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SHORT COMMUNICATION

Effect of hypothermic shock as a method of euthanasia for wild fish from the Yucatan coast

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Abstract The present study evaluated the effect of hypothermic shock as a method of euthanasia for some genus of wild fish from the Yucatan coast: *Eucinostomus, Floridichthys, Mayaheros, Parachromis, Poecilia*, and *Trachinotus*. Two indirect post-mortem indicators were used to evaluate the method: muscle pH and *rigor mortis* (RM) index. These indicators were measured after the death of the fish at different time intervals: 0, 2, 6, 12, 24, 48, and 72 hours. The study revealed no correlation between pH and RM index in the fish analyzed ($P > 0.05$), excluding the genus *Trachinotus*, where this correlation was found significant ($P < 0.05$). In most genera, muscle pH decreased during the first 24 hours post mortem and subsequently stabilized or increased toward 72 hours. The onset of RM was observed from 2 hours post mortem, reaching maximum values between 6 and 24 hours post mortem, depending on the genus. In the context of the experimental conditions that were assessed, hypothermic shock produced post-mortem changes in muscle pH and RM that were consistent with those that had been previously reported for fish subjected to rapid cooling. However, given the indirect nature of these variables as *post-mortem* indicators, the present results do not support direct conclusions regarding pain, distress, or consciousness during euthanasia. Subsequent studies incorporating direct physiological and behavioral indicators, as well as increased sample sizes, are necessary to evaluate the welfare implications of this method.

Keywords Hypothermic shock. Fish. pH. Rigor mortis. Bioethics

Introduction

The use of animals as experimental models has led to significant contributions to disciplines such as physiology, parasitology, virology, toxicology, pharmacology, microbiology, and immunology, among others (Barrios et al. 2011). However, aquatic species have also been used as experimental models for the study of these and other disciplines. In the specific case of fish, the advantages they offer for research and education are related to their wide diversity at the taxonomic and environmental levels, as well as their ease of adaptation in captivity and their low cost of maintenance, feeding, and reproduction (Fabacher and Little 2000). The use of wild fish in experimental research has been employed to develop methodologies for the rescue and conservation of species classified as at risk. These methodologies have also been used in genetic improvement studies, the development and/or modification of diets, and the study of food preferences

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through the examination of digestive content. Furthermore, otoliths have been examined to determine the age and growth of organisms, and reproductive studies have been conducted to determine the stage of gonadal development.

In this context, the refinement of techniques is imperative to ensure the ethical management of aquatic organisms in research. This refinement should prioritize minimizing the suffering of animals used during experimental procedures, including at the end of their lives. Consequently, in the context of research involving the collection of biological samples (e.g., biopsies, blood, whole organs), it is imperative to establish euthanasia protocols to ensure the humane end of life for the captured organisms. It is imperative to acknowledge that the practice of euthanizing any organism, specifically for the purposes of research or education, is subject to stringent ethical guidelines that are meticulously delineated for each species. These ethical principles are designed to minimize pain or suffering, both prior to and during the euthanasia procedure (Huntingford et al. 2006; NHMRC 2004).

According to the American Veterinary Medical Association (AVMA 2020), two types of euthanasia methods can be used on fish: non-inhalation methods (immersion, injection) and physical methods (decapitation followed by pithing, cervical transection, cranial contusion followed by pithing or exsanguination, penetrating captive bolt, maceration, and hypothermic shock). However, selecting the most suitable method can be challenging due to the absence of universal standards and the potential for variation in international guidelines. This challenge is further compounded by the unique anatomical, physiological, behavioral, and environmental characteristics that are distinct for each species (ACEC 2015).

One of the most widely used and recommended euthanasia methods for fish is hypothermic shock, which consists of immersing the fish in a container with ice and water (ratio 1:2) at a temperature of 2 to 4°C to ensure optimal exposure and rapid cooling of the fish (AVMA 2020). This method ensures a temperature difference between the water where the fish are caught and/or farmed and the container where the procedure will be carried out (at least 10°C).

This method has proven effective in small-bodied tropical and subtropical stenothermic species with a lethal temperature range below 4°C, such as sea bass (*European seabass*) (Zampacavallo et al. 2015), sea bream (*Sparus aurata*) (Huidobro et al. 2000), and zebrafish (*Danio rerio*) (Wallace et al. 2018; Wilson et al. 2009). It has been determined that other species are also suitable candidates for the application of this method, including hardhead catfish (*Ariopsis felis*), silver perch (*Bairdiella chrysoura*), paguara (*Chaetodipterus faber*), silver mojarra (*Eucinostomus argenteus*), gray snapper (*Lutjanus griseus*), red drum (*Sciaenops ocellatus*), yellowtail (*Urobatis jamaicensis*), ladyfish (*Elops saurus*), broad-striped anchovy (*Anchoa hepsetus*), scaly sardine (*Harengula jaguana*), lizardfish (*Synodus foetens*), toadfish (*Opsanus beta*), white mullet (*Mugil curema*), needlefish (*Strongylura notata*), white jack mackerel (*Caranx latus*), head-biter fish (*Chriodorus atherinoides*), lamparosa (*Selene vomer*), yellow pomfret (*Trachinotus carolinus*), toadfish (*Sphoeroides testudineus*), gilthead seabream (*Sparus aurata*), and Amazonian arowana (*Osteoglossum bicirrhosum*) (Bonilla-Gómez et al. 2013; De Freitas et al. 2025; Mace and Rozas 2018; Ntzimani et al. 2022; Vega-Cendejas et al. 2023).

The employment of hypothermic shock has been demonstrated to effectively reduce brain function and rapidly induce consciousness in fish, concomitant with a decline in metabolic rate, oxygen consumption, and immobilization until death (Bagni et al. 2007; Hovda and Linley 2000; Poli 2009; Robb and Kestin 2002). This method of euthanasia offers several advantages: it can be performed on multiple organisms simultaneously, it requires minimal handling and there is minimal risk of error when preparing the immersion bath, and it also reduces health risks for the person performing the procedure compared to other chemical and physical methods of euthanasia used on fish (Wilson et al. 2009). Moreover, it is regarded as an eco-friendly approach, as it does not generate waste that could be toxic. It is also cost-effective and aligns with bioethical procedures when performed in accordance with established guidelines. However, observations have revealed the formation of ice crystals and prolonged cooling times in certain species or body sizes, as well as species-dependent responses to rapid cooling (Reilly 2001).

A variety of indicators can be measured in blood or tissue to assess the efficiency and level of stress caused in fish when they are subjected to a method of euthanasia. These indicators include cortisol (Reddy and Leatherland 1998), glucose and lactate concentrations in blood (Daskalova 2019; Roque et al. 2021; Rotllant and Tort 1997; Simontacchi et al. 2008), ATP levels (Poli et al. 2005; Pottinger 2001; Tejada et al. 2001), pH, and the onset of RM in muscle (Acerete et al. 2009; Izquierdo-Pulido et al. 1992; Nakayama et

al. 1992; Sigholt et al. 1997).

Post-mortem variables, such as muscle pH and RM index, can offer indirect insights into pre-slaughter muscular activity and metabolic status. This is due to the potential modification of glycogen utilization, post-mortem glycolysis, muscle acidification, ATP depletion, and the onset of RM by stress and intense muscular activity prior to death. However, it is important to note that these variables do not directly measure pain, distress, or consciousness. This study had two objectives: first, to characterize post-mortem changes in muscle pH and RM following hypothermic shock in wild fish from the Yucatan coast; and second, to evaluate the applicability of this method under field conditions.

Materials and methods

Ethics statement

The protocol employed in this study was performed in accordance with the ethical guidelines established by the AVMA (2020), with hypothermic shock serving as the method of euthanasia. The protocol in question was subjected to a rigorous evaluation and approval process. This process was conducted in accordance with the established procedures recommended by two prominent ethical review committees: the Comité de Ética en Investigación y Docencia (CEID) (approval no. 004/INV/2023, October 12th, 2023) of the Universidad Nacional Autónoma de México (UNAM), and the Comité de Responsabilidad Científica y Ética Académica (CEAR) (approval no. PD_21_06_2023_Gaxiola, June 21th, 2023) of the Facultad de Ciencias at UNAM.

Origin of experimental organisms

The capture of wild fish was recorded in October 2023 in Sisal, Yucatan, Mexico (21°9’55.22” N, 90°1’54.93” W). The collection was carried out under the Protection for Small Boat Fishing Permit (PPF/DGOPA-036/23), issued by the Direccion General de Ordenamiento Pesquero y Acuicola, Comision Nacional de Acuacultura y Pesca (CONAPESCA). The capture methods employed included cast nets with a radius of 0.70 m and a mesh size of 1 cm, as well as a 20 m long gill net with a drop of 1 m and a mesh size of ½ inch. The total number of fishes used in this study was 29, and they belonged to the following genera: *Eucinostomus, Floridichthys, Mayaheros, Parachromis, Poecilia,* and *Trachinotus.*

Euthanasia method

The procedures carried out were similar for all organisms captured: the time elapsed between capture and immersion in the ice water bath was approximately 2–3 minutes. During this period, fish were maintained in water collected from the capture site (19 L) at approximately 28°C while health condition, body weight, total length, and life stage were recorded. Subsequently, the fish were transferred to a cooler with a capacity of 142 L, which was filled with a mixture of crushed ice and water from the site in a proportion of 1:2. A pocket temperature gauge (Hanna Instruments®, model H198121) was employed to verified that the water temperature remained between 2–4°C, ensuring the efficacy of the method.

During exposure to the ice-water bath, fish were continuously monitored for loss of equilibrium, absence of escape responses, loss of the vestibulo-ocular reflex, and cessation of opercular movements. The absence of spontaneous movement, in and of itself, was not regarded as adequate evidence to substantiate the determination of death. The operational confirmation of death was determined by the persistent absence of opercular movements for a minimum of 10 minutes, in conjunction with the absence of observable reflex responses.

Measurement of pH and *rigor mortis*

To characterize post-mortem changes following the euthanasia method, muscle pH and RM index were measured. These were monitored at intervals of 0, 2, 6, 12, 24, 48, and 72 hours post mortem (hpm), according to previously described methodologies (Daskalova 2019; Roque et al. 2021).

The pH of the muscle tissue was measured *in situ* using a potentiometer with a penetration electrode specifically designed for use with meat (Hanna Instruments®, model HI99163), ensuring an accurate reading directly in the muscle tissue. The RM index was determined using a modified protocol from Roque et al. (2021). Each fish was carefully placed on a flat surface, leaving two-thirds of its length protruding beyond the edge, i.e., without structural support. Subsequent to a five-second interval, the degree of caudal fin descent relative to the horizontal plane was recorded. The RM index was subsequently calculated as a percentage using the following formula:

RM index (%) = 100 ((current height - height before rigor mortis set in)) / (current height)

Between measurements for both indicators, the fish were placed individually in a polystyrene bag and stored at 4°C (Roque et al. 2021).

Final disposal of organisms

Once the pH and RM index measurements were completed, the specimens were taxonomically identified and donated to the Coleccion Ictiologica Regional de Referencia UMDI-Sisal (registration DGVS YUC-PEC-239-01-11) and the Coleccion de Otolitos de Peces del Golfo de Mexico (registration DGVS CC-305-18), both of which are housed in the Laboratorio de Biología de la Conservación (BioCon), Facultad de Ciencias, UNAM.

Statistical analysis

The results for each variable analyzed were expressed as mean ± standard error. Spearman's correlation coefficient was used as an exploratory analysis to assess the association between muscle pH and RM index across the post-mortem observation period, considering $P < 0.05$ as statistically significant. Given the repeated-measures structure of the dataset and the small and uneven sample sizes among genera, temporal patterns were interpreted descriptively. All statistical analyses and graph generation were performed in R using RStudio (version 4.4.1) (R Core Team 2024).

Results

Morphometric parameters

Table 1 shows the genera and life stages of the fish captured in this study, as well as their mean body length (cm) and body weight (g).

Measurement of pH and *rigor mortis*

As illustrated in Table 2, the results of the exploratory Spearman correlation analysis between muscle pH and RM index across the post-mortem observation period are presented. No statistically significant association between these variables was detected for *Eucinostomus, Floridichthys, Mayaheros, Parachromis*, and *Poecilia* ($P > 0.05$). For *Trachinotus,* a statistically significant negative association was observed ($P <$

Table 1 Genera and mean ± standard error of body length (cm), body weight (g), and life stage of the fish captured in this study.

Genera	Body length (cm)	Body weight (g)	n	
			Juveniles	Adults
Eucinostomus	9.18 ± 2.78	10.65 ± 4.42	6	1
Floridichthys	8.26 ± 1.55	11.06 ± 2.84	3	-
Mayaheros	15.05 ± 4.41	70.31 ± 21.98	3	4
Parachromis	15.96 ± 1.07	55.23 ± 13.74	3	-
Poecilia	8.01 ± 0.58	6.71 ± 0.71	-	6
Trachinotus	8.06 ± 3.69	9.90 ± 7.70	3	-

Note: cm: centimeters; g: grams; n: number of organisms

0.05); however, this result should be interpreted with caution. This is due to the small number of individuals represented in this genus and the exploratory nature of the analysis where repeated measurements were obtained from the same individuals.

Figure 1 shows the temporal changes in muscle pH observed during the 72 hpm period. In genera *Eucinostomus, Floridichthys, Mayaheros*, and *Trachinotus,* the lowest mean pH values were recorded at 24 hpm, followed by an increase toward 72 hpm (Table 3). A similar pattern was observed in *Mayaheros* and *Poecilia*, although their highest mean pH values were recorded at 0 hpm. In *Parachromis*, the lowest mean pH values were observed at 2 and 12 hours hpm, whereas the highest values were recorded at 72 hpm

Table 2 Exploratory Spearman correlation analysis between muscle pH and RM index across the post-mortem observation period in the fish genera evaluated in this study.

Genera	R	P value
Eucinostomus	0.29	0.08
Floridichthys	-0.37	0.13
Mayaheros	-0.003	0.98
Parachromis	-0.02	0.91
Poecilia	-0.16	0.34
Trachinotus	-0.50	< 0.05

P values reported as "< 0.05" indicate a statistically significant association between muscle pH and RM index

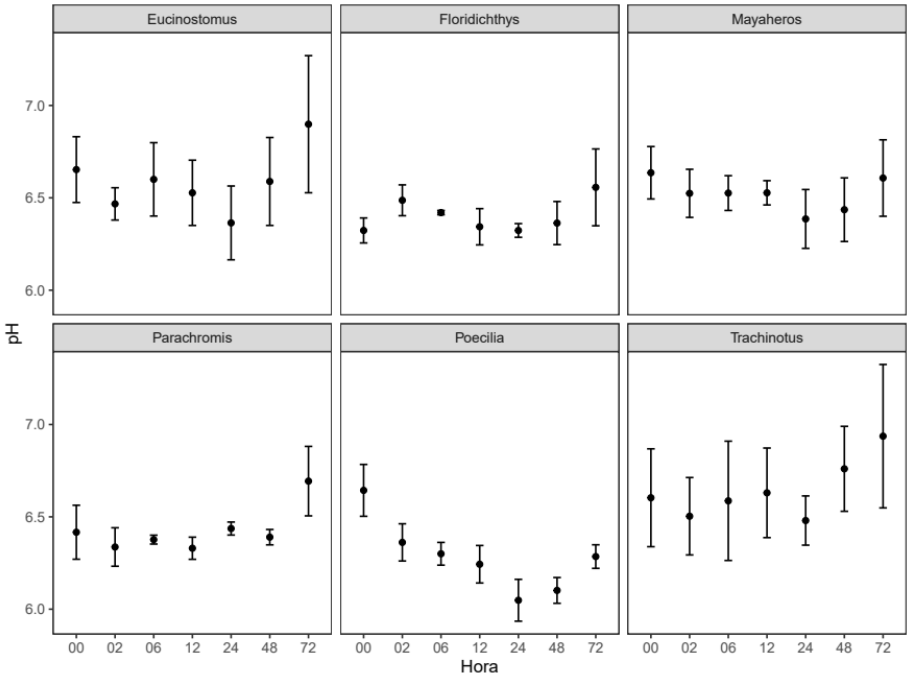


Fig. 1 Comparison of the mean ± standard error of post-mortem changes in muscle pH during different measurement times in wild fish of the genera *Eucinostomus, Floridichthys, Mayaheros, Parachromis, Poecilia,* and *Trachinotus*.

Table 3 Mean ± standard error of muscle pH values during different measurement times in wild fish of the genera *Eucinostomus*, *Floridichthys*, *Mayaheros, Parachromis, Poecilia*, and *Trachinotus.*

Genera	Time (hpm)						
	0	2	6	12	24	48	72
Eucinostomus	6.65 ± 0.08	6.46 ± 0.04	6.60 ± 0.09	6.52 ± 0.08	6.36 ± 0.09	6.58 ± 0.11	6.89 ± 0.18
Floridichthys	6.32 ± 0.03	6.48 ± 0.04	6.42 ± 0.005	6.34 ± 0.04	6.32 ± 0.01	6.36 ± 0.05	6.55 ± 0.10
Mayaheros	6.63 ± 0.07	6.52 ± 0.06	6.52 ± 0.04	6.52 ± 0.03	6.38 ± 0.07	6.43 ± 0.08	6.60 ± 0.10
Parachromis	6.41 ± 0.07	6.33 ± 0.05	6.37 ± 0.01	6.33 ± 0.03	6.43 ± 0.01	6.39 ± 0.02	6.69 ± 0.09
Poecilia	6.64 ± 0.07	6.36 ± 0.05	6.30 ± 0.03	6.24 ± 0.05	6.04 ± 0.05	6.10 ± 0.03	6.28 ± 0.03
Trachinotus	6.60 ± 0.13	6.50 ± 0.10	6.58 ± 0.16	6.63 ± 0.12	6.48 ± 0.06	6.76 ± 0.11	6.93 ± 0.19

(Figure 1, Table 3). The temporal pattern of post-mortem muscle pH exhibited variability among the genera evaluated.

Rigor mortis developed progressively during the post-mortem period, although the temporal pattern varied among the genera evaluated. The maximum mean RM index values were recorded at 6 hpm in *Eucinostomus, Floridichthys, Mayaheros, Parachromis,* and *Trachinotus*, whereas the maximum values occurred later in *Trachinotus* (12 hpm) and *Poecilia* (24 hpm) (Figure 2, Table 4). Following the peak values, the RM index typically exhibited a decline or reached a state of stability during the ensuing post-mortem interval.

Discussion

Currently, Mexico lacks specific guidelines or regulations that establish the euthanasia methods that can be applied to aquatic organisms. This includes species used in aquaculture production, wild species, and species in captivity, such as those found in educational centers, aquariums, and zoos. Consequently, studies that involve the implementation of any method of euthanasia must be supported by international regulations or by the technical specifications for the use of laboratory animals stipulated in the Norma Oficial Mexicana NOM-062-ZOO-1999 (Abeja-Pineda et al. 2023; Hernández-Sandoval et al. 2022; López-López et al. 2011; Paredes-Trujillo et al. 2020). However, this particular study focuses on the use of rodents, lagomorphs, carnivores, non-human primates, and pigs, thereby leaving a gap in relation to the use of aquatic

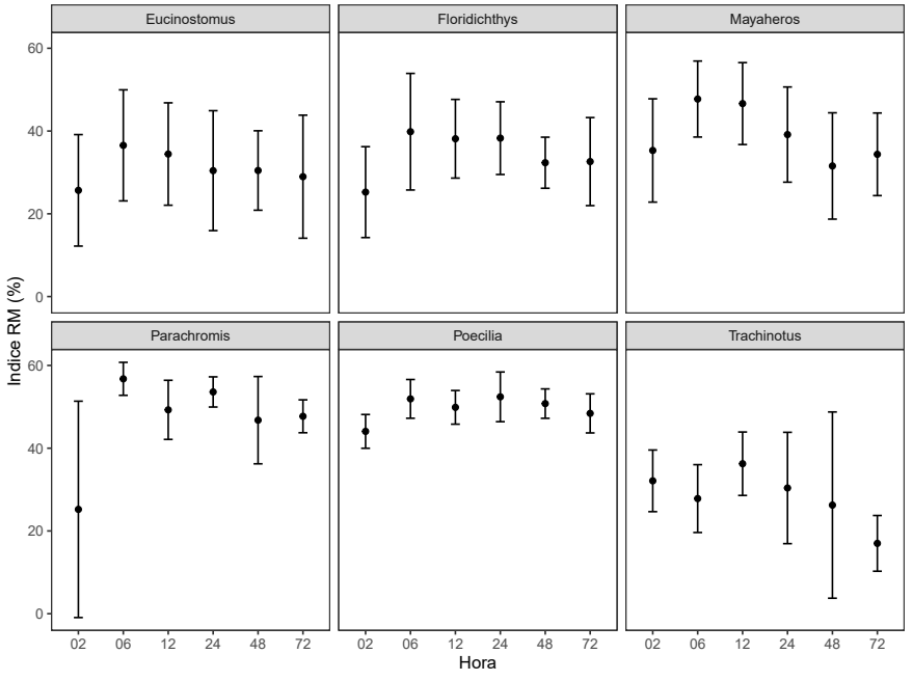


Fig. 2 Comparison of the mean ± standard error for post-mortem changes in the RM index (%) during different measurement times in wild fish of the genera *Eucinostomus, Floridichthys, Mayaheros, Parachromis, Poecilia*, and *Trachinotus*.

Table 4 Mean ± standard error of RM index values (%) during different measurement times in wild fish of the genera *Eucinostomus*, *Floridichthys*, *Mayaheros, Parachromis, Poecilia*, and *Trachinotus.*

Genera	Time (hpm)					
	2	6	12	24	48	72
Eucinostomus	25.66 ± 6.73	36.53 ± 6.71	34.44 ± 6.18	30.42 ± 7.24	30.46 ± 4.79	28.96 ± 7.43
Floridichthys	25.23 ± 5.49	39.83 ± 7.04	38.13 ± 4.75	38.28 ± 4.39	32.34 ± 3.08	32.61 ± 5.31
Mayaheros	35.30 ± 6.24	47.73 ± 4.59	46.63 ± 4.94	39.14 ± 5.75	31.55 ± 6.42	34.38 ± 4.99
Parachromis	25.21 ± 13.07	56.76 ± 1.99	49.26 ± 3.57	53.59 ± 1.82	46.77 ± 5.27	47.71 ± 1.98
Poecilia	44.06 ± 2.04	51.92 ± 2.34	49.87 ± 2.03	52.41 ± 3.00	50.77 ± 1.77	48.41 ± 2.36
Trachinotus	32.11 ± 3.72	27.82 ± 4.09	36.24 ± .83	30.38 ± 6.72	26.25 ± 11.5	17.00 ± 3.36

organisms. In this context, the present study offers significant insights by establishing the scientific basis for the development of future national guidelines and regulations.

In the context of practical teaching or the development of any research project involving the use and sacrifice of animals, it is imperative to acknowledge the necessity of selecting an effective euthanasia method that is capable of inducing rapid and irreversible loss of consciousness, along with a reduction in sensitivity prior to and during its application. This approach ensures that the animals experience minimal suffering (Lambooij et al. 2002). In addition, adherence to the bioethical procedures stipulated by current regulations is crucial.

Among the most commonly used methods of euthanasia in teaching and research involving aquatic organisms, particularly fish, are immersion in substances made from natural products (eugenol) or chemicals (benzocaine, CO_2, ethanol, isoflurane, methanesulfonate tricaine, lidocaine), electrical stunning, percussion, and hypothermic shock (AVMA 2020; Poli et al. 2005). The application of a euthanasia method in the context of wild fish is often a complex process, particularly when considering the potential challenges posed by field conditions. These conditions may include the logistics of transporting necessary materials to the sampling site, the spatial limitations of the work area, the use of personal protective equipment, waste management, and the preservation of instruments and substances to prevent spills. Additional factors that contribute to the complexity of euthanasia methods in wild fish include the cost of the procedure and the lack of training in the use of instruments and substances.

The use of hypothermic shock as a euthanasia method is a contentious subject due to its potential consequences for fish, both physically and physiologically (NHMRC 2008). These consequences may include the formation of ice crystals on the skin and within the body (Reilly 2001); the potential for a prolonged treatment time before causing the animal's death (Van De Vis et al. 2003), due to the low thermal conductivity of the tissues surrounding the brain (European Commission 1997); and an accelerated decrease in ATP and muscle pH levels during the post-mortem stage, possibly associated with increased activity caused by stress before and during the application of the method (Özogul and Özogul 2004). However, a significant proportion of these findings have been derived from large fish species inhabiting cold or temperate climate regions (Blessing et al. 2010), suggesting that this controversy may be associated with both the size and environmental characteristics of the organisms.

Nevertheless, hypothermic shock as a euthanasia method in fish is supported by various studies, regulations, and international committees that endorse its advantages from a bioethical point of view, provided that it is practiced on small to medium-sized fish from warm climates (AVMA 2020; Institutional Animal Care and Use Committee 2022; McGill University Animal Policy and Welfare Oversight Committee 2024; NHMRC 2008; NIH Intramural Research Program 2009; Wallace et al. 2018). In the present study, behavioral alterations induced by hypothermic shock were recorded during the initial two minutes in smaller fish species, including *Eucinostomus, Floridichthys, Poecilia,* and *Trachinotus*. In contrast, larger fish species, with body lengths exceeding 15 centimeters, exhibited these changes after approximately three minutes, with *Mayaheros* and *Parachromis* being the species observed. It is noteworthy that the fish genus examined in this study are classified as tropical and subtropical organisms based on their environmental characteristics, thereby rendering them suiTable candidates for the euthanasia method proposed in this study.

One post-mortem variable evaluated in this study to characterize the response to hypothermic shock in the wild fish genera captured was *rigor mortis*, defined as a post-mortem physical process related to a decrease in pH and ATP concentration in the muscle below a critical level. Furthermore, *rigor mortis* has been linked to acidification induced by lactic acid production within muscle tissue. This process depends on the stress levels experienced by the subjects before and during slaughter (Bagni et al. 2007; Thomas et al. 1999; Watabe et al. 1989). However, if the method of euthanasia and/or the handling of the organisms is stressful for them, the onset and development of *rigor mortis*, as well as the decrease in muscle pH, is faster (Sigholt et al. 1997).

The resolution of *rigor mortis*, defined as the stage in which the muscles enter a state of relaxation, is caused by endogenous (cathepsins) and microbial proteolytic enzymes, which destroy myofibrillar proteins (Sebastio et al. 1996). In the present study, *rigor mortis* resolution became increasingly evident after a 24-hour observation period, indicating loss of muscle rigidity and subsequent gradual relaxation. This result aligns with findings reported by Roque et al. (2021), who, in their study comparing various methods of euthanasia for *Sparus aurata*, observed that the resolution of *rigor mortis*, induced by hypothermic shock,

occurred after a 24-hour period following the fish's demise. In consideration of the aforementioned results, it can be posited that the progression and resolution of *rigor mortis* occurred in a manner that was consistent with the anticipated ranges for non-traumatic fatalities. The onset of *rigor mortis* occurred approximately six hours post-mortem, with resolution occurring around 24 hours later, as evidenced by the present data.

However, the differences observed in the timing of maximum *rigor mortis* among genera should be interpreted with caution and should not be attributed to differences in the effectiveness of hypothermic shock. The development and resolution of *rigor mortis* can be influenced by several biological factors, including species-specific characteristics, body size, life stage, metabolic status, pre-mortem activity, and stress produced before applying the euthanasia method (Abe and Okuma 1991; Bagni et al. 2007; Thomas et al. 1999; Watabe et al. 1989). Consequently, the subsequent occurrence of maximum RM values observed in this study in *Trachinotus* and *Poecilia* may reflect biological distinctions among the fish evaluated rather than a divergent response to the euthanasia method.

The decline in muscle pH that occurs subsequent to the death of fish is attributable to an increase in the generation of H+ ions. These ions are also associated with lactic acid production and the decrease in ATP reserves characteristic of stress (Poli et al. 2005). These alterations can lead to a decline in the physical characteristics of the fish, including muscle texture, which impacts its quality when sold as fillets for human consumption (Iwamoto et al. 1987; Izquierdo-Pulido et al. 1992; Nakayama et al. 1992; Sigholt et al. 1997). Muscle pH declined from the second hour post mortem in all fish species examined in this study, except *Floridichthys*, where the decrease occurred after six hours post mortem. These results are consistent with those reported by Roque et al. (2021), who observed a decrease in muscle pH two hours after the death of fish subjected to hypothermic shock as a freezing method. Studies conducted to determine changes in muscle pH during the post-mortem process have found that this value remains around 6.5 and tends to decrease over time after the animal's death. In the present study, muscle pH exhibited relatively moderate changes during the 72-hour monitoring period, with differences in temporal patterns among genera.

The post-mortem changes in muscle pH observed in the present study were generally gradual and within the range reported in previous studies evaluating post-mortem muscle metabolism in fish. However, several factors influence muscle pH, including pre-slaughter activity, glycogen reserves, handling, capture method, species, body size, and storage conditions. Consequently, the pH patterns observed in this study should not be interpreted as direct evidence of the absence of stress or suffering during euthanasia.

Although no correlation was found between muscle pH and *rigor mortis* for most of the fish analyzed, the exploratory Spearman analysis indicated a significant negative association between muscle pH and RM index in *Trachinotus*; however, this finding should be interpreted with caution. Only three individuals of this genus were evaluated, and repeated observations from the same individuals were included in the correlation analysis. Therefore, this association should be considered preliminary and should be confirmed in future studies using larger sample sizes and statistical approaches specifically designed for repeated-measures data.

From a bioethical perspective, the development of practical euthanasia procedures for wild fish used in research and teaching remains an important consideration. The present study provides preliminary information on post-mortem changes in muscle pH and *rigor mortis* following hypothermic shock under field conditions. However, given that these variables are indirect post-mortem indicators that do not directly assess pain, distress, or consciousness, it is not possible to establish the welfare implications of this method solely from the present results. The findings must be interpreted considering several factors. Firstly, the relatively small and uneven sample sizes among genera must be considered. Secondly, potential differences in species, body size, life stage, and metabolism must be considered. Thirdly, the possible influence of capture and handling must be considered. Consequently, future studies should incorporate larger and more balanced sample sizes, evaluate seasonal and species-specific variability, compare hypothermic shock with alternative euthanasia methods, and incorporate direct behavioral and physiological indicators of stress and welfare. This information will be necessary to more comprehensively determine whether hypothermic shock can be considered an appropriate euthanasia alternative for wild fish used in research and teaching.

Competing interests The authors declare no conflict of interest

Authors' contributions Data curation and formal analysis, R.M.G.C.B.; conceptualization, research, methodology, supervision, formal analysis, writing, review and editing, D.A.R.; research, methodology, data collection, writing-revision, review and

editing, D.AC., M.B.A. and A.G.T.; project management, G.G.C. All authors have read and agreed to the published version of the manuscript.

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References

Abe H, Okuma E (1991) *Rigor mortis* progress of carp acclimated to different water temperatures. Nippon Suisan Gakkaishi 57:2095–2100

Abeja-Pineda O, Figueroa-Lucero G, Hernández-Rubio MC, Perozzi LF (2023) Acute effect of carbofuran in *Chirostoma humboldtianum* juveniles (Atheriniformes: Atherinopsidae). Hidrobiológica 33(3):281–290. https://doi.org/10.24275/guqb9526

ACEC (2015) A guide to acceptable procedures and practices for aquaculture and fisheries research. New South West, Australia

Acerete L, Reig L, Alvarez D, Flos R, Tort L (2009) Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*). Aquaculture 287:139–144. https://doi.org/10.1016/j.aquaculture.2008.10.012

American Veterinary Medical Association (2020) AVMA guidelines for the euthanasia of animals: 2020 edition. American Veterinary Medical Association, Schaumburg

Bagni M, Civitareale C, Priori A, Ballerini A, Finoia M, Brambilla G, Marino G (2007) Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). Aquaculture 263:52–60. https://doi.org/10.1016/j.aquaculture.2006.07.049

Barrios EE, Espinoza M, Leal U, Ruiz N, Pinto V, Jurado B (2011) Bioética y el empleo de animales de experimentación en investigación. Salus 15:50–63

Blessing JJ, Marshall JC, Balcombe SR (2010) Humane killing of fishes for scientific research: A comparison of two methods. J Fish Biol 76:2571–2577. https://doi.org/10.1111/j.1095-8649.2010.02633.x

Bonilla-Gómez JL, Badillo-Alemán M, Gallardo-Torres A, Chiappa-Carrara X (2013) Temporal variation, growth and natural mortality of two species of mojarras (Perciformes: Gerreidae) from a tropical coastal lagoon: La Carbonera, Yucatan, Mexico. Rev Cienc Mar Costeras 5:57–67

Daskalova A (2019) Farmed fish welfare: stress, post-mortem muscle metabolism, and stress-related meat quality changes. Int Aquat Res 11:113–124. https://doi.org/10.1007/s40071-019-0230-0

De Freitas PHL, Bussons MRFM, Bussons INB, Dias FVN, Garcês LM, De Oliveira AT (2025) Feeding ecology of *Osteoglossum bicirrhosum* (Osteoglossiformes: Osteoglossidae) in a Central Amazon Reserve. An Acad Bras Cienc 97(2): e20241121. https://doi.org/10.1590/0001-3765202520241121

European Commission (1997) Euthanasia of experimental animals. Publications Office of the European Union. Available: https://op.europa.eu/en/publication-detail/-/publication/b0ecd88f-8c31-4a5f-9ebd-2c8ad4da4c14. Accessed 2 Feb 2025

Fabacher DL, Little EE (2000) Introduction. In: Ostrander GK (ed) The laboratory fish. Academic Press, San Diego

Hernández-Sandoval P, Timaná-Morales M, Robles-Ravelero M, Peraza-Gómez V, Ascencio F, Jiménez-Ruíz E (2022) Increase in the survival of whiteleg shrimp (*Litopenaeus vannamei*) infected with white spot virus and fed a diet supplemented with coconut (*Cocos nucifera*) oil. Abanico Vet 12:1–21. https://doi.org/10.21929/abavet2022.2

Hovda J, Linley TJ (2000) The potential application of hypothermia for anesthesia in adult Pacific salmon. N Am J Aquac 62:67–72. https://doi.org/10.1577/1548-8454(2000)062<0067:TPAOHF>2.0.CO;2

Huidobro A, Pastor A, Tejada M (2000) Quality index method developed for raw gilthead seabream (*Sparus aurata*). J Food Sci 65(7):1202–1205. https://doi.org/10.1111/j.1365-2621.2000.tb10265.x

Huntingford F, Adams C, Braithwaite V, Kadri S, Pottinger T, Sandøe A, Turnbull JF (2006) Current issues in fish welfare. J Fish Biol 68:332–372. https://doi.org/10.1111/j.0022-1112.2006.001046.x

Institutional Animal Care and Use Committee (2022) Guidelines on euthanasia of fish, amphibians and reptiles. Available: https://vpr.tamu.edu/wp-content/uploads/2024/12/TAMU-G-048-Guidelines-on-Euthanasia-of-Fish-Amphibians-and-Reptiles.pdf. Accessed 19 Nov 2024

Iwamoto M, Yamanaka H, Watabe S, Hashimoto K (1987) Effect of storage temperature on rigor-mortis and ATP degradation in plaice *Paralichthys olivaceus* muscle. J Food Sci 52(6):1514–1517. https://doi.org/10.1111/j.1365-2621.1987.tb05867.x

Izquierdo-Pulido ML, Hatae K, Haard NF (1992) Nucleotide catabolism and changes in texture indices during ice storage of cultured sturgeon, *Acipenser transmontanus*. J Biochem 16:173–192. https://doi.org/10.1111/j.1745-4514.1992.tb00444.x

Lambooij E, Van De Vis JW, Kloosterboer RJ, Pieterse C (2002) Evaluation of captive needle stunning of farmed eel (*Anguilla anguilla* L.): suitability for humane slaughter. Aquaculture 212:141–148. https://doi.org/10.1016/S0044-8486(01)00872-9

López-López E, Sedeño-Díaz JE, Soto C, Favari L (2011) Responses of antioxidant enzymes, lipid peroxidation, and Na +/K +-ATPase in liver of the fish *Goodea atripinnis* exposed to Lake Yuriria water. Fish Physiol Biochem 37:511–522. https://doi.org/10.1007/s10695-010-9453-0

Mace III MM, Rozas LP (2018) Fish predation on juvenile penaeid shrimp: Examining relative predator impact and size-selective predation. Estuaries Coasts 41(7):2128–2134. https://doi.org/10.1007/s12237-018-0409-4

McGill University Animal Policy and Welfare Oversight Committee (2024) Fish and aquatic amphibian euthanasia. Available: https://www.mcgill.ca/research/files/research/303-fish_and_aquatic_amphibian_euthanasia.pdf. Accessed 15 Apr 2025

Nakayama T, Liu DJ, Ooi A (1992) Tension change of stressed and unstressed carp muscles in isometric rigor contraction and resolution. Nippon Suisan Gakkaishi 58(8):1517–1522. https://doi.org/10.2331/suisan.58.1517

NHMRC (2004) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Canberra

NHMRC (2008) Guidelines to promote the wellbeing of animals used for scientific purposes. Available: https://www.nhmrc.gov.au/about-us/publications/guidelines-promote-wellbeing-animals-used-scientific-purposes. Accessed 15 Dec 2024

NIH Intramural Research Program (2009) Guidelines for animal study proposals using zebrafish in the NIH intramural research program. Available: https://oacu.oir.nih.gov/system/files/media/file/2023-08/b17_zebrafish.pdf. Accessed 24 Sept 2024

Ntzimani A, Angelakopoulos R, Stavropoulou N, Semenoglou I, Dermesonlouoglou E, Tsironi T, Moutou K, Taoukis P (2022) Seasonal pattern of the effect of slurry ice during catching and transportation on quality and shelf life of gilthead sea bream. J Mar Sci Eng 10:443. https://doi.org/10.3390/jmse10030443

Özogul Y, Özogul F (2004) Effects of slaughtering methods on sensory, chemical and microbiological quality of rainbow trout (*Onchorynchus mykiss*) stored in ice and MAP. Eur Food Res Technol 219(3):211–216. https://doi.org/10.1007/s00217-004-0951-0

Paredes-Trujillo A, Martínez-Aquino A, Rodiles-Hernández R, González-Solís D (2020) Metazoan parasite communities of three endemic cichlid fish species from the upper Grijalva River, Chiapas, Mexico. Helminthologia 57(4):344–352. https://doi.org/10.2478/helm-2020-0041

Poli BM (2009) Farmed fish welfare-suffering assessment and impact on product quality. Ital J Anim Sci 8(1):139–160. https://doi.org/10.4081/ijas.2009.s1.139

Poli BM, Parisi G, Scappini F, Zampacavallo G (2005) Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquac Int 13:29–49. https://doi.org/10.1007/s10499-004-9035-1

Pottinger TG (2001) Effects of husbandry stress on flesh quality indicators in fish. In: Kestin SC, Warriss PD (eds) Farmed fish quality. Fishing News Books, Oxford

R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

Reddy PK, Leatherland JF (1998) Stress physiology, fish diseases and disorders. In: Leatherland JF, Woo P (eds) Fish diseases and disorders, non-infectious disorders, vol 2. CABI Publishing, Oxfordshire

Reilly JS (ed) (2001) Euthanasia of animals used for scientific purposes. Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), South Australia

Robb DHF, Kestin SC (2002) Methods used to kill fish: Field observations and literature reviewed. Anim Welf 11:269–282. https://doi.org/10.1017/S0962728600024854

Roque A, Gras N, Rey-Planellas S, Fatsini E, Pallisera J, Duncan N, Muñoz I, Velarde A, Hernandez MD (2021) The feasibility of using gas mixture to stun seabream (*Sparus aurata*) before slaughtering in aquaculture production. Aquaculture 545:737168. https://doi.org/10.1016/j.aquaculture.2021.737168

Rotllant J, Tort L (1997) Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. J Fish Biol 51:21–28. https://doi.org/10.1111/j.1095-8649.1997.tb02510.x

Sebastio P, Ambroggi F, Baldrati G (1996) Influenza del sistema di sacrificazione su trote iridee di allevamento. I Considerazioni biochimiche. Industria Conserve 71:37–49

Sigholt T, Erikson U, Rustad T, Johansen S, Nordtvedt TS, Seland A (1997) Handling stress and storage temperature affect meat quality of farmed-raised Atlantic Salmon (*Salmo Salar*). J Food Sci 62(4):898–905. https://doi.org/10.1111/j.1365-2621.1997.tb15482.x

Simontacchi C, Poltronieri C, Carraro C, Bertotto D, Xiccato G, Trocino A, Radaelli G (2008) Alternative stress indicators in sea bass *Dicentrarchus labrax*, L. J Fish Biol 72:747–752. https://doi.org/10.1111/j.1095-8649.2007.01717.x

Tejada M, Huidobro A, Pastor A (2001) Slaughter methods affecting adenosine triphosphate and derivates in chilled stored gilthead seabream (*Sparus aurata*). In: Kestin SC, Warriss PD (eds) Farmed Fish Quality. Fishing News Books, Oxford

Thomas PM, Pankhurst NW, Bremner HA (1999) The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. J Fish Biol 54:1177–1796. https://doi.org/10.1111/j.1095-8649.1999.tb02047.x

Van De Vis H, Kestin S, Robb D, Oehlenschläger J, Lambooij B (2003) Is humane slaughter of fish possible for industry? Aquac Res 34:211–220. https://doi.org/10.1046/j.1365-2109.2003.00804.x

Vega-Cendejas ME, Hernández de Santillana M, Palacios-Sánchez S (2023) Length–weight relations of 44 fish species (Actinopterygii) inhabiting an unprotected tropical coastal biological corridor of Yucatan, Mexico. Acta Ichthyol Piscat 53:201–207. https://doi.org/10.3897/aiep.53.110519

Wallace CK, Bright LA, Marx JO, Andersen RP, Mullins MC, Carty AJ (2018) Effectiveness of rapid cooling as a method of euthanasia for young zebrafish (*Danio rerio*). J Am Assoc Lab Anim Sci 57(1):58–63

Watabe S, Ushio H, Iwamoto M, Kamal M, Ioka H, Hashimoto K (1989) Rigor-mortis progress of sardine and mackerel in association with ATP degradation and lactate accumulation. Nippon Suisan Gakkaishi 55(10):1833–1839

Wilson JM, Bunte RM, Carty AJ (2009) Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as method of euthanasia in zebrafish (*Danio rerio*). J Am Assoc Lab Anim Sci 46(6):785–789

Zampacavallo G, Parisi G, Mecatti M, Lupi P, Giorgi G, Poli BM (2015) Evaluation of different methods of stunning/killing sea bass (*Dicentrarchus labrax*) by tissue stress/quality indicators. J Food Sci Technol 52(5):2585–2597. https://doi.org/10.1007/s13197-014-1324-8

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