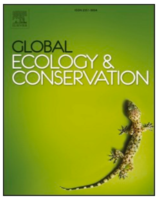
Global Ecology and Conservation 54 (2024) e03086

Contents lists available at ScienceDirect

Global Ecology and Conservation

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



A novel method to measure the impact of water quality on judgement bias in wild juvenile fish

Rafael Freire[a,*], Christine J. Nicol[b]

[a] Gulbali Institute, Charles Sturt University, Elizabeth Mitchell Drive, Albury, NSW 2640, Australia
[b] Royal Veterinary College, University of London, 4 Royal College Street, London NW1 0TU, United Kingdom

ARTICLE INFO

Keywords:
Fish
Behavior
Affect
Welfare
Decision-making

ABSTRACT

Methods to examine judgement bias in free-living animals *in situ* are required to address ecological, conservation and animal welfare questions. Wild animals make behavioural decisions based on complex information, and judgement bias is an experience-induced adjustment in the cognitive appraisal of ambiguous information. Following on from recent research showing judgement bias in fish, we developed a novel approach to measure population-level judgement bias using the natural tendency of juvenile Murray cod (*Maccullochella peelii*) to approach a light-source, but move away from larger, potentially predator fish. Population-level judgement bias was determined from the number of Murray cod caught in three slightly different light traps containing; 1) a light-only (positive stimulus), 2) a predator-model (negative stimulus) and 3) an ambiguous-model (ambiguous stimulus). Ten water quality parameters were also recorded at each site. All combinations of water quality parameters were included in models to examine how well they explained (i) the presence of Murray cod and (ii) in sites where Murray cod were present the population-level judgement bias. The best models were selected using Akaike's Information Criterion. We caught 113 Murray cod at 19 out of 33 sites and modelling highlighted the importance of dissolved oxygen (P=0.02–0.05 in top logistic models) to explain presence/absence of fish, confirming the threat of low dissolved oxygen for this species. More Murray cod were caught in light-only (positive stimulus) traps than in predator-model (negative stimulus) traps (P=0.04). Population-level judgement bias was overall negative, indicating a general tendency to avoid the ambiguous-model light trap. The top linear model (AICc=57.71, R^2=0.63, P=0.025) indicated that in combination, there was greater avoidance of the ambiguous stimulus (i.e. a more pessimistic response) as salinity (P=0.043) and filterable reactive phosphorous increased (P=0.055) and pH decreased (P=0.013). The above water quality parameters were not near known lethal levels, indicating a need to better understand the sub-lethal effects of water parameters on fish behaviour and physiology. Our findings indicate that methods to measure population-level judgement bias can support research on the function of judgement bias and its possible relation to affect in fish. More generally, the method provides a potentially useful tool to bring together conservation biology and animal welfare disciplines.

---

* Correspondence to: Gulbali Institute, Charles Sturt University, Elizabeth Mitchell Drive, Albury, NSW 2640, Australia.
E-mail address: rfreire@csu.edu.au (R. Freire).

https://doi.org/10.1016/j.gecco.2024.e03086
Received 6 April 2024; Received in revised form 17 June 2024; Accepted 9 July 2024

1. Introduction

Here we apply concepts and methods from captive animal welfare science to investigate the mental processes that wild animals use to make behavioural decisions when presented with diverse and often ambiguous information. Judgement bias is an experience-induced adjustment in the cognitive appraisal of ambiguous information. In animals, judgement bias can result in a shift to a positive (optimistic) or negative (pessimistic) behavioral response (recent review by Lagisz et al., 2020). Judgement biases have been hypothesised to serve an evolutionary function by directing an individual's behavior towards the most fitness-relevant activities (Bateson, 2016). In a typical go/no-go judgement bias test (Harding et al., 2004), animals are first trained to distinguish between a positive and a negative stimulus and, following experimenter-applied experience, the animal's subsequent response to intermediate, ambiguous stimuli is assessed. The typical go/no-go judgement bias test involves extensive, time-consuming training in a carefully controlled laboratory environment (Bethell, 2015). The challenge, as we seek to expand our understanding of the function of judgement biases is that research methods currently used are poorly suited to studying free-living wild animals (but see Brilot et al., 2009 for an exception). Therefore, in order to examine the function and ecological significance of judgement biases, new methods are required that allow testing of judgement bias in free-living animals *in situ* with minimal experimenter interference.

In addition, the study of judgement bias in free-living animals can be expected to support conservation efforts and activities. Although not frequently considered, threats to the survival and fitness of wild animals in many cases also influence the welfare of individuals. Assessment of wild animal welfare can help quantify the challenge that wild animals face and may provide a useful early-warning indicator of animals at risk (Kirkwood et al., 1994). A wide range of indicators can be used to assess animal welfare though the positive and negative affective experiences of an animal have been considered a central determinant of animal welfare (Mellor and Beausoleil, 2015). In recent years, much discussion has centred on whether judgement biases, and the corresponding changes in decision-making, are modulated by changes in affective state (Nettle and Bateson, 2012; Bateson, 2016; Gygax, 2017; Lagisz et al., 2020; Mendl and Paul, 2020). Objectively assessing affective state in animals is an important aim of animal welfare science (Mellor and Beausoleil, 2015), but in the absence of methods to examine it in free-living wild animals, research on the welfare of wild animals has become dissociated from both other areas of animal welfare science and conservation biology (Beausoleil et al., 2018). One area where such methods could be particularly beneficial is in the conservation of freshwater fish, many of which are threatened (Closs et al., 2016).

Anthropogenic factors such as flow modification, overexploitation, water pollution, destruction or degradation of habitat and invasion by exotic predators have caused severe decline in freshwater fish populations around the world (See Darwall and Freyhof 2016 for a global overview). Conservation and fisheries research report considerable variation in the proportion of larval fish that transition to older life stages capable of reproducing, a process called recruitment, even within the same freshwater system (Ludsin et al., 2014). The causes of this variation have proved elusive (King et al., 2003) but changes in water quality mediated by dissolved oxygen, pH, turbidity, nitrogen and phosphorous can impact fish behavior and physiology (Jensen, 2003; Alabaster and Lloyd, 2013).

Although the impact of water quality on fish judgement bias has not been studied, negative judgement biases have recently been reported in fish paired with non-preferred partners (convict cichlid, *A. sequia*, Laubu et al., 2019), after aggressive encounters (Murray cod, *Maccullochella peelii,* Rogers et al., 2020) and in fish with shorter telomere lengths (zebrafish, *D. rerio*, Espigares et al., 2021). Since shorter telomeres, often associated with reduced welfare, have been reported in European chub (*Squalius cephalus)* living in polluted rivers (Molbert et al., 2021), this raises the yet unexplored possibility that environmental pollution may lead to judgement bias in wild animals. In contrast, there is already clear evidence that environmental pollutants are linked to both shorter telomeres (Zhao et al., 2018) and impaired mental health and negative mood in humans (Klompmaker et al., 2019; Ventriglio et al., 2021), though to our knowledge the impact of pollution on judgement bias in humans has not been studied. In addition, the causal link, if any, between telomere length and pollution in animals and humans is unclear. In order to begin to disentangle the possible causal links between environmental pollution and affective state, in humans and animals, research in the effect of environmental pollution on judgement bias would be particularly useful.

Murray cod is a keystone species that lives in the Murray Darling Basin (MDB) in Southeast Australia. In common with many large freshwater ecosystems around the world, fish populations in this system have declined due to anthropogenic factors by >90 % over the past 150 years with ~50 % of species now of conservation concern (Morrongiello et al., 2011). Low dissolved oxygen (hypoxic events) has been implicated in fish kills in the MDB (Koehn, 2004), and is often the result of an increase in dissolved organic carbon due to unusual inundation patterns, river regulation and bushfires (Whitworth et al., 2012). Turbidity has also increased in the MDB due in part to bank erosion caused by irrigation flows and the invasive, bottom-feeding carp (*Cyprinus carpio,* Gell et al., 2009). Murray cod are particularly impacted by nitrite levels and extremes in pH (Ingram et al., 2005) and low oxygen levels (McPhee et al., 2023), though are less affected by turbidity (Allen-Ankins et al., 2012). The MDB therefore provides a useful system in which to study the impact of environmental challenges on Murray cod behavior.

This innovative study attempts for the first time to measure judgement bias in wild fish *in situ* in response to variation in water quality. Our proposed method is based on the natural approach/avoidance responses in juvenile Murray cod and builds on our laboratory work showing judgement bias in this species (Rogers et al., 2020). Juvenile Murray cod have highly sensitive vision, able to detect prey in low-light environments and predominantly feed at night by swimming towards a light source (usually moonlight, Humphries, 2005). However, juvenile Murray cod also avoid and swim away from larger, potential predator fish (Sales et al., 2023) but are attracted to similar-sized fish as a form of anti-predator response (Allen-Ankins et al., 2012). By altering the attractiveness of a light-source with the use of nearby different-sized fish models, we used the number of juvenile fish caught in different types of light-traps as an indicator of decision-making.

We predicted that fewer Murray cod would be caught in light-traps with a model 12 cm predator lure suspended near the entrance of the light-trap compared to light-traps without the predator model. The ambiguous stimulus, necessary to examine judgement bias, was a similar light-trap but with a 5 cm model fish near the entrance. By selecting sites with differing water quality for trap placement we were able to model the influence of water quality parameters on the population-level response to the ambiguous stimulus. Our hypothesis was that poorer water quality would be associated with a more negative judgement bias. We consider our findings within the broader contexts of complex decision making in animals and implications for wild animal welfare.

2. Materials and methods

We sampled from 33 locations (coordinates in Supplementary material Table A1) in the Murray Darling Basin (MDB) which were anticipated to be suitable for Murray cod and within 100 km of Deniliquin (New South Wales, Australia, Fig. 1a) in the Spring (September to November) of 2023. The MDB is a large basin draining an area of 1.07 million km^2 westward and flow into our sampling area originates from a variety of sources proving variation in waterways and source of water. The area we selected encompassed the Edward and Wakool rivers, two major anabranches of the Murray river and a large network of inter-connecting smaller rivers, creeks

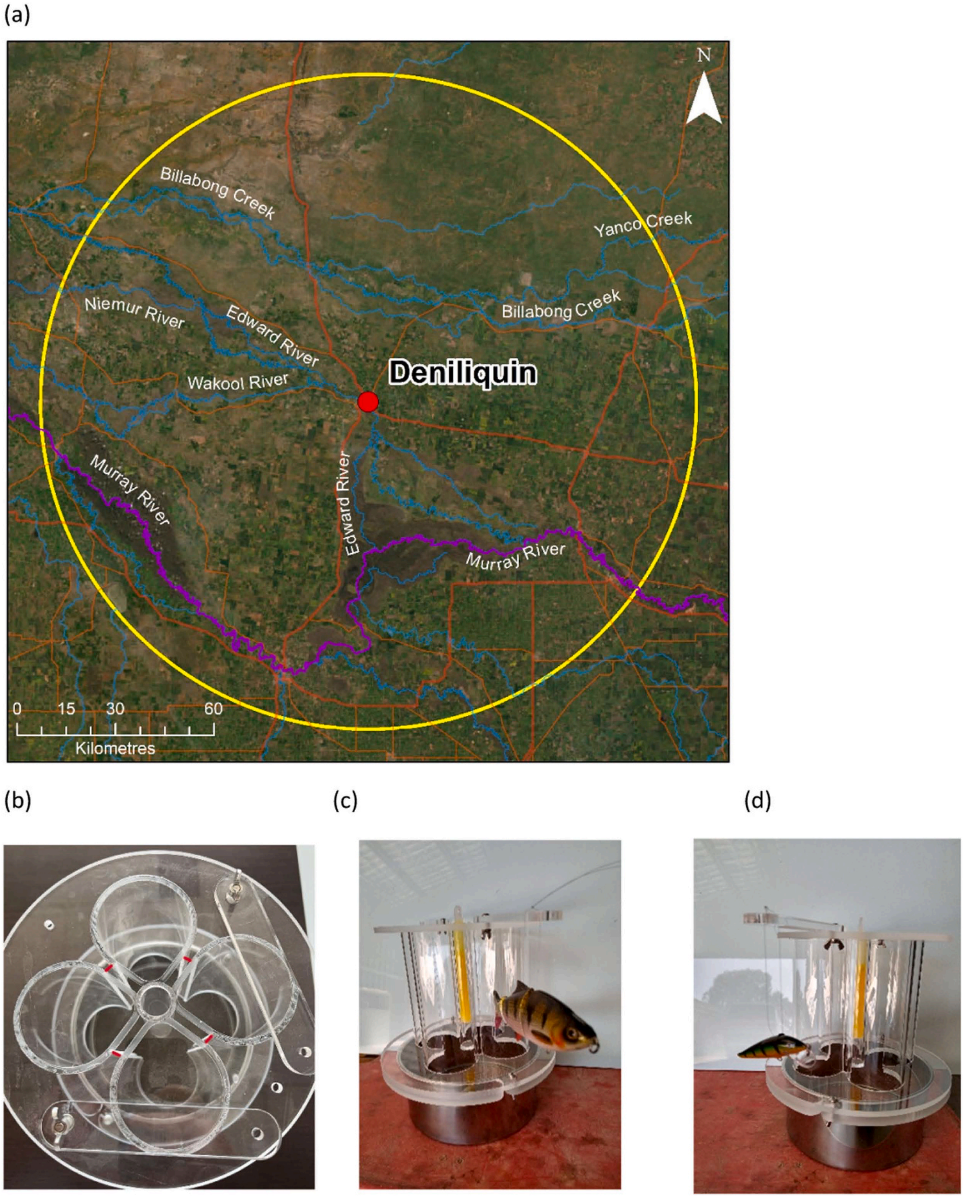


Fig. 1. (a) Map showing the area where fish were sampled; (b) Quatrefoil light trap from above showing 5 mm gaps for fish to enter (marked in red) and central tube for placement of light stick; (c) predator-model trap (negative stimulus) and (d) ambiguous-model trap. Note that only one lure is shown for each trap (c and d), although there were two lures placed at opposite sides of the trap.

and wetlands that were expected to vary in water quality parameters.

At each site, we used a HORIBA® MultiParameter Water Quality Meter (Horiba group, Japan) to record water temperature (^{0}C), pH, conductivity (mS/cm), turbidity (NTU), dissolved oxygen (mg/L) and salinity (ppt). Water parameter measurements were taken between 6 and 8 am to minimise temporal confounding factors. Water samples were also collected at this time from each site and stored in 70 ml sterile jars. The collection method involved passing the water through a 0.45 µm filter following a standard protocol before collection (see Supplementary material for water collection procedure). Water samples were kept frozen until chemical analysis to measure ammonia nitrogen (NH_3-N, µgN/L), oxides of nitrogen (NOx-N, µgN/L), nitrite nitrogen (NO_2-N, µgN/L) and filterable reactive phosphorous (FRP, µgP/L). Chemical analysis was undertaken by the CSIRO laboratory (Albury, NSW; National Association of Testing Authorities No 1400) using a Lachat QuickChem 8500 Flow Injection Analyser (see Supplementary material for analysis of water samples). Methods were based on the Standard Method for the Examination of Water and Waste (Rice et al., 2012) and include running two quality control samples of known concentration, sample duplicates and spiked samples with each batch.

At each site, three Perspex quatrefoil light traps were placed 10 m apart in approximately 50 cm deep, slow-flowing water between 6 and 8 pm. The relative position of each light trap was randomised on each occasion. The traps had a removable 200 µm aluminium mesh tray at the bottom to remove fish. The traps were approximately 30 cm high x 25 cm x 25 cm, and between each of the four tubes were 5 mm gaps so that the fish could enter (Fig. 1b). A yellow Cyalume® 12 h light stick (Omniglow Corporation, W. Springfield, MA, USA), which has been found to be superior at attracting native fish in this area (Gehrke, 1994), was placed in the centre of the tube. The traps were retrieved between 6 and 8 am the following morning, and Murray cod identified and counted. Fish were then transferred to a transparent tray and photographed above a grid for secondary identification and sizing. Fish were then returned to the location where they were caught unharmed. Fish were collected under a NSW Fisheries Scientific collection permit (s37 Research permit FP23/52) and all procedures were approved by Charles Sturt University's Animal Ethics Committee (A22337).

The three traps placed at each site were altered to provide the positive, negative and ambiguous stimuli necessary for assessment of judgement bias. The light-only trap was as described above and provided the positive stimulus (Fig. 1b). For the predator-model trap, two 12 cm European perch (*Perca fluviatilis*) hard-body lures (Rapala, Rapala.com) were attached 10 cm from opposite corners of the light trap via nylon, to provide the negative stimulus (Fig. 1c). The ambiguous stimulus (ambiguous-model trap) involved placing two 5 cm European perch hard-body lures (Rapala countdown, Rapala.com) at opposite corners of the light trap, also 10 cm from the corners (Fig. 1d). Please note that hooks were removed from the lures to avoid harming other fish.

2.1. Statistical analysis

First, with total number of Murray cod caught at each site as the response variable, we initially fitted models using R (R Core Team, 2023) based on all possible combinations of 10 water quality parameters. However, despite trying various distributions including zero-inflated model families, we did not achieve a respectable distribution of residuals. We therefore expressed Murray cod presence as a binomial (presence/absence) variable; 1 for fish caught at site and 0 for fish not caught at site. All combinations of water quality parameters to explain the presence/absence of Murray cod were modelled using multiple logistic regression and ranked according to second-order Akaike's Information Criterion (AICc; Burnham and Anderson, 2002; Hurvich and Tsai, 1989). Models within 2 AICc of the best ranked model are considered to have substantial empirical support and are reported (Burnham & Anderson, 2002). These models were checked for goodness of fit and significance measures (p value, pseudo R^2 and classification table). Due to a problem with our cooler, five water samples were lost and were not analysed, so are treated as missing values.

Second, a key requirement of the judgement bias test is that fish must show a difference in responding to the positive and negative stimuli. It is important to note that "response" here is considered at the population level, with the number of fish caught in each trap indicative of responses of all fish at that site. That is, we expected to catch more fish in the light-only trap (i.e. the positive stimulus) than in the predator-model trap (i.e. the negative stimulus). The number of fish caught in the predator-model and light-only traps at each site was compared using a Wilcoxon signed rank test with continuity correction non-parametric test.

There are two conditions that need to be met before we can determine if water quality influences judgement bias. First, there must be fish at the site. Second, fish at the site must be able to discriminate between the positive and negative stimuli. For these reasons we first removed from the dataset 1) all sites where no fish were caught ($N=14$) and 2) all sites where the number of fish caught in the light-only trap was not greater than the number of fish caught in the predator-model trap ($N=6$). This resulted in a sample of 13 sites to examine population-level judgement bias.

In a judgement bias test, a response to the ambiguous-model stimuli similar to the response to the negative stimuli indicates a stronger tendency to avoid risk (a "pessimistic" response), whereas a response to the ambiguous-model stimuli similar to the response to the positive stimulus indicates more risk-taking (an "optimistic" response). In order to calculate population-level judgement bias (PLJB), we first plotted the number of fish (y axis) caught at each site in the light-only, ambiguous-model and predator-model traps with corresponding x axis values of 1, 2 and 3 respectively. The light-only and predator-model points were joined by a line and the midpoint calculated. The distance of the ambiguous-model point to the midpoint of the line can be positive (indicating a positive PLJB) or negative (indicating a negative PLJB), and was calculated using the formula;

$$\left(PLJB = N(ambiguous - model) - \frac{1}{2}[N(predator - model) + N(light - only)]\right.$$

We fitted models using R of all combinations of water quality parameters with population-level judgement bias (PLJB) as the dependent variable using multiple linear regression and ranked models according to second-order Akaike's Information Criterion (AICc). These models were checked for goodness of fit and significance measures (p value, pseudo R^2 and classification table).

3. Results

3.1. *Presence/absence of juvenile Murray cod*

We collected 113 juvenile Murray cod ranging in length from 1 to 4 cm from 19 sites. We caught 56 (range 0–12), 32 (range 0–9) and 25 (range 0–8) Murray cod in light-only, ambiguous-model and predator-model light traps respectively (Table 1). Mean and range of water quality parameters is provided in Supplementary materials (Table A2).

Ten models were within 2 AICc of the best model for predicting the presence/absence of Murray cod (Table 2). Dissolved oxygen was included in all 10 models, with a coefficient range of 0.89–1.19 and was significant in 8 of the 10 models (P range 0.02–0.05). The best model was perhaps number 8, with the highest pseudo R^2 (0.41) and highest correct fitting of absence (83.3 %) and presence (90.0 %) of Murray cod. This latter model included dissolved oxygen (coefficient 1.11±0.51, z=2.12, P=0.03), ammonia nitrogen (coefficient 0.12±0.09, z=2.08, P=0.16), nitrite nitrogen (coefficient 0.83±0.69, z=1.21, P=0.23) and conductivity (coefficient 18.30 ±0.13.92, z=2.14, P=0.19). Details of 10 top models in Supplementary material.

3.2. *Population-level judgement bias*

Across all 19 sites where fish were caught, we collected 1.7±0.48, 0.97±0.38 and 0.76±0.35 Murray cod from the light-only, ambiguous-model and predator-model traps respectively. We collected significantly more Murray cod from the light-only trap than the predator-model trap (Wilcoxon, V=108, N=33, P=0.040; Fig. 2).

Thirteen sites met both conditions for inclusion in our judgement bias modelling, and at these sites population-level judgement bias was on average −0.96 (range −4 to +4), with a negative PLJB recorded at 12 of the 13 sites (Table 1). Although there were 9 models within 2 AICc of the best model (see Supplementary material), the high R^2 and significant P value (AICc=57.71, R^2=0.63, P=0.025) suggests that the top model was superior to others. This latter model included FRP (coefficient −0.19±0.0.09, t=-2.98, P=0.055), pH (coefficient 5.11±0.1.66, t=3.09, P=0.013) and salinity (coefficient −14.93±6.33, t=-2.36, P=0.043). FRP varied between 7 and 21 μgP/L, pH between 6.98 and 7.72 and salinity between 0 and 0.2 ppt. Coefficients indicate that in combination, fish were more likely to avoid the ambiguous-model stimuli (i.e. showed a more pessimistic response) as salinity and FRP increase, and pH decreases (Fig. 3).

4. Discussion

We found that significantly more Murray cod were caught in the light-only (positive stimulus) than in the predator-model (negative stimulus) light-trap, fulfilling an important requirement of a judgement bias task. Our results support previous work showing that juvenile Murray cod are attracted to light (Humphries, 2005) and extend laboratory work by showing that wild juvenile Murray cod also avoid model predators. In this respect, the population-level measure of approach and avoidance of the positive and negative stimuli respectively was comparable to the responses to positive and negative stimuli in individual fish (Laubu et al., 2019; Espigares

Table 1
Number of juvenile Murray cod caught in the three light-traps at 19 sites and population-level judgement bias (PLJB). More fish were caught in the light-only trap than the predator-model trap at 13 sites and were included in the judgement bias (JB) analysis. The six sites where more fish were not caught in the light-only trap than in the predator-model trap that were excluded from the JB analysis is also shown.

Site	Light-only trap	Ambiguous-model trap	Predator-model trap	PLJB	JB
2	7	0	1	-4	Yes
4	1	0	0	-0.5	Yes
10	4	2	1	-0.5	Yes
23	3	0	0	-1.5	Yes
24	3	1	0	-0.5	Yes
25	5	0	1	-3	Yes
11	4	1	0	-1	Yes
14	12	9	8	-1	Yes
29	4	1	0	-1	Yes
20	6	1	1	-2.5	Yes
21	1	0	0	-0.5	Yes
28	1	0	0	-0.5	Yes
33	2	5	0	+4	Yes
Sub-total	**53**	**20**	**12**	**-0.96**	
3	3	1	8	-4.5*	No
5	0	0	4	-2*	No
13	0	2	0	2*	No
19	0	1	0	1*	No
30	0	8	0	8*	No
36	0	0	1	-0.5*	No
Sub-total	**3**	**12**	**13**	**+0.67**	
Total	**56**	**32**	**25**	**-0.45**	

Footnote: * Population-level judgement bias is shown for the six sites excluded from the analysis for completeness, but care should be taken in interpreting these figures since there were few fish caught in the light-only trap.

Table 2

Ten models within 2 AICc of the best model to predict presence/absence of juvenile Murray cod at 33 sites.

Model	AICc	Pseudo R^2	% Correct	P
1) FRP+DO	34.03	0.30	78.6	0.009
2) NH_3-N+Conductivity+DO	34.21	0.36	82.1	0.007
3) FRP+NO_2-N, DO	34.93	0.36	85.7	0.01
4) FRP+turbidity+DO	34.95	0.34	78.6	0.01
5) NO_2-N+Conductivity+DO	35.12	0.34	85.7	0.01
6) FRP+Conductivity+DO	35.20	0.34	78.6	0.01
7) NH_3-N+FRP+DO	35.43	0.34	82.1	0.01
8) NH_3-N+ NO_2-N+ Conductivity+DO	35.67	0.41	85.7	0.009
9) NH_3-N+DO	35.73	0.25	71.4	0.02
10) NH_3-N+turbidity+DO	35.93	0.32	78.6	0.02

Footnote: Conductivity (mS/cm), turbidity (NTU), dissolved oxygen (DO, mg/L), ammonia nitrogen (NH_3-N, μgN/L), nitrite nitrogen (NO_2-N, μgN/L) and filterable reactive phosphorous (FRP, μgP/L). The % correct is overall percentage of zeros and ones correctly predicted by the model. The P value is representative of the significant difference to the null model.

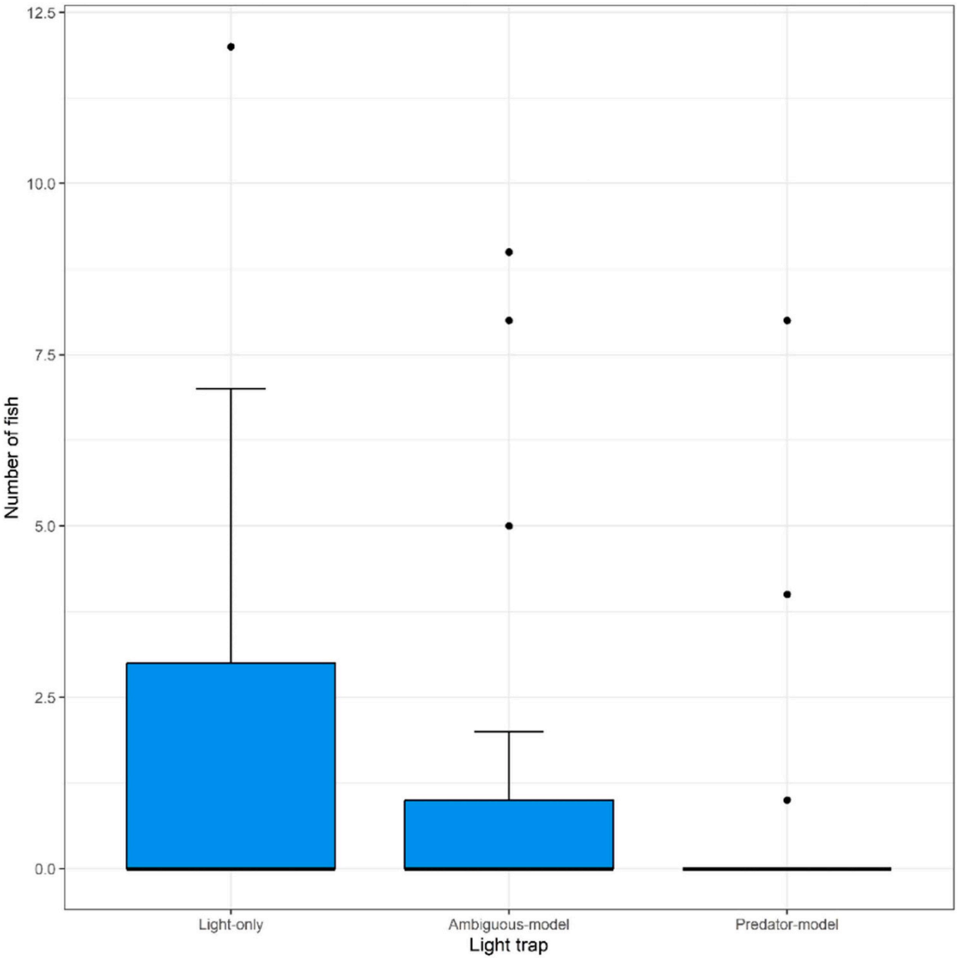


Fig. 2. Boxplot of the number of juvenile Murray cod caught in each of the three light-traps.

et al., 2021; Rogers et al., 2020). The negative score for population-level judgement bias in 12 out of 13 eligible sites, and mean PLJB of −0.96, indicated an overall tendency to avoid the ambiguous stimuli (i.e. a "pessimistic" response). Juvenile Murray cod are predated by other fish, and our findings suggest that they considered the ambiguous stimulus a threat. It is usual in judgement bias tests in the laboratory to have several ambiguous stimuli, ranging from more negative to more positive (Bethell, 2015), to account for variation in the level of ambiguity posed by the ambiguous stimuli. It seems likely, in our study, that the ambiguous stimulus was generally interpreted as a potential predator rather than an approachable fish, explaining the relatively high level of avoidance of the

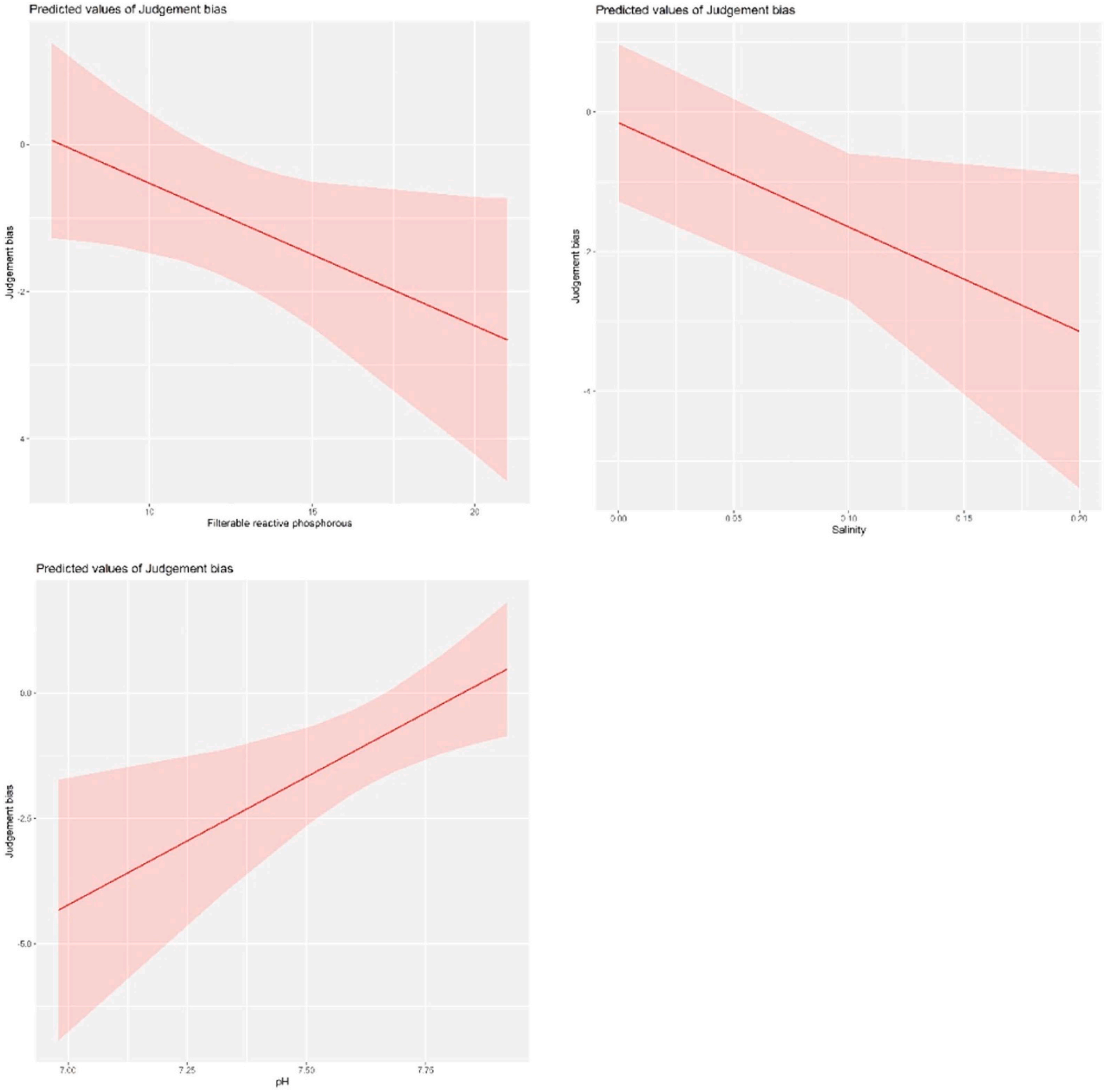


Fig. 3. Partial plots of best-ranked model on the influence of water quality parameters on population-level judgement bias.

ambiguous-model light-trap. In order to facilitate comparison between population-level and individual judgement bias tasks, future tests of population-level judgement bias should consider more than one ambiguous stimulus in order to construct a more informative discrimination curve.

At six sites, we did not catch more fish in the light-only than in the predator-model light trap, and these sites were excluded from judgement bias analysis since they did not display the required difference in response to the positive and negative stimuli. One possible reason for this unexpected finding is that fish do not always avoid potential predators, but instead sometimes approach and inspect them (Brown and Warburton, 1999), and this may have led to them being more likely to be caught in the predator-model trap. Alternatively, since the traps were left in the water for around 12 hours, fish may have altered their behavior with time to be more likely to approach the predator model, whose behavior (i.e. not chasing prey) would have been very unusual. Another important consideration with our population-level measure of judgement bias is that in our study trapped fish may have attracted other fish, thereby reducing the level of perceived “aversiveness” of these traps. This may also explain our finding that at six sites more fish were caught in the predator-model or ambiguous-model light traps than in the light-only trap. Future methods to assess population-level judgement bias should attempt to reduce animals influencing each other’s behavior, as well as reducing changes in response to the stimuli through prolonged exposure. Nonetheless, our findings suggest that population-level measures of judgement bias offer a promising method as we begin to study the evolutionary function of judgement bias. In addition, environmental and anthropogenic factors are likely to impact individuals in a roughly similar manner and our method to examine judgement bias in a free-living wild animal allows us to explore questions of wild animal affect and welfare, thereby aligning conservation and animal welfare objectives (Beausoleil et al., 2018).

Although there was a general tendency to avoid the ambiguous-model light trap, population-level judgement bias score of −4 to +4 suggested sufficient variation between sites to justify further analysis of the role of water quality on PLJB. The top model to explain judgement bias indicated a stronger tendency to avoid risk (i.e. pessimistic response) with increasing FRP and salinity and decreasing pH. Although our sample size was 13 sites, the model that emerged to explain population-level judgement bias was significant with a high R-squared value (0.63). The role of pH in our model to explain judgement bias is perplexing, since pH varied within 6.98 and 7.92; levels suitable for juvenile Murray cod (Ingram et al., 2005). Nonetheless, it is important to note that it was in combination that FRP, pH and salinity yielded a significant model; in isolation these three factors were not significant (Supplementary material).

Filterable reactive phosphorous at the sites where we analysed judgement bias ranged from 7 to 21 μgP/L. These levels of FRP correspond well with previous levels found in permanent waterways in the region (McInerney et al., 2017; Holland et al., 2020) and are generally attributed to leaching and decomposition of forest litter. Although phosphorous is essential for the health of freshwater ecosystems, elevated concentrations can lead to increased algae growth and eutrophication (Hilton et al., 2006). The levels of FRP at our sites have not previously been considered a threat to Murray cod, and total phosphorous has been recorded at 50–3000μgP/L in Murray cod aquaculture (Palmeri et al., 2008). It is however unknown how phosphorous levels impact juvenile Murray cod, with little known about the impact of sub-lethal levels of phosphorous on freshwater fish. Understanding how water quality parameters, particularly in sub-lethal ranges, influence species-specific fish physiology and behavior would be important aims for future research as we begin to explore the welfare of fish in the wild.

The region of this study has a reasonably low annual rainfall (<500 mm) and salinity generally shows an increasing trend, possibly due to saline irrigation returns to the rivers (Jolly et al., 2001; White et al., 2009). Salinity at our sites ranged from 0 to 0.2ppt, well below the estimated isosmotic point for juvenile Murray cod of 5.8ppt (Mellor and Fotedar, 2005). Again, further research is necessary to understand the impact of salinity on freshwater fish behavior and physiology at sub-lethal levels. Freshwater teleost fish respond to increasing salinity by increasing drinking rates in order to maintain osmoregulation (Marshall and Grosell, 2005). One impact of the higher drinking rate is that the gut and gills become more exposed to waterborne chemicals and other water parameters. Our model on the impact of water quality on judgement bias again highlights the importance of interpreting explanatory variables in combination, since it is only when salinity is higher, and perhaps causes increased drinking, that FRP is significant.

It is important to note that water quality parameters may not be acting on judgement bias directly, but instead they may influence other factors of the environment that in turn influence judgement bias. One possible important mediating factor on judgement bias in our study could be prey availability. The diet of young Murray cod consists mainly of microcrustacea such as *cladocerans* and chironomid larvae, and prey availability can be significantly influenced by water flow and its effects on water velocity and quality (Ingram and De Silva, 2007; Kaminskas and Humphries, 2009; King et al., 2009). It is therefore possible that the increased risk-taking behavior we observed at some sites (i.e. the more "optimistic" response) is a result of increased food deprivation and a behavioral adjustment in response to the difficulty in acquiring sufficient food. It has been recognised for some time that food deprivation alters the appraisal of stimuli (Thomas and King, 1959), and more recent work indicates that chronic food restriction in sheep leads to a more "optimistic" response in a judgement bias test (Verbeek et al., 2014). Our findings highlight the importance of obtaining a complete understanding of the impact of all possible factors on the animal, and whether these impacts are direct or indirect, in order to avoid misleading conclusions about the function, mechanism and causes of judgement bias.

A pertinent question as we consider the mechanism and function of judgement bias in fish is whether shifts in the appraisal of ambiguous stimuli arise from changes in affective state. As discussed above, it is first necessary to discount whether the more pessimistic judgement bias we observed in water with higher PRP and salinity, and lower pH, may be related to other effects of water quality. Affective states have been implicated as an important mechanism assisting animals with decisions-making (Mendl and Paul, 2020). In particular, juvenile Murray cod can move considerable distances under their own control (Humphries, 2005), though little is known about the decision-making process determining the timing and distance they move. In addition, if judgement bias, as a possible correlate of affective state, is an indicator of a species struggling in its environment, then selecting a keystone species such as Murray cod (Koehn, 2004) could provide a valuable early-warning indicator of emerging conservation threats to ecosystems. Even if judgement bias in fish is found to reflect affective state, an additional question is whether fish are aware of these states (Mendl et al., 2011). Recent research in bees, for example, indicates that judgement bias may arise through other processes such as shifts in learning and stimulus generalisation (Strang and Muth, 2023). Clearly, uncovering the underlying mechanisms resulting in judgement bias in fish, and possible role of affective states, is essential to avoid inaccurate interpretations of observed judgement bias. Future work revealing the mechanism resulting in judgement bias is also likely to inform the current debate around the ability of fish to experience affective states (Cerqueira et al., 2017).

Very low concentrations of dissolved oxygen have previously been reported in rivers within the distribution range of Murray cod (Dyer et al., 2016), and we observed considerable variation in dissolved oxygen, ranging from 3.56 to 10.12 mg/L (40.9–99.9 %). Murray cod are particularly sensitive to low oxygen levels, possibly related to adaptations to lotic river channels and habitats that are less susceptible to hypoxic events (Small et al., 2014). This sensitivity to low dissolved oxygen was perhaps reflected in our presence/absence analysis since dissolved oxygen was in eight out of 10 of our top models explaining the presence of fish at each site. Absence of juvenile Murray cod could result from parents not accessing those sites and does not imply that juvenile fish did not survive or moved away from these sites. It is also important to note that since our analysis of population-level judgement bias relied on the presence of fish, our failure to find links between dissolved oxygen and judgement bias could be a result of not being able to obtain data from fish experiencing low levels of dissolved oxygen. The possibility that low dissolved oxygen impacts judgement bias in freshwater fish therefore remains an important question for future research.

4.1. Conclusion

In conclusion, the method proposed here shows promise in being able to measure population-level judgement bias in a wild animal *in situ*. In addition, by comparing population-level judgement bias between different sites that vary in water quality parameters, we were able to begin to understand how water quality impacts wild animal judgement bias. Our findings suggest that high phosphorous levels in the sub-lethal range may lead to a more negative judgement bias, though further research is required to elucidate the relationship between water quality and decision-making. We propose that more methods are necessary to measure judgement bias in free-living wild animals, in order to reveal the evolutionary function of judgement bias and align conservation biology and animal welfare efforts to address threats to wild animals.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rafael Freire reports financial support was provided by Wild Animal Initiative. If there are other authors, they 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.

Acknowledgement

This work was supported by a Wild Animal Initiative grant (C-2022-00002) and part of the publication costs were covered by a Charles Sturt open access publishing grant. We thank M Gill, M Clark, A Jones and A Buckley for assistance with fieldwork, J Pengelly for water analysis and S McDonald and A Freire for applied mathematics guidance.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2024.e03086.

References

Alabaster, J.S., & Lloyd, R.S. (2013). *Water quality criteria for freshwater fish* (No. 3117). Elsevier.

Allen-Ankins, S., Stoffels, R.J., Pridmore, P.A., Vogel, M.T., 2012. The effects of turbidity, prey density and environmental complexity on the feeding of juvenile Murray cod Maccullochella peelii. J. Fish. Biol. *80* (1), 195–206.

Bateson, M., 2016. Optimistic and pessimistic biases: a primer for behavioural ecologists. Curr. Opin. Behav. Sci. *12*, 115–121.

Beausoleil, N.J., Mellor, D.J., Baker, L., Baker, S.E., Bellio, M., Clarke, A.S., Zito, S., 2018. "feelings and fitness" not "feelings or fitness"–the raison d'être of conservation welfare, which aligns conservation and animal welfare objectives. Front. Vet. Sci. *5*, 296.

Bethell, E.J., 2015. A "how-to" guide for designing judgment bias studies to assess captive animal welfare. J. Appl. Anim. Welf. Sci. *18* (sup1), S18–S42.

Brilot, B.O., Normandale, C.L., Parkin, A., Bateson, M., 2009. Can we use starlings' aversion to eyespots as the basis for a novel 'cognitive bias' task? Appl. Anim. Behav. Sci. *118* (3-4), 182–190.

Brown, C., Warburton, K., 1999. Differences in timidity and escape responses between predator-naive and predator-sympatric rainbowfish populations. Ethology *105* (6), 491–502.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2 ed. Springer Verlag, New York.

Cerqueira, M., Millot, S., Castanheira, M.F., Félix, A.S., Silva, T., Oliveira, G.A., Oliveira, R.F., 2017. Cognitive appraisal of environmental stimuli induces emotion-like states in fish. Sci. Rep. *7* (1), 13181.

Closs, G.P., Angermeier, P.L., Darwall, W.R., Balcombe, S.R., 2016. Why are freshwater fish so threatened. Conserv. Freshw. Fish. *37*.

Darwall, W.R., Freyhof, J.Ö.R.G., 2016. Lost fishes, who is counting? The extent of the threat to freshwater fish biodiversity. Conserv. Freshw. Fish. 1–36.

Dyer, F., Sangston, D., & Webb, J.A. (2016, January). Water quality and murray cod: Temperature and dissolved oxygen in pools of the upper murrumbidgee river. In *11th International Symposium on Ecohydraulics 2016* (pp. 1-7). Engineers Australia.

Espigares, F., Abad-Tortosa, D., Varela, S.A.M., Ferreira, M.G., Oliveira, R.F., 2021. Short telomeres drive pessimistic judgement bias in zebrafish. Biol. Lett. *17* (3), 20200745.

Gehrke, P., 1994. Influence of light intensity and wavelength on phototactic behaviour of larval silver perch Bidyanus bidyanus and golden perch Macquana ambigua and the effectiveness of light traps. J. Fish. Biol. *44* (5), 741–751.

Gell, P., Fluin, J., Tibby, J., Hancock, G., Harrison, J., Zawadzki, A., Walsh, B., 2009. Anthropogenic acceleration of sediment accretion in lowland floodplain wetlands, Murray–Darling Basin. Aust. *Geomorphol. 108* (1-2), 122–126.

Gygax, L., 2017. Wanting, liking and welfare: The role of affective states in proximate control of behaviour in vertebrates. Ethology *123* (10), 689–704.

Harding, E.J., Paul, E.S., Mendl, M., 2004. Cognitive bias and affective state. Nature *427* (6972), 312-312.

Hilton, J., O'Hare, M., Bowes, M.J., Jones, J.I., 2006. How green is my river? A new paradigm of eutrophication in rivers. Sci. Total Environ. *365* (1-3), 66–83.

Holland, A., McInerney, P.J., Shackleton, M.E., Rees, G.N., Bond, N.R., Silvester, E., 2020. Dissolved organic matter and metabolic dynamics in dryland lowland rivers. Spectrochim. Acta Part A: Mol. Biomol. Spectrosc. *229*, 117871.

Humphries, P., 2005. Spawning time and early life history of Murray cod, Maccullochella peelii (Mitchell) in an Australian river. Environ. Biol. Fish. *72*, 393–407.

Hurvich, C.M., Tsai, C.L., 1989. Regression and time series model selection in small samples. Biometrika *76* (2), 297–307.

Ingram, B.A., De Silva, S.S., 2007. Diet composition and preference of juvenile Murray cod, trout cod and Macquarie perch (Percichthyidae) reared in fertilised earthen ponds. Aquaculture *271* (1-4), 260–270.

Ingram, B.A., Gavine, F.M., & Lawson, P. (2005). *Fish health management guidelines for farmed Murray cod*. Department of Primary Industries.

Jensen, F.B., 2003. Nitrite disrupts multiple physiological functions in aquatic animals. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. *135* (1), 9–24.

Jolly, I.D., Williamson, D.R., Gilfedder, M., Walker, G.R., Morton, R., Robinson, G., McNeill, V., 2001. Historical stream salinity trends and catchment salt balances in the Murray–Darling Basin. Aust. *Mar. Freshw. Res. 52* (1), 53–63.

Kaminskas, S., Humphries, P., 2009. Diet of Murray cod (Maccullochella peelii peelii)(Mitchell) larvae in an Australian lowland river in low flow and high flow years. Hydrobiologia *636*, 449–461.

King, A.J., Humphries, P., Lake, P.S., 2003. Fish recruitment on floodplains: the roles of patterns of flooding and life history characteristics. Can. J. Fish. Aquat. Sci. *60* (7), 773–786.

King, A.J., Tonkin, Z., Mahoney, J., 2009. Environmental flow enhances native fish spawning and recruitment in the Murray River, Australia. River Res. Appl. *25* (10), 1205–1218.

Kirkwood, J.K., Sainsbury, A.W., Bennett, P.M., 1994. The welfare of free-living wild animals: methods of assessment. Anim. Welf. *3* (4), 257–273.

Klompmaker, J.O., Hoek, G., Bloemsma, L.D., Wijga, A.H., van den Brink, C., Brunekreef, B., Janssen, N.A., 2019. Associations of combined exposures to surrounding green, air pollution and traffic noise on mental health. Environ. Int. *129*, 525–537.

Koehn, J.D., 2004. The loss of valuable Murray cod in fish kills: a science and management perspective. Manag. Murray Cod Murray–Darling Basin Statement, Recomm. Support. Pap. 3–4.

Lagisz, M., Zidar, J., Nakagawa, S., Neville, V., Sorato, E., Paul, E.S., Løvlie, H., 2020. Optimism, pessimism and judgement bias in animals: a systematic review and meta-analysis. Neurosci. Biobehav. Rev. *118*, 3–17.

Laubu, C., Louâpre, P., Dechaume-Moncharmont, F.X., 2019. Pair-bonding influences affective state in a monogamous fish species. Proc. R. Soc. B *286* (1904), 20190760.

Ludsin, S.A., DeVanna, K.M., Smith, R.E., 2014. Physical–biological coupling and the challenge of understanding fish recruitment in freshwater lakes. Can. J. Fish. Aquat. Sci. *71* (5), 775–794.

Marshall, W.S., Grosell, M., 2005. Ion transport, osmoregulation, and acid-base balance in homeostasis and reproduction. In: Evans, D.H., Claiborne, J.B. (Eds.), In The Physiology of Fishes. CRC Press, Boca Raton, pp. 177–210.

McInerney, P.J., Stoffels, R.J., Shackleton, M.E., Davey, C.D., 2017. Flooding drives a macroinvertebrate biomass boom in ephemeral floodplain wetlands. Freshwater Science 36 (4), 726–738.

McPhee, D., Watson, J.R., Harding, D.J., Prior, A., Fawcett, J.H., Franklin, C.E., Cramp, R.L., 2023. Body size dictates physiological and behavioural responses to hypoxia and elevated water temperatures in Murray cod (Maccullochella peelii). Conserv. Physiol. *11* (1), coac087.

Mellor, D.J., Beausoleil, N.J., 2015. Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states. Anim. Welf. *24* (3), 241–253.

Mellor, P., Fotedar, R., 2005. Physiological responses of Murray cod (Maccullochella peelii peelii)(Mitchell 1839) larvae and juveniles when cultured in inland saline water. Indian J. Fish. *52* (3), 249–261.

Mendl, M., Paul, E.S., 2020. Animal affect and decision-making. Neurosci. Biobehav. Rev. *112*, 144–163.

Mendl, Michael, Paul, Elizabeth S., Chittka, Lars, 2011. Animal behaviour: emotion in invertebrates? Curr. Biol. 21 (12), R463–R465.

Molbert, N., Angelier, F., Alliot, F., Ribout, C., Goutte, A., 2021. Fish from urban rivers and with high pollutant levels have shorter telomeres. Biol. Lett. *17* (1), 20200819.

Morrongiello, J.R., Beatty, S.J., Bennett, J.C., Crook, D.A., Ikedife, D.N., Kennard, M.J., Rayner, T., 2011. Climate change and its implications for Australia's freshwater fish. Mar. Freshw. Res. *62* (9), 1082–1098.

Nettle, D., Bateson, M., 2012. The evolutionary origins of mood and its disorders. Curr. Biol. *22* (17), R712–R721.

Palmeri, G., Turchini, G.M., Keast, R., Marriott, P.J., Morrison, P., De Silva, S.S., 2008. Effects of starvation and water quality on the purging process of farmed Murray cod (Maccullochella peelii peelii). J. Agric. Food Chem. *56* (19), 9037–9045.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rice, E.W., Bridgewater, L., & American Public Health Association (Eds.). (2012). *Standard methods for the examination of water and wastewater* (Vol. 10). Washington, DC: American public health association.

Rogers, L., Sales, E., Shamsi, S., Kopf, R.K., Freire, R., 2020. Aggressive encounters lead to negative affective state in fish. Plos One *15* (4), e0231330.

Sales, E., Rogers, L., Freire, R., Luiz, O., Kopf, R.K., 2023. Bold-shy personality traits of globally invasive, native and hatchery-reared fish. R. Soc. Open Sci. *10* (8), 231035.

Small, K., Kopf, R.K., Watts, R.J., Howitt, J., 2014. Hypoxia, blackwater and fish kills: experimental lethal oxygen thresholds in juvenile predatory lowland river fishes. PLoS One *9* (4), e94524.

Strang, C., Muth, F., 2023. Judgement bias may be explained by shifts in stimulus response curves. R. Soc. Open Sci. *10* (4), 221322.

Thomas, D.R., King, R.A., 1959. Stimulus generalization as a function of level of motivation. J. Exp. Psychol. *57* (5), 323.

Ventriglio, A., Bellomo, A., di Gioia, I., Di Sabatino, D., Favale, D., De Berardis, D., Cianconi, P., 2021. Environmental pollution and mental health: a narrative review of literature. CNS Spectr. *26* (1), 51–61.

Verbeek, E., Ferguson, D., Lee, C., 2014. Are hungry sheep more pessimistic? The effects of food restriction on cognitive bias and the involvement of ghrelin in its regulation. Physiol. Behav. *123*, 67–75.

White, I., Macdonald, B.C., Somerville, P.D., Wasson, R., 2009. Evaluation of salt sources and loads in the upland areas of the Murray–Darling Basin, Australia. Hydrol. Process.: Int. J. *23* (17), 2485–2495.

Whitworth, K.L., Baldwin, D.S., Kerr, J.L., 2012. Drought, floods and water quality: drivers of a severe hypoxic blackwater event in a major river system (the southern Murray–Darling Basin, Australia). J. Hydrol. *450*, 190–198.

Zhao, B., Vo, H.Q., Johnston, F.H., Negishi, K., 2018. Air pollution and telomere length: a systematic review of 12,058 subjects. Cardiovasc. Diagn. Ther. *8* (4), 480.