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REVIEW ARTICLE

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New indices for the diagnosis of fish welfare and their application to the grass carp (*Ctenopharyngodon idella*) reared in earthen ponds

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Abstract

Animal welfare is an issue of increasing importance in global aquaculture. However, the incorporation of this issue into routine aquaculture operations is a challenge. A possible path to change this is the recognition, identification and addressing of the ecological, physiological, nutritional, behavioural and psychological needs of fish. The present study uses grass carp (*Ctenopharyngodon idella*), the world's most widely produced fish species, and earth ponds, the world's most widely used production system in fish farming worldwide, as the basis for developing indices to assess and monitor the welfare of farmed fish. The proposed indices were structured based on specific welfare indicators, reference values, individual weights and associated scores to address the Animal Welfare Five Freedoms and a Life Worth living concepts. Two types of indices were proposed: (1) partial welfare indices (PWI_x), specific to each of the four observed/analysed freedoms; (2) general welfare index (GWI), which simultaneously summarizes the addressees' freedoms in a single variable. Both indices range from 0 (critical welfare impairment) to 1.0 (minimal risk of welfare impairment). The study was based on a comprehensive systematic review of the literature using the PRISMA method. The proposed indices were based on 10 environmental indicators, nine indicators for health, five for nutrition and four for behaviour. The PWI_x can be used to determine how each category of indicators contributes to the GWI, which defines the level of fish farm welfare at a given point in time and which needs are affected or met during a production cycle.

KEYWORDS
aquaculture, grow-out, PRISMA method, scores, welfare index, welfare indicators

1 | INTRODUCTION

A growing number of scientific literature on anatomical, physiological, behavioural and pharmacological aspects has provided solid evidence that fish have nociceptive and cognitive abilities; they can feel pain, anxiety and fear, similar to other vertebrates (Braithwaite, 2010; Broom, 2007; Brown, 2015; Chandroo et al., 2004; Kristiansen et al., 2020; Sneddon et al., 2018). The scientific community's recognition of the sentience of fish is reflected in society's stance calling for greater control over how animals are kept and managed in captivity, particularly when they are intended for human consumption (Huntingford & Kadri, 2009). These demands have led, for example, to changes in technical regulations and aquaculture standards that now consider animal welfare as a prerequisite for production (Branson, 2008; Medaas et al., 2021; Seibel et al., 2020).

Animal welfare has been defined by Broom (1986) as 'the state of the individual as regards its attempts to cope with its environment', where welfare is poor when coping capacity is low. More recently, the World Organization for Animal Health and Welfare (OIE, 2021) has defined animal welfare as the psychological state of an animal in relation to the conditions in which it lives and dies. According to the five freedoms of animals established by the Farm Animal Welfare Council (FAWC, 1979), animals must be kept free from hunger, discomfort, disease, behavioural disorders and psychological suffering to ensure their welfare (Broom, 2007).

Many factors threaten the welfare of farmed fish, but deterioration in water quality is the most visible face of this process, along with external factors that can directly affect the aquatic environment and thus animal homeostasis (Ashley, 2007; Conte, 2004; Håstein et al., 2005; Noble et al., 2012). When animals are kept in unfavourable environmental conditions, their immunity decreases, making them susceptible to parasitic and bacterial infections, reducing food intake and growth rate and even increasing mortality rates (Lieke et al., 2020; Lymbery, 2002). Improper distribution or excessive amounts of food—natural or artificial—can lead to water quality degradation (Boyd & Tucker, 2019), while providing feed of inadequate quantity or quality can lead to increased fish aggressiveness, causing injury and promoting the spread of disease (Almazán-Rueda et al., 2004, 2005; Ghosh et al., 1984; Goddard, 1996; Liang & Chien, 2013; López-Olmeda et al., 2012; Tørud & Håstein, 2008). Physical manipulation of fish during routine procedures such as classification, vaccination, biometrics, harvesting and invasive procedures, such as tagging, are activities that can cause pain, physical stress and psychological distress, apart from harmful exposure of animals to air (Branson, 2008; Höglund et al., 2022; Noble et al., 2018; Santurtun et al., 2018; Saraiva et al., 2019; Sørum & Damsgård, 2004). Targeted measurements and continuous monitoring of fish welfare during their rearing have gradually become indispensable tools for optimizing aquaculture techniques and practices (Barreto et al., 2022) and aquaculture itself as a commercial activity.

Since it is impossible to directly ask a fish how it feels, it is assumed that the welfare of the animal is directly related to the fulfilment of its basic needs. Any measure or observation that provides information on the extent to which the fish's needs are being met is considered a potential indicator of its welfare. Such indicators should also describe the fish's quality of life (Speare, 2006; Stien et al., 2020; van de Vis et al., 2020).

Currently, attempts are being made to measure fish welfare using different types of indicators, e.g. input or output (van de Vis et al., 2020); direct or indirect (Duncan, 2005; Noble et al., 2018); behavioural or physiological (Magalhães et al., 2020; Segner et al., 2012; Seibel et al., 2021) and even with continuous indicators that propose to monitor the welfare of farmed fish practically in real time, for example through heart rate (Brijs et al., 2018). For indicators to be applied and interpreted under operational conditions, they should use qualitative and quantitative measures and be organized in standardized protocols, as proposed by Grøntvedt et al. (2015), Pedrazzani et al. (2020) and RSPCA (2020). The advantage of using qualitative and quantitative protocols and measures is that they are largely independent of the system and management in cultivation and can be used or adapted for most situations. The protocols then act as an early warning system, alerting the aquaculture operator that something may be wrong and deserves further investigation, preferably before mortality occurs (Noble et al., 2018).

Furthermore, these indicators, when interpreted individually, even when determined using standardized protocols, may not give the aquaculture operator or evaluator an accurate picture of the risk/deterioration in husbandry conditions for farmed fish, which may result in problems not being mitigated in time to prevent them from becoming too severe (Toni et al., 2019). There may be two reasons for this: (1) because it is difficult to identify and define the factors to which different fish species are exposed in different production systems and regimes under a variety of management conditions; (2) because these factors and parameters establish very complex relationships among themselves with varying degrees of severity and impact on fish welfare. Therefore, indicators need to be analysed individually and collectively using indices.

According to FAO (2020a), the grass carp, *Ctenopharyngodon idella*, was the most produced fish in the world in the last decade. In 2018, this species accounted for 10.5% of the total volume of fish produced in aquaculture worldwide, totaling 5704 thousand tonnes. However, as of 2021, less than five scientific articles have been published on the welfare of this species (Franks et al., 2021). This study aims to use *C. idella* reared in earthen ponds as the basis for proposing a new set of indices to assess and monitor the welfare of farmed fish.

2 | MATERIAL AND METHODS

2.1 | Animal welfare indicators

The indicators selected to assess the welfare of grass carp during the grow-out phase (juveniles with an initial weight of about 2.1 g and adults weighing more than 150 g) in earthen ponds were defined based on existing protocols developed for other farmed fish species, such as Atlantic salmon (Stien et al., 2013) and Nile tilapia (Pedrazzani et al., 2020). These indicators were grouped according to four of the five freedoms defined by the Farm Animal Welfare Council (FAWC, 1979): (1) environmental, (2) sanitary, (3) nutritional and (4) behavioural (Table 1). The indicators related to psychological freedom were not considered as a separate category, and the other proposed indicators assessed this freedom indirectly.

Environmental indicators included physical and chemical factors related to water quality and terrestrial or aquatic predators, and interspecific inhabitants. Nutritional indicators were defined according to the target species' dietary needs and metrics related to access to food and applied food management. Nine health indicators were

TABLE 1 Selected indicators to assess the welfare of *Ctenopharyngodon idella* during grow-out phase

Freedom			
Environment	Nutrition	Health	Behaviour
Alkalinity (mg/L $CaCO_3$)	Crude protein (%)	Abdomen	Anaesthesia—surgical stage (reduction in opercular rate, absence of swimming), during vaccination
Dissolved oxygen (% of saturation)	Feeding frequency (times/day)	Anus	Feed intake (minutes)
Aquatic interspecific inhabitant	Fish condition factor (k)[a]	Eyes	Respiratory frequency (opercular rate/min) during acclimatization, classification or transfers
Nitrite (mg/L NO_2)	Food distribution (% of the surface area reached)	Fins	Swimming behaviour during massive capture
Nonionized ammonia (mg/L NH_3)	Amount of food (% biomass)[b]	Gills	
pH		Jaws/lips	
Transparency (cm)		Operculum	
Salinity (psu)		Skin	
Predators and other aquatic interspecific inhabitants		Mortality (%)	
Terrestrial predators			

[a]Set to $K = (W{\cdot}L^{-3}){\cdot}100$, where W is the weight (g) and L is the length (cm).
[b]Feed and/or forage.

based on direct clinical examination of the farmed fish and were defined according to the main clinical signs of disease in grass carp. Finally, four indicators were related to the behaviour of the animals during routine aquaculture management.

2.2 | Reference values of indicators of grass carp welfare

Based on the information available in the literature, three scores were assigned (1, 2 and 3). Score 1 can be interpreted as covering the ideal limits of variation for the target species. Score 2 refers to variations within the limits that animals normally tolerate. Even if such variations cause or show adverse effects on the animals, these are sublethal. Score 3 refers to reference levels that significantly affect the physiological, health and behavioural status of the animals.

2.3 | Identification and selection of documents for systematic review and reference levels

The ideal, tolerable and unacceptable qualitative and quantitative thresholds for each indicator of grass carp welfare listed in Table 1 were determined using a systematic literature review. This review was conducted using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) method (Moher et al., 2009), with Google Academic serving as the search platform (Figure 1).

The search focussed on books, technical and scientific articles, case studies, manuals, theses and dissertations published between 1976 and 2021 that contained in their title, abstract or keywords the terms listed in Table 2 in five groups. Group I included the terms selected for the search for the most comprehensive literature on grass carp cultivation at the global level. Groups II, III, IV and V contained the terms selected for searching and selecting the bibliography according to the specific indicators previously selected.

With these combinations of terms from group I with those found from groups II, III, IV and V, 46,430 bibliographic entries were found in the search. The titles and abstracts of the documents found were evaluated according to the following criteria: (1) the approach to the breeding of grass carp in the world; (2) the information on husbandry methods used for breeding this species; (3) studies related to breeding in ponds; (4) information on its description, distribution and biology. The adopted exclusion criteria related to (1) the focus of the studies on fish species other than grass carp; (2) studies that did not relate to the management or rearing of grass carp; (3) studies that dealt only with the rearing of grass carp at the hatchery, larval or juvenile stages; and (4) articles that presented technically questionable methods or data. There were no language restrictions in the selection of studies. A total of 265 documents were preselected based on the title and abstract according to the inclusion and exclusion criteria. All bibliographic references and PDF documents were imported using the automatic reference manager Mendeley 1.19.2 (Elsevier, The Netherlands). Duplicates were then removed, and in the third phase of the review, the preselected texts were read, resulting in the final selection of 54 documents of interest (including scientific articles, book chapters, technical reports, manuals, dissertations and theses). They fully met the criteria set for this review.

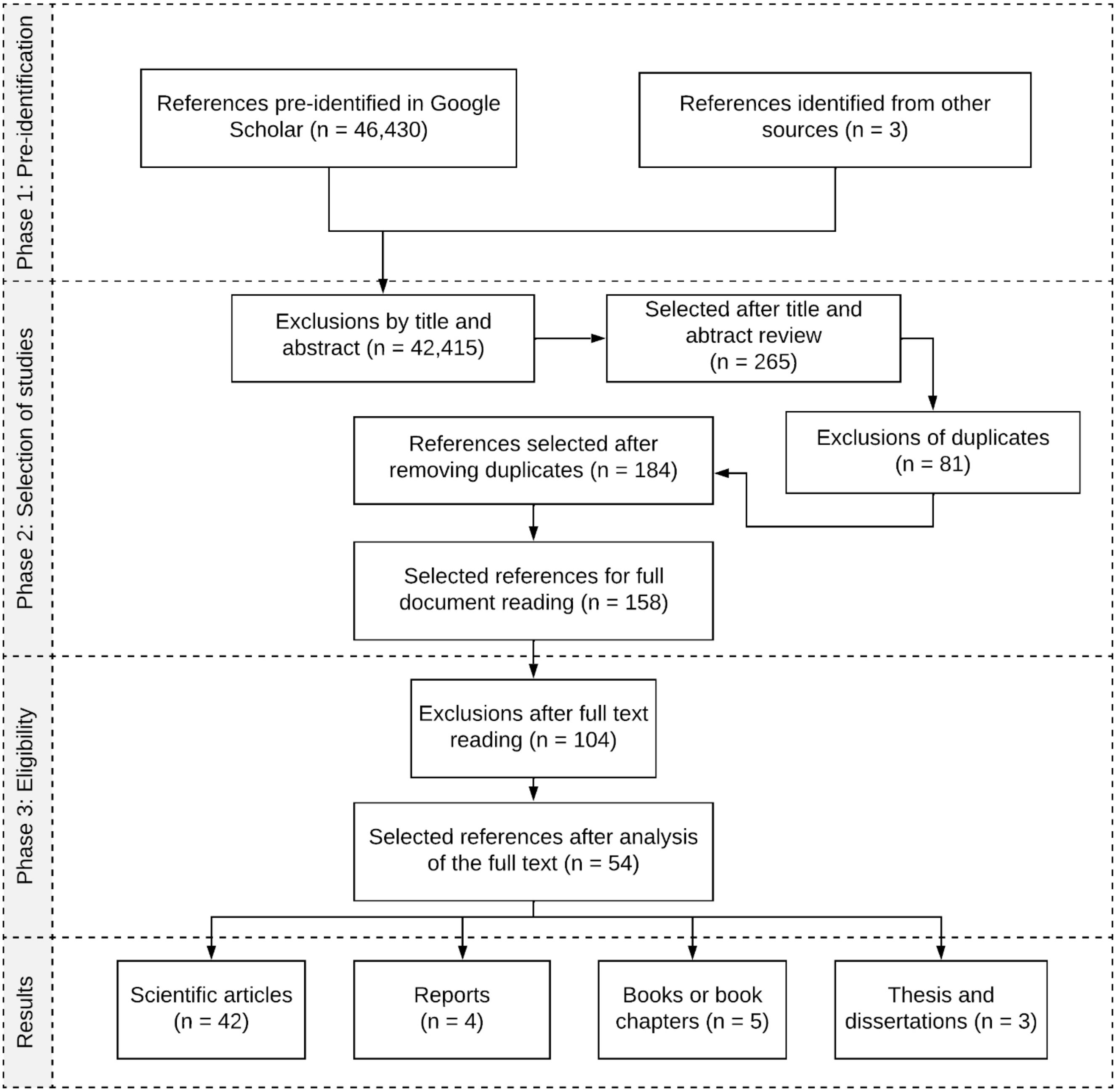

FIGURE 1 Flowchart diagram of the protocol for searching and retrieving the bibliographical references analysed in the systematic review, based on the PRISMA method.

2.4 | Weight (*Y*)

The weight, specific indicators and reference values are variables that must be defined for each species studied. These variables show the estimated importance that each specific indicator has in defining the welfare of the species. For the grass carp, the weight of each indicator was defined based on a literature search that was also conducted using the Google Academic platform. This variable (*Y*) was defined as the integer part of the natural logarithm of the number (*n*) of articles identified directly by searching with the following keywords: *Ctenopharyngodon* AND *idella* AND welfare AND ' the specific indicator' (Equation 1 and Table 2).

$$Y = \mathrm{INT}(\ln(n)). \tag{1}$$

2.5 | Partial and total welfare indices for grass carp

'Indices' are defined here as real numbers derived from mathematical functions obtained from measurements of the application of fish welfare protocols in aquaculture farms. Two types of indices are proposed: (1) the partial welfare indices, specific to each of the four observed/analysed freedoms; (2) a General Welfare Index, covering these four freedoms simultaneously in the same variable.

TABLE 2 Terms and combinations used to access bibliographic data defining the reference values of the grass carp welfare indicators

Groups	Combination of terms	Number of hits on Google Scholar
I	Ctenopharyngodon AND idella AND aquaculture	17,600
II	Ctenopharyngodon AND idella AND aquaculture AND 'specific indicators' Specific indicators: water quality; temperature; pH; transparency; dissolved oxygen; ammonia; nitrite; salinity; alkalinity; predator; interspecific inhabitants	5360
III	Ctenopharyngodon AND idella AND aquaculture AND 'specific indicators' Specific indicators: health; diseases; eyes; jaw; operculum; skin; purposes; gills; abdomen; anus; mortality	14,700
IV	Ctenopharyngodon AND idella AND aquaculture AND 'specific indicators' Specific indicators: protein; feeding frequency; amount of feed; food distribution; condition factor	6970
V	Ctenopharyngodon AND idella AND aquaculture AND 'specific indicators' Specific indicators: behaviour; respiratory frequency; swimming; feed intake; anaesthesia	1800

The partial welfare indices were calculated using Equation (2), based on the farmed fish welfare indicators (Table 1); the weights assigned to each indicator and the respective scores to be obtained through specific measurements and analyses in the monitored aquaculture farm (Tables 4–7).

$$PWI_x = \left(\sum Y / \sum (S \times Y) \times (1.4925 - 0.4925) \right) \qquad (2)$$

Where:

PWI: Partial welfare index standardized to vary continuously between 0 (critical risk of harm to farmed fish welfare) and 1 (maximum welfare or, otherwise, minimum risk of harm to animal welfare), regardless of the number of indicators used in each freedom.

X: Freedom (Environmental—En; Behavioural—Be; Nutritional—Nu or Health—He).

Y: Weight assigned to the specific indicator.

S: Score assigned to the indicators in the analysed fish farm.

The General Welfare Index (WGI, Equation 3) was calculated from the arithmetic mean of the PWI, multiplied by the factor KL (knockout level), as described by Stien et al. (2013).

$$WGI = \frac{\left(\left(PWI_{En} + PWI_{Be} + PWI_{Nu} + PWI_{He} \right) \times kl \right)}{4} \qquad (3)$$

According to Stien et al. (2013), some animal welfare indicators (WI) can reach a level that is so harmful to animals that the variation of other indicators is irrelevant. In the present case, we assume that the mortality rate is the most critical factor for the welfare of farmed fish and outweighs all others. Therefore, we arbitrarily assume that if the mortality rate is 30% or more of the total animals kept in a grass carp rearing system, the knockout value is zero (kl = 0), and the WGI is classified as 'critical'. This value means that irreparable impairment of the welfare of the fish has occurred. If mortality is less than 30%, the knockout value is equal to 1 ($k = 1$) and fish welfare is defined based on the analysed indicators, their scores and weights. We also arbitrarily defined the confidence level (CL_X) for each PWI and GWI based on the weights of the analysed indicators (Equation 4):

$$CL_x = \left(\frac{\sum W_{An}}{\sum W_{max}} \right) \qquad (4)$$

Where:

CL_X: PWI_x confidence level.

$\sum W_{An}$: Sum of the weights of the indicators analysed for the freedom *x*.

$\sum W_{max}$: Sum of the weights of all the defined indicators for the freedom *x*.

The General Confidential level (GCL) was calculated using the arithmetic mean of the CLX (Equation 5).

$$GCL = \frac{\left(IR_{En} + IR_{Be} + IR_{Nu} + IR_{He} \right)}{4} \qquad (5)$$

For classification and interpretation purposes, PWIx, GWI, CLX and GCL were classified according to the values stated in Table 3.

TABLE 3 Ranking values for the Partial Welfare Indexes (PWIx), the General Welfare Index (GWI) and the respective Confidence Level (CL) and General Confidence Level (GCL) determined for grass carp *Ctenopharyngodon idella*, adopting 0.5, 0.75 and 0.7 as the basis for rank

Classification	PWIx and GWI	CL_X and GCL
Critical	0	-
Low	>0 and ≤0.50	>0 and ≤0.50
Medium	>0.50 and <0.75	>0.50 and <0.70
High	≥0.75	≥0.70

3 | RESULTS

3.1 | Indicators and reference values

The environmental indicators used to assess the welfare of juvenile and adult *C. idella* during the rearing phase in earthen ponds were determined using quantitative data following a systematic review of eight indicators routinely assessed in aquaculture farms through water quality analyses with instruments or colorimetric kits (temperature, pH, transparency, nonionized ammonia, nitrite, dissolved oxygen and water salinity). In addition, the other two indicators (presence of terrestrial predators and aquatic predators or other interspecific inhabitants) are qualitative and should be analysed by direct observation by the evaluator. The reference values have been established on the basis of studies or recommendations by different authors for the species at this stage of the production cycle (Table 4).

When defining the indicators for assessing the nutritional status of juvenile and adult *C. idella*, the particularity that they can be fed either dry (artificial feed) or wet with natural food (different forage plants; Table 5) was taken into account. Therefore, when filling in the worksheets to calculate the PWI_{Nu} scores, the evaluator should choose the leading feed in each case. If it is a dry feed, he should indicate both the crude protein content of the feed (an indicator that can be taken from the feed manufacturer's information) and the amount of feed supplied in relation to the stored biomass of the fish. If natural food (forage) is the primary food source for the fish, the evaluator must record the fresh food biomass supplied together with the total biomass of the fish in the pond. However, the evaluator may also choose to record both the feed and the forage provided. In this case, the mean value resulting from the analysis can be used to calculate the PWI_{Nu}. Two other indicators (frequency of feeding and distribution of food) assess the welfare of the animals regardless of the type of food used (forage

TABLE 4 Environmental indicators for juvenile and adult grass carp reared in grow-out earthen ponds

Indicators	Score (*S*)	Reference values	Bibliographic references
Temperature (°C)	1	20–33	Bettoli et al. (1985), Cudmore et al. (2004), Faria et al. (2013), Díaz et al. (1998), Kristan et al. (2019), Poli et al. (2004), Zhao, Zhu, et al. (2020), Zhao, Zhang, et al. (2020)
	2	16–19 or 34–37	
	3	≤15 or ≥38	
pH	1	7.0–8.5	Froese and Pauly (2017), Hamackova et al. (2000), Huang (2018), Tiwary et al. (2013), Zhao, Zhu, et al. (2020), Zhao, Zhang, et al. (2020)
	2	6.0–6.9 or 8.6–9.0	
	3	≤5.9 or ≥9.1	
Transparency (cm)	1	30–60	Huang (2018)
	2	20–29 or 61–70	
	3	≤19 or ≥71	
Nonionized ammonia (mg/L NH_3)	1	0.000–0.025	Ijaz et al. (2010)
	2	0.026–0.059	
	3	≥0.060	
Nitrite (mg/L NO_2)	1	0–0.9	Alcaraz and Espina (1997, 1994), Alcaraz and Rangel (2004), Espina and Alcaraz (1993), Williams et al. (1997), Xiao et al. (2017)
	2	1.0–1.5	
	3	≥1.6	
Alkalinity (mg/L as $CaCO_3$)	1	25–100	Cudmore et al. (2004)
	2	≥101	
	3	≤24	
Dissolved oxygen (% of saturation)	1	≥49	Cudmore et al. (2004), Gan et al. (2013), Poli et al. (2004), Zhao, Dong, and Xu (2018)
	2	43–48	
	3	≤42	
Salinity (psu)	1	0–0.7	Ahmed and Jaffar (2013), Cudmore et al. (2004), Froese and Pauly (2017), Jaafar and Ahmed (2011), Kilambi (1980), Kilambi and Zdinak (1980); Maceina et al. (1980), Maceina and Shireman (1980, 1979), Makvandi et al. (2010, 2012), Xiao-qin et al. (2007), Yavuzcan-Yıldız and Kırkavgaç-Uzbilek (2001)
	2	0.8–8.0	
	3	≥8.01	
Terrestrial predators	1	Absence	Cudmore et al. (2004)
	2	Controlled presence	
	3	Uncontrolled presence	
Predators and other aquatic interspecific inhabitants	1	Absence	Cudmore et al. (2004)
	2	Controlled presence	
	3	Uncontrolled presence	

TABLE 5 Nutritional indicators for juveniles and adults of grass carp

Indicators	Score (S)	Juvenile 2.1–150 g	Adults >150 g	Bibliographic references
Crude protein (%)[a]	1	33–44	28–35	Du et al. (2009), FAO (2021), Khan et al. (2004), Köprücü (2012), Towers (2010), Veiverberg (2009), Xu et al. (2016)
	2	29–32	25–27	
	3	≤28 or ≥45	≤24 or ≥36	
Dry feed amount (% biomass)[a]	1	3.0–6.0	2.0–3.0	Cerva (2003), Cui et al. (1994), Du, Liu, Tian, He, et al. (2005), Du, Liu, Tian, Wang, et al. (2005), FAO (2021), Huisman and Valentijn (1981), Marques et al. (2004), Muelbert (2012), Towers (2010)
	2	1.0–2.9	1.0–1.9	
	3	≤0.9 or ≥6.1	≤0.9 or ≥3.1	
and/or				
Natural food amount (% biomass)[b]	1	30–44	100–200	Cerva (2003), Cui et al. (1994), Du, Liu, Tian, He, et al. (2005), Du, Liu, Tian, Wang, et al. (2005), FAO (2021), Huisman and Valentijn (1981), Muelbert (2012), Shireman and Smith (1983), Towers (2010)
	2	10–29	50–99	
	3	≤9 or ≥45	≤49 or ≥201	
Feeding frequency (times/day)	1	≥3	≥2	FAO (2021), Nekoubin and Sudagar (2012), Wu et al. (2021)
	2	2	1	
	3	≤1	<1	
Food distribution (% of surface area reach)	1	>75% of surface area		Pedrazzani et al. (2020)
	2	50%–75% of surface area		
	3	<50% of area surface		
Fish condition factor (K)	1	≥1.20		Bogutskaya et al. (2017), Chitrakar and Parajuli (2017), Taher (2021)
	2	1.00–1.19		
	3	≤0.99		

[a]Values do not apply to natural food.

[b]Amount of food based on vegetation such as grass and other plants.

or natural food) through direct observation by the evaluator during feed management. All these are indirect indicators of the degree of nutritional welfare of grass carp. The condition factor (*k*) of the fish is a direct measure of the nutritional status of the farmed animals.

For the eight health indicators identified, the welfare scores were determined using qualitative data and should be analysed by the evaluator based on a clinical examination of the external morphology of the animals (eyes, jaws, operculum, skin, fins, gills, abdomen and anus, Table 6). The evaluation should be carried out during management activities, when the evaluator has direct access to the animals, i.e. during vaccination, classification and biometric examination of the farmed fish. Mortality, a quantitative indicator, refers to the total percentage of dead individuals recorded up to that point or at the end of the rearing cycle analysed.

The proposed animal behavioural welfare indicators have been subdivided according to farm management. Individual respiratory frequency should be analysed during acclimatization, classification and transfer of fish between ponds (Table 7). Swimming behaviour can be analysed during massive, partial or complete harvesting procedures. During invasive procedures requiring anaesthesia, such as vaccinations, induction (reduction in surgical movement and swimming) and recovery times from anaesthesia (restoration of normal swimming position and swimming ability) should be measured. The analysis of feeding behaviour follows the same logic and method already explained for nutritional indicators, with the possibility of scoring according to the type of food—dry feed or forage—or the average between the two.

3.2 | Weighting the indicators of grass carp welfare

Table 8 shows the keywords used in the literature review to define the weighting (*Y*) of each indicator used to define PWI_x. The environmental indicators recorded the highest number of documents and among them, the term 'temperature' was the most cited with 5430 references. The least cited term was 'feeding area' with only 36 entries. Converting the respective number of citations by their natural logarithm reduces these discrepancies and makes the assumed weights mathematically and conceptually proportional to each other.

3.3 | Simulation of the welfare indices of grass carp

To test the welfare indices of farmed fish, we ran two simulations with hypothetical data on grass carp. In the first scenario (Figure 2), the GWI was 0.75, indicating that the fish were bred with likely high welfare. However, the GCL in this case was 0.68, so confidence in this

TABLE 6 Health indicators for juveniles and adults of carp

Indicators	Scores (S)	Reference values	Bibliographic references
Eyes	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Bleeding, unilateral exophthalmos or traumatic injury	
	3	Bleeding, bilateral exophthalmos or traumatic injury; chronic condition, impaired vision	
Jaws/lips	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Bleeding, redness or mild injury or deformity (without affecting eating)	
	3	Bleeding, redness or severe injury or deformity (affecting eating)	
Operculum	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Absence of tissue (<25%)	
	3	Bleeding, redness, absence of tissue (≥25%)	
Skin	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Scar tissue, punctual loss of scales, ulcers or superficial lesions <1 cm^2	
	3	Rising or general loss of scales, ulcers or superficial lesions >1 cm^2, redness, necrosis, darkening or lightening, bleeding, swelling, parasites	
Fins	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Light lesion or splitting	
	3	Severe bleeding, ray exposure, necrosis, foreign body or parasite	
Gills	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Injury, mild necrosis, splitting or thickening	
	3	Bleeding, redness, pallor, severe necrosis, excess mucus, spots, swelling, deformation, adhered foreign body, parasite	
Abdomen	1	Normal and healthy appearance	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	Discreet distension, redness	
	3	Bleeding, severe distension with or without the presence of fluid, weight loss	
Anus	1	Normal and healthy appearance	Hoole et al. (2008)
	2	Faecal residue, swelling and redness	
	3	Necrosis, bleeding, severe injury	
Mortality (%)	1	≤10	Hoole et al. (2008), Pedrazzani et al. (2020)
	2	11–24	
	3	≥25	

information was only medium, as the average number of indicators effectively analysed for each freedom was below 70%. In the second scenario (Figure 3), we simulated a case where the GWI was only 0.41, indicating that the fish were exposed to low welfare during their rearing. In this case, the GCL of 0.75 would confirm the high level of confidence in this information. For illustration, in this second case, we also simulated that the feed offered consisted of artificial and natural food and that the average score for the amount of feed offered was 1.5.

4 | DISCUSSION

This study is based on specific evidence of the welfare of *C. idella* farmed in earthen ponds. The grass carp has been named the most widely produced aquaculture species in the world (FAO, 2020b), and although data on aquaculture production systems are not fully traceable (Tidwell, 2012), earthen ponds are certainly the most widely used system for producing farmed fish on the planet. Only China, the largest aquaculture producer in the world, has more than 2,64,000 ha of ponds for freshwater fish farming (FBMA, 2020).

4.1 | Freedoms and their indicators

The indices were constructed to directly cover four of the five freedoms identified by the Farm Animal Welfare Council (FAWC, 1979). Only psychological freedom, which relates to fear and suffering and aims to ensure conditions and treatments for fish that avoid mental suffering, was not analysed using indices. But there are good reasons for this. The concept of animal welfare based on allostasis (Korte et al., 2007), which presents itself as an alternative to the 'five freedoms', argues that behaviour and physiology should be interpreted in terms of animal perceptions and not only in terms of human values. The problem with this is that the domestication of most fish species only began in the mid-20th century (Balon, 2004; El-Sayed, 2006; Teletchea, 2015; Teletchea & Fontaine, 2014). Thus,

TABLE 7 Behavioural indicators for juvenile and adult grass carp

Procedure	Indicators	Scores (S)	Reference values	Bibliographic references
Acclimatization classification transfers	Respiratory frequency (opercular rate/min)	1	40–70	Zhao, Xia, et al. (2018), Zhao, Dong, and Xu (2018)
		2	20–39 or 71–90	
		3	≤19 or ≥91	
Invasive procedure (vaccination)	Anaesthesia—surgical stage (reduction in opercular rate, absence of swimming)	1	Induction or recovery in 2–4	Hamackova et al. (2006)
		2	Induction or recovery ≤1 or ≥5	
		3	No induction or no recovery; death	
Massive capture (partial or total)	Swimming behaviour	1	Regular swimming, no or few body parts on the surface	Noble et al. (2018), Pedrazzani et al. (2020)
		2	Restless swimming behaviour, swimming in different directions and or jumping	
		3	Decreasing activity; fish trapped against the net or swimming sideways; exposure of the body to air; exhaustion	
Food intake	Feed intake (minutes)	1	3–5	Pedrazzani et al. (2020)
		2	2–3 or 5–7	
		3	≤1 or ≥8	
	and/or	1	120–180	Cui et al. (1993), FAO (2021), Ni and Wang (1999)
	Forage intake (minutes)	2	60–119 or 181–240	
		3	≤59 or ≥241	

TABLE 8 The number of documents identified by Google Academic search on 4 March 2022, with the terms *Ctenopharyngodon* AND *idella* AND Welfare AND '*keyword*' and their weighting ($W = \text{Int}(\ln(n))$)

Freedom	Keyword	Number of documents (*n*)	Weight (*Y*)
Environmental	Temperature	5430	9
	pH	4730	8
	Oxygen	4150	8
	Salinity	1930	8
	Ammonia	1860	8
	Predators	1320	7
	Nitrite	848	7
	Alkalinity	638	6
	Transparency	556	6
	Interspecific interactions	82	4
Health	Mortality	3090	8
	Skin	2240	8
	Gills	1460	7
	Fins	813	7
	Eyes	516	6
	Abdomen	273	6
	Jaw	254	6
	Operculum	171	5
	Anus	149	5
Nutrition	Condition factor	730	7
	Amount of food	275	6
	Feed protein	103	5
	Feeding area	36	4
Behavioural	Swimming	1480	7
	Food intake	643	6
	Anaesthesia	210	5
	Respiratory rate	43	4

the recognition and study of the mental dimensions of fish are still very recent and incipient in terms of scientific development, especially compared to what happens with animals used by land farming. In particular, the effects of domestication on the welfare of farmed fish are more complex to study, as fish differ significantly from terrestrial animals in terms of genetics, physiology and behaviour, and they experience completely different sensory world (Foster, 2018; Saraiva, Castanheira, et al., 2019). Therefore, empathizing with fish and understanding their needs is even more challenging than in studies of the welfare of terrestrial animals that serve as food sources for humans (IGN, 2020).

Another important point is that it is usually not useful to analyse the different freedoms individually, as the effects of the different biotic and abiotic stressors are usually interactive and even cumulative. It is therefore difficult to distinguish between the underlying and immediate causes of mortality in a fish farming system (Ellis et al., 2012). In more extreme cases, although not uncommon, factors responsible for increased stress can lead to outbreaks of disease and even death in animals. Most causes of death can be assumed to be related to the previous suffering. This has led some authors to suggest that mortality rates should be used along with other indicators in regulations and legislation related to the keeping of farmed fish (Ellis et al., 2012; Gåsnes et al., 2021; Santurtun et al., 2018). We agree with this proposal and believe that the mortality rate recorded in an aquaculture farm should be the first indicator considered when characterizing the welfare of farmed fish. In the case of *C. idella* reared in ponds, we suggest that a mortality rate of 30% or more, in the short or long term, should automatically trigger the knockout threshold so that the GWI is classified as 'critical'. The loss of almost 1/3 of the herd, from whatever cause, means that the animals have been exposed to excessive stress factors that have irreversibly affected their welfare. If the final rate is below this threshold, the mortality is considered in the other indicators of fish welfare with the corresponding score.

Dead or moribund fish in the husbandry system should therefore automatically be a warning sign for fish farmers. The following avenues should be explored: identify and adequately quantify mortality of stocked fish; conduct analyses to determine the causes; take measures to eliminate them. The fish farmer should also bear in mind that certain diseases are classified as notifiable by national or international legislation (Dixon & Longshaw, 2005).

4.1.1 | Environmental indicators

The greatest strength of environmental indicators of fish welfare is that they are observational and quantitative. They allow the construction of sub-indices of robust and reliable welfare rather than an assessment based on the opinion of the evaluator or their qualifications and specialization. Caution is needed in determining the most appropriate indicators for each species and farming system, and especially in the reference values used to characterize the indicators. An example is fish stocking density, an issue usually assessed as central to welfare in aquaculture (Carbonara et al., 2019; Frisso et al., 2020; Liu et al., 2019; Lu et al., 2022; Santurtun et al., 2018).

It is well known that high stocking density is a chronic source of stress that negatively affects growth rates, changes the physical condition and fish health (Baldwin, 2011). However, there are also significant and even critical differences between life stages of a species that directly affect potential stocking density and fish behaviour, physiology and health. Rui et al. (2006) postulated that fish mortality at high stocking densities may be due to an autoregulatory mechanism of the population, a self-protective measure of the species to avoid more intense intraspecific competition. Furthermore, the definition of the density of organisms used in aquaculture also depends on the intrinsic characteristics of the particular husbandry system, the management practices applied and, more recently, the energy supplied to the system (Cozer et al., 2020), either in the form of dissolved oxygen (Burke et al., 2021; Li, Shen, et al., 2021), as food

General Walfare Index (GWI) for the grass carp (*Ctenopharyngodon idella*)

Environmental (En)	***Score (S)***	***Weight (Y)***	***S X W***		
				ΣY_{An}	56
Temperature	2	9	18	ΣY_{Max}	71
Ammonia	1	8	8	$\Sigma(SxY)$	73
Dissolved oxygen	2	8	16	*nS*	8
pH	1	8	8	*tS*	10
Salinity	0	8	0	PWI_{En}	**0,65**
Nitrite	0	7	0	CL_{En}	0,80
Predators	1	7	7		
Alkalinity	1	6	6		
Transparency	1	6	6		
Interspecific inhabitants	1	4	4		

Behavioral (Be)	***Score (S)***	***Weight (Y)***	***S x Y***		
				ΣY_{Be}	17
Swimming behavior	1	7	7	ΣY_{Max}	22
Food intake	2	6	12	$\Sigma(SxY)$	23
Anaesthesia	0	5	0	*nS*	3
Respiratory rate	1	4	4	*tS*	4
				PWI_{Be}	**0,61**
				CL_{Be}	0,75

Nutritional (Ne)	***Score (S)***	***Weight (Y)***	***S x Y***		
				ΣY_{Nu}	15
Condition factor (*k*)	0	7	0	ΣY_{Max}	27
Amount of food supplied	1	6	6	$\Sigma(SxY)$	15
Feeding Frequency	0	5	0	*nS*	3
Crude protein in feed	1	5	5	*tS*	5
Food/Feed distribution area	1	4	4	PWI_{Nu}	**1,00**
				CL_{Nu}	0,60

Health (He)	***Score (S)***	***Weight (Y)***	***S x Y***		
				ΣY_{He}	35
Mortality	1	8	8	ΣY_{Max}	35
Skin	1	8	8	$\Sigma(SxY)$	42
Gills	2	7	14	*nS*	5
Fins	0	7	0	*tS*	9
Abdomen	0	6	0	PWI_{Be}	**0,75**
Jaw	1	6	6	CL_{Be}	0,56
Eyes	1	6	6		
Anus	0	5	0		
Operculum	0	5	0		

Mortality (%)	**5**		
kl	**1**		
General Walfare Index (*GWI*)	**0,75**	(min= 0, máx =1)	**High**
General Confidence Level (*GCL*)	**0,68**		**Medium**

FIGURE 2 Simulation of the calculation of the partial indices (PWIx) for environmental, behavioural, nutritional health freedoms, with their respective confidence levels (CL_X), of the General Welfare Index (GWI) for grass carp (*Ctenopharyngodon idella*) in a hypothetical aquaculture farm with high welfare, although the General Confidence Level (GCL) has only a medium value.

General Walfare Index (GWI) for the grass carp (*Ctenopharyngodon idella*)

Environmental (En)	Score (S)	Weight (Y)	S X W
Temperature	2	9	18
Ammonia	1	8	8
Dissolved oxygen	3	8	24
pH	1	8	8
Salinity	0	8	0
Nitrite	1	7	7
Predators	1	7	7
Alkalinity	2	6	12
Transparency	2	6	12
Interspecific inhabitants	1	4	4

ΣY_{An}	63
ΣY_{Max}	71
$\Sigma(SxY)$	100
nS	9
tS	10
PWI_{En}	0,45
CL_{En}	0,90

Behavioral (Be)	Score (S)	Weight (Y)	S x Y
Swimming behavior	2	7	14
Food intake	2	6	12
Anaesthesia	0	5	0
Respiratory rate	1	4	4

ΣY_{Be}	17
ΣY_{Max}	22
$\Sigma(SxY)$	30
nS	3
tS	4
PWI_{Be}	0,35
CL_{Be}	0,75

Nutritional (Ne)	Score (S)	Weight (Y)	S x Y
Condition factor (*k*)	2	7	14
Amount of food supplied	1,5	6	9
Feeding Frequency	0	5	0
Crude protein in feed	1	5	5
Food/Feed distribution area	1	4	4

ΣY_{Nu}	22
ΣY_{Max}	27
$\Sigma(SxY)$	32
nS	4
tS	5
PWI_{Nu}	0,53
CL_{Nu}	0,80

Health (He)	Score (S)	Weight (Y)	S x Y
Mortality	3	8	24
Skin	2	8	16
Gills	2	7	14
Fins	0	7	0
Abdomen	0	6	0
Jaw	1	6	6
Eyes	1	6	6
Anus	0	5	0
Operculum	0	5	0

ΣY_{He}	35
ΣY_{Max}	35
$\Sigma(SxY)$	66
nS	5
tS	9
PWI_{Be}	0,30
CL_{Be}	0,56

Mortality (%)	26		
kl	1		
General Walfare Index (*GWI*)	0,41	(min= 0, máx =1)	Low
General Confidence Level (*GCL*)	0,75		High

FIGURE 3 Simulation of the calculation of the partial indices (PWI_x) for environmental, behavioural, nutritional and health freedoms, with the respective confidence levels (CL_X) of the General Welfare Index (GWI) for grass carp (*Ctenopharyngodon idella*) in a hypothetical aquaculture farm with low welfare and high General Confidence Level (GCL) of this information.

(Adineh et al., 2021; Liu et al., 2019) or even as energy to remove or process toxic substances produced during the rearing cycle (Fayed et al., 2019; Gesto et al., 2020), such as renewing water or using probiotics. Due to the many variables and uncertainties, we assume that the effects of stocking density can be better assessed indirectly by using other indicators of farmed fish welfare, as we did for grass carp.

Fish depend on water to perform all their vital functions such as respiration, feeding, reproduction, osmoregulation and excretion. (Boyd & Tucker, 2019; Cudmore et al., 2004; Djiba et al., 2021; Goddard, 1996). Although *C. idella* is a rheophilic species, known for its great tolerance and adaptability to different environmental conditions, its welfare in breeding is inextricably linked to relatively well-established water quality conditions (Cudmore et al., 2004; Ijaz et al., 2010; Xie et al., 2019; Zhao et al., 2011).

For example, temperature is one of the most critical indicators for grass carp (as well as for most farmed fish species). Its fluctuations, even within species tolerance limits, can be associated with metabolic changes that have significant effects on feeding, respiration and growth rate (Cudmore et al., 2004; Galloway & Kilambi, 1984; Gerlach et al., 1990; Li, Shen, et al., 2021; Li, Li, & Wu, 2021; Poli et al., 2004; Zhao et al., 2011). Temperature also has a pronounced and direct influence on other variables that determine water quality, such as dissolved oxygen concentration (Boyd & Tucker, 2019) and the proportion of nonionized (NH_3) and ionized (NH_4^+) ammonia in water (Huu & Duc, 2021). The same logic was used to define the other environmental indicators of water quality (alkalinity, dissolved oxygen, nitrite, nonionized ammonia, pH, salinity and transparency). All these variables influence fish welfare and the zootechnical indices achieved (da Silva et al., 2006; Ding, 1991). Another important factor in determining these indicators is that they can all be measured directly in the fish farms, with relatively simple and inexpensive equipment and materials.

Interspecific inhabitants and especially aquatic or terrestrial predators are also robust environmental indicators of farmed grass carp welfare (Cudmore et al., 2004). There are reports of cases where grass carp losses due to predators can reach 60%–80% of the herd (Balami & Pokhrel, 2020; Domiciano, 2015). Terrestrial predators, such as herons, hawks and otters, can efficiently capture and kill fish. Even when attacks are unsuccessful, predators often cause severe injuries, causing the animals to suffer and die (Cudmore et al., 2004).

4.1.2 | Health indicators

Fish can be affected by various diseases, due to infections by microorganisms, neoplasms, nutritional deficiencies or environmental problems (Abowei et al., 2011). Inadequate environmental or management conditions are often the trigger of stress, the most important aetiological agent of disease in farmed fish (Harper & Wolf, 2009; Martos-Sitcha et al., 2020; Rottmann et al., 1992; Yada & Tort, 2016).

The grass carp may show various signs that indicate health problems. The main organs affected are the eyes, lips, operculum, skin, swimmers, gills, abdomen and anus (Hoole et al., 2008; Luo et al., 1993, 2015; Ni & Wang, 1999; Zeng et al., 2021). Ocular disease is common and can occur as a primary or secondary manifestation of systemic disease. Exophthalmos is a clinical sign of major bacterial diseases affecting *C. idella* breeding, such as *Aeromonas hydrophila* (Hoole et al., 2008). Swelling in the abdominal cavity is usually the result of fluid accumulation (dropsy), usually associated with bacterial infections caused by *Aeromonas* or opportunistic *Pseudomonas*, which manifest under unfavourable growth conditions (Hoole et al., 2008). The decline in immunity in this scenario, associated with improper food handling, also favours the occurrence of bacterial infections or the exacerbation of viral infections.

The grass carp is an herbivorous fish that has no stomach (Du, Liu, Tian, He, et al., 2005; Du, Liu, Tian, Wang, et al., 2005) and can consume many times its weight in forage, mainly aquatic macrophytes (Camargo et al., 2006) but also accepts artificial feeds very well (Taher, 2021; Zhao, Xia, et al., 2018). When no preferred food is available, the species may eat small fish, benthic invertebrates and insects (Lopinot, 1972). Maintaining good nutritional status supports metabolic processes, enables better response to physiological and pathogenic stressors, and ensures fish gut health (Akter et al., 2016; Lall & Tibbetts, 2009). Natural foods can also be used to enrich the environment. They serve as a strategy to provide social, sensory and cognitive stimuli to grass carp and encourage the animals to perform behaviours similar to those found in nature (Santos, 2018), which helps to reduce their stress levels (Collymore et al., 2015; Giacomini et al., 2016).

Regarding nutritional aspects, we also emphasize the use of quantitative indicators to assess fish welfare. The ratio between length and weight (expressed by the body condition factor K) of animals of the same species and age can vary according to feeding and reproductive activities and provides important information on the health status of the animals. The grass carp shows a negative allometric growth pattern (Du et al., 2009). Dietary protein is one of the essential factors in fish growth performance and in determining feed costs (Lovell, 1989), although in excess it can lead to increased excretion of nitrogen residues in the water. The type, quantity and quality of food are directly related to the amount of energy consumed by the fish and, consequently, to the energy available for their growth (Cui et al., 1994). Feeding frequency is a crucial factor in determining feed intake and affects fish appetite control, nutrient utilization, feed conversion rate (FCR) and specific growth rate (SGR) achieved in the aquaculture farm (Haina Amin et al., 2018; Nekoubin & Sudagar, 2012; Wu et al., 2021). Finally, the food distribution area is related to a feature of fish feeding behaviour reflected in the movement patterns (horizontal and vertical) of individuals in the pond (Li et al., 2020) and their access to food. Anecdotal information suggests that carp quickly learn where food is easiest to find and adapt their habitat use accordingly, concentrating their activity in these locations (Jurajda et al., 2016). The aggregation of fish in limited feeding areas may reduce the use of other available habitats and even existing natural food resources. Finally, feeding strategies that lead to a concentration of food supply in limited pond areas may increase competition, resulting in less homogeneous group performance and more aggression and injury between animals (Kaushik, 2013). Since farmed fish are selected for rapid growth and food intake is a critical factor in growth rate, limited feeding areas usually affect body condition and growth rates themselves (Huntingford et al., 2010).

4.1.3 | Behavioural indicators

Behavioural indicators of welfare reflect both internal and external conditions and provide a holistic approach to the animal (Volpato, 2009). These indicators should be analysed at specific points in the management to which the fish are subjected in an aquaculture facility (e.g. biometrics, acclimation, classification, transfer, vaccination, feeding or harvest).

The daily variation in food intake and the time taken for animals to eat may represent a combination of endogenous and exogenous influences on appetite (Carter et al., 1992) and consequently on the intake of nutrients and energy. Therefore, the feeding behaviour of individuals may describe changes in the general state of grass carp welfare and feeding opportunities in the face of disturbance (Mangano et al., 2017).

A slightly more specific situation arises with stunning. Although not a common procedure in a fish farm, anaesthesia of fish may be necessary to reduce stress and avoid injury during manipulations, especially during management such as vaccination, artificial propagation, tagging, biometric assessment, biopsy, application of hormonal preparations, health check and others (Hamackova et al., 2006). Criteria used to define ideal anaesthesia include rapid initiation of the surgical procedure and gradual recovery of the animal (Aydın & Barbas, 2020; Marking & Meyer, 1985).

Measuring oxygen consumption in fish is considered an efficient energy indicator, allowing them to detect a variety of biological processes, including anaesthetic effects and stress behaviour under different challenging conditions (Remen et al., 2016). Direct measurement of oxygen consumption rate under field conditions is not trivial. But such a measure can easily be determined indirectly, for example, by recording the animals' respiratory rate (Ferrer et al., 2020; Martos-Sitcha et al., 2019).

4.2 | The partial and general indices of farmed fish welfare

Any stress inflicted on an animal triggers metabolic, physiological or behavioural responses aimed at restoring a new state of equilibrium (Korte et al., 2007). Some researchers believe that aquaculture still lacks scientifically validated and proven methods to assess fish welfare under farmed conditions (Segner et al., 2012; Tschirren et al., 2021). Although some authors have studied and proposed indicators of fish welfare (Martins et al., 2012; Noble et al., 2018; Parma et al., 2020; Pedrazzani et al., 2020; Segner et al., 2012), there are few studies that propose mathematical indices and models for a more comprehensive and conclusive analysis of the results of these indicators.

Stien et al. (2013) developed a semantic model (Swim 1.0) to assess the welfare of Atlantic salmon in cages. The authors proposed to assess all aspects that are relevant from the animals' point of view. For this purpose, 16 indicators (water temperature, salinity, oxygen saturation, flow rate, stocking density, lighting, daily mortality rate, appetite, sea lice infestation rate, condition factor, smoltification status, vertebral deformation, maturity stage, fin and skin condition) were used. These indicators were weighted based on literature research and factors defined in a semantic modelling table. Pettersen et al. (2013) developed an extended version of this model called swim 2.0. The authors also aimed to provide aquaculture health professionals with a formal and standardized assessment of fish welfare based on the selected indicators. In this case, the indicators are the condition of the eyes, heart and other abdominal organs, gills, operculum, skeletal muscles, vaccine-related diseases, morphological malformations and other parameters determined both at necropsy of dead fish and active euthanasia. In both cases, the indices range from 0 (minimum welfare) to 1.0 (maximum welfare). According to the authors, the indicators used were chosen because they are practical, can be measured in the aquaculture farms themselves and are not very complex.

Müller-Belecke (2019), used an Excel application to create an index to assess the welfare of rainbow trout and pikeperch farmed in a recirculating system. Although the proposed index includes environmental, nutritional and behavioural indicators, as well as mortality recorded during farming, the number of indicators used is small, which may affect the quality of the final analysis. Tschirren et al. (2021) have developed the application MyFishCheck to assess and document the welfare of fish in different production systems. According to the authors, the application enables simple and standardized measurement of relevant, feasible and reliable parameters from which the model derives intuitive welfare scores. In this case, the general welfare of fish is assessed using five modules (aquaculture management, water quality, fish group behaviour, morphology and anatomy). Each module includes at least 10 specific indicators.

In addition to the various aspects associated with our indices, we are also concerned with the reliability of the analyses. Under operational conditions, it is not always possible to analyse all the proposed indicators. Sometimes this happens because the activity was simply not carried out (e.g. vaccination), in other cases for purely circumstantial reasons. In the first simulation carried out (Figure 2), the CL_X was found to vary between 0.56 and 0.80, with an average value of 0.68, while the minimum threshold that would be considered a high confidence level is 0.70. The main positive points related to the welfare of the carps in the simulated breeding were their nutrition ($PWI_{Nu} = 1.0$) and the health aspects of the fish ($PWI_{He} = 0.75$). Although the GWI = 0.75 indicates a high level of welfare of the farmed fish, this information should be interpreted with caution, precisely because the number of indicators analysed was not ideal. In the second simulation (Figure 3), only one set of indicators (health) CL was below the desirable value, so the MWI of 0.41 almost certainly indicates that the welfare of the fish was severely compromised in this scenario. A more detailed analysis shows that the main problems in this case were health ($PW_{He} = 0.30$) and behaviour ($PW_{Be} = 0.35$). The whole step-by-step process involving the construction of these indices, the concepts and the methods proposed here could be applied to many fish species and farming systems, as long as the appropriate indicators for the species/system in question are defined beforehand, the respective reference values are adjusted and their relative weights are determined. With the method proposed here, the results obtained in different scenarios can be directly compared, even if the indicators to be used, the species or the farming systems are not the same. In addition, the indices identified can show the risks of compromising fish welfare at certain points in a production cycle or be used to assess the cumulative data over the whole cycle.

5 | CONCLUSION

Assessing the welfare of farmed fish is, by definition, a means to optimize the production process and a way to value farmed animals and spare them pain, fear, hunger and unnecessary suffering, but it cannot and should not be seen as a goal. Therefore, we understand the result of our work as just another step on the way to an increasingly technical, scientific and nontechnical assessment of farmed fish welfare.

One difference between the indices and the method proposed here is that they fully meet the six criteria proposed by Noble et al. (2012), so that fish welfare indicators can be classified as 'operational': (1) they give an accurate picture of fish welfare; (2) they are easy to apply on the farm; (3) they are reliable; (4) they are repeatable; (5) they are comparable; (6) they are appropriate and fit the purposes of the farming system or routines of the aquaculture farm.

Furthermore, in this paper, we have used the grass carp as an example to evaluate what the indices and models developed by other authors to assess the welfare of farmed fish have to offer, while trying to overcome their shortcomings and limitations. However, the developed set of modular welfare indices can be adapted and applied to any farmed fish and production system, thus expanding the scope and applicability of the method. The reduced number of only three scores per indicator and the adoption of noninvasive quantitative indicators have reduced the subjectivity of the semantic data models, increasing the chances of replicating results from the field and the protocol adaptation for many species. The robust indices developed can reduce subjectivity in the interpretation of results by combining scientific information from the literature with the application of field protocols. Finally, the protocol and indices proposed here are dynamic and allow the number of publications on a given topic increases, the respective weights of each indicator can be reviewed and changed without losing comparability with previously calculated analyses and indices. The integration of all these features makes this work an important step towards a highly trustworthy assessment of fish welfare.

The next step in our work is to develop an application integrated into a cloud database for direct application of the indices to aquaculture farms, their documentation and validation under operational conditions. The system should enable better traceability of products and processes and allow problems related to the management of farmed fish to be identified as early as possible. As a result, the method proposed here can help to find solutions to the problems identified, thus minimizing the commitment to fish welfare and the risks of technical and financial damage to fish farmers. However, such validation must of course take place over time and requires analysis and acceptance of the results by experts and fish farmers, as well as confirmation of their usefulness by the different sectors involved in the aquaculture production chain.

AUTHOR CONTRIBUTIONS

Ana Pedrazzani involved in conceptualization, data curation, funding acquisition, project administration and writing—original draft, review and editing. *Camila Tavares* and *Nathieli Cozer* involved in data curation, formal analysis, methodology, software and writing—original draft. *Murilo Quintiliano* involved in funding acquisition, supervision and writing—review and editing. *Antonio Ostrensky* involved in conceptualization, data curation, funding acquisition, project administration, supervision, validation and writing—original draft, review and editing.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the content of the manuscript, and there is no financial interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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