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Conference Paper in *Frontiers in Marine Science* · July 2014
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Rui E. Silva^{1*}, Hugo A. Morais^{2,3}, Nuno V. Rodrigues^{2,3}, Tiago Reis¹, João P. Correia^{2,3}

¹ ESTM – School of Tourism and Sea Technology, Polytechnic Institute of Leiria, Peniche, Portugal
² GIRM – School of Tourism and Sea Technology, Marine Resources Research Group, Polytechnic Institute of Leiria, Peniche, Portugal
³ Flying Sharks, Flying Sharks, Lisboa, Portugal
 * raages@gmail.com

Introduction

In recent years the zoo and aquarium industry experienced a major shift in their mission, which was reciprocated by the public's perception towards these organizations.

Many public aquaria throughout the world desire to engage the public in protecting marine shorelines and therefore exhibit intertidal pools to demonstrate the incredible mechanisms these organisms have developed to adapt to such a harsh and constantly changing environment.

Lepadogaster lepadogaster, *Lipophrys pholis*, and *Gobius paganellus* are cryptic species regularly found amidst intertidal areas (Rodrigues et al. 2008). *Diplodus sargus* (Linnaeus, 1758) and *Gobiusculus flavescens* (Fabricius, 1779) are benthopelagic fish abundant along rocky bottoms near shore, with juveniles often found in intertidal pools (D'Anna et al. 2012).

Small ornamental fish have been shipped throughout the world for many years inside plastic bags, typically half-filled with water and half-filled with pure oxygen. This proportion may change slightly according to the nature, and size, of the animals transported but this technique has proved extremely effective for trips up to 48 hours. The small plastic bags are then sealed, double-bagged, and placed inside a styrofoam container, which is acknowledged by the International Air Transport Association (IATA) as adequate means for transporting fish and labeled accordingly as "Live Animal Regulations container type 51".

Description of the Process

Collections and husbandry

All specimens were collected at low tide with small hand nets, or hook and line, on the rocky coast of Peniche, Portugal.

Specimens were kept in a multi-tank system (1 specie per tank) with mechanical and biological filtration. The bioload in each tank was inferior to 1 kg / m³ and shelter was provided, to avoid aggressive behavior between animals. Water parameters were monitored daily and kept within a narrow range, specifically: temperature (± 18°C), salinity (35 ppt), pH (± 8,30), dissolved oxygen (> 90%) and nitrogen compounds (0,00 ppm). All fish were fed daily with an assortment of finely chopped mussels, shrimp and pellet food but were fasted for 48 hours before each trial.

Trials

- "Mix" : System water + Amquel ® (25 mg / L) (Novalek, Inc., Hayward, CA) + sodium bicarbonate (50 mg / L) + sodium carbonate (50 mg / L) (Correia et al. 2011).
- Bioloads were individually adapted to each species in accordance to their social behavior and ecological characteristics. Size, number and weight of the animals were similar per trial.
- Each trial consisted of nine 3 L bags (3 replicates):
 - Control:** 3 bags (1 L of system water)
 - Normal transport:** 3 bags (1 L of system water + animals)
 - Mix transport:** 3 bags (1 L of "mix" + animals)

- All bags were filled with ±1,5 L of pure O₂ (Gasin, Lisbon, Portugal), sealed and then packed in styrofoam box. Water parameters (pH, dissolved oxygen, ammonia, temperature) were measured prior to each trial.
- Each of the 3 bags (control, normal and mix) were opened in 24 hours, 48 hours and 72 hours. Survival rate, dissolved oxygen, pH, ammonia and temperature were registered. Surviving animals were monitored during 48 hours to discard side effects.
- Trials were repeated for all species using increasing bioloads.

Demonstration of Efficacy

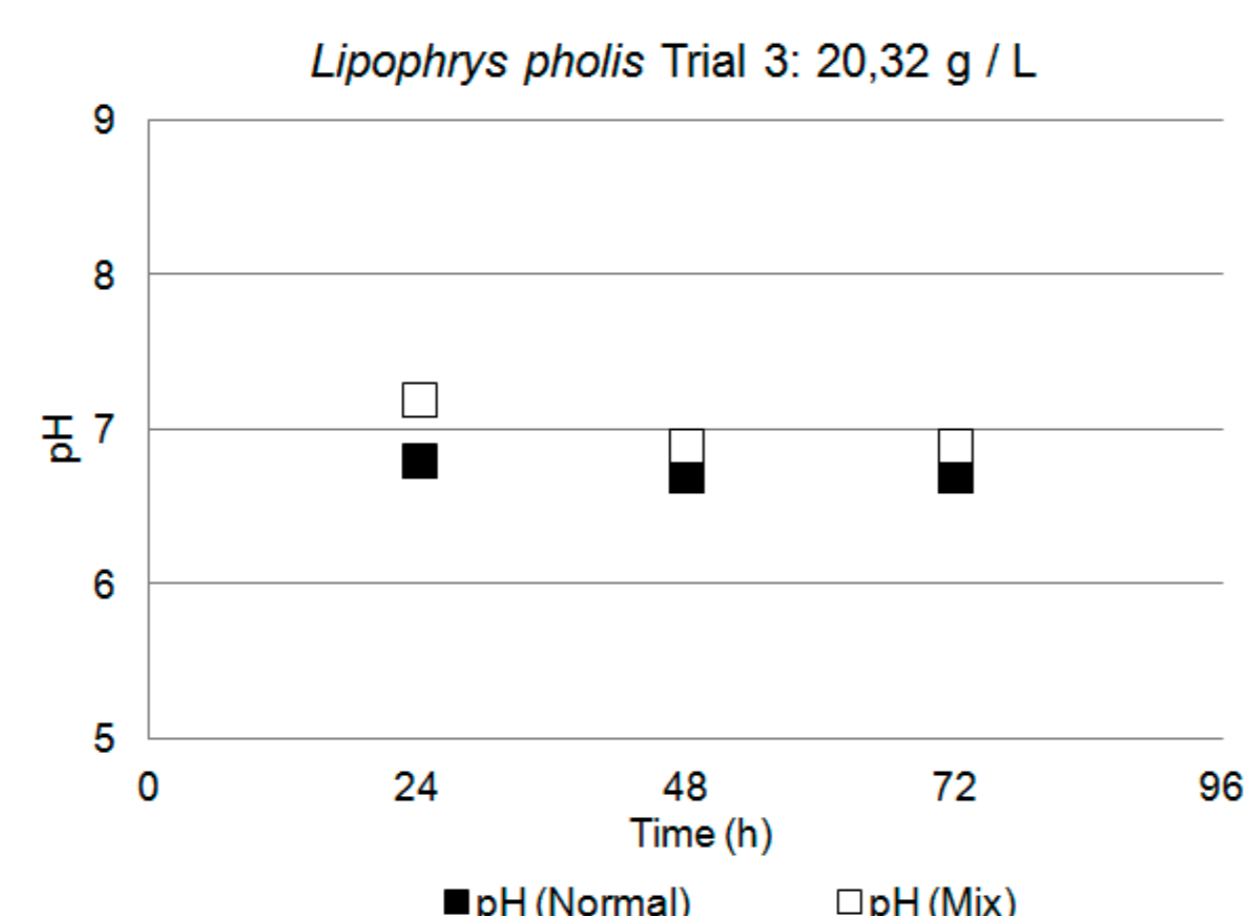
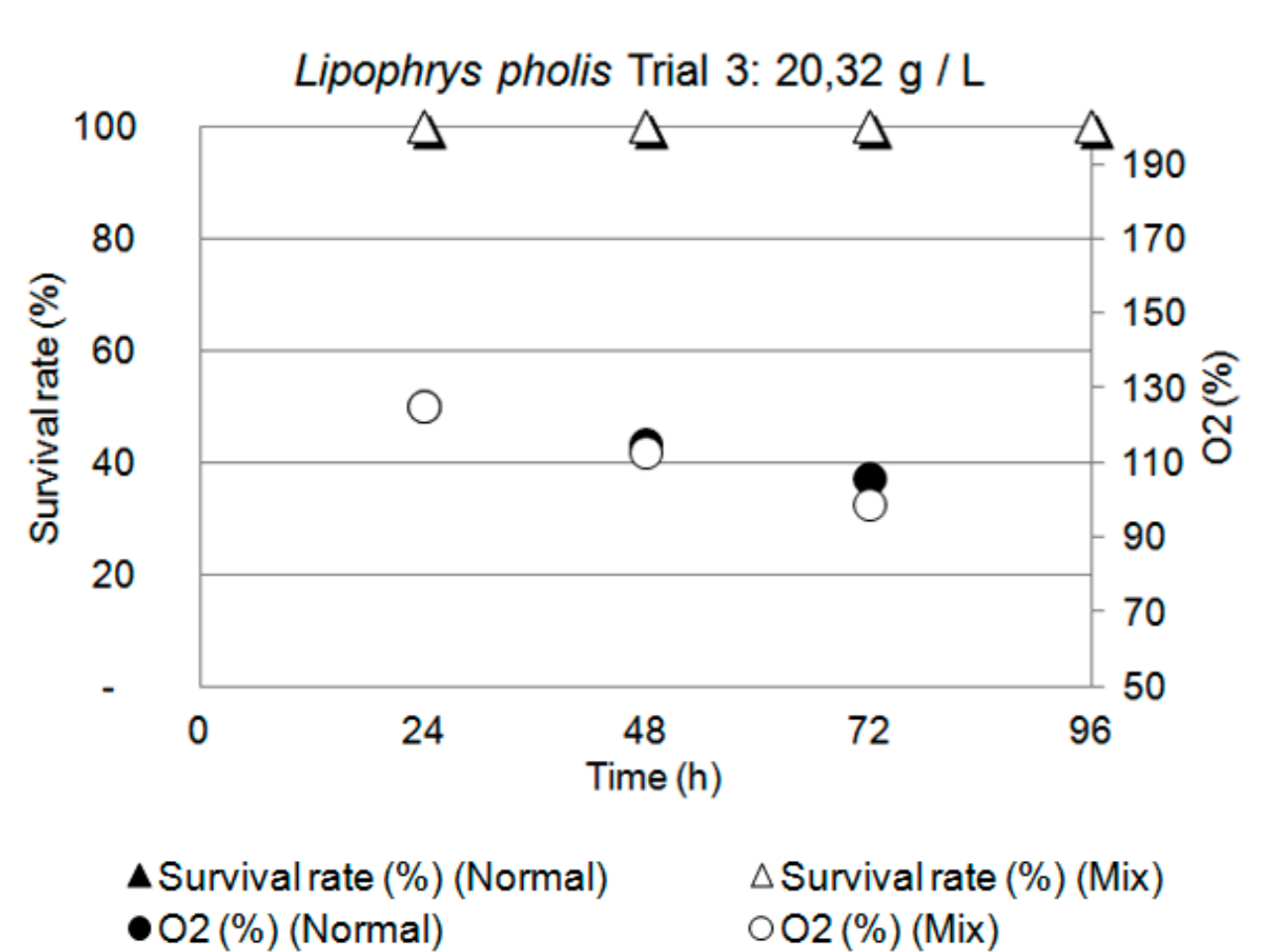
These trials results proved very enlightening and allowed to conclude what the appropriate bioload is for each species. Regrettably, data from ammonia analysis (Salicylate method) were inconclusive and were therefore discarded.

Lipophrys pholis

The use of a bioload above 20 g / L will most likely result in animal losses after 48 hours due to lack of oxygen;

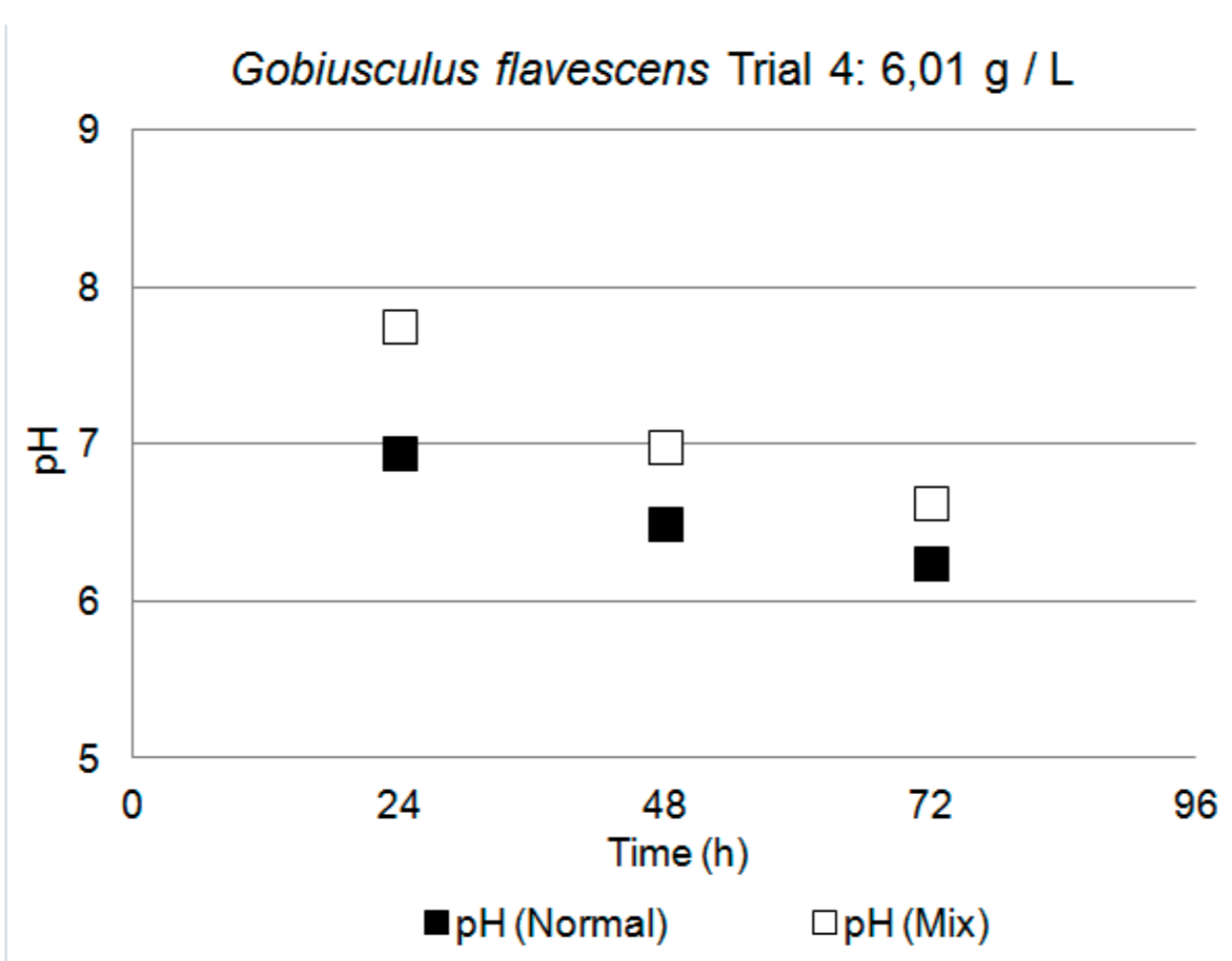
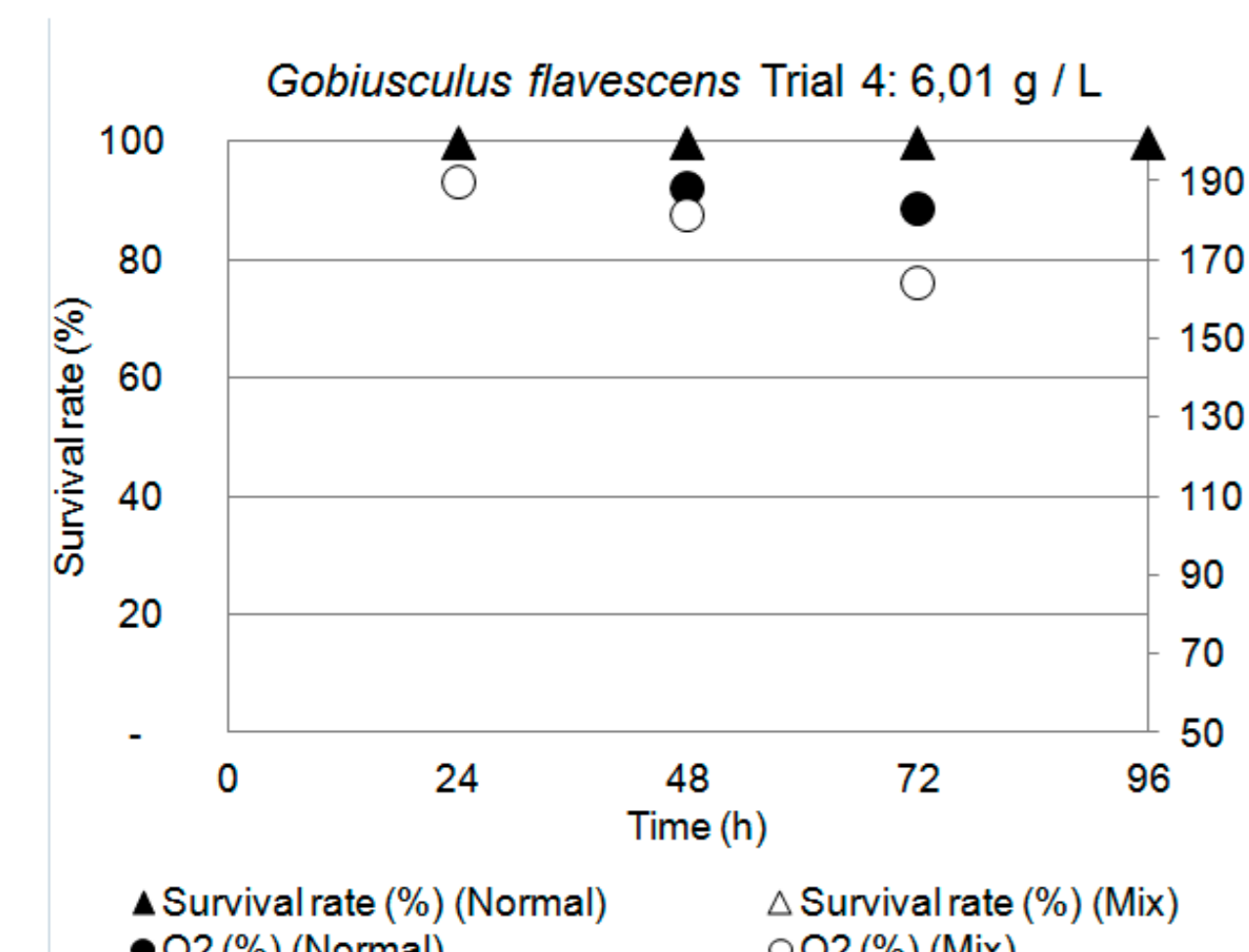
The use of a bioload of 10 to 15 g / L will ensure 100% survivorship up to 72 hours but will not fully maximize the economical potential of the shipment. In other words, more fish may be bagged without any losses occurring;

For shipments of 48 to 72 hours a bioload of 15 to 20 g / L seems to yield 100% survivorship with the highest bioload.



Gobiusculus flavescens

A 72 hours transport can be successful with up to 10 specimens, or 6 g / L, and "normal" water is recommended. Higher bioload is not recommended, as the authors observed aggressiveness occasionally occurred.

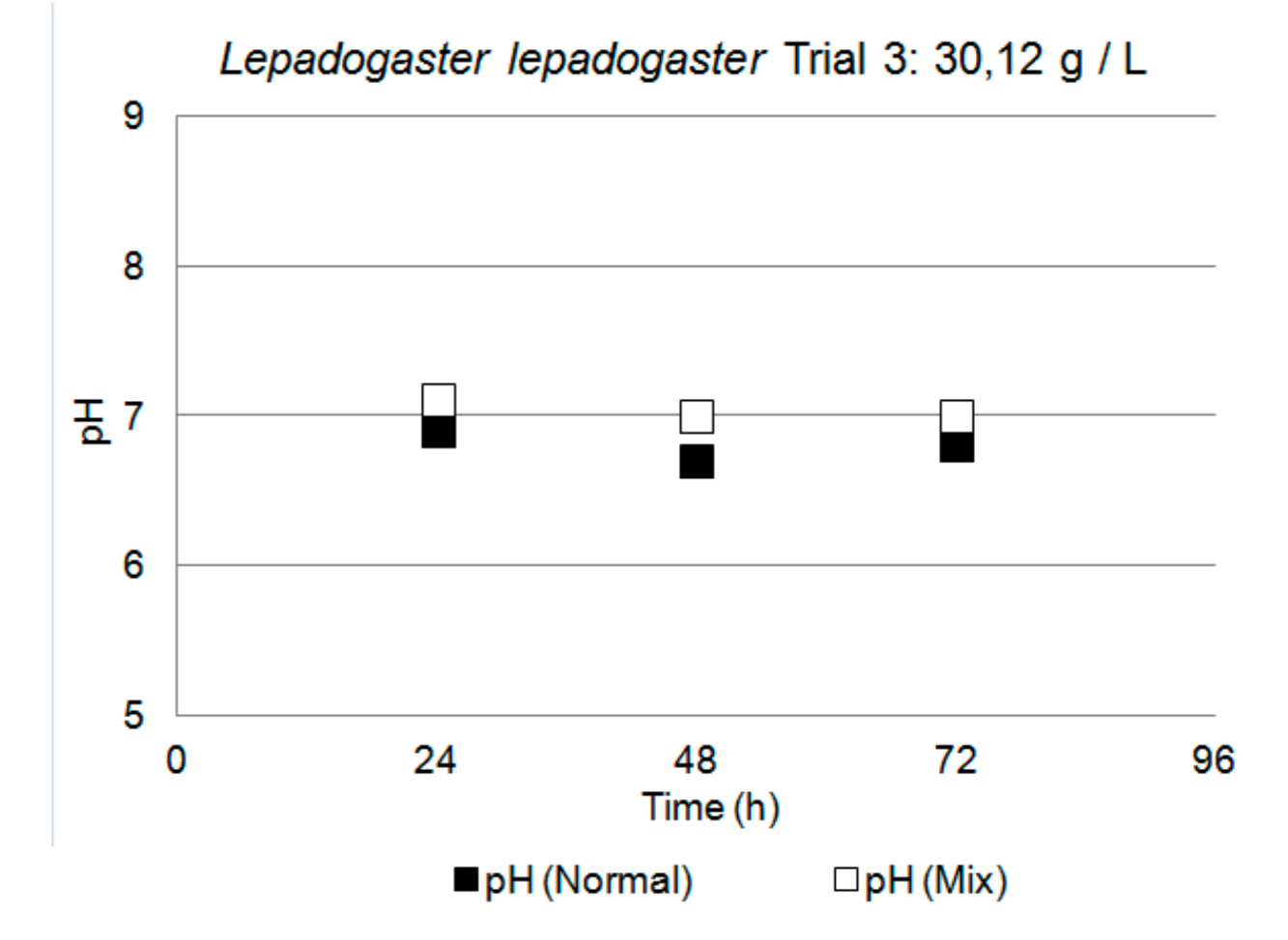
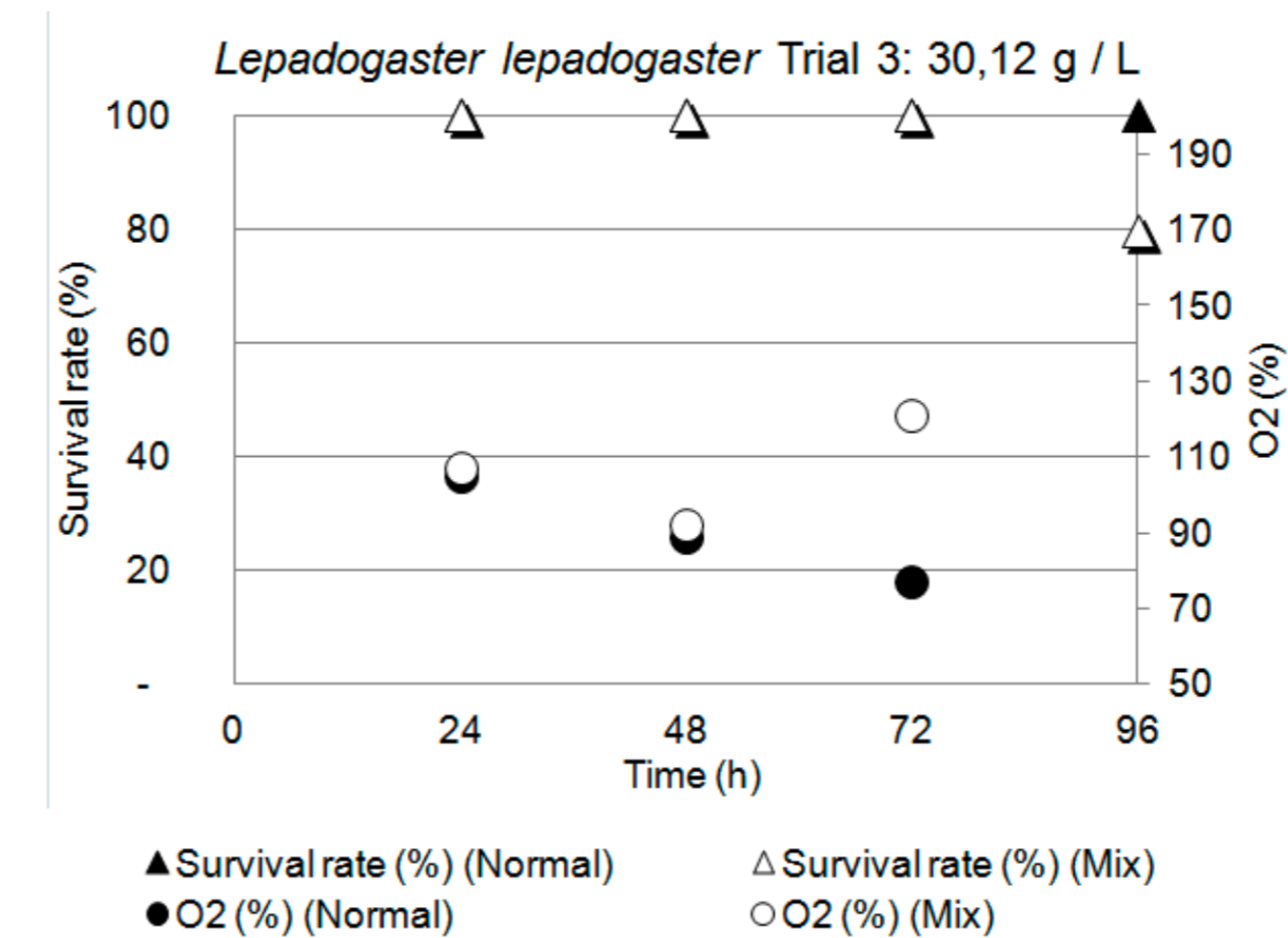


Lepadogaster lepadogaster

The use of a bioload above 40 g / L will most likely result in animal losses after 72 hours due to lack of oxygen and / or decreasing pH;

The use of a bioload as high as 30 g / L will ensure 100% survivorship up to 72 hours but will not fully maximize the economical potential of the shipment. In other words, more fish may be shipped without any losses occurring;

For shipments of 48 to 72 hours a bioload of 30 to 40 g / L seems to yield 100% survivorship with the highest bioload.

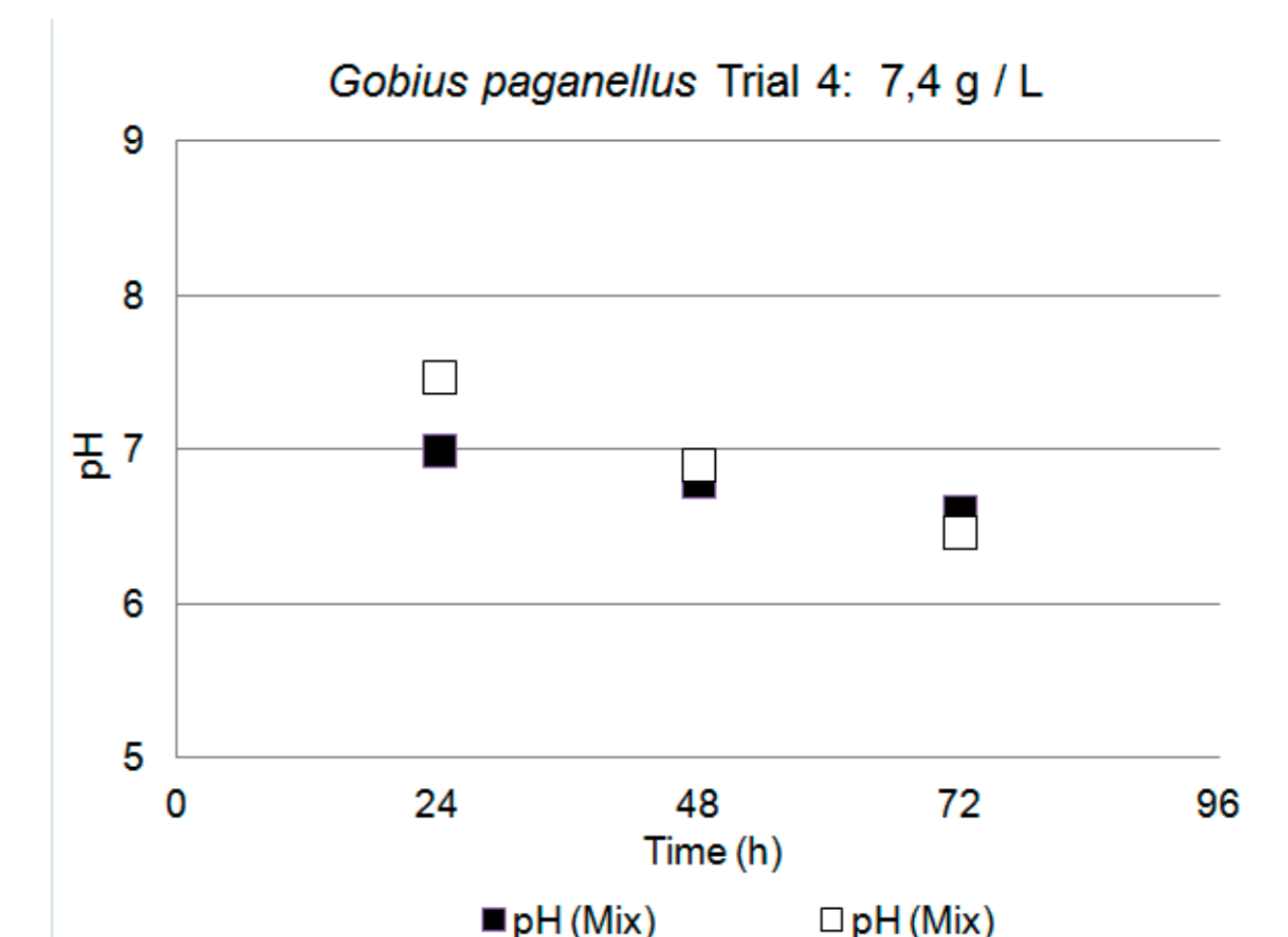
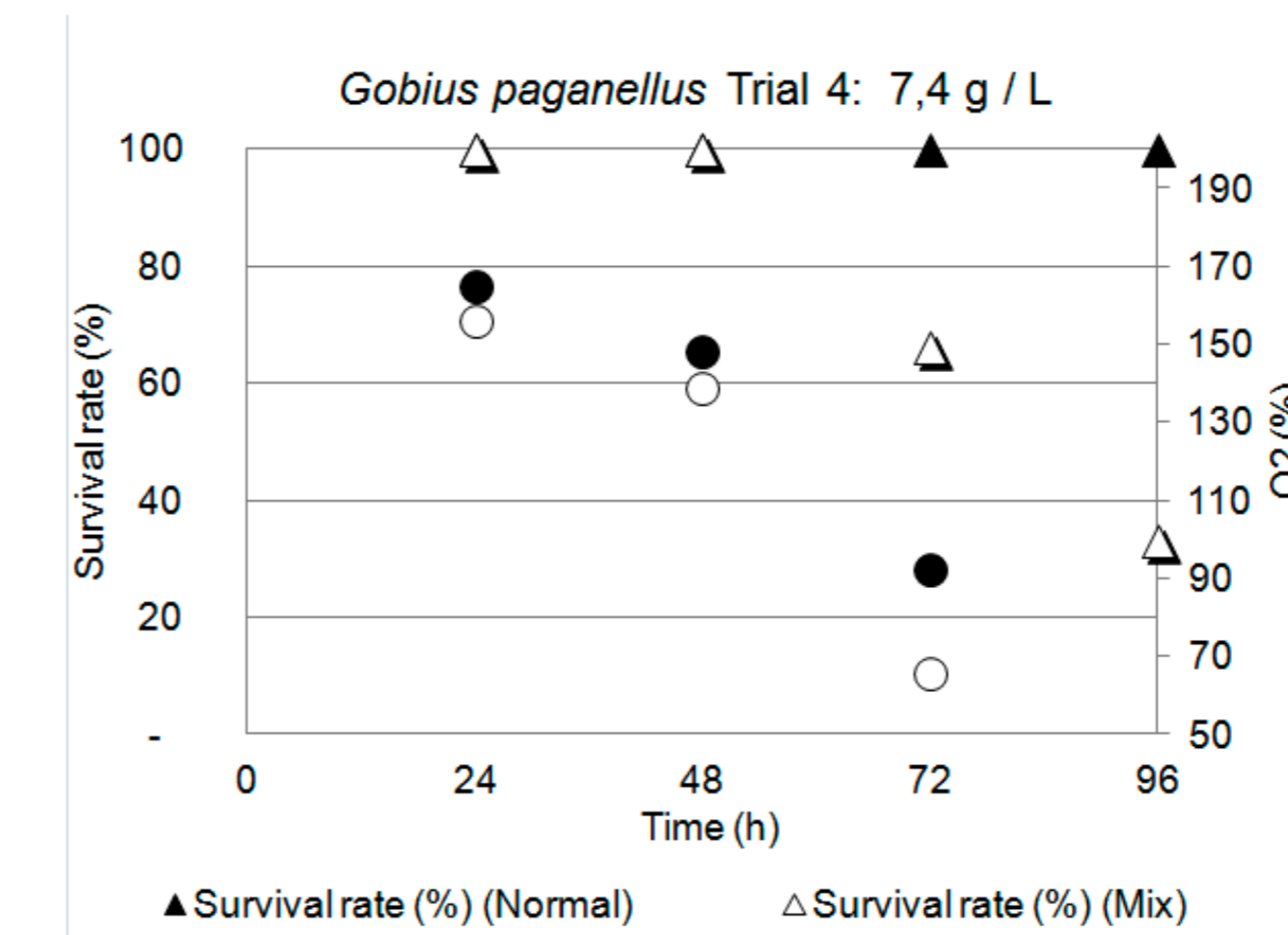


Gobius paganellus

The somewhat less "benthic" nature of this species, especially when compared to the much more sessile *Lipophrys pholis* and *Lepadogaster lepadogaster*, renders them substantially more susceptible to transport related stress;

The use of a bioload as low as 6,69 g / L will most likely result in animal losses after 48 hours due to decreasing pH;

Transports with a duration higher than 48 hours should therefore not surpass a bioload of 7 g / L (correspondent to a minimum of 3 animals) and although buffering is advised to keep pH high, its use remains inconclusive because survival rates with "mix" water were substantially lower.

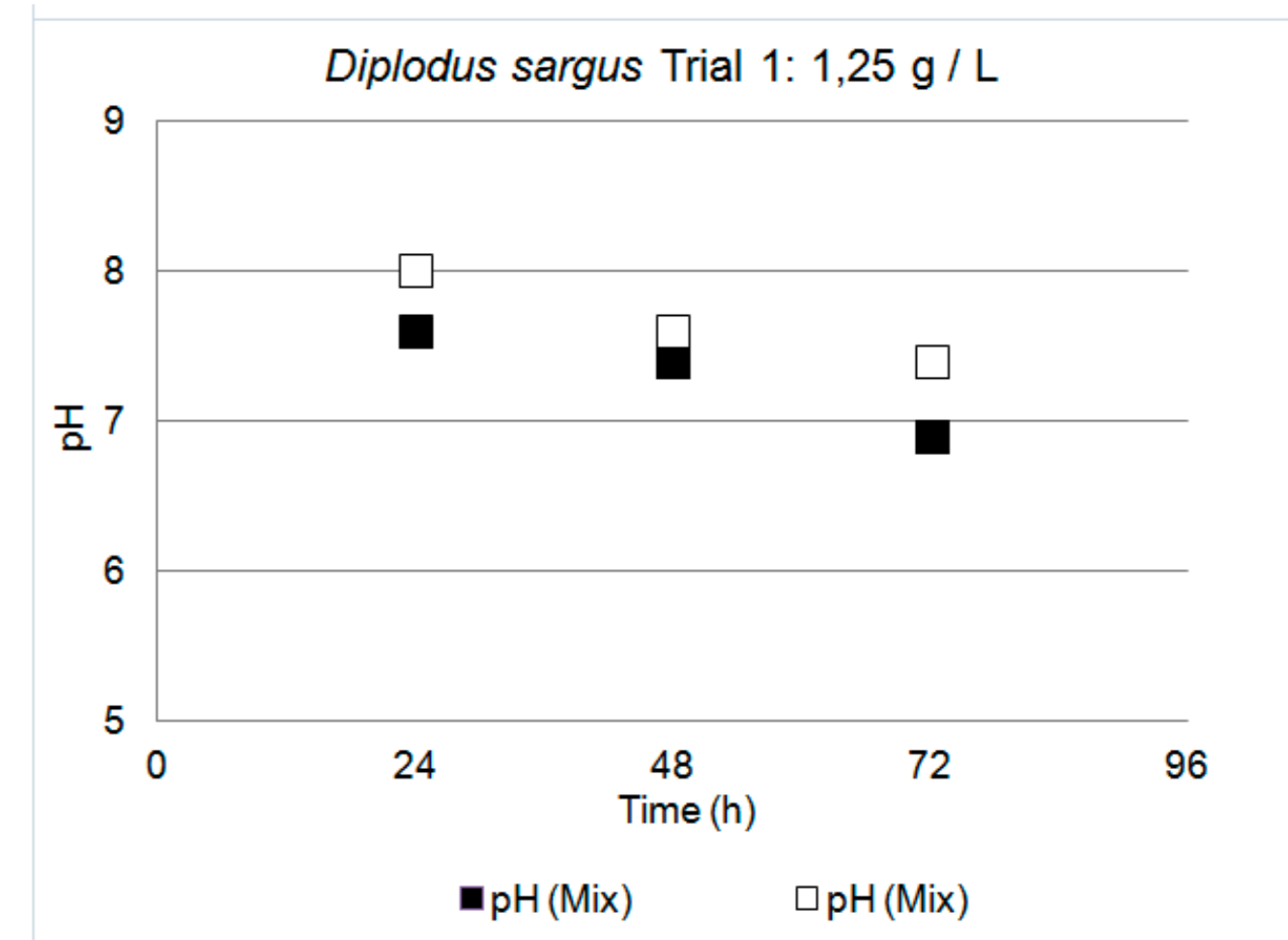
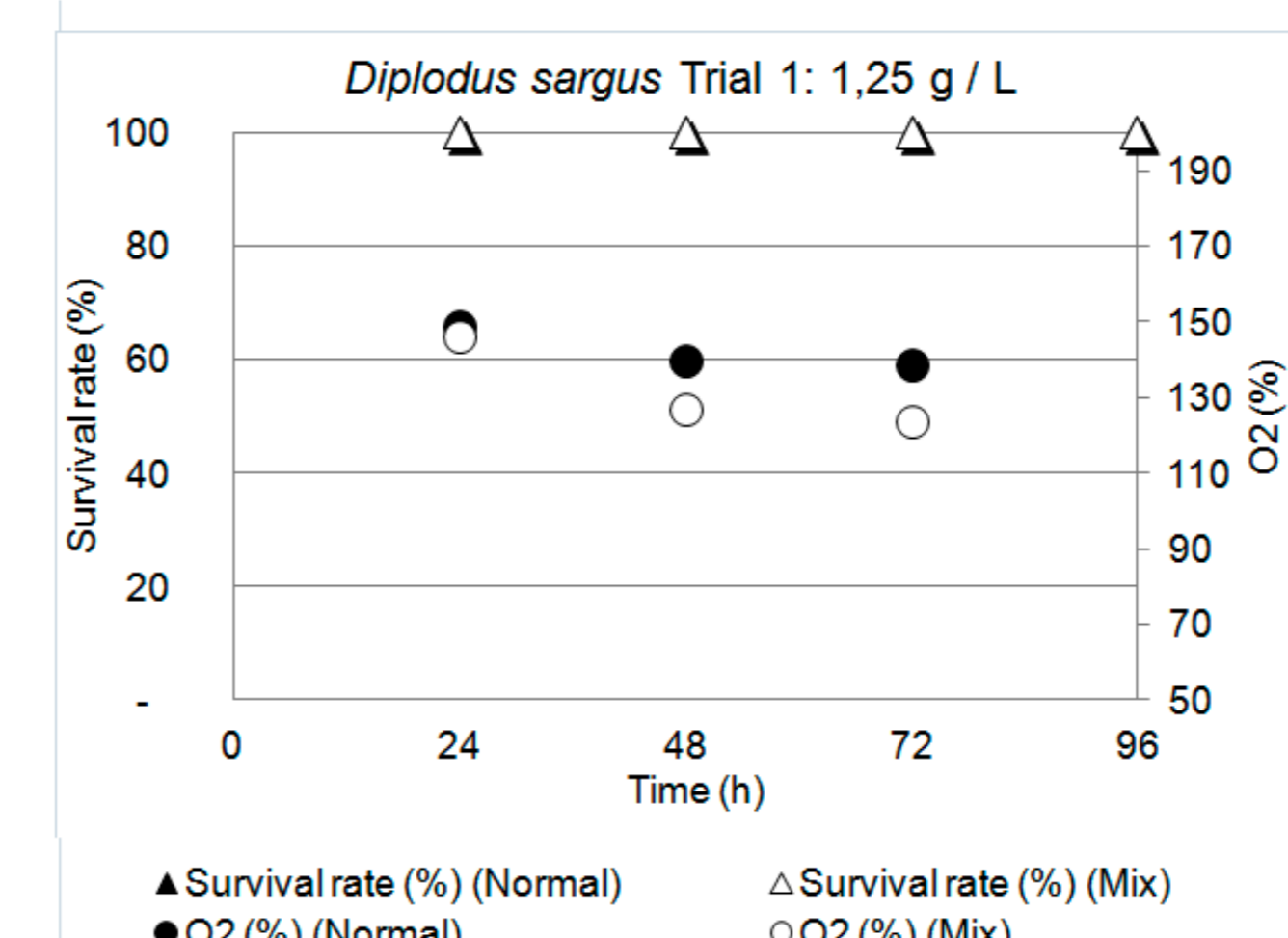


Diplodus sargus

The much more "pelagic" nature of this species, especially when compared to the much more sessile *Lipophrys pholis* and *Lepadogaster lepadogaster*, renders them substantially more susceptible to transport related stress;

The use of a bioload as low as 3,23 g / L (trial 2) will most likely result in animal losses after 48 hours due to decreasing pH;

Transports with a duration higher than 48 hours should therefore not surpass a bioload of 1,25 (trial 1) g / L and buffering is highly advised to keep pH high; a concentration higher than the used 50 g / m³ (for both sodium bicarbonate and sodium carbonate) is in fact recommended.



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