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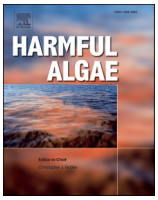

A non-microcystin-producing *Microcystis wesenbergii* strain alters fish food intake by disturbing neuro-endocrine appetite regulation

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ABSTRACT

Cyanobacterial harmful algal blooms (cHABs) are pervasive sources of stress resulting in neurotoxicity in fish. A member of the widely distributed Microcystis genus of bloom-forming cyanobacteria, *Microcystis wesenbergii* can be found in many freshwater lakes, including Dianchi Lake (China), where it has become one of the dominant contributors to the lake's recurrent blooms. However, unlike its more well-known counterpart *M. aeruginosa*, the effects of dense non-microcystin-containing *M. wesenbergii* blooms are seldom studied. The disturbance of appetite regulation and feeding behaviour can have downstream effects on the growth of teleost fish, posing a significant challenge to aquaculture and conservation efforts. Here we examined the effects of *M. wesenbergii* blooms on the food intake of *Acrossocheilus yunnanensis*, a native cyprinid in southern China. This fish species has disappeared in Dianchi Lake, and its reintroduction might be negatively affected by the presence of this newly-dominant *Microcystis* species. We co-cultured juvenile *A. yunnanensis* with a non-microcystin-producing strain of *M. wesenbergii* at initial densities between 5×10^4 and 1×10^6 cells/mL and monitored fish feeding behaviour and changes in neurotransmitter and hormone protein levels. High-density *M. wesenbergii* cultures increased the feeding rate of co-cultured fish, elevating concentrations of appetite-stimulating signalling molecules (Agouti-related protein and γ-aminobutyric acid), while decreasing inhibitory ones (POMC). These changes coincided with histopathological alterations and reduced somatic indices in brain and intestinal tissues. Given this potential for detrimental effects and dysregulation of food intake, further studies are necessary to determine the impacts of chronic exposure of *M. wesenbergii* in wild fish.

1. Introduction

Cyanobacterial harmful algal blooms (cHABs) are pervasive sources of stress within freshwater environments, harming an array of taxa including fish. Increased turbidity, benthic oxygen depletion, and the production of foul surface scum during heavy bloom events degrade both pelagic and near-shore habitats (Havens, 2008; Pick, 2016). At the same time, fish in bloom-affected areas must contend with a variety of toxins and other metabolites produced and released by cyanobacterial species, many of which are known to have hepatotoxic (Malbrouck and Kestemont, 2006), immunotoxic (Lin et al., 2021), neurotoxic (Faltermann et al., 2014), and teratogenic effects (Li et al., 2021).

In fish, regulation of growth through the growth hormone (GH) /insulin-like growth factor (IGF) axis (GH/IGF) is strongly influenced by physiological responses to environmental stressors, including those induced by cHABs (Canosa and Bertucci, 2023), and monitoring effects of algal blooms on somatic growth regulation has been integrated into current toxicological research. Indeed, in both larval and adult fish,

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exposure to the cyanobacterial species *Microcystis aeruginosa* decreased expression of both GH and IGF and their associated receptors (Chen et al., 2017; Li et al., 2021). However, regulation of somatic growth and the GH/IGF axis are also interconnected with neuroendocrine and endocrine pathways which control feeding behaviours and appetite (Canosa and Bertucci, 2020). These pathways include both orexigenic and anorexigenic compounds, which stimulate and inhibit food intake, respectively. The impact of cHABs, particularly those of bloom-associated species that produce a diverse array of metabolites, on these compounds and systems remains largely unexplored, as does the consequences of these effects on the health of affected fish populations.

Conversely, the interactions between these compounds and the nervous system have been of significant interest in cyanobacterial research and studies have highlighted their potential to disturb neurological systems and related endocrine pathways (Zi et al., 2023). In early life stages of fish, exposure to *M. aeruginosa* alters concentrations of critical neurotransmitters, disturbs biomarkers of developmental neurotoxicity, and alters larval swimming behaviour and activity (Cai et al., 2022; Qian et al., 2018; Sergi et al., 2022). Similarly, *M. aeruginosa* extract exposure in juvenile fish also alters neurotransmitter levels in the brain and spinal cord and disturbs neurotransmission by altering both mitochondrial membrane potential and Ca^{2+} levels in the same tissues (Nájera-Martínez et al., 2022). Modulation of key neurotransmitters, such as serotonin and dopamine, also drives changes in food intake and appetite in fish (Conde-Sieira et al., 2018).

Dianchi Lake in Yunnan Province is the sixth largest lake in China and suffers from extensive cHABs driven largely by cultural eutrophication and the favourable local climate (Wu et al., 2016). The economic and ecological importance of Dianchi Lake makes it a focal point of cyanobacterial research and remediation efforts. Among these efforts is the re-introduction of extirpated species to the lake, including the Yunnan glossy lipfish *Acrossocheilus yunnanensis,* a native cyprinid fish species that inhabits rivers and lakes of eastern Yunnan. Persistent cHAB blooms present in Dianchi Lake pose significant hurdles for the re-establishment of sustainable fish populations. These blooms are primarily dominated by members of the *Microcystis* genus, including *Microcystis wesenbergii* (Shan et al., 2019). Analysis of the planktonic community of Dianchi Lake has found that *M. wesenbergii* has become the most abundant *Microcystis* species within the lake, eclipsing the often prolific, toxin-producing *M. aeruginosa* (Xiang et al., data unpublished).

Some strains of *M. wesenbergii* lack the ability to produce the major cyanotoxin microcystin (MC), including those of Dianchi Lake. While formerly known as 'non-toxic,' these non-MC-producing groups are capable of significant disruptive effects on aquatic fauna (Pham et al., 2015; Le Manach et al., 2018) and produce toxic effects rivalling those of MC toxin-producing strains (Harshaw et al., 2024). As observed in Dianchi Lake and other Chinese lakes (Xu et al., 2008), these strains can also make up substantial portions of freshwater blooms (Rinta-Kanto et al., 2009). Despite the significant presence of non-microcystin-producing cyanobacteria in numerous freshwater environments, their impacts are rarely addressed. Dianchi Lake holds significant ecological and economic importance to the adjacent city of Kunming and to Yunnan Province, and the intensity and frequency of the lake's blooms has fueled major on-going remediation and research efforts (Wang et al., 2020). However, *M. wesenbergii* is broadly distributed in the North American Laurentian Great Lakes (Murphy et al., 2003), as well as those in Europe (Via-Ordorika et al., 2004) and Japan (Ozawa et al., 2005), making the interactions between *M. wesenbergii* and key fish species critical to aquaculture and conservation efforts in freshwater systems beyond Yunnan Province.

Acrossocheilus yunnanensis is a member of the Cyprinidae family, a wide-ranging family encompassing species found in Eurasia, Africa, and North America (Winfield and Nelson, 2012). Cyprinids include major research model species such as zebrafish and goldfish, as well as critically endangered species like the razorback sucker (*Xyrauchen texanus*, IUCN, 2023) and highly invasive taxa such as bighead carp (*Hypophthalmichthys nobilis*, Herborg et al., 2007). Although studies have been conducted on the regulation of food intake and appetite in teleost fish and a few cyprinids (Volkoff, 2016), there exists no published information on the regulation of feeding in *A. yunnanensis*, and little is known about the effects of cyanobacteria on feeding in fish. Therefore, our study offers an opportunity to expand on these knowledge gaps by exploring the influence of the cyanobacteria *M. wesenbergii* on *A. yunnanensis* food intake.

To explore how cHABs can impact feeding regulation in freshwater fish and potentially produce deleterious downstream health outcomes, we investigated interactions between *M. wesenbergii* and neurological and endocrine signalling pathways of juvenile *A. yunnanensis.* We assessed effects on feeding behaviour well as changes in levels of neurotransmitters and appetite-regulating factors from both major and peripheral tissues – brain and gastrointestinal tract – involved in food intake regulatory systems (Volkoff, 2016). We sought to assess the potential role of impaired appetite regulation in the ongoing failure of re-introduction efforts of Yunnan glossy lipfish and other imperiled fish species back in environments subject to major cHAB blooms.

2. Materials and methods

2.1. Cyanobacterial cultures

Pure cultures of *M. wesenbergii* (FACHB-1112) were purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-Collection, Wuhan, China). Stock cultures were grown in 2000 mL Erlenmeyer flasks in 600 mL of COMBO medium and incubated at 24 ± 1 °C and a 12:12 h light-dark cycle at 2000 lx. Flasks were gently shaken twice daily. We confirmed the lack of microcystin production by this strain via liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) as previously described by Xu et al. (2023) prior to their use in experimental procedures. Microcystin concentrations in these cultures were determined to be below the limit of detection for this method (1 ng/mL).

2.2. Fish husbandry and exposure conditions

Captive-bred 16-month-old *A. yunnanensis* (7.7 ± 0.1 cm in length) were provided by Yunnan Provincial Aquatic Technology Promotion Station (Yunnan, China) from their artificial broodstock populations. We acclimated fish in tanks of aerated COMBO medium at 24 ± 1 °C and a 12:12 h light-dark cycle for 7 days prior to exposure. Fish were fed twice daily with commercial fish pellets at a feeding rate of 1 % weight/body weight at each feeding. Fish were fasted for 24 h prior to the start of treatments and during the last 24 h of the exposure period. All experimental procedures involving fish in this study were approved by the Experimental Animal Welfare Ethics Committee at the Kunming University (China, Ref. No. KMU2023039).

Once acclimated, juvenile *A. yunnanensis* were randomly allocated to one of five experimental treatment groups, consisting of a media control of COMBO medium (Control) or *M. wesenbergii* cultures at densities of 5×10^4 cells/mL (C1), 1×10^5 cells/mL (C2), 5×10^5 cells/mL (C3), and 1×10^6 cells/mL (C4). We conducted experiments in glass 15-L tanks containing 10 L of either exposure media or cyanobacterial culture under the same temperature and day-night cycle as the acclimation period. In between uses, tanks were disinfected with a potassium permanganate ($KMnO_4$) solution, soaked for 24 h, and rinsed thoroughly. Each treatment group comprised four tanks of fish at densities of ~2 g/L. Temperature, pH, dissolved oxygen, and total dissolved solids were monitored in each tank daily throughout the exposure period (Supplementary Table 1); however, growth media was not renewed.

2.3. Food consumption analysis

During the exposure period, we fed fish commercial fish pellets (1.5

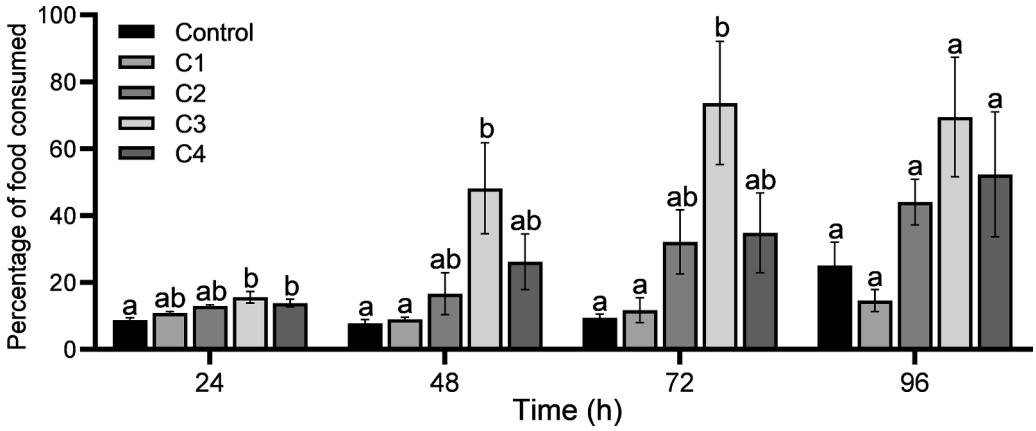

Fig. 1. Mean (± SEM) percentage of pellets consumed by *A. yunnanensis* during co-culture exposure with *M. wesenbergii,* calculated as pellets eaten/total pellets provided x 100. Treatment groups included a growth media-only control (Control) and cyanobacteria cultures at initial densities of 5×10^4 cells/mL (C1), 1×10^5 cells/mL (C2), 5×10^5 cells/mL (C3), and 1×10^6 cells/mL (C4). Differences in letters indicate significant differences in food intake of each treatment group within each 24-hour period ($p < 0.05$).

mm) in the same manner as during the acclimation period (twice daily, up to 2% w/bw). At each feeding, after 30 min, excess food was removed and counted to calculate the percentage of food consumed for each experimental tank. Food consumption percentage was determined by:

$$\text{Food consumption percentage} = \frac{(\textit{Total pellets} - \textit{pellets remaining})}{\textit{Total pellets}} \times 100$$

and presented as a percentage of total food provided.

2.4. Body size and tissue analysis

After 96 h, we euthanized fish using 0.02 % tricaine methanesulphonate (MS-222) and total length, body length, and total weight of each fish were recorded. Brain and intestine tissues were excised and weighed to determine organ somatic indexes, which was determined as:

$$\textit{Somatic index } (SI) = \frac{\textit{weight of tissue } (g)}{\textit{body weight } (g)} \times 100,$$

before being stored at −80 °C for future analysis. For the intestinal tissue weight, gut contents were flushed and weighed to determine empty intestinal mass prior to calculating intestinal somatic index. Relative body condition (K_n) was calculated for each group using the method described by Le Cren (1951) using the equation:

$$K_n = \frac{W}{aL^b}$$

where W is weight, L is length, and a and b are the intercept and slope, respectively, of the linear regression of the log-transformed length-weight relationship of all sampled fish.

2.5. Enzyme-linked immunosorbent assays

We quantified concentrations of both orexigenic (appetite-stimulating) and anorexigenic (appetite-inhibiting) regulatory factors in the brain and gut tissues using enzyme-linked immunosorbent assays (ELISAs) purchased from Wuhan Moshake Biotechnology Co., Ltd (MSK, China), according to the manufacturer's protocols. Tissues were homogenized in phosphate-buffered saline (PBS) in a SCIENTZ-48 high-throughput tissue grinder (Scientz Biotechnology Co., Ltd, Zhejiang, China) before being centrifuged in a TGL-1650 benchtop refrigerated centrifuge (Sichuan Shuke Instrument Co., Ltd., Chengdu, China) at 5000 rpm. The supernatant of each sample was then collected and stored at −80 °C prior to analysis. Concentrations were determined from absorbance values measured using a SpectraMax iD3 microplate reader (Molecular Devices, Shanghai, China). Appetite regulating factors measured in the brain included the orexigenic factors Agouti-related protein (AgRP, Cat. No. KT20444), neuropeptide Y (NPY, Cat. No. KT36655), and orexin (Cat. No. KT20447, the anorexigenic factors cocaine- and amphetamine-regulated transcript (CART, Cat. No. KT20446), and pro-opiomelanocortin (POMC Cat. No. KT20623), and the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT, Cat. No. KT20284), acetylcholine (ACh, Cat. No. KT21180), γ-aminobutyric acid (GABA, Cat. No. KT20525), norepinephrine (NE, Cat. No. KT20303), and dopamine (DA, Cat. No. KT20521). In the intestinal tissues, ELISA was used to assess concentrations of the stimulatory ghrelin (Cat. No. KT20448) and the inhibitory factors cholecystokinin (CCK, Cat. No. KT20461), peptide YY (PYY, Cat. No. KT20532) and glucagon-like-peptide-1 (GLP-1, Cat. No. KT20468).

2.6. Statistical analysis

All data were presented as mean ± standard error of the mean (SEM) and assessed for normality of distribution and homogeneity of variances using the Shapiro-Wilks test and Brown-Forsythe test, respectively. When both tests passed, differences between treatment groups were evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Otherwise, results were assessed using the non-parametric Kruskal-Wallis test with Dunn's post hoc or Welch's ANOVA with Games-Howell's post hoc tests when appropriate. All statistical analysis was performed using GraphPad Prism 8.0.2 software. P-values of ≤ 0.05 were considered statistically significant.

3. Results

3.1. Effects of cyanobacteria on feeding rates

Under co-culture conditions, food intake by juvenile *A. yunnanensis* was significantly increased by initial densities of *M. wesenbergii* of 5×10^5 cells/mL (C3 treatment) and 1×10^6 cells/mL (C4) during the first 24-hour monitoring period (Fig. 1). However, after 48 and 72 h, only the C3 treatment continued to display higher percentages of food consumed compared to the control – up to 7.8 times the control rate – and after 96 h differences between all treatments were not significant.

Table 1
Mean (± SEM) body length, width, and weight as well as relative body condition of *A. yunnanensis* following 96 h exposure to *M. wesenbergii*. Differences in letters down each column indicate significant differences between each treatment group ($p < 0.05$).

Treatment	Total body length (cm)	Body width (cm)	Weight (g)	Relative body condition (K_n)
Control	7.28 ± 0.12[a]	1.35 ± 0.11[a]	3.29 ± 0.18[a]	0.95 ± 0.02[a]
C1	7.43 ± 0.08[a]	1.35 ± 0.09[a]	3.55 ± 0.14[a]	0.97 ± 0.01[ab]
C2	7.37 ± 0.10[a]	1.35 ± 0.10[a]	3.54 ± 0.15[a]	1.01 ± 0.01[bc]
C3	7.32 ± 0.10[a]	1.41 ± 0.11[a]	3.67 ± 0.15[a]	1.05 ± 0.01[c]
C4	7.38 ± 0.11[a]	1.40 ± 0.12[a]	3.77 ± 0.16[a]	1.04 ± 0.01[c]

3.2. Effects on A. yunnanensis body size and somatic indices

Following 96 h of exposure to co-culture conditions, there were no significant differences in total body length, body width, or weight between treatment groups and the control (Table 1). In terms of relative body condition, there was a trend of increasing body condition with increasing cyanobacterial density with treatment groups at initial densities of 1×10^5 cells/mL or higher (C2 through C4) relative to control.

Among the somatic indices examined post-exposure, both brain (BSI) and intestinal somatic indices (ISI) decreased significantly in *M. wesenbergii* treatments. For brain, the C3 treatment decreased BSI to 84.4 % of that of control values (Fig. 2A). For intestinal tissues, all but the lowest density of *M. wesenbergii* significantly reduced ISI values as compared to the control, with the highest density (C4) treatment reducing mean ISI to 63.4 % of the control (Fig. 2B). Comparing the somatic indices changes under *M. wesenbergii* treatment, intestine was the most sensitive organ in *A. yunnanensis* to this stress.

3.3. Effects on appetite regulators

Following exposure to *M. wesenbergii* treatment, the concentration of AgRP was significantly upregulated in *A. yunnanensis* from the 5×10^5 cells/mL density treatment group (C3) as compared to the control (Fig. 3A), with up to 1.6 times higher values observed in the treatment group. Conversely, orexin concentration decreased significantly to 55.6 % of control values in the C2 treatment (Fig. 3D). There were no significant effects observed in the other two orexigenic factors examined (Figs. 3B, C).

Pro-opiomelanocortin, an anorexigenic factor, was significantly downregulated following *M. wesenbergii* exposure, with POMC concentration in fish from the C4 treatment down to 76.6 % of control values (Fig. 4E). We observed no significant differences between treatment and control groups for the remaining anorexigenic factors studied (Figs. 4A,

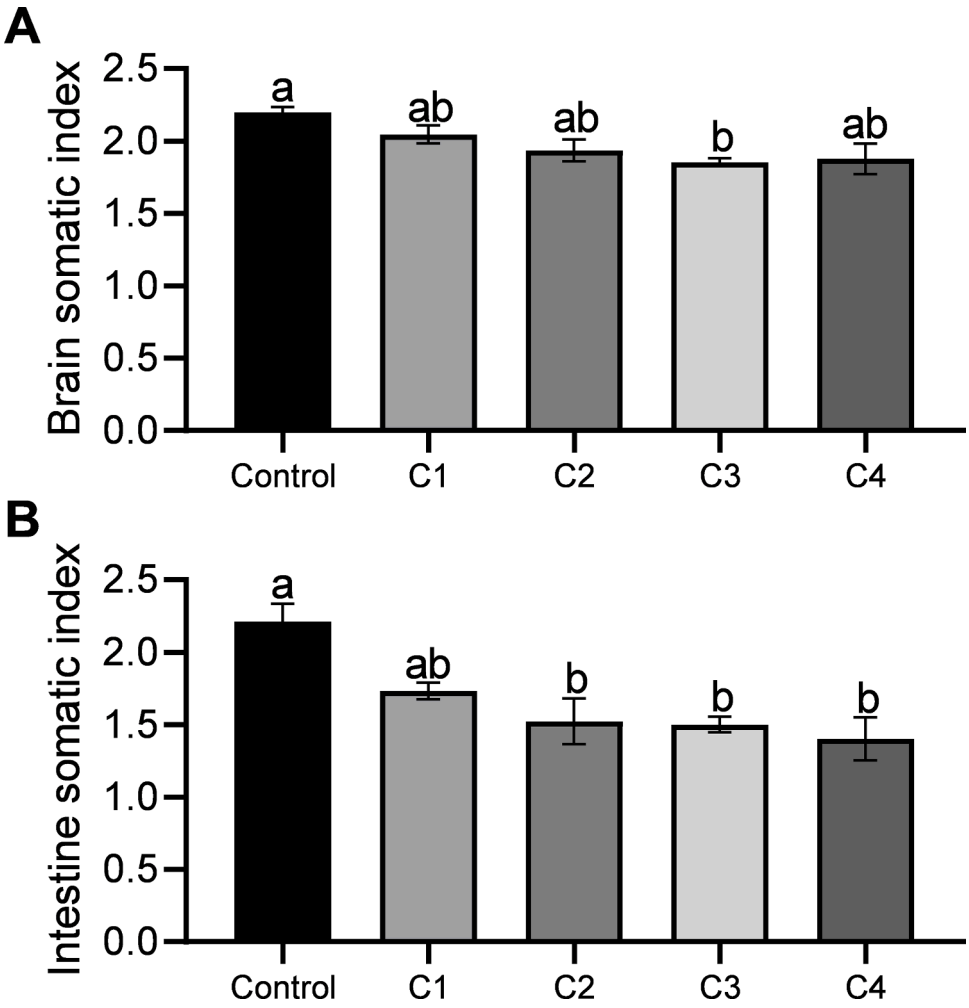

Fig. 2. Mean (± SEM) somatic indices for the brain (A) and intestines (B) for *A. yunnanensis* following 96 h co-culture with *M. wesenbergii* at initial densities of 5×10^4 cells/mL (C1), 1×10^5 cells/mL (C2), 5×10^5 cells/mL (C3), and 1×10^6 cells/mL (C4). Differences in letters indicate significant differences between treatment and control groups ($p < 0.05$).

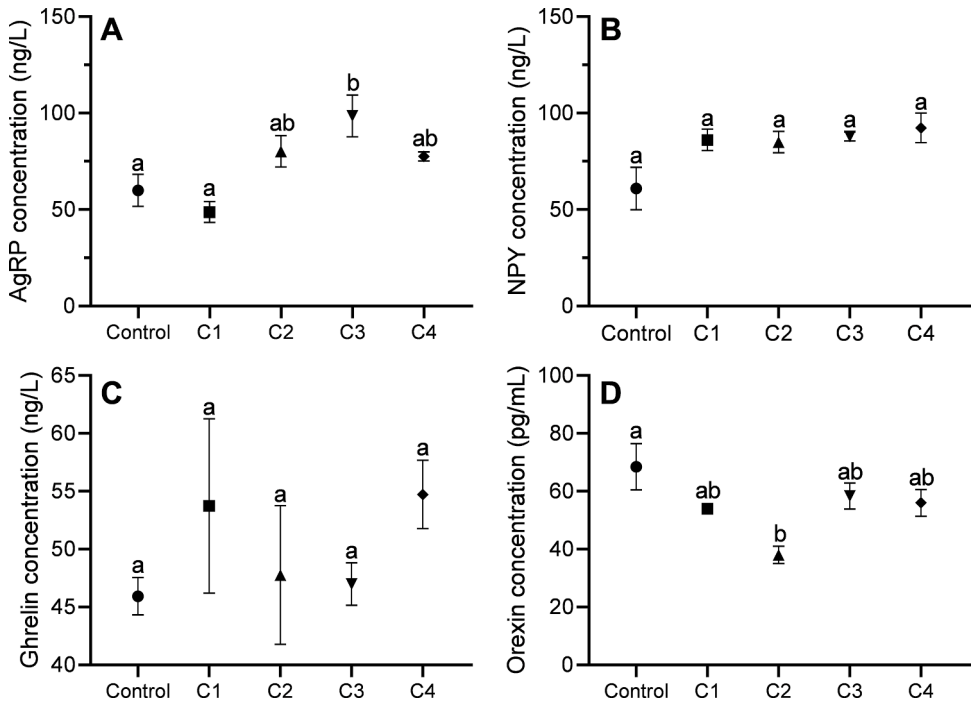

Fig. 3. Mean (± SEM) concentrations of major orexigenic regulatory factors AgRP (A), NPY (B), ghrelin (C) and orexin (D) following 96 h co-culture exposure to *M. wesenbergii* at initial densities of 5×10^4 cells/mL (C1), 1×10^5 cells/mL (C2), 5×10^5 cells/mL (C3), and 1×10^6 cells/mL (C4). Differences in letters indicate significant differences between treatment and control groups ($p < 0.05$).

B, C, D).

3.4. Changes in neurotransmitter concentrations

Following co-culture exposure, GABA concentrations in the treatments were elevated in a density-dependent manner, with fish exposed to *M. wesenbergii* at initial densities of 5×10^5 cells/mL (C3) and 1×10^6 cells/mL (C4) exhibiting significantly higher GABA levels compared to the control group – up to 1.3 times control values in the C4 treatment (Fig. 5C). Norepinephrine concentration was also significantly elevated in *A. yunnanensis* from the C3 treatment, to 126.4 % of that of the control (Fig. 5D). Conversely, DA concentration was elevated significantly only in the lower density (C1 and C2) treatments, higher *M. wesenbergii* densities were not significantly different than controls (Fig. 5E).

4. Discussion

Our findings indicate that co-culture exposure of the cyprinid *A. yunnanensis* with *M. wesenbergii* cyanobacterial cells altered feeding behaviour and associated signalling pathways, potentially driving a maladaptive feeding response in bloom-affected juvenile fish. Increased cyanobacterial density resulted in a stimulatory shift of neurotransmitter and endocrine hormone concentrations, with significant increases in food consumption and relative body condition evident in our second highest density treatment (C3) over much of the exposure period (Fig. 1). Following exposure, fish from this treatment group had elevated concentrations of the orexigenic peptide AgRP in the brain, expression of which is increased during periods of fasting (Volkoff, 2016) and decreased in response to elevated nutrient levels – particularly fatty acids - to alter food intake appropriately (Delgado et al., 2017). Similarly, concentration of the neurotransmitter GABA also increased in brain in fish in the high-density *M. wesenbergii* treatment. GABAergic inputs in the central nervous system increase feeding behaviour (Snigirov and Sylantyev, 2018) in fish, possibly by enhancing stimulatory activity of AgRP, as previously reported in mammals (Sohn et al., 2013).

In previous studies, dietary exposure to *M. aeruginosa* cells increased feeding rates in both Nile tilapia (*Oreochromis niloticus*) and gibel carp (*Carassius auratus gibelio*) (Zhao et al., 2006a, 2006b). Similarly, diets comprised of *M. wesenbergii* in a 1:1 mixture with *M. aeruginosa* also elevated feeding rates in hybrid tilapia (*O. niloticus x O. aureus*) (Dong et al., 2009), although effects of *M. wesenbergii* diets alone have yet to be studied. In contrast to dietary exposures, which rely on the direct supplementation of cyanobacterial cells into fish feed, co-culture exposure replicates natural bloom conditions, integrating both exposure through the gills, integument, and gastrointestinal tract. In the wild, *Microcystis* spp. have been recorded as only a small component of the diet of *A. yunnanensis* (Zhang et al., 2020) and likewise, *M. wesenbergii* cells made up only a small percentage of gut contents observed in treated fish (Long & Chang et al., unpublished data). However, in studies of the related *M. aeruginosa,* cell-free exudates alone were capable of significant toxicity in both larval and adult fish (Li et al., 2021; Zhao et al., 2024).

Uptake of MCs through the gastrointestinal tract may result in both GI tissue damage and facilitatation of transport and accumulation of MCs in other major organs – such as the liver (Malbrouck and Kestemont, 2006). However, the effects of non-MC metabolites of *Microcystis* species on the gut and other GI tract tissues is poorly understood. In tilapia, mixed *Microcystis* diets containing both *M. aeruginosa* and *M. wesenbergii* impaired growth and digestion efficiency (Dong et al., 2009). In this study, both the brain and intestinal tissues of fish co-cultured with *M. wesenbergii* exhibited significantly reduced somatic indices, with the ISI reduced in all but the lowest density treatment. Further histopathological analysis of intestinal tissues of *A. yunnanensis* exposed to *M. wesenbergii* found significant alterations to the structures of villi and tissue layers (Long, W. et al., unpublished data). While intestinal damage and inflammation have been observed in fish exposed to *M. aeruginosa* cultures and lyophilized cells containing MCs (Preeti et al., 2016; Qian et al., 2019; Zhao et al., 2024), the potential damage present in studied *A. yunnanensis* tissues represents a novel effect of non-MC-producing *M. wesenbergii*. Extracts of similar "non-toxic" *M. wesenbergii* cultures induce significant toxicity to the zooplankton *Daphnia magna* (Luu, 2019), though this cyanobacterial species remains

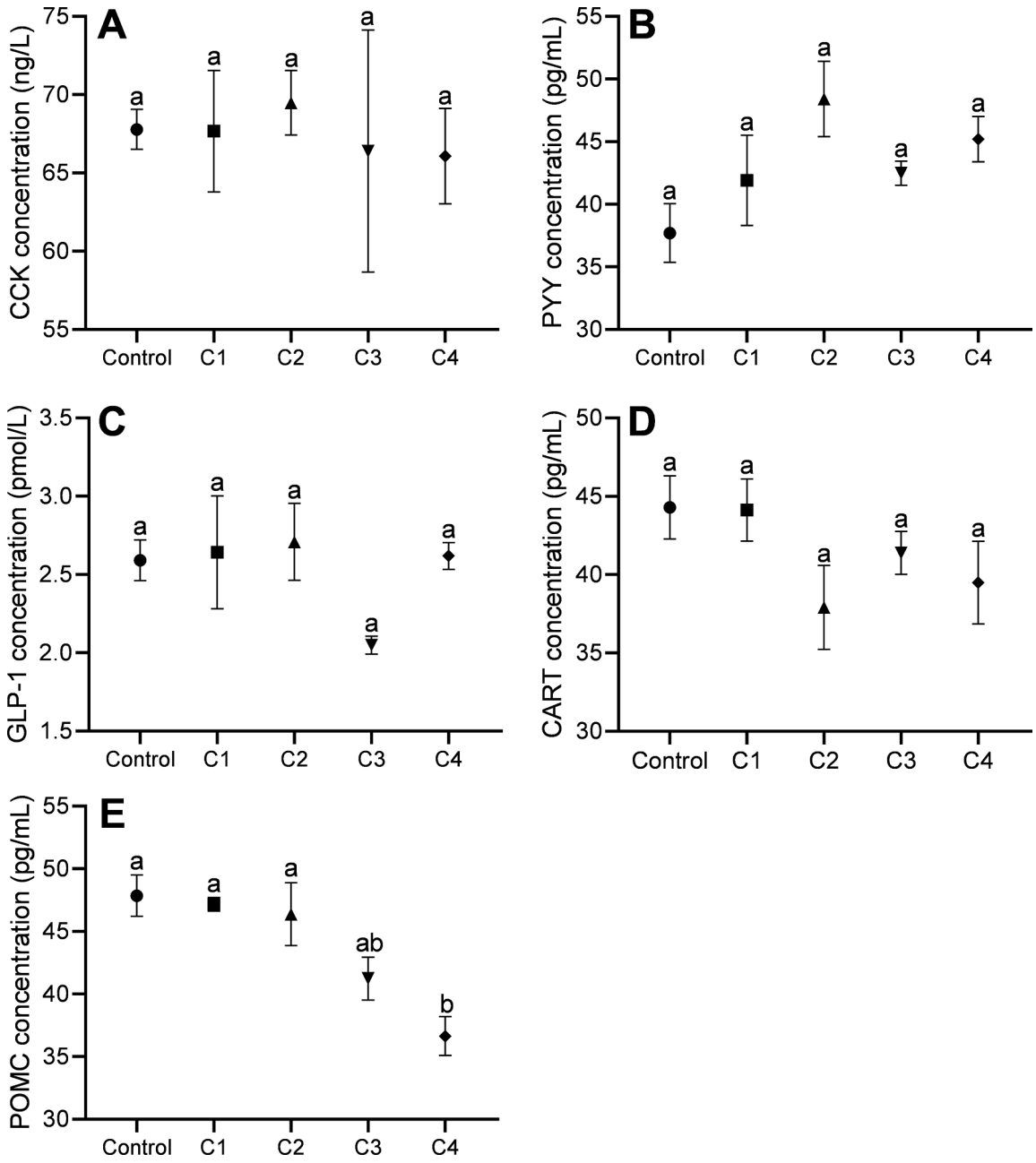

Fig. 4. Mean (± SEM) concentrations of major anorexigenic regulatory factors CCK (A), PYY (B), GLP-1 (C), CART (D), and POMC (E) following 96 h co-culture exposure to M. wesenbergii at initial densities of 5 × 104 cells/mL (C1), 1 × 105 cells/mL (C2), 5 × 105 cells/mL (C3), and 1 × 106 cells/mL (C4). Differences in letters indicate significant differences between treatment and control groups ($p < 0.05$).

understudied, and its potential metabolome remains uncharacterized.

Investigations into the toxic effects of the non-microcystin metabolites of the ubiquitous *M. aeruginosa* found that these compounds alter regulation of proteins associated with key metabolic and homeostatic processes, including carbohydrate and lipid metabolism (Le Manach et al., 2016; Sotton et al., 2017, 2018). It is possible that alongside histopathological changes to tissue integrity, dysregulation of metabolic processes may also contribute to reduced feed utilization observed in previous *Microcystis* diet studies (Dong et al., 2009). Further characterization of potential bio-active compounds produced by *M. wesenbergii* is critical to understanding the whole-body impacts of fish feeding within high density blooms.

In addition to putative tissue damage, norepinephrine concentration was also elevated in brain of fish from the C3 treatment. Together with increasing heart rate and oxygen uptake, the catecholamines norepinephrine and epinephrine are involved in the mobilization of energy reserves in the acute stress response of fish (Wendelaar Bonga, 1997). However, it is unclear how *M. wesenbergii* exposure may affect other aspects of the teleost stress response. Additionally, the neurotransmitter GABA dampens actions of the hypothalamus-pituitary-adrenal axis through the inhibition of the release of corticotropin-releasing hormone and, subsequently, adrenorticotrophin (Jessop, 1999). Under transportation stress, GABA supplementation reduced cortisol and epinephrine concentrations in koi carp (*Cyprinus carpio*) compared to untreated fish (Zhang et al., 2022).

At an initial density of approximately 5×10^5 *M. wesenbergii* cells/mL, our C3 treatment is similar to that observed during bloom conditions in Dianchi Lake (observed concentrations of between 3×10^5 and 6 x 10^5 cells/mL) for more than 3 months in 2009 (Wu et al., 2014). While our lower density treatments were primarily dominated by appetite

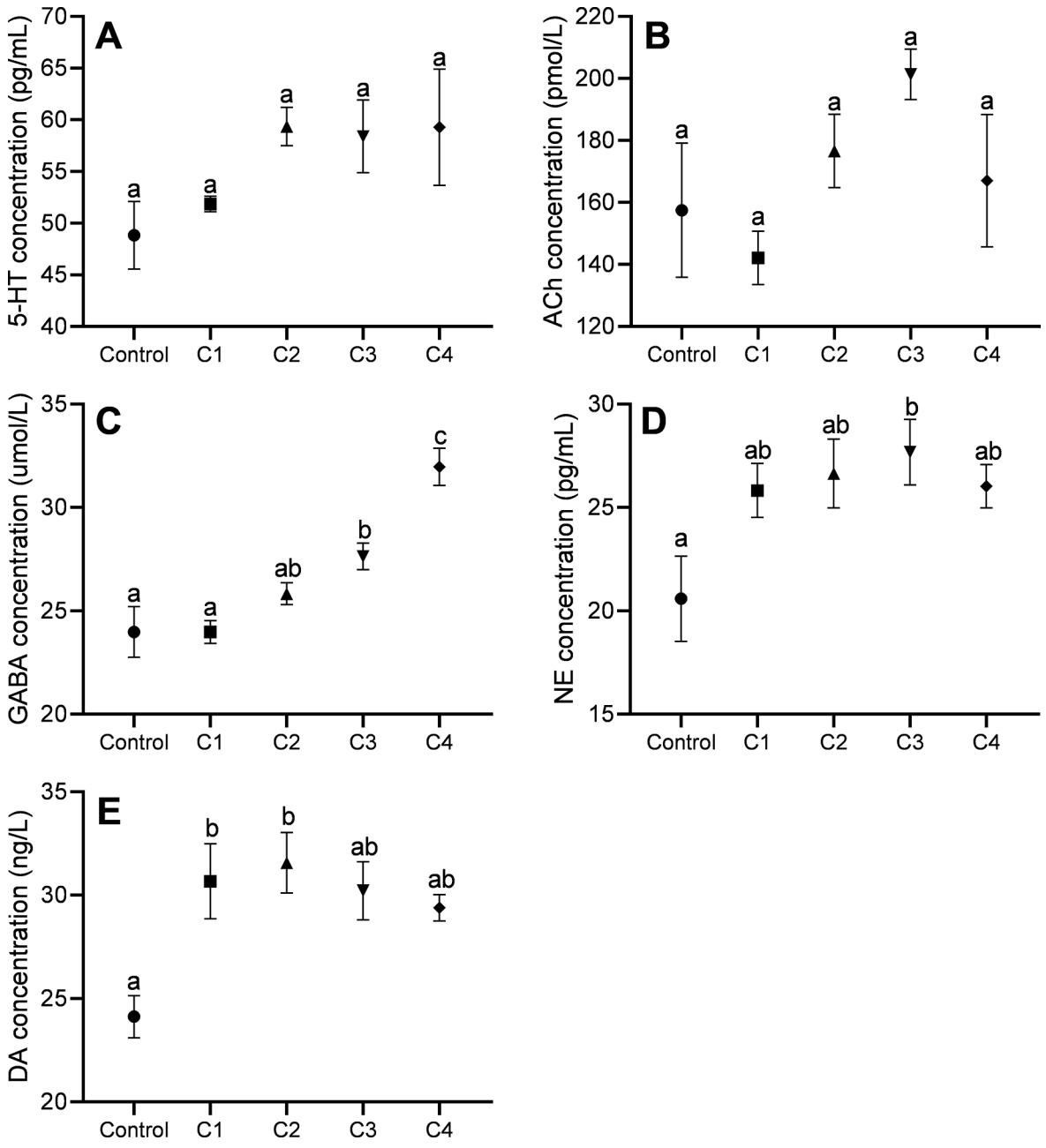

Fig. 5. Mean (± SEM) concentrations of major neurotransmitters 5-HT (A), ACh (B), GABA (C), NE (D), and DA (E) following 96 h co-culture exposure to *M. wesenbergii* at initial densities of 5×10^4 cells/mL (C1), 1×10^5 cells/mL (C2), 5×10^5 cells/mL (C3), and 1×10^6 cells/mL (C4). Differences in letters indicate significant differences between treatment and control groups ($p < 0.05$.

inhibition (e.g., increased dopamine, decreased orexin), there was a strong shift in both food intake and related stimulatory cues toward a heightened feeding response at this environmentally-relevant treatment density – despite coinciding with potentially detrimental tissue-level alterations.

Overall, changes in feeding behaviour, appetite regulation, and histopathological tissue changes observed in our study highlight a significant capacity for *M. wesenbergii*-dominated blooms to dysregulate foraging behaviours in the cyprinid *A. yunnanensis* under acute exposure conditions in which fish may either inadvertently or purposefully ingest cyanobacterial cells (Zhang et al., 2020) and which ultimately may lead to loss of tissue integrity in sensitive tissues of the brain and gut. However, more research is needed to confirm these detrimental effects over chronic exposure periods, especially considering the persistence of high-density blooms in Dianchi Lake.

5. Conclusion

The results of our study highlight the potential for *M. wesenbergii* blooms to disturb regulation of food intake in fish, altering both the expression of key appetite-related peptides such as AgRP and POMC as well as neurotransmitters including GABA, norepinephrine, and dopamine. The dysregulation of these important signalling molecules combined with the potential for significant tissue damage indicate that *M. wesenbergii* can strongly influence the health and feeding behaviour of Yunnan glossy lipfish (*A. yunnanensis)* present in these blooms. The interaction between these two species should be considered in future reintroduction efforts in Dianchi Lake and highlights the need to further characterize lesser-known cyanobacterial species such as *M. wesenbergii*.

CRediT authorship contribution statement

Wenyu Long: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Keira Harshaw:** Writing – review & editing, Writing – original draft, Formal analysis. **Yunfeng Wang:** Conceptualization. **Qianqian Xiang:** Methodology, Conceptualization. **Yuanyan Zi:** Methodology, Formal analysis. **Helene Volkoff:** Writing – review & editing, Conceptualization. **Hugh J. MacIsaac:** Writing – review & editing. **Runbing Xu:** Methodology, Conceptualization. **Minmin Niu:** Methodology. **Qiwen Xi:** Methodology. **Xuexiu Chang:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2024.102647.

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