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Fish Welfare: the use of CO_2 to avoid fish suffering during preparation to processing

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Abstract

Fish welfare has become a significant concern during the last decade. The issue of slaughter is getting the interest of many consumer organizations and fish growers, and suppliers. We need to take careful consideration to food fish slaughtering, and not only for an ethical reason. Numerous quality traits are in place already at the end of fish life. Nonetheless, other traits can be developed while the post mortem muscles are turning into consumer produce. Therefore, it is necessary to investigate ways to avoid the suffering of the fish before their processing. To improve the efficiency of the immobilizing and stunning process of the fish at the entrance of the fish plant, we investigated the use of CO_2 in combination with low temperature. When combining CO_2 to 2°C water temperature, fish were stunned and permanently immobilized within 20 min or less, while their core temperature was 3°C. Our results suggest that combining low temperature and bubbling CO_2 in the fish receiving tank of the processing plant might be more appropriate for animal welfare. Therefore, we suggest continuing our experiments in the fish processing plant.

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Introduction

Nowadays, both fish consumers and aquaculture producers are concerned about animal welfare. Therefore, a request for an investigation was submitted to our laboratory via a Ministry of Agriculture and Rural Development Veterinarian Services representative, prompted by signs of life in a carp in the packing plant. The regulations require that the fish must be stunned before the sorting and processing starts, for welfare reason, while the fish's core temperature much not be higher than 4°C for concerns related to the consumer's health. It appears that the reported case was during midwinter, during which the average water temperature in the fish ponds is 12°C.

Despite the hot climate of the Northern Valleys, the fish center of the Israeli fish industry, the aquaculture farmers also produce fish species such as various carps and striped bass, which originate from the earth's middle latitudes (40° to 60° north and south of the Equator). Carp, for example, can live at the bottom of lakes in Europe submerged at 4°C during the winter. The routine procedures of permanently immobilizing fish before sorting and packing in the Israeli processing plants are hosting them in a 2°C pool of tap water for about 20 min. In contrast, the fish ponds' water temperature during the summer can reach 31°C. So naturally, fish species of northern origin, such as carp, die due to the over 20°C temperature immediate reduction.

The adaptation of fish to low temperatures is the result of long-term evolution. For example, amur carp (*Cyprinus carpio haematopterus*) survives low temperatures (0-4°C) for six months per year (Liang et al., 2015). Moreover, Liu and coworkers (2009) characterized the antifreeze protein during embryogenesis and hatched larvae in the gibel carp (*Carassius auratus gibelio*). Also, the precursor of the antifreeze protein concentration varies with the season; it is at its peak in November and lowest in May (Hew et al., 1999). Thus, it is not surprising that by introducing carps to a 2°C pool of tap water for about 20 min during the cold winter, they stay alive. Therefore, we had to consider adding a factor to the cold water that will help avoid the suffering of the fish before their sorting and packing process but will not affect the consumers' health or change the fish smell and taste.

In Israel, after harvesting the fishponds, the farmers transfer the fish to cool, oxygenated water tanks, carrying the fish to the 2°C water introductory tank in the process plant. As a result, the fish body temperature decreases rapidly, and the fish lose consciousness because of anoxia. This easy and quick procedure became the standard in Mediterranean Countries and the UK for rainbow trout (Poli et al., 2005). Carbon dioxide was the only anesthetic allowed for food fish. Usually, the fish, such as salmon, turned unconscious within a few minutes (Robb, 2001; Roth et al., 2006; Wall, 2001). Wall (2001) offered to refine the method and modify it to speed up stunning using CO2. Therefore, we investigated the use of CO2 to successfully avoid the suffering of the fish by shortening its immobilizing time.

Materials and Methods

Fish

One hundred of each species, adult, ~500-gr common carp (*Cyprinus carpio*), mullet (Mugil cephalus) and hybrid striped bass (striped bass, *Morone saxatilis* crossed with white bass, *M. chrysops*) were supplied by the Nir David aquaculture farm. The fish were let to adapt in flow through 1000-L tanks, with water temperature of 18°C for a week.

Experiment procedures

Fish of each species was divided into a set of 5-100L tanks, five fish per tank. Ten tanks had fish in 2°C water, five tanks with bubbling CO2 and five tanks without, and ten tanks with 20°C water temperature, 5 with bubbling CO2, and five without bubbling CO2. Carbon dioxide was bubbled at the bottom of the tanks via air stones at the flow rate of 20-L/min. The fish's core temperature was taken immediately after 20 min, and the fish was placed on crushed ice for macroscopic and behavior observations.

Carbon dioxide (CO_2) measurements

Water samples were taken by submersing 500-ml clear sampling bottle that was capped close tight under the surface of the water. Temperature, pH, conductivity and alkalinity were measured with a few minutes and CO_2 values were calculated.

Ethic committee

The authors received certification to conduct the experiments mentioned here by the Ethic Committee of the Israeli Ministry of Agriculture and Rural Development. Each of the authors is a certified researcher by the Ethic Committee of the Agriculture Research Organization of the Israeli Ministry of Agriculture and Rural Development.

Results

The results of our experiments are shown in **Table 1**. Three fish species, common carp, hybrid striped bass, and flathead grey mullet, were tested in at temperatures 20°C versus 2°C, with and without CO_2. Bubbling CO_2 shortens the time of immobilizing and euthanizing while achieving full stillness of the fish. However, only a water temperature of 2°C in concert with CO_2 achieved 100% success. This treatment also provided the needed fish's core temperature of less than 4°C.

Table 1 Average values of CO_2 in the experimental tanks after 20 min.

	CO_2 Values (mg/L)			
	20°C air [1]	*20°C+CO_2* [2]	*2°C+air* [3]	*2°C+CO_2* [4]
Carp	8	1198	11	2149
Striped Bass	5	1553	8	1819
Mullet	4	1127	9	2043

[1] No euthanized fish.
[2] 75% euthanized with ambient fish core temperature.
[3] 67% euthanized with 3.5°C fish core temperature.
[4] 100% euthanized with 3.5°C fish core temperature.

Discussion

Poikilothermic (cold-blooded) animals have developed several biochemical and physiological adaptations to survive exposure to diurnal or seasonal low temperatures and chronic cold. Numerous investigations mentioned the adaptive changes for cold survival of the Antarctic notothenioid. A breakthrough identified antifreeze glycoprotein (AFGP) is considered one of the significant factors in expanding polar fish populations into harsh environments (Devries, 1971). Also, discovering the loss of many genes, including hemoproteins in the icefish family Channichthyidae, seems to respond to constant cold water (Sidell and O'Brien, 2006). However, eurythermal fish such as the common carp (Cyprinus carpio) use different strategies to survive low temperatures. One famous hypothesis regarding the survival mechanism at cold temperature is "homeoviscous adaptation," which suggests that fish can avoid freezing by decreasing the saturation of membrane phospholipids to offset the cold-induced rigidification of lipid bilayers during cold periods (Tiku et al., 1996).

Those fishes in low-temperature environments have evolved some unique physiological mechanisms to adapt to icy conditions (Chen et al., 2008; Fletcher et al., 2001; Kim et al., 2017). For example, the production of antifreeze protein, lack of hemoglobin, and tubulin synthesis can improve the adaptability of Antarctic fishes to low-temperature environments (Fletcher et al., 2001; Parker and Detrich, 1998). However, eurythermal freshwater fishes are exposed to a wide range of fluctuations in water temperature rather than in a relatively stable temperature environment. Therefore, eurythermic fish must adjust physiological processes quickly to respond to the acute cooling when severe cold fronts approach in winter. Interestingly enough, Raymond (1992) reported that glycerol is a colligative antifreeze in some northern fishes.

Carbon dioxide was first mentioned as a fish anesthetic since it could immobilize fish (Fish, 1943). Either bubbling CO_2 gas can produce carbon dioxide in the fish tank or the soda-acid technique (Post, 1979; Summerfelt and Smith, 1990). It is safe for humans, and its use is unrestricted (Summerfelt and Smith, 1990). CO_2 does not induce analgesia and has a "shallow effect" compared to MS-222 (Anderson et al., 1997). Physiological responses to CO_2, such as no decrease in blood PO2 and lowered plasma pH during anesthesia, differ from benzocaine, 2-phenoxyethanol, MS-222, and metomidate (Iwama et al., 1989). Acerete et al. (2009) show that both CO_2 and asphyxia in ice are suitable for processing fish. However, the CO_2 treatment showed lower lactate and cortisol response compared to asphyxia in ice. In terms of quality, differences between methods are minor. Cortisol concentration revealed that MS-222, clove oil, and CO_2 did not cause significant stress to steelhead trout (Pirhonen and Schreck 2003). Our results suggest that combining low temperature with bubbling CO_2 in the fish receiving tank of the processing plant might be a more satisfactory strategy for animal welfare.

Moreover, only a water temperature of 2°C in concert with CO_2 achieved 100% successful results. It was the only treatment that provided the needed fish's core temperature of less than 4°C. Therefore, we suggest validating our results in a field study in the fish processing plant conditions.

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