	model 1b	0.0021725	0.0003787	0.0039472	<0.05*
Only Nile tilapia	*Intercept*	model 1c	1.4856444	-0.6394159	3.6902818	0.1769
	Temperature	model 1c	0.0640836	-0.0112230	0.1506045	0.1153
	Weight	model 1c	0.0022322	0.0003322	0.0041366	<0.05*

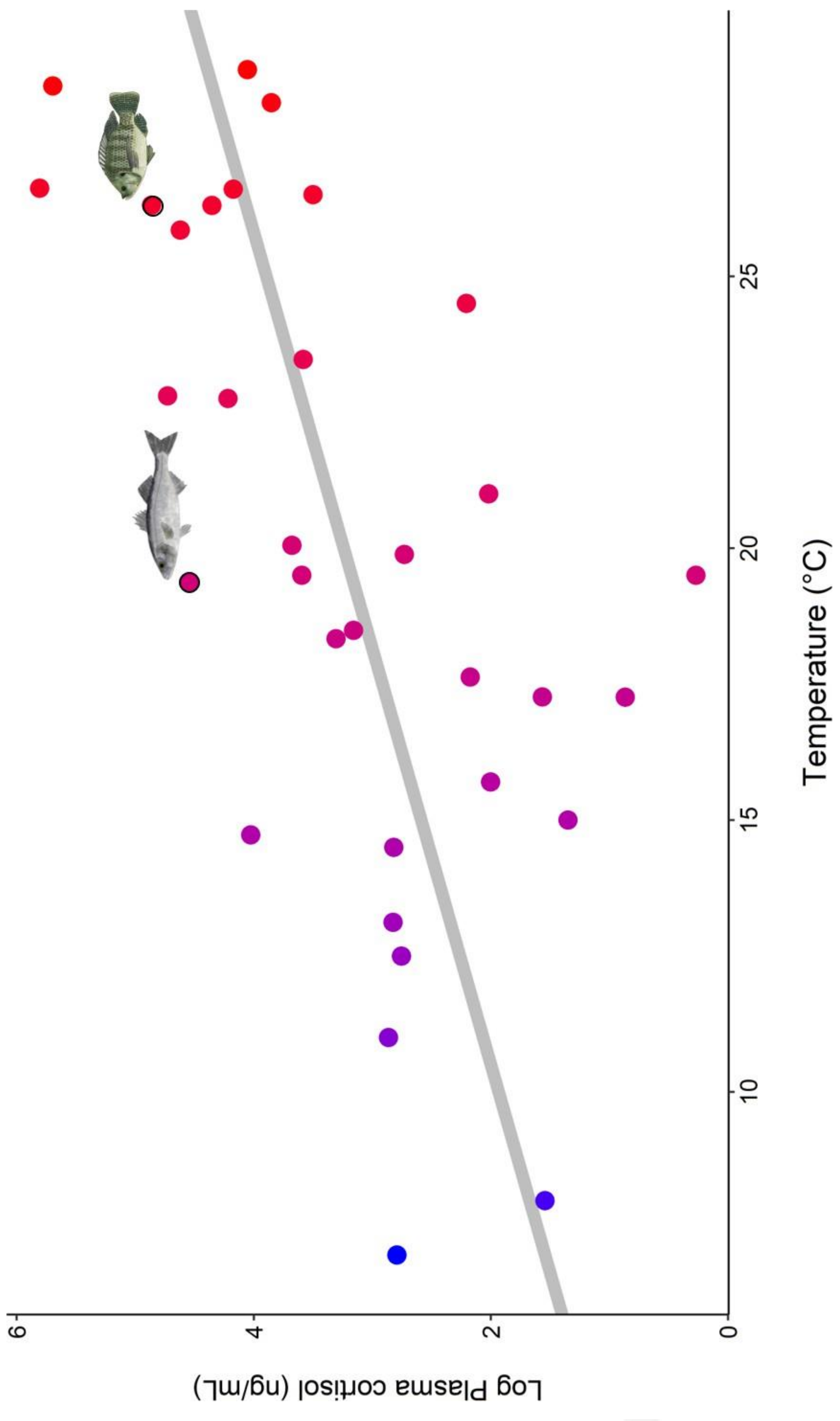
Figure legends:

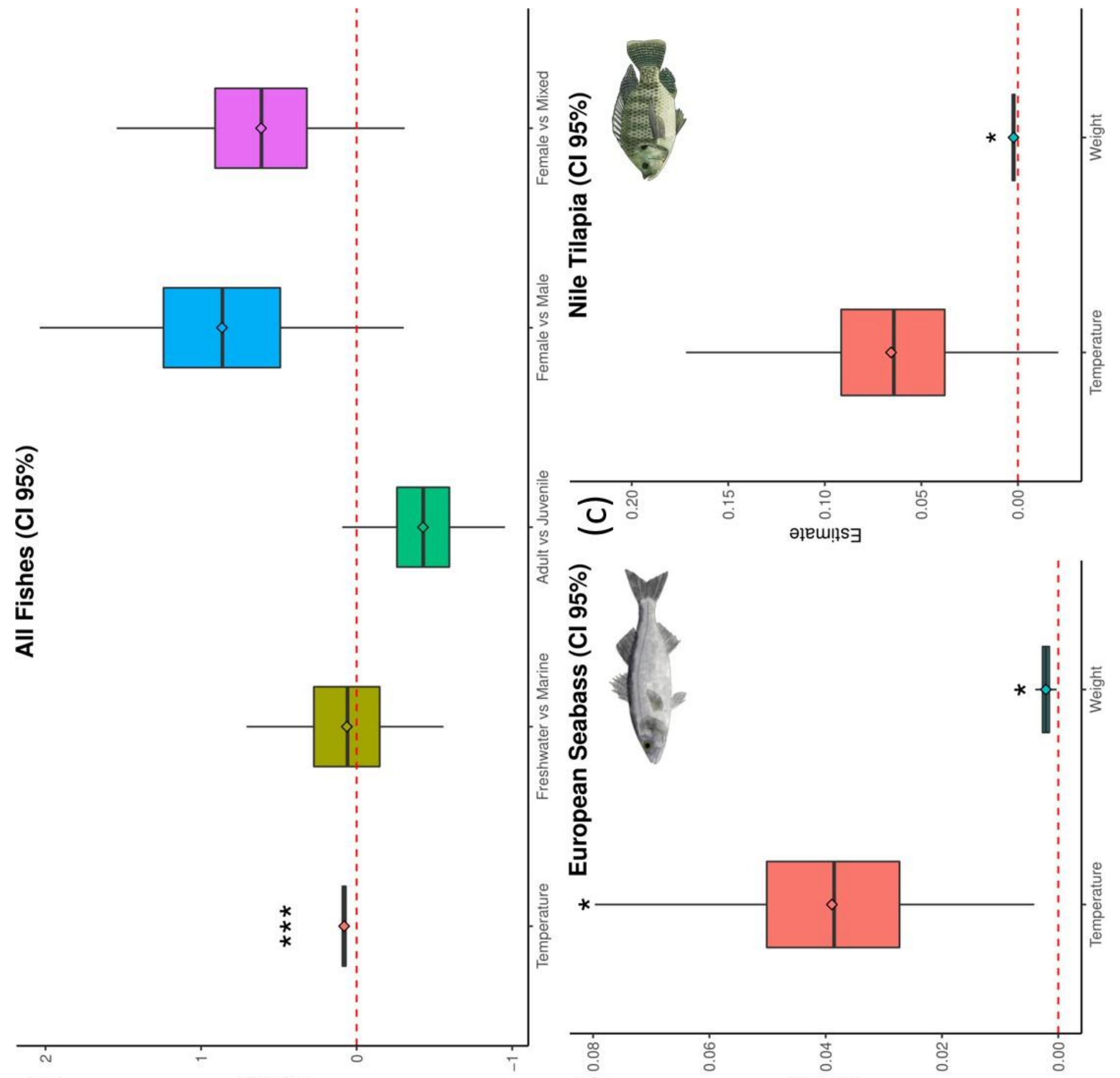
Fig 1. Basal cortisol level of the 33 species investigated in the study (ng/mL; mean ± SE). Red bars indicate the two species for which we made a focus to analyse intra-species effects of temperature on cortisol level (*Dicentrarchus labrax* and *Oreochromis niloticus*).

Fig 2. Mean log plasma cortisol (ng/mL) in relation to temperature (°C) of the 33 species investigated. Each dot represents one value and the grey line represents the linear regression between the two variables estimated by the MCMCglmm model (intercept=0.65 and slope=0.13). A colour gradient from blue to red was added to illustrate the temperature experienced by each fish. Black circle surrounding the dots are referring to the two species for which we made a focus to analyse intra-species effects of temperature on cortisol level (*Dicentrarchus labrax* and *Oreochromis niloticus*).

Fig 3. Boxplots of the estimates for the fixed factors computed using MCMCglmm models for **(a)** Model1a, **(b)** 1b and **(c)** 1c (**Table 1**) for the "all fishes", "only sea bass" (*Dicentrarchus labrax*) and "Only Nile tilapia" (*Oreochromis niloticus*) datasets respectively. The central line of the boxplot indicates the estimates of the model and the boxes the quartiles, with the whiskers covering 95% of the values. Estimates with 95% CIs not spanning zero are considered statistically significant (significance codes: ***: <0.001; *: < 0.05).


Cortisol (ng/mL)
0
100
200
300
Argyrosomus regius
Salmo trutta
Perca flavescens
Gadus morhua
Scophthalmus maximus
Pagrus pagrus
Pagrus major
Ictalurus punctatus
Clarias gariepinus
Sparus aurata
Salvelinus fontinalis
Salvelinus alpinus
Salmo salar
Oncorhynchus mykiss
Anguilla anguilla
Solea senegalensis
Perca fluviatilis
Ctenopharyngodon idella
Cyprinus carpio
Channa punctata
Oncorhynchus tshawytscha
Colossoma macropomum
Salmo caspius
Piaractus mesopotamicus
Brycon cephalus
Morone saxatilis
Carassius gibelio
Dicentrarchus labrax
Oreochromis mossambicus
Prochilodus lineatus
Oreochromis niloticus
Tachysurus fulvidraco
Megalobrama amblycephala


Log Plasma cortisol (ng/mL)
0
2
4
6
Temperature (°C)
10
15
20
25


(a)
All Fishes (CI 95%)
Estimate
2
1
0
−1
***
Temperature
Freshwater vs Marine
Adult vs Juvenile
Female vs Male
Female vs Mixed
(b)
European Seabass (CI 95%)
Estimate
0.08
0.06
0.04
0.02
0.00
*
*
Temperature
Weight
(c)
Nile Tilapia (CI 95%)
Estimate
0.20
0.15
0.10
0.05
0.00
*
Temperature
Weight