Fisheries Research 266 (2023) 106776

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Relationships of scale cortisol content suggest stress resilience in freshwater fish vulnerable to catch-and-release angling in recreational fisheries☆

J.R. Britton [a], [*], D. Andreou [a], M. Lopez-Bejar [b], [c], A. Carbajal [b]

[a] Fish Ecology & Conservation Cluster, Bournemouth University, Poole, Dorset BH12 5BB, UK
[b] Department of Animal Health and Anatomy, Veterinary Faculty, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain
[c] College of Veterinary Medicine, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766, USA

ARTICLE INFO

Handled by B. Morales-Nin

Keywords:
Stable isotope analysis
Fishery
Angling baits
Angling induced selection

ABSTRACT

The capture by angling of an individual fish is recognised as a short-term physiological stressor. In fish populations exploited by catch and release angling (C&R), there is potential for some individual fishes to be captured on multiple occasions, but the longer term physiological consequences of this remain uncertain. Using scale cortisol content as a biomarker of chronic stress and scale samples from two fish populations exploited by C&R angling, we developed proxies of angling capture vulnerability before testing these proxies against scale cortisol content. In a riverine population of European barbel *Barbus barbus*, fish with the highest scale cortisol content were predicted as those sampled by angling rather than electric fishing, as angled fish had significantly smaller home ranges and diets based primarily on angling baits. In a population of common carp *Cyprinus carpio* in a small pond fishery, we predicted that fish with the highest scale cortisol content would be those with higher proportions of angling bait in their diet. In both species, however, the fish predicted to be most vulnerable to angling capture had the lowest levels of scale cortisol content. We suggest that this is through fish that are captured regularly being highly stress resilient (with this independent of other traits) or fish with traits that suggest high capture vulnerability being able to minimise their recapture rates through developing hook avoidance behaviours after an initial capture. Overall, these results suggest that scale cortisol content is a useful biomarker for measuring chronic stress from C&R angling.

1. Introduction

Global estimates of the number of recreational anglers vary, but suggestions are of up to 700 million recreational anglers in the world who capture 12% of the global fish harvest, primarily from freshwaters and inshore areas (Arlinghaus and Cooke, 2009). Harvesting by angling can result in exploited populations comprising of low-activity, highly stress-responsive phenotypes due to ‘angling induced selection’ for specific traits, with the most vulnerable individuals to capture often being active phenotypes of high stress resistance (Koeck et al., 2019; Monk et al., 2021). Catch and release angling (C&R) is increasingly being adopted in many world regions as it minimises angling impacts on target populations as captured fish are returned alive, so maintaining phenotypic diversity and providing conservation benefits in exploited populations (Arlinghaus et al., 2007).

In populations exploited by C&R, the capture vulnerability of individuals is also non-random, with some individuals rarely being captured, but with others re-captured on a regular basis (Lennox et al., 2017). For example, across 46 tagged European catfish *Silurus glanis* in a pond fishery, 30 individuals went uncaptured across an entire year, but 8 individuals were captured between 10 and 26 times, with some being recaptured on successive days (Britton et al., 2007). Differences in the capture vulnerability of individuals is associated with intra-population variability in behavioural traits, although the phenotypes most vulnerable to C&R can be species-specific (Alos et al., 2012; Lennox et al., 2017) and, in some species, behaviours such as boldness are not a good predictor of capture vulnerability (Vainikka et al., 2016).

The capture by angling of an individual fish is considered as a short-term physiologically stressful event, with the probability of sub-lethal effects occurring being influenced by a range of individual and interacting variables, including fish size, hook damage extent, fight time, air exposure, general fish handling, and environmental conditions

---

☆ Please note this manuscript is available as a pre-print at BioRxiv (https://doi.org/10.1101/2022.11.15.516354).

* Corresponding author.
E-mail address: rbritton@bournemouth.ac.uk (J.R. Britton).

https://doi.org/10.1016/j.fishres.2023.106776
Received 17 November 2022; Received in revised form 4 June 2023; Accepted 8 June 2023

(Muoneke and Childress, 1994; Cooke and Suski, 2004; Pinder et al., 2019). Stress responses to single capture events have been detected through changes to blood chemistry (e.g. Cooke et al., 2013) and the application of simple reflex action impairment indicators as a 'whole-body' stress response (e.g. Pinder et al., 2019). However, the longer-term physiological effects (i.e. chronic stress effects) of an individual fish being repeatedly captured by C&R angling remain unclear, especially in large, open systems where knowledge on previous capture events of an individual fish might be unknown. In these situations, proxies of individual vulnerability to angling capture could be used to infer capture history. For example, it could be assumed capture vulnerability will be increased in individual fish that have diets more heavily subsidised by angling baits (increasing their probability of encountering a baited hook) (Britton et al., 2022) and/or that have smaller home ranges (increasing their spatial encounters with anglers) (Gutmann Roberts et al., 2019).

The level of cortisol in an individual fish is increased when they are exposed a stressor (Carbajal et al., 2018, 2019a; 2019b). Circulating cortisol levels in fish correlate strongly with scale cortisol content, but with rates of accumulation and clearance in scales being much slower than for blood plasma (Laberge et al., 2019). Although exposing individual goldfish *Carassius auratus* to a single acute air emersion stressor did not influence their scale cortisol content, high and sustained circulating cortisol levels produced from unpredictable chronic stressors did increase it, with this content being homogenous in scales across the whole body surface (Laberge et al., 2019). Carbajal et al., (2018, 2019a, 2019b); revealed that scale cortisol levels are influenced by energetically intense periods of intermediate duration (e.g. up to 30 days) rather than long-term stressors (e.g. exposure of several months). Thus, scales can provide retrospective stress hormone measurements from fishes and across time periods that are usually difficult or impossible to obtain. Scales have recently been applied to provide a retrospective measure of past stress experience in fishes as diverse as rainbow trout *Oncorhynchus mykiss* (Carbajal et al., 2019a), goldfish *Carassius auratus* (Carbajal et al., 2018; Laberge et al., 2019), Catalan chub *Squalius laietanus* (Carbajal et al., 2019b), sea bass *Dicentrarchus labrax* (Lebigre et al., 2022), and dab *Limanda limanda* (Vercauteren et al., 2022). These studies all suggest that fish of relatively low scale cortisol content have higher resilience to chronic stress than those with relatively high content.

The aim here was to apply scale cortisol levels as a biomarker of chronic stress to fish exposed to C&R angling in recreational fisheries. This biomarker was applied to two fish populations exploited by C&R angling, European barbel *Barbus barbus* ('barbel') in a riverine fishery and common carp *Cyprinus carpio* ('carp') in a pond fishery. As the previous capture history of these fishes were unavailable, the initial objective was to develop proxies of vulnerability to angling capture. For barbel, these proxies were based on the sampling method, dietary reliance on angling bait (from stable isotope analysis, SIA), and home range size (from acoustic telemetry). For carp, the proxies were based only on their dietary reliance on angling bait (from SIA). The second objective was to then test these proxies against the corresponding scale cortisol levels. We posit that fish with proxies that suggest high vulnerability to angling (re)capture will have significantly higher scale cortisol content (due to repeated capture events) than fish of low vulnerability.

2. Materials and methods

2.1. Study species

Barbel is a rheophilic and omnivorous species encountered in many European rivers and is a popular angling target species due to their aggregative behaviours that facilitates the capture of multiple individual fish during a single angling event, where fish might be captured between 1 and 8 kg (Britton and Pegg, 2011). Their populations are mainly comprised of individuals that have relatively small home ranges (< 1 km), although a small proportion of individuals are usually more mobile, with home ranges exceeding 10 km (Britton and Pegg, 2011). Carp is also an omnivorous species and is generally encountered in slow flowing rivers and lakes (Britton et al., 2022). Introductions by both aquaculture and angling have resulted in their domesticated strains being invasive in many of the world's freshwaters (Vilizzi et al., 2015). Their popularity for angling results from their relatively large size and fighting qualities (Britton et al., 2022). Lentic recreational fisheries tend to be stocked either with very high abundances of smaller fish (e.g. 1–7 kg) (North, 2002) or smaller numbers of relatively large fish (>10 kg) (Žák, 2021).

In the last 20 years, angling methods for both of these species have focused on the use of manufactured baits (Imbert et al., 2022). For barbel, the baits used in many rivers in England often have a strong marine fishmeal base, with anglers often releasing relatively large amounts of these baits into the water to attract fish into the area, as well as using these baits on the hook. Consequently, the release of these baits into the water results in their consumption by the fish (Gutmann Roberts et al., 2017; De Santis et al., 2019). These baits, and those also based on plants (such as maize), tend to be highly enriched in the ^{13}C stable isotope ($> -22.0‰$), whereas the putative prey resources of these fishes tend to highly depleted ($< -28.0‰$). They are often also depleted in the ^{15}N stable isotope ($< 6.0‰$) versus the putative prey resources that usually have much higher values ($> 10.0‰$). These differences in stable isotope values thus provide the ability to assess the extent to which fish are consuming these different resources (Britton et al., 2022). Accordingly, SIA has indicated that the diet of some larger barbel (> 400 mm) is primarily comprised of angling baits, whilst other fish of similar size have diets that remain based mainly on natural prey resources (Gutmann Roberts et al., 2017; De Santis et al., 2019). (Britton et al., 2022). Regarding carp angling, high quantities of manufactured baits are also regularly released by anglers to attract fish into the angling area and increase the probability of capture. Where these fisheries have a high stock density of carp that are fished for regularly, their diet (and the diets of other species present) tend to be dominated by these baits (Britton et al., 2022).

2.2. Sampling sites and methods

The barbel was sampled from the lower River Severn basin (western England) between 2015 and 2020, with fish sampled from both the main River Severn and its River Teme tributary. The lower River Severn is relatively wide (> 25 m width), deep (>2 m) and slow flowing due to being impounded by a series of navigation weirs. It also features the River Teme, a Severn tributary that is narrower (<15 m) and comprised of pool and riffle sequences. A full site description is available in Gutmann Roberts et al. (2019).

The scale samples collected in September 2015 were from barbel used in an acoustic telemetry study in which fish were sampled by both electric fishing and C&R angling; the angled fish had significantly smaller home ranges than those electric fished (Gutmann Roberts et al., 2019). Barbel scale samples were then also collected in summer 2016, 2018 and 2020, although the home range sizes were not measured for these fish. The full methodology of fish capture, tagging and scale removal are outlined in Gutmann Roberts et al. (2019). To summarise, the sampling method (angling or electric fishing) and fork length (mm) were recorded for each individual, and up to 5 scales were taken from the area between the dorsal fin and the lateral line. All fish were then returned alive to the river. For details on acoustic tagging and estimation of individual home ranges, see Gutmann Roberts et al. (2019). At the same time as the fish sampling, amphipod samples (gammarids) were collected as a putative fish prey resource, given they are consumed naturally by barbel (Sheath et al., 2018). The length range of barbel used was 394–800 mm (Table 1).

Carp were sampled in summer 2020 from a small (1.5 ha), shallow (maximum depth 2 m) pond in Southern England that is managed for intensive recreational angling. Multiple anglers are present at this

Table 1
Meta-data of the groups of European barbel that were pooled for the purposes of scale cortisol content analyses (EF = electric fishing).

Group	Year	Method	n	Mean length (range) (mm)	Mean δ^{13}C (range) (‰)	Mean δ^{15}N (range) (‰)	Scale mass (mg)	Scale cortisol content (pg mg^{-1})
1	2015	Angling	6	604 ± 47 (557 – 721)	-20.52 ± 0.28 (−20.98 to −20.10)	11.50 ± 0.24 (11.03–11.90)	45.5	2.71
2	2015	Angling	8	600 ± 41 (529 – 680)	-22.04 ± 0.46 (−22.79 to −21.02)	12.11 ± 0.59 (10.74–13.30)	50.7	2.49
3	2015	Angling	5	633 ± 40 (585 – 698)	-24.47 ± 1.18(−26.66 to −23.37)	12.37 ± 0.52 (11.81–13.27)	46	1.60
4	2015	EF	8	584 ± 75 (394 – 717)	-22.08 ± 0.43 (−22.82 to −20.98)	12.03 ± 0.57 (10.71–13.37)	50.7	4.32
5	2015	EF	7	534 ± 95 (397 – 770)	-24.85 ± 0.91 (−26.50 to −23.25)	13.27 ± 0.78 (11.81–14.88)	46.3	3.73
6	2016	Angling	5	724 ± 88 (600 – 800)	-20.03 ± 0.50 (−20.55 to −19.37)	11.33 ± 0.67 (10.48–12.31)	50.2	1.64
7	2016	Angling	4	600 ± 74 (520 – 690)	-22.50 ± 0.72 (−22.95 to −21.40)	11.85 ± 0.34 (11.47–12.25)	47.9	1.71
8	2018	EF	3	642 ± 86 (576 – 725)	-25.03 ± 0.25 (−25.27 to −24.84)	13.37 ± 0.45 (13.01–13.79)	38.2	5.89
9	2018	EF	4	537 ± 14 (520 – 550)	-23.95 ± 0.47 (−24.36 to −23.26)	12.65 ± 0.75 (11.70–13.36)	28.2	2.63
10	2020	Angling	3	618 ± 119 (555 – 739)	-21.53 ± 1.00 (−22.53 to −20.86)	11.47 ± 0.66 (10.81–11.89)	20.4	3.43
11	2020	Angling	5	707 ± 47 (557 – 721)	-24.64 ± 0.89 (−25.54 to −23.12)	12.87 ± 0.74 (11.86–13.72)	50.4	3.47
12	2020	Angling	5	626 ± 41 (529 – 680)	-25.45 ± 0.51 (−26.12 to −24.78)	13.77 ± 0.22 (13.42–14.03)	50.4	4.42

fishery on a daily basis for much of the year (e.g. > 20 anglers per day), including participating in competitions that generally last for 5 h, and where captured fishes are held in keep-nets until the end of the competition before being batch-weighed and then returned alive to the water. This fishery had been stocked with fish to provide a very high fish biomass to support angler catches that reach over 10 kg h^{-1} (North, 2002), and where relatively high volumes of manufactured angling baits are released to enable these catch rates to be maintained during the entirety of the angling session (Britton et al., 2022). The scale samples used here were all from fish captured by angling; the fish were identified to species, measured (fork length, nearest mm), and up to three scales removed (taken from below the dorsal fin but above the lateral line). The carp used in analyses were between 530 and 700 mm fork length. After sampling, the fish were returned alive to the pond. Concomitantly, amphipod samples were also collected as a putative fish prey resource, as these are part of the natural diet of carp and provided a putative prey resource consistent with the sampled barbel.

All of the fish sampling was completed following ethical review and under UK Home Office licence P47216841 and 70/8063 (under the UK Animals (Scientific Procedures) Act 1986 and associated guidelines).

2.3. Stable isotope analysis

The proxies of angling vulnerability that related to fish diet (dietary reliance on angling bait) were developed from stable isotope analysis (SIA) of scales of the sampled fishes (Sections 2.5, 3.1; Britton et al., 2022). This involved the removal of tissue from the outer edge of the scale (i.e., scale tissue that had been produced in recent months; Hutchinson and Trueman, 2006), which was then dried to constant mass at 60 °C. The amphipod samples were also dried in this manner. All samples were then analysed at the Cornell University Stable Isotope Laboratory (New York, USA) for δ^{13}C and δ^{15}N in a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Analytical precision of the δ^{13}C and δ^{15}N sample runs was estimated against an internal standard sample of animal (deer) material every 10 samples, with the overall standard deviation estimated at 0.08 and 0.04‰ respectively. Ratios of C:N indicated no requirement for lipid normalisation (Winter and Britton, 2021).

As differences in the mean values of δ^{13}C and δ^{15}N of the amphipod samples from the River Severn were minor across the sampling years (< 1‰), the barbel SI data were used in subsequent analyses without correction. Analysed groups of barbel that has increasingly enriched values of δ^{13}C and depleted values of δ^{15}N were interpreted as having diets more reliant on angling baits (Gutmann Roberts et al., 2017).

For carp, to indicate the extent to which individuals had diets based on angler baits, then their δ^{13}C data were converted to corrected carbon ($\delta^{13}C_{corr}$) (Olsson et al., 2009; Britton et al., 2022):

$$\delta^{13}C_{corr} = (\delta^{13}C_{fish} - \delta^{13}C_{meanMI}) / CR_{MI}$$

wherein $\delta^{13}C_{fish}$ is the δ^{13}C value of each fish, $\delta^{13}C_{meanMI}$ is the mean δ^{13}C of the macroinvertebrate prey and CR_{MI} is the carbon range ($\delta^{13}C_{max} - \delta^{13}C_{min}$) of the same macroinvertebrates (Olsson et al., 2009). To complement $\delta^{13}C_{corr}$, carp δ^{15}N data were converted to trophic position (TP) (Olsson et al., 2009):

$$TP = (\delta^{15}N_{fish} - \delta^{15}N_{prey} / 3{\cdot}4) + 2$$

where TP and $\delta^{15}N_{fish}$ are the trophic positions and the nitrogen ratios of each individual fish, $\delta^{15}N_{prey}$ is the mean nitrogen ratio of the putative macroinvertebrate prey resource, 2 is the trophic position of these prey resources (as primary consumers) and 3.4 is the generally accepted fractionation factor between adjacent trophic levels (Post, 2002). The $\delta^{13}C_{corr}$ and TP data were then used to assess the extent to which the individual fish were consuming angling baits versus natural prey. Regarding $\delta^{13}C_{corr}$, as discrimination factors of δ^{13}C between prey and fish predators are generally 1–2‰, but can be higher for scale (e.g. up to 5‰ on invertebrate based diets; Busst and Britton, 2016), then fish values of $\delta^{13}C_{corr}$ outside of these ranges (e.g. > 5‰) indicate the fish were feeding on alternative dietary items (i.e. angling baits). Similarly, had the fish been foraging on the putative macroinvertebrate prey groups used in the TP equation, the resultant TP values would be expected to be 2.5–4.5 (with variation resulting from differences in dietary proportions between individual fish) (Busst and Britton, 2016; Winter and Britton, 2021). Values outside of this range (< 2.5 or > 4.5) would indicate the consumption of alternative dietary items, such as angling baits.

2.4. *Scale cortisol content*

Scale cortisol content was determined from scales by enzyme immunoassay (Cortisol EIA KIT; Neogen® Corporation, Ayr, UK). Although the scale mass required to provide reliable cortisol content estimates from this method has not been determined specifically, in other taxa, material from feathers and faeces has required a minimum of = /> 20 mg of material (e.g. Millspaugh and Washburn, 2004; Lattin et al., 2011). For carp, their relatively large scale size and mass meant that the method could be successfully applied on individual fish (scale mass range: 38.7–50.9 mg; n = 10). For barbel (n = 68), their smaller scales meant the scale mass available for individual fish was not sufficient for the analysis and so fish had to be pooled in order to provide enough material. To provide groups of fish that were considered to be consistent in their traits and behaviours, they were pooled according to their sampling method (electric fishing or angling), then sampling year (2015/ 2016/ 2018/ 2020) and then by $\delta^{13}C$ values (< -23.00/ -22.99 to -21.0/ -20.99 to -19.00), resulting in 12 groups of barbel comprising between 3 and 8 fish that provided 20.4 and 50.7 mg of material for analysis (Table 1).

Cortisol extraction from fish scales was performed following the methodology of Carbajal et al. (2018). In brief, scales were first washed with isopropanol three times to remove any hormone sources that could have been on the external surface of the scales. Once dry, the scale samples of each fish/ group of fish were mechanically pulverized with a ball mill (Retsch, MM2 type, Germany), and each powdered sample was incubated overnight in methanol. After extraction, samples were centrifuged, and the supernatant was evaporated. Dried extracts were reconstituted with enzyme immunoassay buffer provided by the assay kit and immediately stored at − 20 °C until analysis.

2.5. *Data analyses*

2.5.1. *Proxies of vulnerability of fish to angling capture*

To develop the proxies for angling vulnerability of barbel, data from the fish sampled in 2015 were used (Gutmann Roberts et al., 2019). Generalised linear models (GLM) tested the relationship of sampling method (electric fishing or angling; independent variable) versus the dependent variables of home range size, $\delta^{15}N$ (both gamma distribution, log link function), and $\delta^{13}C$ (normal distribution), with fish length included in models as a covariate. The data distribution and link function of models were finalised following model validation through plotting residuals against fitted values and the covariate, which revealed no problem in the final models (i.e. no pattern or relationship evident) (Zuur and Ieno, 2016). For carp, the proxies were developed from their relationships between $\delta^{13}C_{corr}$ and TP according to linear regression.

2.5.2. *Capture vulnerability-scale cortisol relationships*

For barbel, testing differences in scale cortisol content between the groups was then based on the sampling method (Table 1) in GLMs (normal distribution), where in the full model, the dependent variable was scale cortisol content, the independent variable was sampling method, and the covariates were year, mean $\delta^{15}N$, mean $\delta^{13}C$ and mean fork length. A further five candidate models were also run to identify the best fitting model by including only the following parameters in models: (i) stable isotope data (mean $\delta^{15}N$, mean $\delta^{13}C$); (ii) sampling method; (iii) stable isotope data and fish length; (iv) year; and (v) fish length. In all cases, the data distribution and link function of models were finalised following model validation, which involved plotting residuals against fitted values and covariates, and revealed no problems (i.e. no pattern or relationship evident) (Zuur and Ieno, 2016). The best fitting model was determined according to the lowest AIC value, but where models within 2 AIC of the top-ranked model were also considered to have strong support. For carp, linear regression was used to test the scale cortisol levels against $\delta^{13}C_{corr}$ and TP.

3. Results

3.1. *Proxies of vulnerability of fish to angling capture*

In European barbel, the mean home range size (± 95% CI) of fish sampled by angling was significantly smaller than of fish sampled by electric fishing (2793 ± 1352 m vs 6100 ± 2745 m; $P < 0.01$; Table 2A; Gutmann Roberts et al., 2019). Angler caught fish had significantly enriched ^{13}C values versus the fish sampled by electric fishing (-21.74 ± 0.74 vs -22.91 ± 0.70‰; $P = 0.03$; Table 2B), but this was not evident in $\delta^{15}N$ (11.88 ± 0.54 vs 12.44 ± 0.53‰; $P = 0.16$; Table 2C). We thus suggest groups of barbel sampled by angling probably have higher vulnerability to angling capture due to smaller home ranges and so higher spatial encounters with anglers, and through their higher contributions of angling baits to their diets. Accordingly, we posit these barbel will have significantly higher scale cortisol content (due to multiple capture events in preceding months).

In carp, values of $\delta^{13}C_{corr}$ ranged between 6.22‰ and 8.10‰, where higher values indicate a higher dietary proportion of angling bait (Britton et al., 2022). These $\delta^{13}C_{corr}$ values were highly correlated with TP (range 1.5–1.9), with fish of enriched $\delta^{13}C_{corr}$ being of higher TP (linear regression: $R^2 = 0.75$, $F_{1,8} = 24.35$, $P < 0.01$). We thus suggest that fish with enriched $\delta^{13}C_{corr}$ and of higher TP have higher angling recapture probabilities due to their higher reliance on angling baits in the diet. Accordingly, we posit these carp will have significantly higher scale cortisol content (due to multiple capture events in preceding months).

3.2. *Capture vulnerability - scale cortisol relationships*

Across the 12 barbel groups, differences in fork length between the groups of angled and electric-fished fish were minor (639 ± 34 vs 575 ± 49 mm), with this also the case in $\delta^{13}C$ (-22.65 ± 1.39 vs -23.98 ± 1.32‰) and $\delta^{15}N$ (12.16 ± 0.58 vs 12.83 ± 0.61‰). Scale cortisol concentrations per group ranged between 1.60 and 5.89 pg mg^{-1} (mean 3.17 ± 0.74 pg mg^{-1}), with concentrations being substantially lower in groups that were captured by angling versus groups captured by electric fishing (2.68 ± 0.71 vs 4.14 ± 1.33 pg mg^{-1}). The best fitting GLM was the full model, with high support also for the stable isotope model (Table 3A). In the full model, the significant variables were sampling method (significantly lower scale cortisol concentrations in groups of angled barbel) and $\delta^{15}N$ (significantly increased scale cortisol as $\delta^{15}N$ increased), but with all other covariates being non-significant, including $\delta^{13}C$ ($P = 0.08$) (Table 3B; Fig. 1).

In common carp, scale cortisol concentrations were 1.26–5.40 pg mg^{-1} (mean 3.23 ± 1.26 pg mg^{-1}). Linear regression indicated that as scale cortisol levels increased in individual carp, their values of both $\delta^{13}C_{corr}$ and TP decreased significantly ($\delta^{13}C_{corr}$: $R^2 = 0.57$, $F_{1,8} = 10.41$, $P = 0.01$; trophic position: $R^2 = 0.55$, $F_{1,8} = 9.82$,

Table 2

Results of GLMs testing the effect of sampling method (SM) on the home range size, $\delta^{13}C$ and $\delta^{15}N$ of European barbel in 2015, where fish fork length (FL) was a covariate in the model (in column 'Model', the text refers to the response variable, with the distribution and link function (if needed) provided in brackets).

Model	Variable	Estimate	SE	Z	P
Home range (gamma, log link)	Intercept	9.23	0.89	10.37	< 0.001
	SM	-0.77	0.27	-2.85	0.005
	FL	-0.001	0.02	-0.05	0.569
$\delta^{13}C$ (normal)	Intercept	-25.09	1.69	-14.85	< 0.001
	SM	1.18	0.54	2.19	0.029
	FL	< 0.01	< 0.01	1.00	0.21
$\delta^{15}N$ (gamma, log link)	Intercept	2.61	0.10	26.10	< 0.001
	SM	-0.05	0.03	-1.67	0.155
	FL	0.01	0.01	1.00	0.386

Table 3
Results of GLMs testing the effect of sampling method (SM), fish fork length (FL), the year the fish was sampled (Yr) and stable isotope data ($\delta^{13}C$, $\delta^{15}N$) on the scale cortisol levels of European barbel, where: (A) Structure and summary of candidate generalised linear models (GLM; linear, identity link function), where the best fitting model has ΔAIC of 0 and models with ΔAIC < 2 having strong support; and (B) Results of best fitting GLMs by parameters.

(A)

Model	Structure	AIC	ΔAIC	Log-likelihood
Full model	SM + FL + Yr + $\delta^{13}C$ + $\delta^{15}N$	38.92	0	-12.46
Stable isotope only	$\delta^{13}C$ + $\delta^{15}N$	39.23	0.31	-15.62
Sampling method only	SM	40.99	2.07	-17.50
Fish data only	FL + $\delta^{13}C$ + $\delta^{15}N$	41.24	2.32	-15.62
Year only	Yr	43.16	4.24	-18.58
Fish length only	FL	45.22	6.30	-19.61

(B)

Model	Parameter	Estimate	SE	Z	P
Full	Intercept	-447.90	229.66	-1.95	0.051
	SM	1.35	0.56	2.41	0.016
	FL	0.03	0.01	3.00	0.587
	Yr	0.22	0.12	1.83	0.058
	$\delta^{13}C$	0.58	0.33	1.76	0.076
	$\delta^{15}N$	1.74	0.77	2.26	0.024
Stable isotope only	Intercept	-11.65	4.38	-2.66	0.008
	$\delta^{13}C$	0.63	0.41	1.54	0.132
	$\delta^{15}N$	2.36	0.96	2.46	0.014

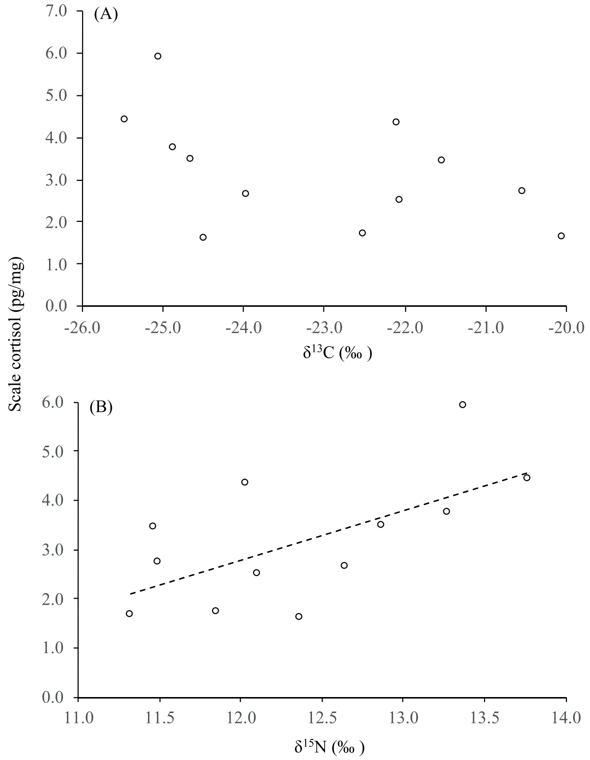

Fig. 1. Relationship of (A) mean $\delta^{13}C$ and (B) mean $\delta^{15}N$ versus scale cortisol concentration in pooled European barbel. In (B), the dashed line is the significant relationship between the variables according to linear regression ($R^2 = 0.40$, $F_{1,10} = 6.54$, $P = 0.03$). In (A), the relationship between the variables was not significant ($R^2 = 0.23$, $F_{1,10} = 3.03$, $P = 0.11$).

$P = 0.01$; Fig. 2). However, the relationship between fish length and scale cortisol concentration was not significant ($R^2 = 0.01$, $F_{1,8} = 0.09$, $P = 0.77$).

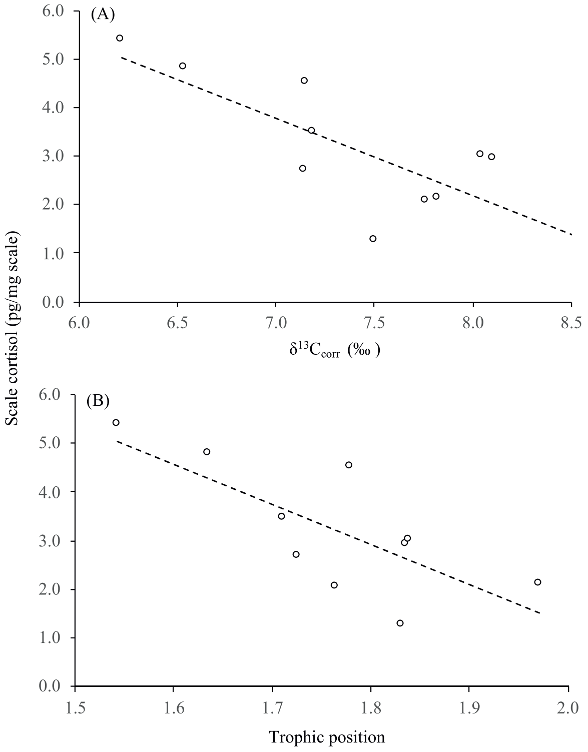

Fig. 2. Relationship of (A) corrected carbon ($\delta^{13}C_{corr}$) and (B) trophic position versus scale cortisol concentration in common carp. The dashed line is the significant relationship between the variables according to linear regression (Section 3.2).

4. Discussion

Scale cortisol content provides a reliable biomarker of chronic stress across a range of different fish species from both freshwater and marine environments (e.g. Carbajal et al., 2018, 2019a; b; Laberge et al., 2019; Lebigre et al., 2022; Vercauteren et al., 2022). Applying this biomarker here to two freshwater fishes exposed to catch-and-release angling, we initially developed proxies of angling capture vulnerability for the two study populations. We then assumed that fish with higher capture vulnerabilities would have had higher recapture rates in preceding months and, given individual angling capture events represent a short-term physiologically stressful event (Cooke et al., 2013), we suggested that these fishes would then have higher accumulations of cortisol in their scales. However, in both barbel and carp, significantly lower scale cortisol content was measured in the fishes that had been suggested as most vulnerable to angling capture. This result could have related to several factors, including fish captured regularly by angling having high stress resilience to repeated capture that is independent of other behaviours, fish with high capture vulnerability developing high hook avoidance behaviours following a capture event, fish feeding on angling baits experiencing lower chronic stress due to reduced foraging times, and/ or the proxies developed here not adequately describing angling capture vulnerability in the two species.

Angling is recognised as a non-random method of fish capture, where angling induced selection results in population sub-groups with specific trait combinations being most vulnerable to capture. In many species, the most vulnerable trait combinations involve high activity and boldness, which generally align to high stress resilience within proactive-reactive coping styles (Castanheira et al., 2017; Vindas et al., 2017; Villegas-Ríos et al., 2018). For example, a phenotypic syndrome in rainbow trout was evident, whose physiological responses to an experimental stressor (including cortisol levels) was relatively low, with these fish then having a higher vulnerability to angling capture than other phenotypes (Koeck et al., 2019; Monk et al., 2021). The acoustic tracking study of the barbel completed in the lower River Severn basin in 2015 indicated that there was some inter-individual variability in their behaviours, with some individuals having relatively small home ranges (some < 1 km, suggesting a low activity phenotype), with others having home ranges that were substantially larger (> 12 km, suggesting a high activity phenotype) (Gutmann Roberts et al., 2019). Although high activity phenotypes are often associated with higher capture probabilities (Villegas-Ríos et al., 2018), it was the low activity barbel that were primarily sampled by angling here. These angled barbel also had substantially enriched δ^{13}C versus electric fished barbel, indicating a greater dietary reliance on angler baits. Thus, we posited that the low activity barbel sampled by angling would have higher angling recapture rates (and so higher scale cortisol levels) than those electric fished due to: (i) their smaller home ranges increasing their spatial encounters with anglers (Gutmann Roberts et al., 2019), and (ii) their higher dietary reliance on angling bait increasing their likelihood of encountering a baited hook while foraging. However, the higher activity barbel groups captured by electric fishing had significantly higher scale cortisol content than the groups captured by angling. Thus, despite electric fished barbel groups being associated with high activity phenotypes, this high activity was not associated with higher levels of stress resilience.

Although fish capture in catch and release angling is recognised as a stressor that can have considerable short term physiological effects (e.g. Cooke et al., 2013; Pinder et al., 2019), the longer term effects of angling capture (especially multiple capture events) are less apparent. Although knowledge of how multiple captures affect individual fish behaviour and physiology is limited, evidence suggests that fishes which are captured can often demonstrate ‘hook avoidance’ behaviours that reduces their subsequent capture vulnerability. For example, Raat (1985) demonstrated that after carp were hooked once, their vulnerability to subsequent capture was decreased, with experiments by Lovén Wallerius et al. (2020) indicating that in addition to direct experience of angling capture leading to hook avoidance, carp can also develop these behaviours through social learning alone. Although domesticated strains of carp are bolder and more vulnerable to angling capture than wild strains, the development of hook avoidance behaviours are similar across both strains (Klefoth et al., 2013). Thus, while our proxies for angling capture vulnerability were considered as likely to result in higher capture rates (and so higher scale cortisol content), it could be that following an initial capture event, these fishes developed strong hook avoidance behaviours that enabled them to continue foraging on angling baits while reducing their risk of ingesting a baited hook. However, some studies suggest that within populations, only a minority of individual fish within populations are likely to be recaptured on multiple occasions (e.g. European catfish; Britton et al., 2007). In these recaptured individuals, there could be trade-offs between the ease of resource and energy acquisition from feeding on readily available angling baits versus the elevated risk of being repeatedly captured and released. It might be that the costs of angling capture then reduce with capture frequency through habituation, although this is highly speculative in the absence of any supporting evidence.

A limitation of this study was that in the population that had the most appropriate sample size for robust testing of chronic stress responses (barbel, $n = 68$), the method used to determine scale cortisol content required scale mass in excess of that able to be provided by individual fish. The pooling of individual fish into groups according to their capture method, sampling year and stable isotope data was considered as the most appropriate way of dealing with this issue, but it is acknowledged that the reduction from 68 individuals to 12 groups resulted in a relatively coarse analysis that could have resulted in the barbel phenotypes that were most vulnerable to angling capture and so having the highest levels of scale cortisol content being mixed with individuals of lower vulnerability. The relatively low sample size of carp and lack of replication across water bodies also limits our ability to transfer these finding to other fisheries and species. For both species, the evaluation of their results was also inhibited by the lack of control populations that were not exploited by recreational angling. It is recommended that this lack of baseline knowledge on understanding levels of scale cortisol in the absence of angling as a potential chronic stressor is overcome in future studies. In addition, given that fish consuming large amounts of angling baits might not necessarily experience multiple recaptures (e.g. due to hook avoidance; Lovén Wallerius et al., 2020), then it is recommended that recapture rates are also measured directly and tested against both diet and scale cortisol levels (e.g. by using tagged fish). Notwithstanding, there was consistency in the results across both species whereby those fishes that we predicted would be most vulnerable to angling capture being the groups and individuals with the lowest scale cortisol content, suggesting angling high vulnerability correlates with high stress resilience.

In summary, the use of scale cortisol as a biomarker of chronic stress indicated that fish of high angling vulnerability had significantly lower scale cortisol content. In barbel, this was despite these fish being of a low activity phenotype that had been assumed to have lower stress resilience than the high activity phenotype (which had higher scale cortisol content). The reasons for these more vulnerable fish having lower scale cortisol remain unclear, but could relate to fish that are recaptured regularly being able to cope with the physiological demands of angling capture more easily than other fishes or these vulnerable fishes developing hook avoidance behaviours that minimises their recapture rates while enabling them to continue expressing other behaviours.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

JRB and DA conceptualised the study and provided scale samples, AC and MLB designed the laboratory protocols and completed all laboratory analyses, JRB analysed the data and wrote the manuscript, all authors revised the manuscript and all authors agree to its submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

We thank Dr Catherine Gutmann Roberts and all the anglers who helped collect the fish scales used in analyses.

References

Alos, J., Palmer, M., Arlinghaus, R., 2012. Consistent selection towards low activity phenotypes when catchability depends on encounters among human predators and fish. PloS One 7, e48030.

Arlinghaus, R., Cooke, S.J., 2009. Recreational fisheries: Socioeconomic importance, conservation issues and management challenges. In: Dickson, B., Hutton, J., Adams, W.M. (Eds.), Recreational hunting, conservation and rural livelihoods: Science and practice. Blackwell Publishing Ltd, pp. 39–58.

Arlinghaus, R., Cooke, S.J., Lyman, J., Policansky, D., Schwab, A., Suski, C., Sutton, S.G., Thorstad, E.B., 2007. Understanding the complexity of catch-and-release in recreational fishing: an integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. Rev. Fish. Sci. 15, 75–167.

Britton, J.R., Pegg, J., 2011. Ecology of European barbel *Barbus barbus*: implications for river, fishery, and conservation management. Rev. Fish. Sci. 19, 321–330.

Britton, J.R., Pegg, J., Sedgwick, R., Page, R., 2007. Investigating the catch returns and growth rate of wels catfish, *Silurus glanis*, using mark–recapture. Fish. Man. Ecol. 14, 263–268.

Britton, J.R., Cucherousset, J., Dominguez Almela, V., 2022. Novel trophic subsidies from recreational angling transform the trophic ecology of freshwater fishes. J., Appl. Ecol. 59, 2373–2385.

Busst, G.M., Britton, J.R., 2016. High variability in stable isotope diet–tissue discrimination factors of two omnivorous freshwater fishes in controlled ex situ conditions. J. Exp. Biol. 219, 1060–1068.

Carbajal, A., Monclús, L., Tallo-Parra, O., Sabes-Alsina, M., Vinyoles Cartanyà, D., López Béjar, M., 2018. Cortisol detection in fish scales by enzyme immunoassay: biochemical and methodological validation. J. Appl. Ichthyol. 32, 967–970.

Carbajal, A., Reyes-López, F.E., Tallo-Parra, O., Lopez-Bejar, M., Tort, L., 2019a. Comparative assessment of cortisol in plasma, skin mucus and scales as a measure of the hypothalamic-pituitary-interrenal axis activity in fish. Aquaculture 506, 410–416.

Carbajal, A., Tallo-Parra, O., Monclús, L., Vinyoles, D., Solé, M., Lacorte, S., López-Bejar, M., 2019b. Variation in scale cortisol concentrations of a wild freshwater fish: Habitat quality or seasonal influences? Gen. Comp. Endocrin. 275, 44–50.

Castanheira, M.F., Conceição, L.E., Millot, S., Rey, S., Bégout, M.L., DamsgAard, B., Kristiansen, T., Höglund, E., Øverli, Ø., Martins, C.I., 2017. Coping styles in farmed fish: consequences for aquaculture. Rev. Aquacult. 9, 23–41.

Cooke, S.J., Suski, C.D., 2004. Are circle hooks an effective tool for conserving marine and freshwater recreational catch-and-release fisheries? Aquat. Conserv. Mar. Fresh. Ecosys. 14, 299–326.

Cooke, S.J., Donaldson, M.R., O'connor, C.M., Raby, G.D., Arlinghaus, R., Danylchuk, A. J., Hanson, K.C., Hinch, S.G., Clark, T.D., Patterson, D.A., Suski, C.D., 2013. The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. Fish. Man. Ecol. 20, 268–287.

De Santis, V., Roberts, C.G., Britton, J.R., 2019. Influences of angler subsidies on the trophic ecology of European barbel *Barbus barbus*. Fish. Res. 214, 35–44.

Gutmann Roberts, C., Bašić, T., Amat Trigo, F., Britton, J.R., 2017. Trophic consequences for riverine cyprinid fishes of angler subsidies based on marine-derived nutrients. Freshw. Biol. 62, 894–905.

Gutmann Roberts, C., Hindes, A.M., Britton, J.R., 2019. Factors influencing individual movements and behaviours of invasive European barbel *Barbus barbus* in a regulated river. Hydrobiologia 830, 213–228.

Hutchinson, J.J., Trueman, C.N., 2006. Stable isotope analyses of collagen in fish scales: limitations set by scale architecture. J. Fish. Biol. 69, 1874–1880.

Imbert, A., Cucherousset, J., Parthuisot, N., Boulêtreau, S., 2022. Elemental composition and microbial decomposition of different angling baits. Fish. Man. Ecol. https://doi.org/10.1111/fme.12537.

Klefoth, T., Pieterek, T., Arlinghaus, R., 2013. Impacts of domestication on angling vulnerability of common carp, *Cyprinus carpio*: the role of learning, foraging behaviour and food preferences. Fish. Man. Ecol. 20, 174–186.

Koeck, B., Závorka, L., Aldvén, D., Näslund, J., Arlinghaus, R., Thörnqvist, P.O., Winberg, S., Björnsson, B.T., Johnsson, J.I., 2019. Angling selects against active and stress-resilient phenotypes in rainbow trout. Can. J. Fish. Aquat. Sci. 76, 320–333.

Laberge, F., Yin-Liao, I., Bernier, N.J., 2019. Temporal profiles of cortisol accumulation and clearance support scale cortisol content as an indicator of chronic stress in fish. Conserv. Physiol. 7, coz052.

Lattin, C.R., Reed, J.M., DesRochers, D.W., Romero, L.M., 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. J. Avian Biol. 42, 247–252.

Lebigre, C., Woillez, M., Barone, H., Mourot, J., Drogou, M., Goff, R.L., Servili, A., Hennebert, J., Vanhomwegen, M., Aerts, J., 2022. Temporal variations in scale cortisol indicate consistent local-and broad-scale constraints in a wild marine teleost fish. Mar. Env. Res., 105783

Lennox, R.J., Alós, J., Arlinghaus, R., Horodysky, A., Klefoth, T., Monk, C.T., Cooke, S.J., 2017. What makes fish vulnerable to capture by hooks? A conceptual framework and a review of key determinants. Fish Fish 18, 986–1010.

Lovén Wallerius, M., Johnsson, J.I., Cooke, S.J., Arlinghaus, R., 2020. Hook avoidance induced by private and social learning in common carp. Trans. Am. Fish. Soc. 149, 498–511.

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrin. 138, 189–199.

Monk, C.T., Bekkevold, D., Klefoth, T., Pagel, T., Palmer, M., Arlinghaus, R., 2021. The battle between harvest and natural selection creates small and shy fish. Proc. Nat. Acad. Sci. 118, e2009451118.

Muoneke, M.I., Childress, W.M., 1994. Hooking mortality: a review for recreational fisheries. Rev. Fish. Sci. 2, 123–156.

North, R., 2002. Factors affecting the performance of stillwater coarse fisheries in England and Wales. In: Cowx, I.G. (Ed.), Management and Ecology of Lake and Reservoir Fisheries, pp. 284–298.

Olsson, K., Stenroth, P., Nyström, P.E.R., Granéli, W., 2009. Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? Freshw. Biol. 54, 1731–1740.

Pinder, A.C., Harrison, A.J., Britton, J.R., 2019. Temperature effects on the physiological status and reflex impairment in European grayling *Thymallus thymallus* from catch-and release angling. Fish. Res. 211, 169–175.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.

Raat, A.J.P., 1985. Analysis of angling vulnerability of common carp, *Cyprinus carpio* L., in catch-and-release angling in ponds. Aquacult. Res. 16, 171–187.

Sheath, D.J., Dick, J.T., Dickey, J.W., Guo, Z., Andreou, D., Britton, J.R., 2018. Winning the arms race: host–parasite shared evolutionary history reduces infection risks in fish final hosts. Biol. Lett. 14, 20180363.

Vainikka, A., Tammela, I., Hyvärinen, P., 2016. Does boldness explain vulnerability to angling in Eurasian perch *Perca fluviatilis*? Curr. Zool. 62, 109–115.

Vercauteren, M., Ampe, B., Devriese, L., Moons, C.P.H., Decostere, A., Aerts, J., Chiers, K., 2022. Explorative study on scale cortisol accumulation in wild caught common dab (*Limanda limanda*). BMC Vet. Res. 18, 1–12.

Vilizzi, L., Tarkan, A.S., Copp, G.H., 2015. Experimental evidence from causal criteria analysis for the effects of common carp *Cyprinus carpio* on freshwater ecosystems: a global perspective. Rev. Fish. Sci. Aquacult. 23, 253–290.

Villegas-Ríos, D., Réale, D., Freitas, C., Moland, E., Olsen, E.M., 2018. Personalities influence spatial responses to environmental fluctuations in wild fish. J. Anim. Ecol. 87, 1309–1319.

Vindas, M.A., Gorissen, M., Höglund, E., Flik, G., Tronci, V., Damsgård, B., Thörnqvist, P. O., Nilsen, T.O., Winberg, S., Øverli, Ø., Ebbesson, L.O., 2017. How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish. J. Exp. Biol. 220, 1524–1532.

Winter, E.R., Britton, J.R., 2021. Individual variability in stable isotope turnover rates of epidermal mucus according to body size in an omnivorous fish. Hydrobiologia 848, 363–370.

Žák, J., 2021. Diel pattern in common carp landings from angling competitions corresponds to their assumed foraging activity. Fish. Res. 243, 106086.

Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. Method Ecol. Evol. 7, 636–645.