General and Comparative Endocrinology 270 (2019) 18–25

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Cortisol responses of goldfish (*Carassius auratus*) to air exposure, chasing, and increased water temperature

John F. Cockrem[a,*], Mohammad A. Bahry[b,c], Vishwajit S. Chowdhury[d]

[a] School of Veterinary Science, Massey University, Palmerston North 4442, New Zealand
[b] Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, Fukuoka 819-0395, Japan
[c] Department of Animal Science, Faculty of Agriculture, Balkh University, Mazar-e-Sharif, Afghanistan
[d] Laboratory of Stress Physiology and Metabolism, Faculty of Arts and Science, Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, Fukuoka 819-0395, Japan

ARTICLE INFO

Keywords:
Cortisol
Fish
Variation
Heating
Air exposure
Temperature

ABSTRACT

Fish can respond to stimuli from the internal or external environment with activation of the hypothalamo-pituitary-interrenal (HPI) axis and the secretion of cortisol. Stimuli that activate the HPI axis of fish include short term air exposure and increases in water temperature. The present study was conducted to determine how quickly cortisol concentrations increase in goldfish subjected to an increase in water temperature, and to compare the response to an increase in water temperature with responses to other stimuli. Plasma cortisol concentrations varied widely between individual goldfish, with concentrations ranging from 9.1 to 516.0 ng/mL in goldfish on the day of arrival from the supplier. Mean cortisol concentrations in undisturbed goldfish were low (4.5 ± 1.0 ng/mL). Mean cortisol concentrations in fish exposed to air for 3 min and in fish that experienced chasing for 10 min were markedly elevated 15 min after the beginning of the stimuli (132.6 ± 31.0 and 121.1 ± 23.9 ng/mL respectively). Mean cortisol concentrations in fish that experienced an increase in water temperature rose to 22.2 ± 7.6 ng/mL after 15 min, declined to < 10 ng/mL at 30 and 60 min then increased and were elevated (79.0 ± 10.8 ng/mL) at 240 min. Cortisol measurements can be used to indicate the responsiveness of fish to changes in water temperature and goldfish will be a convenient study species for the development of studies of plasticity in responses of fish to increases in water temperature that are happening due to climate change.

1. Introduction

Fish are ectothermic and their body temperature depends on the temperature of the water in which they live. Some species of fish such as trout, salmon and other salmonids are stenothermal and have a relatively narrow temperature tolerance range, whereas other species of fish are eurythermal and can tolerate a wide range of water temperatures. The ability of fish species to adjust to changes in water temperature will be a key factor in determining the likelihood that species can cope with the general increases in water temperature in the oceans, rivers and lakes that are starting to happen as a result of climate change. It is therefore important to consider how fish respond to changes in water temperature, and how these responses vary within and between species.

Fish respond to stimuli from the external environment that are perceived to be a threat or potential threat (emotional stressors) with activation of the hypothalamo-pituitary-interrenal (HPI) axis and the secretion of cortisol (Cockrem, 2013a). Cortisol is a glucocorticoid hormone with metabolic, behavioural and other actions that help fish cope with stimuli that activate the HPI axis. The HPI axis can also be activated when fish experience physical stressors such as short term air exposure or increases in water temperature (see review by Pankhurst (2011)). Fish are sensitive to stressors and can have prolonged cortisol responses after exposure to stressors for periods as short as 15 s (Fast et al., 2008).

Variable relationships between plasma cortisol concentrations and water temperature have been reported for different species of fish. Cortisol concentrations did not differ between juvenile chinook salmon (*Oncorhynchus tshawytscha*) acclimated to 7.5 or 21 °C and did not differ between juvenile cutthroat trout (*Salmo clarki clarki*) acclimated to 9 or 23 °C (Barton and Schreck, 1987; Strange et al., 1977). Cortisol was lower in killifish (*Fundulus heteroclitus*) and Channel catfish (*Ictalurus punctatus*) acclimated to higher water temperatures compared with fish acclimated to lower water temperatures (killifish 61 ± 19 ng/mL at

* Corresponding author.
E-mail address: J.F.Cockrem@massey.ac.nz (J.F. Cockrem).

https://doi.org/10.1016/j.ygcen.2018.09.017
Received 4 July 2018; Received in revised form 19 September 2018; Accepted 29 September 2018
Available online 01 October 2018
0016-6480/

−1 °C and 2 ± 1 ng/mL at 20 °C, (Paxton et al., 1984); Channel catfish 53 ± 6 ng/mL at 10 °C and 20 ± 5 ng/mL at 30 °C, (Strange, 1980)). Conversely, cortisol was higher in Arctic charr (*Salvelinus alpinus*) acclimated to higher water temperatures (2.4 ng/mL at 10.3 °C and 11.5 ng/mL at 18.1 °C; Lyytikäinen et al., 2002).

Goldfish (*Carassius auratus*) are a convenient study species and cortisol responses to a variety of stimuli have been reported for of goldfish. Cortisol responses to an increase in water temperature have not previously been measured in goldfish. The present study was conducted to determine how quickly cortisol concentrations increase in goldfish subjected to an increase in water temperature, and to compare the response to an increase in water temperature with responses to other stimuli. Whilst individual variation in glucocorticoid responses has been widely documented in other vertebrate groups (Cockrem, 2013a), there are few reports of the magnitude of such variation in fish. Further objectives of the study were to measure plasma cortisol concentrations in undisturbed goldfish and to determine the magnitude of variation in cortisol responses of individual fish.

2. Materials and methods

2.1. Study animals and blood sample collection

2.1.1. Fish

Wakin strain goldfish (*Carassius auratus*) were purchased from commercial goldfish suppliers (Aqua shop RARE, Kobe, Japan). The fish were reared in outdoor ponds and were from one to 3 years of age. Goldfish were caught in their home pond, held in large containers of water and then after several hours placed in water in a large plastic bag for transport overnight to the laboratory. After arrival the fish were caught in their transport bag, placed in clean water and then moved to a fish tank. There were 6–8 fish in each tank.

The fish tanks were 60 × 30 × 29 cm and were filled with 52 L of water. The water was tap water that had been conditioned with a commercial goldfish water conditioner (Tetra, Kanagawa, Japan). Water was continuously recirculated separately for each tank by pumping water from the tank through a foam and mesh filter. Sheets of dark plastic were placed between the tanks so that fish could not see fish in other tanks. The air temperature in the fish room was held at 19–22 °C and the daily lighting schedule was 12 h light:12 h dark. Water temperatures in the fish tanks were continuously measured with digital thermometers (Tetra, Tokyo, Japan) and were the same as the air temperature in the room. Fish were provided from the day of arrival with pelleted food (Hikari Baby Gold, Hyogo, Japan) several times daily. The amount of food was adjusted from day to day to ensure that all the food was consumed within several minutes on each feeding occasion. Food was provided on the morning of each experimental day.

2.1.2. Blood sample collection

Blood samples for the measurement of cortisol were collected from goldfish. All fish in each tank were caught with a scoop net and together placed into an anaesthetic solution (MS-222, Ethyl 3-aminobenzoate methanesulfonate, Sigma-Aldrich; concentration of 1 g/L). When opercular movements had ceased the euthanised fish were removed from the MS-222 solution and blood was collected into heparinised capillary tubes after the caudal vein was cut. The time from removal from the tank to the completion of blood sample collection varied from 2.1 to 7.1 min in experiment 1 and from 1.3 to 6.0 min in experiment 2 (mean times 4.2 ± 0.3 min and 3.6 ± 0.1 min). Fish were weighed after the blood sampling. Blood samples were kept cool on ice for up to 2 h before being centrifuged and plasma withdrawn and frozen at −80 °C.

The study was conducted in accordance with the guide for the care and use of laboratory animals of the National Research Council (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

2.2. Experiment design

Two experiments were conducted with goldfish. The aim of experiment one was to determine how long goldfish must be kept after arrival for them to become acclimatised to the experimental tanks and have low cortisol concentrations and hence could be used in experiments. Goldfish arrived in a plastic bag in a cardboard box after overnight travel from Kobe, Japan. The behaviour of the fish changed markedly over the several days after arrival. Initially, the fish were wary of people, whereas by six days after arrival the fish would swim towards a person, presumably seeking food, when a person approached their tank. Six fish were sampled at the time of arrival. The other fish were then transferred in groups of 6 to four tanks in the fish room. All fish in each tank were caught and sampled either 1, 2, 4 or 6 days after arrival.

The aim of experiment two was to measure cortisol responses of goldfish to each of three stimuli. The stimuli were exposure to air for 3 min, chasing in their home tank for 10 min, and a 10 °C increase in water temperature over one hour. Fish were sampled at least six days after arrival from the supplier. The experiment was conducted over a period of five weeks using two batches of fish from the supplier. Five fish from the first batch were sampled on the first sampling day and four fish from the second batch were sampled on the last sampling day. Cortisol concentrations in these fish were combined and used as the 0 min concentrations for comparison with cortisol concentrations in fish sampled in the three stimulus groups. Groups of fish were sampled 15, 30, 60, 120 and 240 min after the start of each stimulus. Sample sizes (4–6 fish per group) differed between treatment groups as occasionally the blood sample collection was not successful.

Fish that were exposed to air were caught in a net and the net held at the water surface so that the upper bodies of the fish were exposed to air. The second group of fish were subjected to continuous gentle stirring of the water with a small net for 10 min. The net was moved throughout the tank so that fish were kept moving and not able to avoid the net during the period of stirring. Four fish tank water heaters (Kotobuki Microdial, Osaka, Japan) were placed in each tank one day before the water temperature stimulus. The heaters steadily increased the water temperature after they were switched on, with a 10 °C increase in temperature over one hour. The higher water temperature was maintained (± 0.5 °C) until fish were removed for sampling. The initial water temperature and the temperature at the time of sampling for each sampling time were: 15 min 19.1–22.1 °C, 30 min 21.6–27.5 °C, 60 min 20.9–31.1 °C, 120 min 21.2–31.1 °C and 240 min 19.3–31.1 °C.

2.3. Plasma cortisol assay

Cortisol concentrations in goldfish plasma diluted in assay buffer were measured by enzyme immunoassay (ELISA) using kits from Enzo Life Sciences (catalogue number ADI-901-071). The 100 ng/mL cortisol standard provided with each kit was diluted in assay buffer to a concentration of 10 ng/mL. Standard curves were prepared by serial dilution of the 10 ng/mL standard in assay buffer to give cortisol concentrations from 10 to 0.156 ng/mL for the standard curve. The kit protocol for the ELISA was followed apart from the preparation of the standard curve. The limit of detection of the cortisol assay was determined as the hormone concentration at the mean minus two standard deviations from the zero hormone point on the standard curves. The assay limit of detection, expressed as ng cortisol per mL plasma, was 2.34 ng/mL. Control solutions of cortisol in assay buffer were prepared with cortisol concentrations that gave approximately 20%, 50% and 80% bound on the standard curve. The inter-assay coefficients of variation for these control solutions were 8.5, 11.2 and 15.8%, and the intra-assay coefficients of variation were 11.5, 13.6 and 12.8%.

2.4. Statistics

Data were analysed using Prism (GraphPad Software, LA Jolla, CA). Relationships between the time taken to collect blood samples and cortisol concentrations were determined by Spearman correlations. Plasma cortisol concentrations were log transformed before analyses of variance were conducted. One way ANOVA was used to compare mean cortisol concentrations between sampling times (0, 1, 2, 4 and 6 days) with post hoc comparisons between days made with Holms-Sidak multiple comparison tests. Mean cortisol concentrations were compared between stimuli and sampling times using two way ANOVA. Comparisons between stimuli were made with Tukey's multiple comparisons tests, and comparisons between sampling times with Dunnett's multiple comparisons tests. Data are presented as individual values or as mean ± S.E.

3. Results

3.1. Experiment one

Goldfish were initially very wary when first placed in a tank after arrival from the supplier. The fish moved away when their tank was approached by a person. This wariness diminished over the following two days, and by day 3 the fish would swim towards a person to seek food. Food was provided in the tank several times each day and goldfish began to eat on the day after arrival. The mean body mass of goldfish at the time of sampling was 12.6 ± 0.4 g and the range of body masses was 9.2–18.6 g.

There was no correlation between sample collection time and cortisol concentrations on the day of arrival and no correlation between body weight and cortisol concentrations on the day of arrival (data not shown). Cortisol concentrations in goldfish on the day of arrival from the supplier varied from 9.1 to 516.0 ng/mL and were high in four of six fish (Fig. 1A). Concentrations varied from 2.2 to 36.8 ng/mL on days 1, 2 and 4, whereas concentrations on day 6 were consistently low (< 7.5 ng/mL). Mean cortisol concentrations changed with time (one way ANOVA; $F_{4,\ 25} = 7.439$, $P = 0.0004$). Mean concentrations decreased from the day of arrival (152.0 ± 77.4 ng/mL) to the next day (9.6 ± 2.0 ng/mL; $P < 0.01$), and did not change significantly from day to day thereafter (Holms-Sidak multiple comparisons). Mean concentrations on day 6 were 4.8 ± 0.9 ng/mL.

3.2. Experiment two

The mean body mass of goldfish at the time of sampling was 11.0 ± 0.3 g and the range of body masses was 4.5–24.8 g. There was no correlation between sample collection time and cortisol concentrations in blood samples from undisturbed fish sampled at 0 min, and no correlation between body weight and cortisol concentrations in these fish (data not shown). Individual plasma cortisol concentrations for all fish are shown in Fig. 2. Cortisol concentrations were low (0.34–9.07 ng/mL) in undisturbed fish sampled at 0 min.

A two-way ANOVA showed that there were significant overall effects of time and stimulus on plasma cortisol concentrations, and a significant interaction ($F_{5,\ 91} = 32.50$, $P < 0.0001$; $F_{2,\ 91} = 28.57$, $P < 0.0001$; $F_{10,91} = 11.46$, $P < 0.0001$ respectively; see Fig. 3). Mean cortisol concentrations differed between fish exposed to an increase in water temperature and fish that experienced air exposure or chasing ($P < 0.0001$ in both cases), and did not differ between fish that experienced air exposure and fish that experienced chasing.

Cortisol concentrations varied considerably between individual fish exposed to air for 3 min, with coefficients of variation from 49.8% to 93.9% at different sampling times. Concentrations were elevated (> 40 ng/mL) in all fish exposed to air and then sampled 15, 30 or 60 min after the beginning of the air exposure. Maximum cortisol concentrations at these times were 255.6, 145.1 and 285.6 ng/mL respectively. Cortisol concentrations at 120 min were lower than at the preceding times, whereas concentrations at 240 min varied from 25.1 to 161.2 ng/mL. Mean cortisol concentrations in undisturbed fish were 4.5 ± 1.0 ng/mL. Mean cortisol concentrations in fish exposed to air for 3 min were markedly increased 15 min after the beginning of air exposure (132.6 ± 31.0 ng/mL; Fig. 3). Concentrations remained elevated at 30 and 60 min and were then lower, whilst still above initial concentrations, at 120 and 240 min. Mean cortisol concentrations were higher than at 0 min at sampling times other than 120 min in fish that experienced air exposure ($P < 0.0001$).

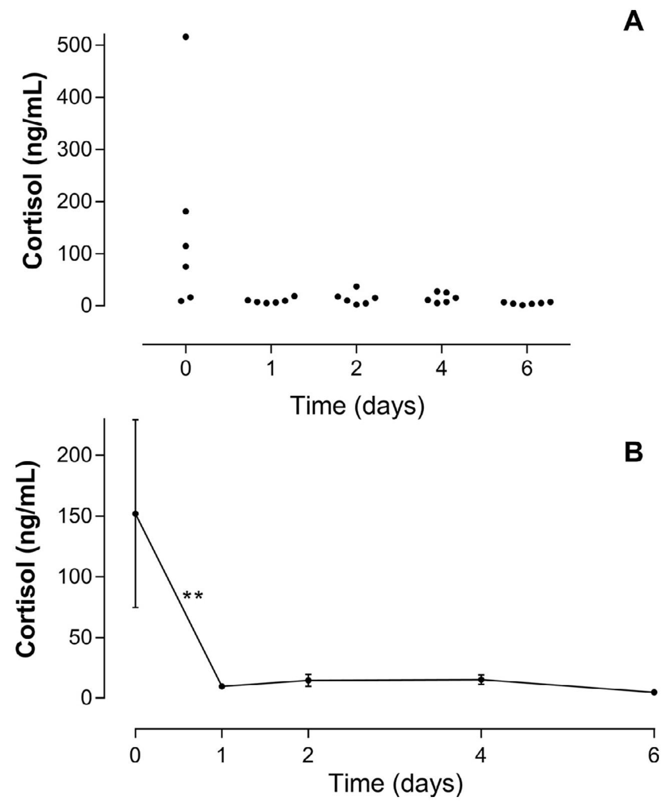

Fig. 1. Individual (A) and mean (± S.E., B) plasma cortisol concentrations in goldfish in relation to time after arrival from supplier. Significant differences in mean cortisol concentrations between successive pairs of days are indicated by asterisks (**$P < 0.01$).

Cortisol concentrations in fish that were chased for 10 min varied from 21.7 to 456.5 ng/mL at sampling times between 15 and 120 min after the beginning of stirring, and from 1.5 to 42.1 ng/mL at 240 min. Coefficients of variation ranged from 32.4% to 92.8% at different sampling times. Mean cortisol concentrations in fish that experienced chasing for 10 min were also elevated 15 min after the beginning of the chasing (121.1 ± 23.9 ng/mL). Concentrations remained elevated at 30, 60 and 120 min then declined at 240 min. Mean cortisol concentrations were higher than at 0 min at all sampling times from 15 min onwards in fish that experienced chasing ($P < 0.05$ at 240 min and $P < 0.0001$ at other times).

Cortisol was elevated (23.0–44.3 ng/mL) 15 min after an increase in water temperature began in 3 fish, whereas concentrations remained low (2.8 and 8.6 ng/mL) in two other fish. Concentrations varied from 1.2 to 15.6 ng/mL at 30 and 60 min, then from 8.2 to 60.4 ng/mL at 120 min and from 52.7 to 115.4 ng/mL at 240 min. Coefficients of variation in fish that experienced an increase in water temperature ranged from 77.4% to 136.96% at 15, 30, 60 and 120 min, whereas the coefficient of variation at 240 min was 33.5%. Mean cortisol concentrations in fish that experienced an increase in water temperature rose to 22.2 ± 7.6 ng/mL after 15 min then declined to < 10 ng/mL at 30 and 60 min. Mean concentrations increased at 120 min and were

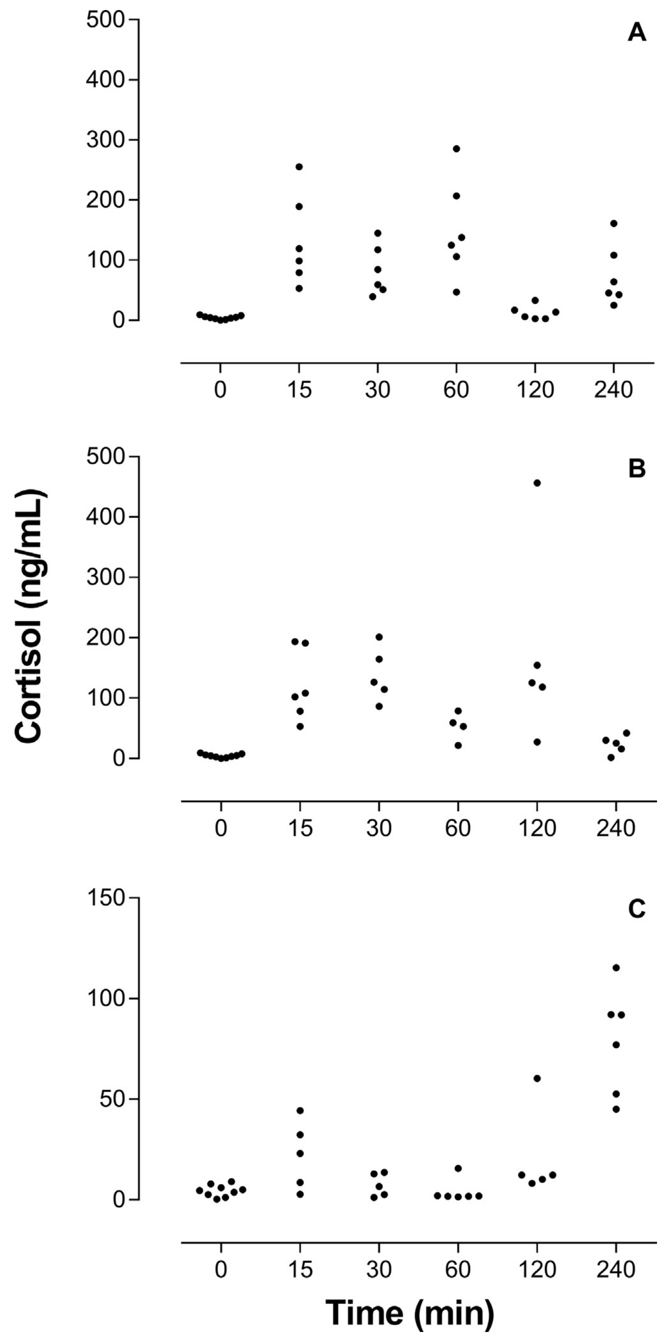

Fig. 2. Individual plasma cortisol concentrations in goldfish exposed to air for 3 min (A), chased in their home tank for 10 min (B), or subjected to an increase in water temperature of 10° over one h (C). The y-axis scale differs between the temperature graph and the air and chase graphs. Cortisol concentrations in five fish sampled on the first sampling day and four fish sampled on the last sampling day were combined and used as the 0 min concentrations for comparison with cortisol concentrations in fish sampled in the three stimulus groups.

elevated (79.0 ± 10.8 ng/mL) at 240 min. Cortisol concentrations at 15, 120 and 240 min were higher than concentrations at 0 min in fish that experienced increased water temperatures ($P < 0.05$).

4. Discussion

This study has shown that goldfish have low plasma cortisol concentrations (< 5 ng/mL) when undisturbed, and that cortisol increases within minutes when goldfish are exposed to a stressor and can reach very high concentrations (> 500 ng/mL). It has also shown that goldfish have rapid cortisol responses both to emotional stressors (stimuli from the external environment that are perceived to be a threat or potential threat) such as chasing and to physical stressors such as increasing water temperature. The increase in cortisol concentrations as the water temperature rose over one hour by 10 °C is the first report of the time course of a cortisol response of goldfish to an increase in water temperature.

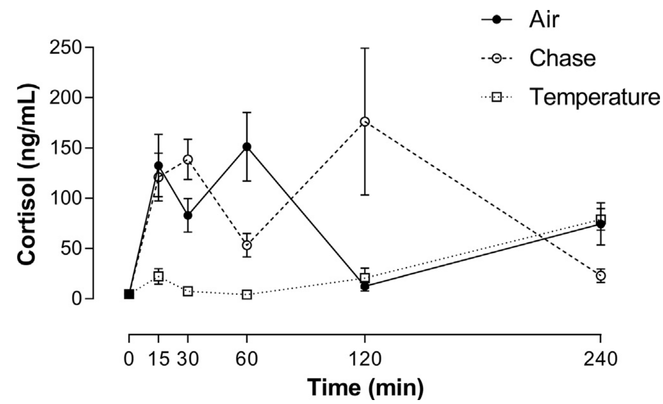

Fig. 3. Mean (± S.E.) plasma cortisol concentrations in goldfish exposed to air for 3 min (— ● —), chased in their home tank for 10 min (- - - ○ - - -), or subjected to an increase in water temperature of 10 °C during one h (··· □ ···) Cortisol concentrations in five fish sampled on the first sampling day and four fish sampled on the last sampling day were combined and used as the 0 min concentrations for comparison with cortisol concentrations in fish sampled in the three stimulus groups. Sample sizes were 4–6 in the treatment groups.

4.1. Individual variation in cortisol concentrations in goldfish

There was a very wide range of plasma cortisol concentrations in individual goldfish, as seen in other species of fish and in animals of other vertebrate groups (Cockrem, 2013a). Some fish had cortisol concentrations of < 10 ng/mL whereas other fish had concentrations > 500 ng/mL on the day of arrival at the laboratory after overnight transport from the supplier. These results show large individual variation in the extent to which individual goldfish perceived the experience of removal from an outdoor pond, confinement with other goldfish in a bag and overnight transport from the supplier to the laboratory to be a stressor.

Variation in glucocorticoid responses in animals has a genetic basis and is also due to differences in early life experiences (Cockrem, 2013a). Genetic variation in cortisol responses in fish has been demonstrated by the selection of lines of rainbow trout with relatively low or high cortisol responses to 3 h of confinement (Pottinger and Carrick, 1999). Individual differences in cortisol responses of fish reflect differences in the sensitivity of the fish to environmental cues. These differences are associated with individual differences in personality, where personality is an individual characteristic of an animal that determines both behavioural and physiological responses to environmental stimuli (Cockrem, 2013b).

The association between physiological and behavioural responses in fish is shown by consistent behavioural differences between low and high cortisol response fish, with low response fish showing a proactive personality and high response fish a reactive personality (Martins et al., 2011; Overli et al., 2007).

Whilst individual cortisol concentrations have not previously been reported in goldfish, individual cortisol concentrations in goldfish in the current study can be compared with concentrations measured in other species of fish. Cortisol concentrations in rainbow trout (*Oncorhynchus mykiss*) after 1 h of confinement varied from 8.0 to 327.7 ng/mL (Pottinger et al., 1992). Largemouth bass (*Micropterus salmoides*) sampled 25 min after 3 min of air exposure had cortisol

Table 1
Plasma cortisol measurements in goldfish.

Treatments	Cortisol (mean ± S.E)	Reference
Mean cortisol concentration in initial sample < 10 ng/mL		
Stirring for 10 min Air exposure for 3 min Water temperature increase of 10 °C	Control 4.5 ± 1.0 ng/mL 15 min after start of stirring 121.1 ± 23.9 ng/mL 15 min after start of air exposure 132.6 ± 31.0 ng/mL 15 min after start of 3 °C water temperature increase 22.2 ± 7.6 ng/mL 2 h after start of 10 °C water temperature increase over 1 h 79.0 ± 10.8 ng/mL	This study
Transfer to new aquaria	Control ~ 8 ± 3 ng/mL 0.5 h after some fish removed for initial samples ~ 14 ± 4 ng/mL Fish moved to new aquaria 3 h after transfer ~ 50 ± 27 ng/mL; 24 h after transfer ~ 9 ± 6 ng/mL	Hoseini and Tarkhani (2013)
Selenium in water	Control ~ 5 ± 1 ng/mL 6 h of selenium treatment ~ 28 ± 2 ng/mL 3 days of selenium treatment ~ 75 ± 2 ng/mL	Choi et al. (2015)
Potassium permanganate added to water Transfer to new aquarium	Control ~ 8 ± 3 ng/mL ~0.5 h of potassium permanganate treatment 130 ± 25 ng/mL Fish moved to new aquarium with clean water ~80 ± 12 ng/mL 3 h after transfer; ~40 ± 12 ng/mL 24 h after transfer	Hoseini and Tarkhani (2013)
Cortisol in food	Control ~ 6.7 ng/mL 3–21 days of cortisol treatment; mean cortisol ~ 50 to ~ 300 ng/mL	Bernier et al. (2004)
Mean cortisol concentration in initial sample > 10 ng/mL		
Control	Diel rhythm in plasma cortisol, concentrations higher during dark than light periods Lowest mean value ~140 ± 120 ng/mL Highest mean value ~560 ± 55 ng/mL	Cook et al. (1980)
Control	Diel rhythm in plasma cortisol, concentrations higher during light than dark periods. Mean cortisol ~49 ± 9 ng/mL during peak period and ~26 ± 4 ng/mL during low period	Singley and Chavin (1975)
Control	Control 58 ± 3 ng/mL	Spieler (1974)
Air exposure	Control ~70 ± 20 ng/mL 1 h after exposure to air for 1 min then return to tank ~315 ± 40 ng/mL 4 h after exposure to air for 1 min then return to tank and exposure to air again for 1 min at 1 and 2 h ~520 ± 30 ng/mL	Dror et al. (2006)
Catch, air exposure for 1 min then return to tank	Control 70 ± 19 ng/mL 16 min after 1 min air exposure 93 ± 16 ng/mL 31 min after 1 min air exposure 72 ± 16 ng/mL 41 min after 1 min air exposure ~71 ± 18 ng/mL	Fryer (1975)
Catch, air exposure and sham injection, return to tank	Control 16 ± 2 ng/mL 15 min after return to tank 94 ± 11 ng/mL	Fryer (1975)
3 min chasing followed by 2 min air exposure at intervals of 8 h for 1 or 3 days	Control ~19 ± 2 ng/mL 1 day of treatment. 12 h after treatment ~120 ± 15 ng/mL; 2 days after treatment ~90 ± 10 ng/mL; 5 days after treatment ~50 ± 10 ng/mL 3 days of treatment. 12 h after treatment ~210 ± 30 ng/mL; 2 days after treatment ~110 ± 20 ng/mL after; 5 days after treatment ~75 ± 10 ng/mL	Eslamloo et al. (2014)
Catch, transfer to bucket with shallow water	Control 16 ± 2 ng/mL 15 min after transfer to bucket 103 ± 17 ng/mL	Fryer (1975)
Water temperature 22 °C or 32 °C	Control fish at 22 °C 41.3, 96.5 and 66.7 ng/mL 32 °C 10 days 96.0 ng/mL; 14 days 106.7 ng/mL; 30 days 56.3 ng/mL	Gandar et al. (2017)
Catch, transfer to tank with water temperature of 35 °C	Control 16 ± 2 ng/mL 15 min after transfer from 20 °C to 35 °C 41 ± 6 ng/mL	Fryer (1975)
Catch, transfer to tank with water temperature of 35 °C, return to original tank after 10 min	Control 16 ± 2 ng/mL 25 min after transfer from home tank at 20 °C to new tank at 35 °C for 10 min then return to home tank at 20 °C 183 ± 35 ng/mL Second experiment with same treatment Control 19 ± 10 ng/mL 25 min after transfer from 20 °C to 35 °C for 10 min 137 ± 27 ng/mL 40 min after transfer from 20 °C to 35 °C for 10 min 163 ± 24 ng/mL 75 min after transfer from 20 °C to 35 °C for 10 min 75 ± 13 ng/mL	Fryer (1975)
Low water temperature	4 weeks at 20 °C 111.0 ± 16.3 ng/mL 4 weeks at 1 °C 7.0 ± 1.0 ng/mL	Paxton et al. (1984)
Low water temperature Short and long photoperiods	A large experiment with male and female goldfish held at 12 °C or 21 °C under LD8:16 or LD16:8 photoperiods. Samples collected every 4 h over 24 h A wide range of patterns of cortisol concentrations over 24 h was seen in fish of different reproductive states. Cortisol was generally higher in fish at 21 °C than in fish at 12 °C. Mean cortisol concentrations varied from ~10 ng/mL to > 300 ng/mL	Peter et al. (1978)
Food restriction	Control ~15 ± 6 ng/mL 80% food restriction 1 day ~10 ± 2 ng/mL; 8 days ~54 ± 9 ng/mL	Bernier et al. (2012)
Food restriction then refeeding	Control ~91 ± 20 ng/mL and ~83 ± 18 ng/mL ~130 ± 20 ng/mL after 50% food restriction for 10 days ~76 ± 10 ng/mL after refeeding for 7 days	Azpeleta et al. (2010)
Starvation High ammonia concentration in water	Control ~36 ± 3 ng/mL; starvation for one week ~34 ± 2 ng/mL (no effect of starvation on mean cortisol) 4 days exposure to high ammonia concentration in water ~47 ± 4 ng/mL (no significant increase in cortisol at 3 h, 12 h or 24 h after start of exposure)	Sinha et al. (2012)
Hypoxia	Control ~15 ± 6 ng/mL 1 day of hypoxia ~29 ± 11 ng/mL; 8 days of hypoxia ~140 ± 60 ng/mL	Bernier et al. (2012)
Varying salinity		Luz et al. (2008)

(*continued on next page*)

Table 1 (*continued*)

Treatments	Cortisol (mean ± S.E)	Reference
	Control 8.1 ± 3.6 ng/mL 3 weeks at 6% salinity 6.8 ± 1.9 ng/mL 3 weeks at 8% salinity 10.3 ± 2.5 ng/mL 3 weeks at 10% salinity 26.6 ± 7.1 ng/mL	
Exposure to predator	Predator fish placed in tank Control ~35 ± 12 ng/mL. After 6 h ~250 ± 30 ng/mL; 12 h ~220 ± 25 ng/mL Predator fish placed in tank with mesh screen between goldfish and predators Control ~12 ± 3 ng/mL. After 2 h ~70 ± 6 ng/mL; 6 h ~10 ± 1 ng/mL; 12 h ~15 ± 3 ng/mL Goldfish fish in tank that sat within larger tank into which predators were placed Control ~75 ± 20 ng/mL. After 6 h ~215 ± 15 ng/mL; 12 h ~130 ± 8 ng/mL after 12 h Water from a tank containing predator fish circulated into goldfish tank Control ~43 ± 10 ng/mL. After 6 h ~28 ± 14 ng/mL; 12 h ~40 ± 8 ng/mL after 12 h	Kagawa and Mugiya (2000)
Loud noise	Control ~35 ng/mL Exposure of fish to loud noise (160–170 dB) did not affect plasma cortisol concentrations measured after 1–21 days of exposure to noise	Smith et al. (2004)
Pesticide added to water	Control fish at 22 °C 41.3, 96.5 and 66.7 ng/mL Pesticide for 6 h 90.1 ng/mL; pesticide for 4 days 138.1 ng/mL; pesticide for 16 days 57.0 ng/mL	Gandar et al. (2017)
Pond water contaminated by oil sand mining	Goldfish placed in cages in outdoor pond for 7 days Control pond ~125 ± 15 ng/mL Contaminated pond ~260 ± 30 ng/mL	Lister et al. (2008)
Cortisol treatment (IP pellet)	Control ~35 ± 2 ng/mL 6 h of cortisol treatment ~500 ng/mL	Bernier et al. (1999)
Cortisol treatment (IP injection)	Control 24.0 ± 10.0 ng/mL 4 days of cortisol treatment at varying doses of cortisol ~70 to ~450 ng/mL	Chasiotis and Kelly (2012)
Saline, CRF, cortisol, and metyrapone treatment	No control 2 h after anaesthesia and ICV injection of saline ~7 ± 3 ng/mL 2 h after anaesthesia and ICV injection of DMSO ~30 ± 10 ng/mL	Kagawa and Mugiya (2002)
Saline and serotonin receptor agonist injection	Control ~17 ± 2 ng/mL and ~7 ± 2 ng/mL 1.5 h after anaesthesia and IP injection of saline ~80 ± 15 ng/mL 8 h after anaesthesia and IP injection of saline ~15 ± 3 ng/mL	Lim et al. (2013)
Melatonin injection	Control ~225 ± 40 ng/mL and ~160 ± 16 ng/mL Daily IP melatonin for 7 days ~93 ± 35 ng/mL Daily ICV melatonin for 4 days ~175 ± 20 ng/mL	Azpeleta et al. (2010)
Melatonin injection Green light (530 nm wavelength) Water temperature 22 °C or 30 °C	Control ~21 ± 1 ng/mL at 22 °C After unknown period of melatonin treatment ~16 ± 1 ng/mL After unknown period of green light treatment ~19 ± 1 ng/mL After unknown period at 30 °C ~50 ± 3 ng/mL Diel rhythm in plasma cortisol, concentrations lower during dark than light periods, amplitude of rhythm ~5 ng/mL at 22 °C and ~20 ng/mL at 30 °C	Jung et al. (2016)

Note: Mean and SE values preceded by ~ have been derived from figures in papers.

concentrations from 28.3 to 1029.9 ng/mL (Cook et al., 2011). The highest individual concentration reported in a fish species is 1927 ng/mL in the chub (*Leuriscus cephalus*; Pottinger et al., 2000).

4.2. Initial cortisol concentrations in goldfish

Fish are very sensitive to stressors. Plasma cortisol concentrations in fish can increase within three minutes when a fish perceives a stimulus to be threatening and cortisol can remain elevated 24 h after a stimulus lasting only 5 min (Cockrem, 2013a). Mean initial cortisol concentrations in goldfish in the current study (4.5 ± 1.0 ng/mL) are the lowest mean cortisol concentrations reported for goldfish. Cortisol concentrations < 10 ng/mL in control fish have been reported in four other studies of goldfish (see Table 1), whereas cortisol concentrations > 10 ng/mL in control fish have been reported in more than 20 goldfish studies (see Table 1). The highest reported mean control concentrations were ~225 ng/mL (Azpeleta et al., 2010).

Clearly the great majority of studies of cortisol in goldfish have been confounded by the collection of initial or control blood samples in conditions where the measured plasma cortisol concentrations did not reflect cortisol concentrations in undisturbed fish. High cortisol concentrations in control samples mean that potential effects of any treatment on cortisol concentrations would be obscured by the sampling conditions. Goldfish are normally housed in tanks containing several fish. Removal of one fish from tank is a stressor for the other goldfish in the tank, so if goldfish are sequentially caught for sampling cortisol will be elevated in fish remaining in the tank.

4.3. Goldfish cortisol responses

Plasma cortisol concentrations have been measured in goldfish subjected to a wide variety of stimuli (Table 1). The stimuli have included air exposure, movement to a new tank, removal from water and sham injection, addition of selenium, potassium permanganate, ammonia, or pesticide to the water, changes in water salinity, changes in water temperature, changes in oxygen content of water, food restriction, starvation, exposure to loud noise, exposure to a predator, and placement in water contaminated by oil sand mining.

4.3.1. Cortisol response to chasing

The current study is the first report of a cortisol response to chasing in goldfish. Goldfish had a large and rapid cortisol response to chasing. Mean plasma cortisol concentrations increased from 4.5 ± 1.0 ng/mL to 138.6 ± 20.1 ng/mL 30 min after the start of a 10 min period of chasing. The cortisol response of goldfish in the current study was greater than responses of some other species of fish to chasing. Juvenile turbot (*Scophthalmus maximus*) chased for 10 min lost equilibrium and no longer responded to chasing. Mean cortisol in these fish increased from 20 ± 4 to 75 ± 9 ng/mL at 30 min after the end of chasing (Van Ham et al., 2003). Cortisol in fish chased for 15 min and then sampled 30 min from the start of chasing increased from 5.7 ± 0.2 ng/mL to 24.4 ± 0.8 ng/mL in rainbow trout (Olsen et al., 2008) and from 4.5 ± 1.8 ng/mL to 59.1 ± 6.8 ng/mL in Atlantic cod (*Gadus morhua*) at 30 min (Tintos et al., 2006).

4.3.2. Cortisol response to air exposure

The magnitude of the cortisol response of goldfish to 3 min air exposure was similar to the magnitude of the response to 10 min stirring. Mean plasma cortisol concentrations increased from 4.5 ± 1.0 ng/mL to 132.6 ± 31.0 ng/mL 15 min after the start of 3 min air exposure and increased further to 151.3 ± 34.2 ng/mL 1 h after the start of exposure. The mean response was smaller than a cortisol increase from ~70 ± 20 ng/mL to ~315 ± 40 ng/mL 1 h after exposure of goldfish to air for 1 min (Dror et al., 2006), and greater than an increase from 70 ± 19 ng/mL to 93 ± 16 ng/mL 16 min after 1 min exposure to air (Fryer, 1975).

Other species of fish also have high cortisol responses to air exposure for as little as 15 s. Cortisol increased from 4 ± 1 ng/mL to 72 ± 10 ng/mL after 1 h in Atlantic salmon (*Salmo salar*) exposed to air for 15 s (Fast et al., 2008), from 8.1 ± 0.7 ng/mL to 398.5 ± 47.1 ng/mL in Sengalese sole (*Solea senegalensis*) exposed to air for 3 min (Silva et al., 2010) and from 35 ± 13 ng/mL to 230 ± 35 ng/mL in red porgy (*Pagrus pagrus*) exposed to air for 5 min (Van der Salm et al., 2006).

4.3.3. Cortisol responses to physical and emotional stressors

Stressors, stimuli that activate the HPA axis, can be classified as physical or emotional (Cockrem, 2007). Physical stressors are signals such as a fall in blood glucose concentrations that arise from the internal or external environment, cause disturbance of physical or chemical tissue parameters and do not involve perception that the signal is a threat. Emotional stressors, also called psychological stressors, are stimuli from the external environment that are perceived to be a threat or a potential threat. Chasing of fish in a tank and exposure to a predator are emotional stressors to which goldfish have been exposed. Goldfish cortisol responses to stirring (~120 to 180 ng/mL from 15 min to 2 h after 10 min chasing; current study) were a little lower than the response to the presence of predator fish in a goldfish tank (control ~35 ± 12 ng/mL, ~250 ± 30 ng/mL after 6 h) and the response to the sight of predators when a goldfish tank was placed in a larger tank containing predator fish (control ~75 ± 20 ng/mL, ~215 ± 15 ng/mL after 6 h; Kagawa and Mugiya, 2000).

Goldfish cortisol responses have been reported to physical stressors that include short term air exposure, increases in water temperature, reductions in water quality, changes in water salinity, food restriction, starvation and hypoxia (see Table 1 for details). The published results show that highest cortisol responses of goldfish to emotional stressors are similar to the highest responses to physical stressors.

4.4. Cortisol and water temperature in goldfish

Goldfish have a wide range of temperature tolerances compared with other fish species. A mean lower critical temperature of 0.3 °C at an acclimation water temperature of 5 °C (Ford and Beitinger, 2005) and mean upper critical temperatures of 37.2 ± 0.7 °C at an acclimation water temperature of 12 °C and 44.7 ± 11.8 °C at an acclimation water temperature of 28 °C (Ferreira et al., 2014) have been reported for goldfish. Plasma cortisol concentrations were lower in cold than in warm acclimated goldfish (water temperatures 1 and 20 °C; cortisol 7 ± 1 cf. 111 ± 16 ng/mL; Paxton et al., 1984). Similarly, the amplitude of a diel rhythm in plasma cortisol concentrations was lower at 22 than 30 °C (~5 cf. 20 ng/mL; Jung et al., 2016). These results are consistent with those from the current study in which cortisol was higher in goldfish after several hours of exposure to increased water temperatures.

Plasma cortisol concentrations in goldfish in the current study increased from 18 ng/ml to 22.2 ± 7.6 ng/ml when the water temperature increased by 3 °C over 15 min. The increase in cortisol 15 min after an increase in water temperature in the current study is the earliest reported increase in plasma cortisol in response to an increase in water temperature in fish. Cortisol in Senegalese sole increased from ~7 to 32 ng/mL after an increase in water temperature over 1 h from 18 to 24 °C (Benitez-Dorta et al., 2017). Rainbow trout that experienced an increase in water temperature over 1.5 h from 13 to 23 °C had a large increase in cortisol from ~20 to 180 ng/mL after 30 min. Cortisol remained high after 1 h at 23 °C and was still high 5 h after the water temperature was lowered back to 13 °C (LeBlanc et al., 2012). Cortisol in juvenile cutthroat trout increased from 20 ± 5 to 71 ± 8 ng/mL 25 min from the start of an increase in water temperature (Strange et al., 1977).

5. Conclusions

Most species of fish are ectothermic so their body temperature varies with changes in water temperature. The extent to which a species of fish can show phenotypic plasticity and increase its critical thermal maximum as the water temperature increases will determine the ability of that species of fish to adjust to increasing water temperatures that are happening due to climate change. Cortisol measurements can be used to indicate the responsiveness of fish to changes in water temperature. Goldfish have rapid cortisol responses both to emotional stressors such as chasing and to physical stressors such as increasing water temperature and will be a convenient study species for the development of studies of plasticity in responses of fish to changes in water temperature.

Acknowledgements

We are grateful to Dr Shunichi Maruno, former Dean of the Faculty of Arts and Science, Kyushu University for support for this research, and to Kyushu University for a Visiting Research Professorship for Dr Cockrem. This study was partly supported by JSPS KHAKENHI (Grant Numbers JP15K07694 and JP18K19271) and by a grant from the Kieikai 2015 Research Foundation, Japan to VSC.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.09.017.

References

Azpeleta, C., Martinez-Alvarez, R.M., Delgado, M.J., Isorna, E., De Pedro, N., 2010. Melatonin reduces locomotor activity and circulating cortisol in goldfish. Horm. Behav. 57, 323–329.

Barton, B.A., Schreck, C.B., 1987. Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 62, 299–310.

Benitez-Dorta, V., Caballero, M.J., Betancor, M.B., Manchado, M., Tort, L., Torrecillas, S., Zamorano, M.J., Izquierdo, M., Montero, D., 2017. Effects of thermal stress on the expression of glucocorticoid receptor complex linked genes in Senegalese sole (*Solea senegalensis*): acute and adaptive stress responses. Gen. Comp. Endocrinol. 252, 173–185.

Bernier, N.J., Lin, X.W., Peter, R.E., 1999. Differential expression of corticotropin-releasing factor (CRF) and urotensin I precursor genes, and evidence of CRF gene expression regulated by cortisol in goldfish brain. Gen. Comp. Endocrinol. 116, 461–477.

Bernier, N.J., Bedard, N., Peter, R.E., 2004. Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. Gen. Comp. Endocrinol. 135, 230–240.

Bernier, N.J., Gorissen, M., Flik, G., 2012. Differential effects of chronic hypoxia and feed restriction on the expression of leptin and its receptor, food intake regulation and the endocrine stress response in common carp. J. Exp. Biol. 215, 2273–2282.

Chasiotis, H., Kelly, S.P., 2012. Effects of elevated circulating cortisol levels on hydromineral status and gill tight junction protein abundance in the stenohaline goldfish. Gen. Comp. Endocrinol. 175, 277–283.

Choi, Y.J., Yang, S.G., Jung, M.M., Kim, B.S., Yun, S.G., Choi, C.Y., 2015. Effects of waterborne selenium on toxic and physiological stress response in goldfish, *Carassius auratus*. Mol. Cell. Toxicol. 11, 35–46.

Cockrem, J.F., 2007. Stress, corticosterone responses and avian personalities. J. Ornithol. 148, S169–S178.

Cockrem, J.F., 2013a. Individual variation in glucocorticoid stress responses in animals. Gen. Comp. Endocrinol. 181, 45–58.

Cockrem, J.F., 2013b. Corticosterone responses and personality in birds: individual

variation and the ability to cope with environmental changes due to climate change. Gen. Comp. Endocrinol. 190, 153–163.
Cook, A.F., Stacey, N.E., Peter, R.E., 1980. Periovulatory changes in serum cortisol levels in the goldfish, *Carassius auratus*. Gen. Comp. Endocrinol. 40, 507–510.
Cook, K.V., O'Connor, C.M., Gilmour, K.M., Cooke, S.J., 2011. The glucocorticoid stress response is repeatable between years in a wild teleost fish. J. Comp. Physiol. A 197, 1189–1196.
Dror, M., Sinyakov, M.S., Okun, E., Dym, M., Sredni, B., Avtalion, R.R., 2006. Experimental handling stress as infection-facilitating factor for the goldfish ulcerative disease. Vet. Immunol. Immunopathol. 109, 279–287.
Eslamloo, K., Akhavan, S.R., Fallah, F.J., Henry, M.A., 2014. Variations of physiological and innate immunological responses in goldfish (*Carassius auratus*) subjected to recurrent acute stress. Fish Shellfish Immunol. 37, 147–153.
Fast, M.D., Hosoya, S., Johnson, S.C., Afonso, L.O.B., 2008. Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short- and long-term stress. Fish Shellfish Immunol. 24, 194–204.
Ferreira, E.O., Anttila, K., Farrell, A.P., 2014. Thermal optima and tolerance in the eurythermic goldfish (*Carassius auratus*): relationships between whole-animal aerobic capacity and maximum heart rate. Physiol. Biochem. Zool. 87, 599–611.
Ford, T., Beitinger, T.L., 2005. Temperature tolerance in the goldfish, *Carassius auratus*. J. Therm. Biol. 30, 147–152.
Fryer, J.N., 1975. Stress and adrenocorticosteroid dynamics in goldfish, *Carassius auratus*. Can. J. Zool. 53, 1012–1020.
Gandar, A., Laffaille, P., Canlet, C., Tremblay-Franco, M., Gautier, R., Perrault, A., Gress, L., Mormede, P., Tapie, N., Budzinski, H., Jean, S., 2017. Adaptive response under multiple stress exposure in fish: from the molecular to individual level. Chemosphere 188, 60–72.
Hoseini, S.M., Tarkhani, R., 2013. Effect of short-term treatment with potassium permanganate on stress markers and blood biochemistry in goldfish *Carassius auratus*. Aquacult. Res. 44, 869–875.
Jung, S.J., Kim, N.N., Choi, Y.J., Choi, J.Y., Choi, Y.U., Heo, Y.S., Choi, C.Y., 2016. Effects of melatonin and green-wavelength LED light on the physiological stress and immunity of goldfish, *Carassius auratus*, exposed to high water temperature. Fish Physiol. Biochem. 42, 1335–1346.
Kagawa, N., Mugiya, Y., 2000. Exposure of goldfish (*Carassius auratus*) to bluegills (*Lepomis macrochirus*) enhances expression of stress protein 70 mRNA in the brains and increases plasma cortisol levels. Zool. Sci. 17, 1061–1066.
Kagawa, N., Mugiya, Y., 2002. Brain HSP70 mRNA expression is linked with plasma cortisol levels in goldfish (*Carassius auratus*) exposed to a potential predator. Zool. Sci. 19, 735–740.
LeBlanc, S., Hoglund, E., Gilmour, K.M., Currie, S., 2012. Hormonal modulation of the heat shock response: insights from fish with divergent cortisol stress responses. Am. J. Physiol. Regul. Integr. Comp. Physiol. 302, R184–R192.
Lim, J.E., Porteus, C.S., Bernier, N.J., 2013. Serotonin directly stimulates cortisol secretion from the interrenals in goldfish. Gen. Comp. Endocrinol. 192, 246–255.
Lister, A., Nero, V., Farwell, A., Dixon, D.G., Van der Kraak, G., 2008. Reproductive and stress hormone levels in goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Aquat. Toxicol. 87, 170–177.
Luz, R.K., Martinez-Alvarez, R.M., De Pedro, N., Delgado, M.J., 2008. Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. Aquaculture 276, 171–178.
Lyytikäinen, T., Pylkkö, P., Ritola, O., Lindström-Seppä, P., 2002. The effect of acute stress and temperature on plasma cortisol and ion concentrations and growth of Lake Inari Arctic charr, *Salvelinus alpinus*. Environ. Biol. Fishes 64, 195–202.
Martins, C.I.M., Silva, P.I.M., Conceicao, L.E.C., Costas, B., Hoglund, E., Overli, O., Schrama, J.W., 2011. Linking fearfulness and coping styles in fish. PLoS One 6, e28084.
National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011. Guide for the Care and Use of Laboratory Animals, eighth ed. National Academies Press, Washington, DC.
Olsen, R.E., Sundell, K., Ringo, E., Myklebust, R., Hemre, G.I., Hansen, T., Karlsen, O., 2008. The acute stress response in fed and food deprived Atlantic cod, *Gadus morhua* L. Aquaculture 280, 232–241.
Overli, O., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers, C.H., Nilsson, G.E., 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neurosci. Biobehav. Rev. 31, 396–412.
Pankhurst, N.W., 2011. The endocrinology of stress in fish: an environmental perspective. Gen. Comp. Endocrinol. 170, 265–275.
Paxton, R., Gist, D.H., Umminger, B.L., 1984. Serum cortisol levels in thermally acclimated goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*) – implications in control of hepatic glycogen metabolism. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 78, 813–816.
Peter, R.E., Hontela, A., Cook, A.F., Paulencu, C.R., 1978. Daily cycles in serum cortisol levels in goldfish – effects of photoperiod, temperature, and sexual condition. Can. J. Zool. 56, 2443–2448.
Pottinger, T.G., Pickering, A.D., Hurley, M.A., 1992. Consistency in the stress response of individuals of 2 strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 103, 275–289.
Pottinger, T.G., Carrick, T.R., 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. Gen. Comp. Endocrinol. 116, 122–132.
Pottinger, T.G., Carrick, T.R., Appleby, A., Yeomans, W.E., 2000. High blood cortisol levels and low cortisol receptor affinity: is the chub, *Leuciscus cephalus,* a cortisol-resistant teleost? Gen. Comp. Endocrinol. 120, 108–117.
Silva, P.I.M., Martins, C.I.M., Engrola, S., Marino, G., Overli, O., Conceicao, L.E.C., 2010. Individual differences in cortisol levels and behaviour of Senegalese sole (*Solea senegalensis*) juveniles: evidence for coping styles. Appl. Anim. Behav. Sci. 124, 75–81.
Singley, J.A., Chavin, W., 1975. Serum cortisol in normal goldfish (*Carassius auratus* L.). Comp. Biochem. Physiol. 50, 77–82.
Sinha, A.K., Liew, H.J., Diricx, M., Kumar, V., Darras, V.M., Blust, R., De Boeck, G., 2012. Combined effects of high environmental ammonia, starvation and exercise on hormonal and ion-regulatory response in goldfish (*Carassius auratus* L.). Aquat. Toxicol. 114, 153–164.
Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). J. Exp. Biol. 207, 427–435.
Spieler, R.E., 1974. Short term serum cortisol concentrations in goldfish (*Carassius auratus*) subjected to serial sampling and restraint. J. Fish. Res. Board Can. 31, 1240–1242.
Strange, R.J., Schreck, C.B., Golden, J.T., 1977. Corticoid stress responses to handling and temperature in salmonids. Trans. Am. Fish. Soc. 106, 213–218.
Strange, R.J., 1980. Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. Trans. Am. Fish. Soc. 109, 298–303.
Tintos, A., Miguez, J.M., Mancera, J.M., Soengas, J.L., 2006. Development of a microtitre plate indirect ELISA for measuring cortisol in teleosts, and evaluation of stress responses in rainbow trout and gilthead sea bream. J. Fish Biol. 68, 251–263.
Van der Salm, A.L., Pavlidis, M., Flik, G., Bonga, S.E.W., 2006. The acute stress response of red porgy, *Pagrus pagrus*, kept on a red or white background. Gen. Comp. Endocrinol. 145, 247–253.
Van Ham, E.H., Van Anholt, R.D., Kruitwagen, G., Imsland, A.K., Foss, A., Sveinsbo, B.O., FitzGerald, R., Parpoura, A.C., Stefansson, S.O., Bonga, S.E.W., 2003. Environment affects stress in exercised turbot. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 136, 525–538.