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Optimising sealed transports of small ornamental fish

Rui E. Silva[1], Hugo A. Morais[1,2], Nuno V. Rodrigues[1,2], Tiago Reis[1] and João P. Correia[1,2*]

[1]MARE – Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, 2520-641 Peniche, Portugal
[2]Flying Sharks, 9900-361 Horta, Portugal
**Correspondence: João P. Correia; info@flyingsharks.eu*

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Abstract
This paper reports on simulated long-term transports of small ornamental fish inside sealed containers. The species involved were *Diplodus sargus*, *Gobius paganellus*, *Gobiusculus flavescens*, *Lepadogaster lepadogaster*, and *Lipophrys pholis*. The objective of such simulations was to determine the maximum bioload that it is possible to move inside a sealed container, while ensuring 100% survivorship, ultimately resulting in financial savings for the end-receiver. Transports were simulated over 24, 48 and 72 hours, with increasing animal bioloads per bag. Fifty percent of the trials were performed with "regular" saltwater while the other half involved seawater buffered with Amquel®, sodium carbonate and sodium bicarbonate, with the objective of keeping ammonia low and pH similar to initial baseline values. At the end of each trial, temperature, dissolved oxygen, pH and ammonia were analysed and survival rates determined. *L. lepadogaster* endured the highest bioloads and displayed 100% survivorship (i.e. up to 30 g/L), which is not surprising given the intertidal nature of this species. *D. sargus* exhibited mortalities with bioloads as low as 3.23 g/L, which echoes its predominantly pelagic nature and relatively lesser ability to endure confinement. The three remaining species showed varying degrees of tolerance to increasing bioloads in transport: *L. pholis*, also an intertidal species, tolerated up to 20 g/L over 72 hours, while *G. paganellus* tolerated up to 7 g/L over 72 hours, and *G. flavescens* (a predominantly pelagic species) could tolerate no more than 6 g/L up to 72 hours.

Background

In recent years, the zoo and aquarium industry has experienced a major shift in its mission, which has been reciprocated by the public's perception of these organisations (Penning et al. 2009). The importance of zoological institutions for both conservation and research efforts is now widely acknowledged (Tlusty et al. 2012). Public aquaria, and zoos, are a relevant, if not the most relevant, conveyor of environmental awareness messages and are also the forerunners of in-situ and ex-situ conservation in various fields of research, such as reproduction, pathology, physiology, nutrition and husbandry.

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. This report focuses on five intertidal species that are relatively abundant along the Portuguese western shore, particularly around the area of Peniche. These species are *Diplodus sargus* (Linnaeus, 1758), *Gobius paganellus* (Linnaeus, 1758), *Gobiusculus flavescens* (Fabricius, 1779), *Lepadogaster lepadogaster* (Bonnaterre, 1788) and *Lipophrys pholis* (Linnaeus, 1758), and are regularly found in the intertidal zone of rocky shores in Peniche (Henriques et al. 2002; Rodrigues et al. 2008; Vinagre et al. 2010; Francisco et al. 2011). *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 of up to 48 hours (Froese 1988; Teo et al. 1989; Lim et al. 2003) . The authors have, in fact, successfully moved small ornamental fish, and invertebrates, using this technique

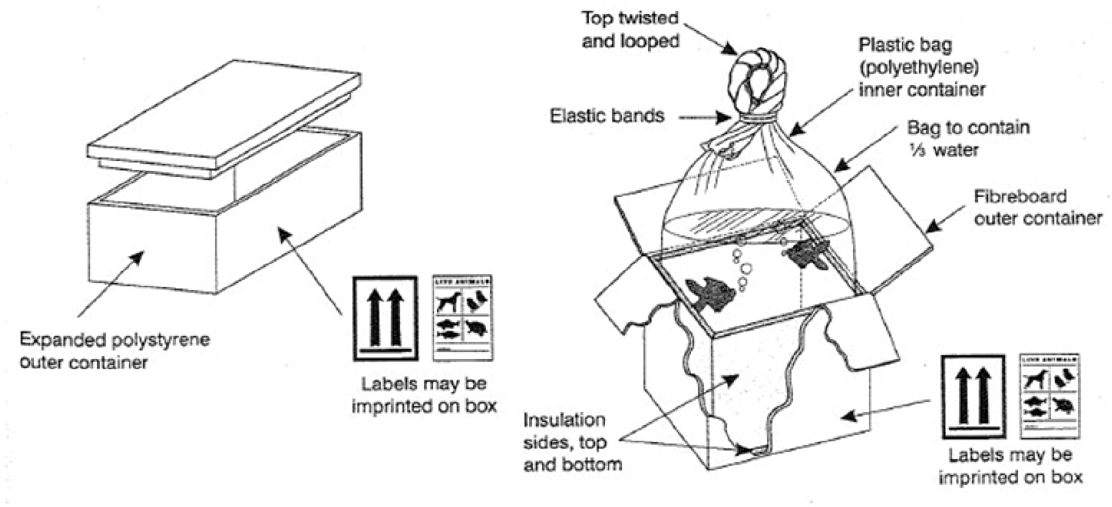

Figure 1. IATA (International Air Transportation Association) Live Animal Regulation number 51, for transporting live fish inside plastic bags, that travel inside styrofoam boxes that, in turn, must travel inside a cardboard box.

for 120 hours, and similar informal reports are abundant in the industry. 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 labelled accordingly as "Live Animal Regulations container type 51" (Figs 1 and 2).

Shipping live marine fish offers a complex mixture of multiple variables and protocols, all sharing the common goal of providing the best possible conditions for the animals involved. Such procedures have been extensively covered in publications such as Correia (2001), Young et al. (2002), Correia et al. (2008, 2011) and Rodrigues et al. (2012). Ammonia and pH rank amongst the main parameters of concern. During transport, pH gradually decreases while ammonia increases as a result of carbon dioxide buildup and the release of nitrogenous waste, along with miscellaneous stress-related metabolites. Both these tendencies have an adverse effect on fish health and therefore need to be counteracted through the use of filtration and/or chemical supplements. The control of pH can be achieved through the use of buffering agents, such as the tribuffer described by McFarland and Norris (1958), common baking soda (i.e. sodium bicarbonate, $NaHCO_3$) and/or soda ash (i.e. sodium carbonate, Na_2CO_3). Ammonia (NH_3 and NH_4^+) may be removed with the assistance of quenching agents, such as AmQuel® ($HOCH_2SO_3$) (Novalek, Inc., Hayward, California, U.S.A.), which binds to ammonia and transforms it into nontoxic aminomethanesulfonate ($H_2NCH_2SO_3^-$) and water.

Figure 2. IATA (International Air Transportation Association) Live Animal Regulation number 51. A – the plastic bags are filled 50% with seawater and 50% with pure oxygen; B – the corners of the plastic bags are then taped, to prevent the possibility of animals being wedged in the corners; C – the bags are placed inside a styrofoam container; D – the styrofoam container is placed inside a cardboard box, with all empty spaces cushioned with soft material, such as newspaper; E – the cardboard box is then taped shut and handed to a courier, or airline, for transport.

Air shipments from Portugal to multiple destinations throughout the world typically last from 24 to 48 hours, but occasional unplanned delays may extend the trip to 72 hours or more. As such, a protocol was devised to investigate shipping conditions of small ornamental fish for 24, 48 and 72 hours, after which key parameters such as oxygen, ammonia and pH were monitored and compared to baseline figures. During this project all aspects related to animal welfare strictly followed the Association of Zoos & Aquariums (AZA) guidelines (Penning et al. 2009).

Action

Collection and husbandry

All specimens were collected at low tide with small hand nets, or hook and line, on the rocky coast of Peniche, Portugal. After collection all animals were kept in transport containers and water was renewed regularly to ensure adequate water quality.

Specimens were then acclimated to 600 L tanks (one species per tank) connected to a TMC 5000 filtration system (Tropical Marine Centre, London, U.K.) consisting of a tower with bioballs for biological filtration, a fluidised bed sand filter and cloth sleeves for mechanical filtration, and a foam fractionator (also known as a protein skimmer) for chemical filtration. The bioload in each tank was up to 1 kg/m^3 and shelter was provided in the form of PVC pipes, to avoid aggressive behaviour between animals. Water parameters were monitored daily and kept within a narrow range of temperature (18±1º C), salinity (35±1 ppt), pH (8.30±0.1), dissolved oxygen (>90%) and total ammonia nitrogen (0.00 ± 0.01 mg/L). 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.

Transport simulations

Preparation of conditioning solution

Prior to each test, 5 L of a solution consisting of system water and the following reagents was prepared: Amquel® (25 mg/L) + sodium bicarbonate (50 mg/L) + sodium carbonate (50 mg/L), as per Correia et al. (2011). This condition solution will hereinafter be referred to as "Mix".

Trials

Each trial consisted of nine 3 L bags:

A. Three bags containing nothing but 1 L of system water – labelled as "control";
B. Three bags containing 1 L of system water plus specimens of one species – labelled as "normal transport";
C. Three bags containing 1 L of "mix" plus specimens of one species – labelled as "mix transport".

Specimens were chosen according to their biomass, to ensure the size, number and weight of the animals were similar in each trial. All bags were then filled with approximately 1.5 L of medicinal oxygen (Gasin, Lisbon, Portugal), sealed with two rubber bands and inserted inside a second 3 L bag to prevent water leaks. During all trials, bags were stored inside a styrofoam box (0.38 m height x 0.37 m width x 0.48 m length), identical to the ones used in "real" transports (Fig. 3). Water parameters (pH, dissolved oxygen, ammonia, and temperature) were monitored at the beginning of each trial and considered baseline parameters for each trial.

Each of the three bags in the three categories ("A" control, "B" normal and "C" mix) were then opened after 24, 48 and 72 hours and the specimens' survival rate, as well as dissolved oxygen, pH, ammonia and temperature were recorded. Surviving animals were acclimated to a holding tank and observed over 48 hours for possible side-effects. Survival rate was calculated (number of survivors/number of individuals per bag x 100%) per trial. Ammonia, oxygen saturation and pH were monitored with the following equipment: Hannah Instruments Oxy-Check HI9147 (O_2 and temperature; Nuşfalău, Romania) and VWR Symphony SP70P (pH; Singapore).

These trials were repeated for all species using increasing bioloads.

Figure 3. Nine trial bags consisting of: A. First vertical row (left): three bags containing nothing but 1 L of system water – labelled as "control"; B. Second vertical row (centre): three bags containing 1 L of system water plus specimens of one species – labelled as "normal transport"; C. Third vertical row (right): three bags containing 1 L of "mix" plus specimens of one species – labelled as "mix transport".

Consequences

The results of these trials allowed us to determine the appropriate bioload for each species during travel.

Lipophrys pholis

Lipophrys pholis trials covered four different bioloads: Trial 1: 9.50 g/L with three specimens; trial 2: 15.39 g/L with four specimens; trial 3: 20.32 g/L with five specimens; trial 4: 30.82 g/L with six specimens.

The first three trials with "normal" water showed a survival rate of 100% after 72 hours; this rate remained after the 24 hours of recovery (Fig. 4). The first death (one of six individuals) occurred at 48 hours with the maximum bioload in trial 4, i.e. 30.82 g/L. Additional deaths (two of six animals) occurred in the 24 hours that followed the 72 hour simulated transport.Results with "mixed" water were unexpected, with 33% mortality occurring at 72 hours with the lowest bioload (i.e. 9.5 g/L) and, again, one in three individuals dying in the 24 hours that followed the end of the 72 hour simulation.

Dissolved oxygen decreased with time, but remained above 85% up to 48 hours, both in "normal" and "mixed" water. At 72 hours, however, in trial 4 (30.82 g/L), oxygen was at 61% and 65% in "normal" and "mixed" water, respectively. Average oxygen in the four trials for "normal" water at 72 hours was 101.8% while average oxygen in the four trials for "mixed" water at 72 hours was 102.4%, in other words, practically identical. pH steadily decreased over time but showed marginally higher numbers in "mixed" (i.e. buffered) water, as expected. Average pH over the four trials for "normal" water at 72 hours was 6.68 while for "mixed" water at 72 hours the average pH was 6.89. As with oxygen, average pH results of all four trials were practically identical at the end of 72 hours.

Lepadogaster lepadogaster

Lepadogaster lepadogaster trials included the following bioloads: Trial 1: 9.39 g/L with four specimens; trial 2: 15.15 g/L with five specimens; trial 3: 30.12 g/L with 10 specimens; trial 4: 40.41 g/L with 15 specimens.

All trials with both "normal" and "mixed" waters showed a survival rate of 100% up to 72 hours, with bioloads as high as 40.41 g/L, which is quite remarkable. L. lepadogaster is an intertidal species, quite accustomed to being in very small volumes of water (if any) during low tide (Briggs 1986), which means this species has a natural ability to withstand extremely adverse conditions (Fig. 5).

Only after 72 hours of simulated transport did any mortality occur, with three of 15 individuals (20%) dying in both the "normal" and "mixed" trials. Dissolved oxygen decreased with time but remained above 77% up to 72 hours, both in "normal" and "mixed" water. At 72 hours, however, oxygen was still 89% and 86% in "normal" and "mixed" water, respectively. Average oxygen over the four trials for "normal" water at 72 hours was 91.0% while for "mixed" water at 72 hours it was 100.8%. Unlike in *Lipophrys pholis*, where there were no noticeable differences in oxygen concentration between "normal" and "mix" water at the end of 72 hours in any trials, *Lepadogaster lepadogaster* results showed, on average, relatively higher oxygen concentration in "mix" water at the end of 72 hours in all trials.

pH steadily decreased over time but showed marginally higher numbers in "mixed" (i.e. buffered) water, as expected. Average pH of the four trials for "normal" water at 72 hours was 6.88 while for "mixed" water at 72 hours it was 7.00.

Gobius paganellus

Trials with *G. paganellus* covered the following bioloads: Trial 1: 4.13 g/L with one specimen; trial 2: 6.69 g/L with two specimens;

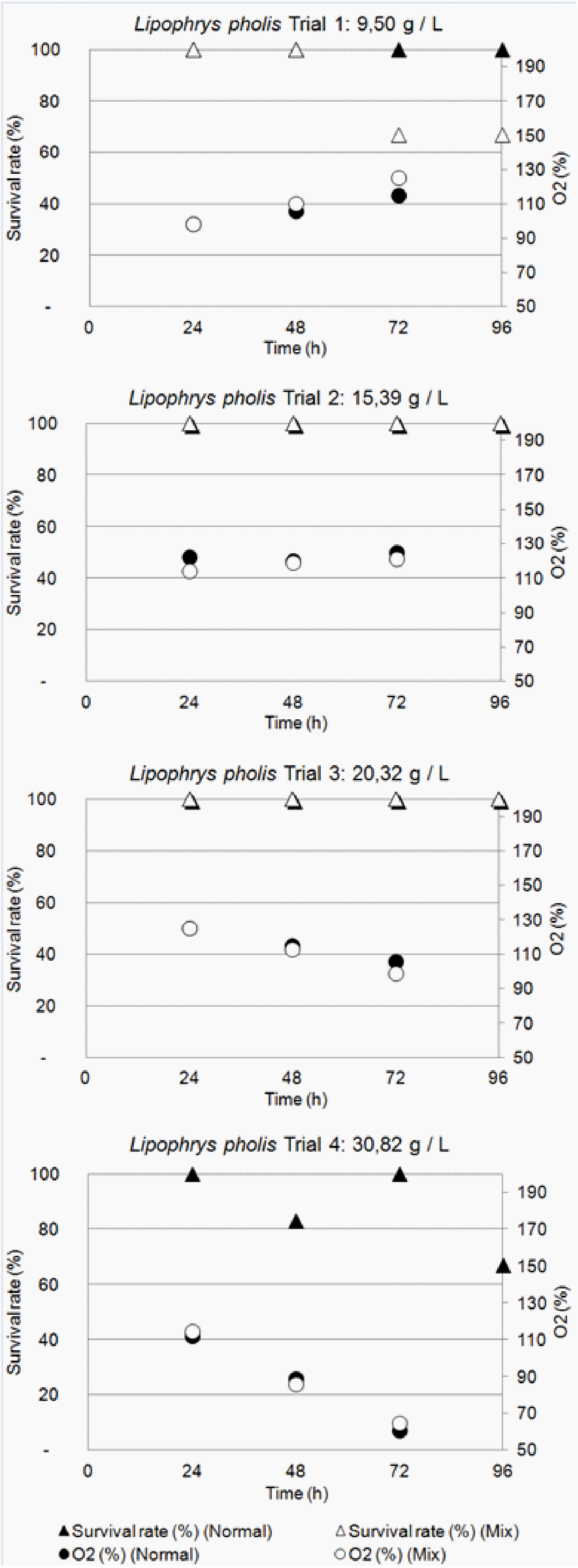

Figure 4a. Survival and oxygen saturation rates of *Lypophris pholis* during simulated sealed transports over 24, 48 and 72 hours, with "normal" and "mixed" water ("mixed" water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

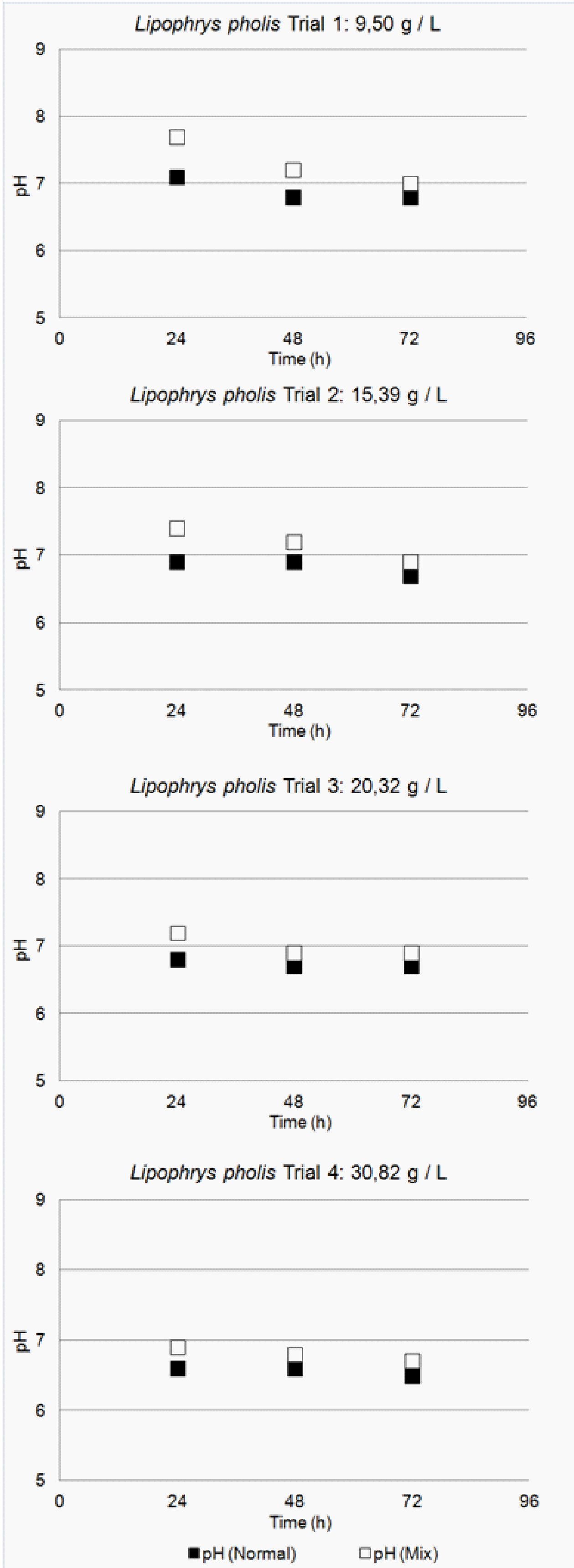

Figure 4b. pH of *Lypophris pholis* during simulated sealed transports over 24, 48 and 72 hours, with "normal" and "mixed" water ("mixed" water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

trial 3: 12.26 g/L with three specimens; trial 4: 7.40 g/L with three specimens.

All trials with both “normal” and “mixed” waters showed a survival rate of 100% up to 24 hours with bioloads as high as 6.69 g/L (Fig. 6). A bioload of 12.26 g/L yielded 66% mortality as early as 24 hours, which was unexpected. After 48 hours of simulated transport further mortalities occurred with one of two individuals (50%) dying in the “mixed” trial at 6.69 g/L and one of three individuals dying in the “normal” trial at 12.26 g/L. Further mortalities occurred at 72 hours in all “mix” bioloads except the lowest.

Dissolved oxygen decreased with time and remained above 52% for up to 72 hours both in “normal” and “mixed” water. At 72 hours oxygen results varied considerably in all three trials conducted with “normal” water: 155% in trial 1 (bioload of 4.13 g/L), 52% in trial 2 (bioload of 6.69 g/L), and 97% in trial 3 (bioload of 12.26 g/L). This disparity strongly suggests that there was a high variability factor amongst the specimens used in the different trials, as one would expect oxygen to decrease with increasing bioload and time. The animals used in trial 2 therefore seemed to use oxygen at a rate substantially higher than expected. Coincidently, similar results were found for trials conducted with “mixed” water: 125% in trial 1 (bioload of 4.13 g/L), 55% in trial 2 (bioload of 6.69 g/L), and 60% in trial 3 (bioload of 12.26 g/L).

pH steadily decreased over time and reached markedly lower values than those reached in trials conducted with *Lipophrys pholis* and *Lepadogaster lepadogaster*. However, as with these two species, pH showed marginally higher numbers in “mixed” (i.e. buffered) water, as expected. Average pH of the three trials for “normal” water at 72 hours was 6.4, while average pH of the three trials for “mixed” water at 72 hours was 6.8.

The fourth trial was conducted with three specimens and a lower bioload (7.4 g/L instead of 12.26 g/L) than in trial 3. The use of smaller animals increased the transport efficiency, as after 48 hours, the oxygen rate was 139% and pH 6.9 in “mix” water, while oxygen was 148% and pH 6.9 in “normal” water.

Diplodus sargus

Diplodus sargus trials covered the following bioloads: trial 1:1.25 g/L with one specimen; trial 2: 3.23 g/L with two specimens; trial 3: 5.4 g/L with three specimens; trial 4: 8.49 g/L with four specimens.

All trials with both “normal” and “mixed” waters showed a survival rate of 100% up to 72 hours with a bioload of 1.25 g/L (Fig. 7). However, in trial 2 (bioload of 3.23 g/L) deaths (one of two individuals) occurred at 48 hours in both the “normal” and “mixed” trials. Further deaths (2/3 to 3/3) occurred at 72 hours in trials 3 and 4 with “mixed” water and 75% in trial 4 with “normal” water (Fig. 7).

Dissolved oxygen decreased with time and remained above 70% up to 72 hours both in “normal” and “mixed” water, except for trial 4 (bioload of 8.49 g/L) with “mix” water, which had 100% mortality at 72 hours and 27% oxygen. Oxygen remained high up to 72 hours in the first three trials with “normal” water and in the first two trials with “mixed” water, (average 125.07% and 112.0%, respectively). The third trial (bioload 5.4 g/L) with “mixed” water, however, was associated to a sudden drop to 70% at 72 hours.

Oxygen results for both “normal” and “mixed” water in trial 4 (bioload 8.49 g/L) averaged 64.3% and 58.7%, respectively, indicating this bioload is excessive for this species, even for short-duration trips.

pH steadily decreased over time and reached substantially lower values than those reached in trials conducted with *Lipophrys pholis* and *Lepadogaster lepadogaster*. However, as with all the species mentioned above, pH showed marginally higher numbers in “mixed” (i.e. buffered) water, as expected. Average pH of the four trials for “normal” water at 72 hours was 6.8 while average pH of the four trials for “mixed” water at 72 hours was 6.9.

Gobiusculus flavescens

Gobiusculus flavescens trials covered the following bioloads: Trial 1: 1.88 g/L with four specimens; trial 2: 3.95 g/L with six specimens; trial 3: 6.08 g/L with 10 specimens; trial 4: 6.01 g/L with 10 specimens.

All trials with “normal” water showed a survival rate of 100% to 72 hours with bioloads as high as 6.01 g/L (Fig. 8). However, trials with “mixed” water had 1 or 2 (out of 10) mortalities at 72 hours in both trials 3 and 4.

Dissolved oxygen remained elevated (above 132%) in all four trials in both “normal” and “mixed” waters up to 72 hours. Oxygen results for both “normal” and “mixed” waters clearly indicate that this is not a conditioning factor for bioloads as high as 6.01 g/L.

pH steadily decreased over time and reached values slightly lower than those reached in trials conducted with *Diplodus sargus*. However, as with this species, pH was marginally higher in “mixed” (i.e. buffered) water, as expected, i.e. average 6.24 versus 6.63 at 72 hours, respectively.

Discussion

The results of this study allowed us to reach the following conclusions:

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 maximise the economical potential of the shipment. For shipments of 48 to 72 hours a bioload of 15 to 20 g/L yielded 100% survivorship with the highest bioload.

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 maximise the economical potential of the shipment. For shipments of 48 to 72 hours, a bioload of 30 to 40 g/L yielded 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 it 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 duration greater than 48 hours should therefore not surpass a bioload of 7 g/L (corresponding to a minimum of three animals) and, although buffering is advised to keep pH elevated, 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 it 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;

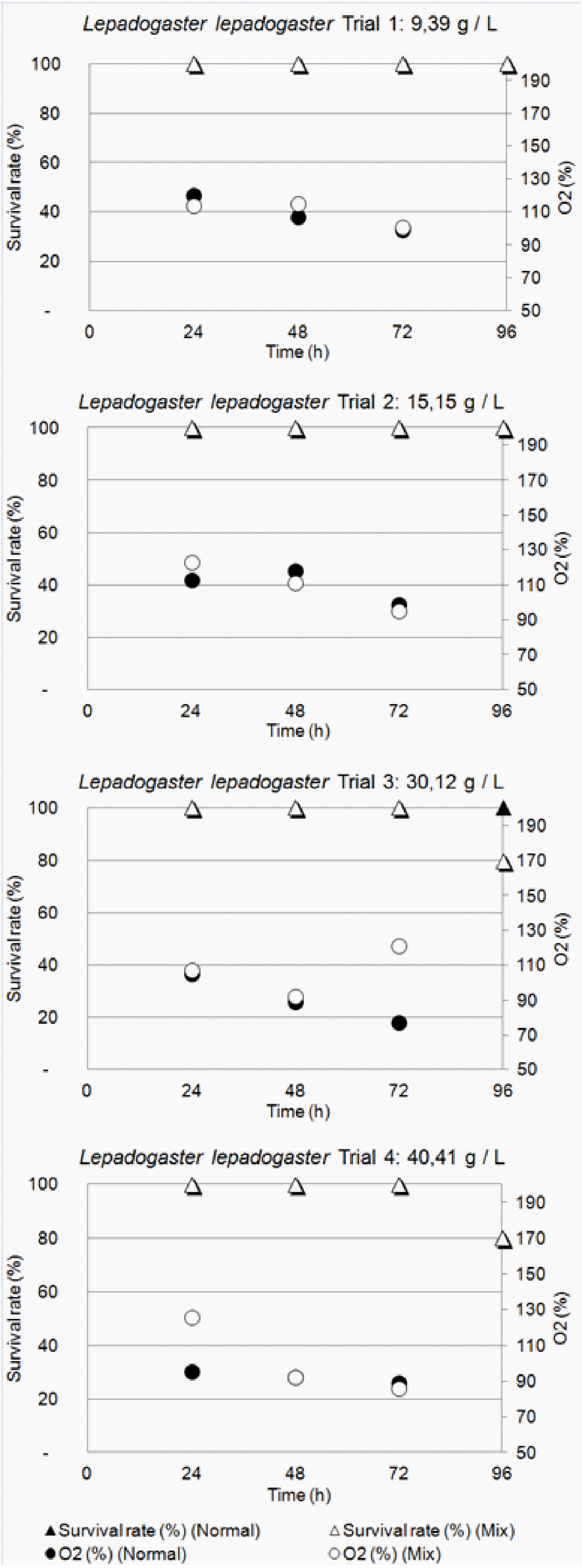

Figure 5a. Survival and oxygen saturation rates of *Lepadogaster lepadogaster* during simulated sealed transports over 24, 48 and 72 hours, with "normal" and "mixed" water ("mixed" water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

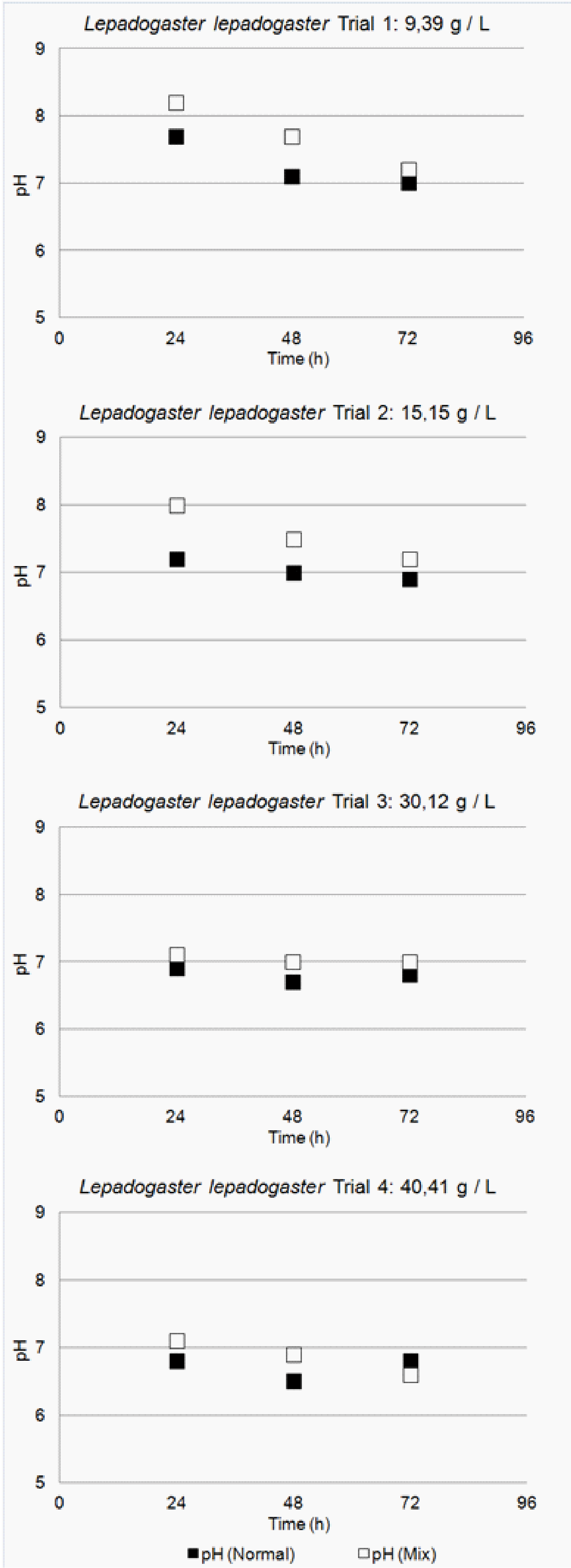

Figure 5b. pH of *Lepadogaster lepadogaster* during simulated sealed transports over 24, 48 and 72 hours, with "normal" and "mixed" water ("mixed" water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

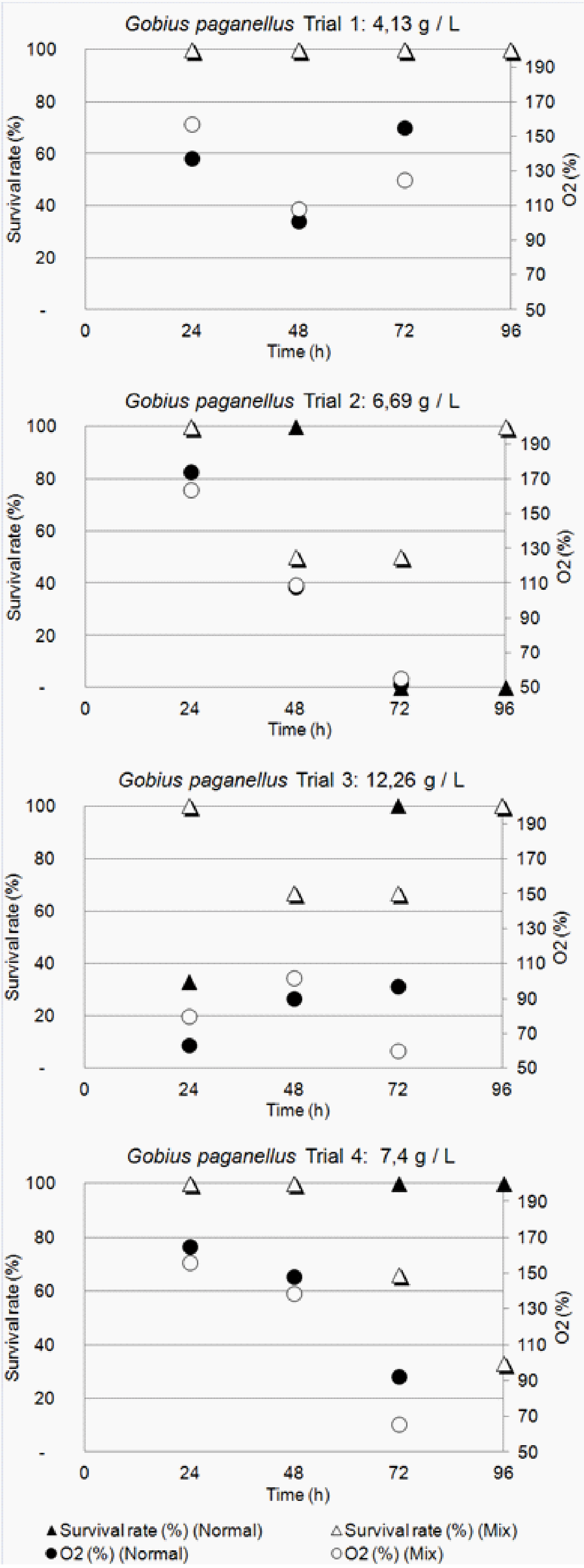

Figure 6a. Survival and oxygen saturation rates of *Gobius paganellus* during simulated sealed transports over 24, 48 and 72 hours, with “normal” and “mixed” water (“mixed” water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

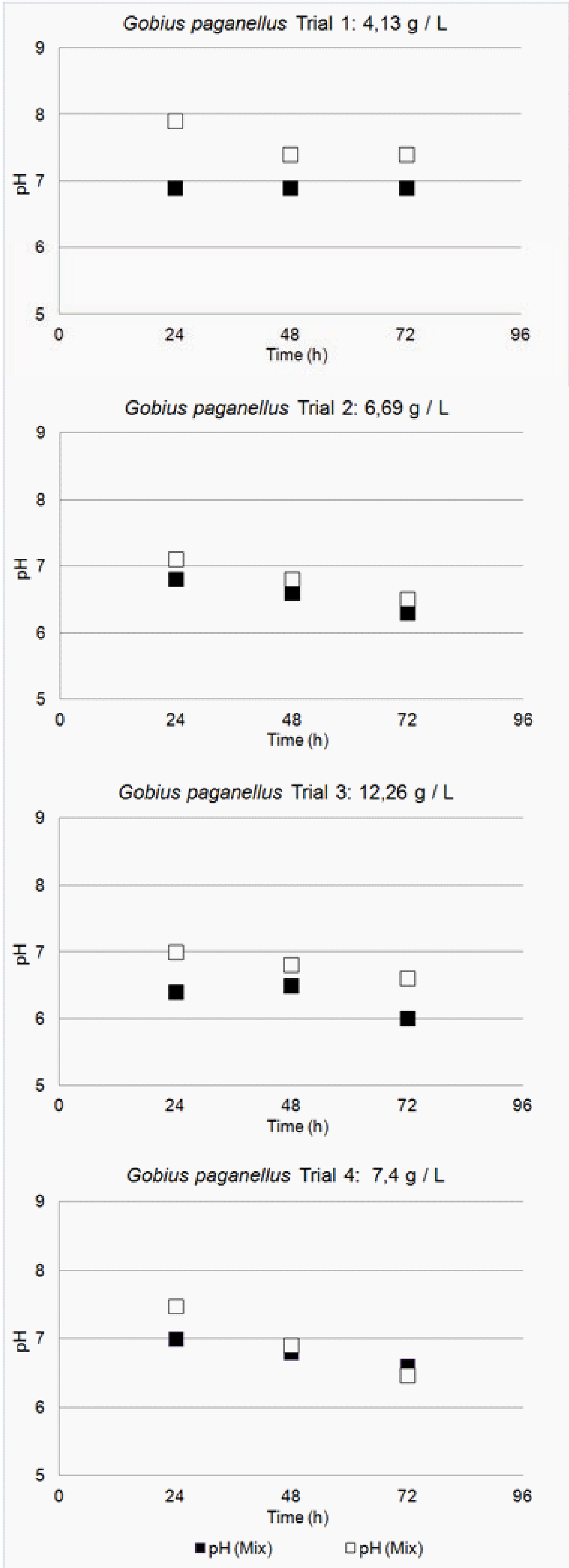

Figure 6b. pH of *Gobius paganellus* during simulated sealed transports over 24, 48 and 72 hours, with “normal” and “mixed” water (“mixed” water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

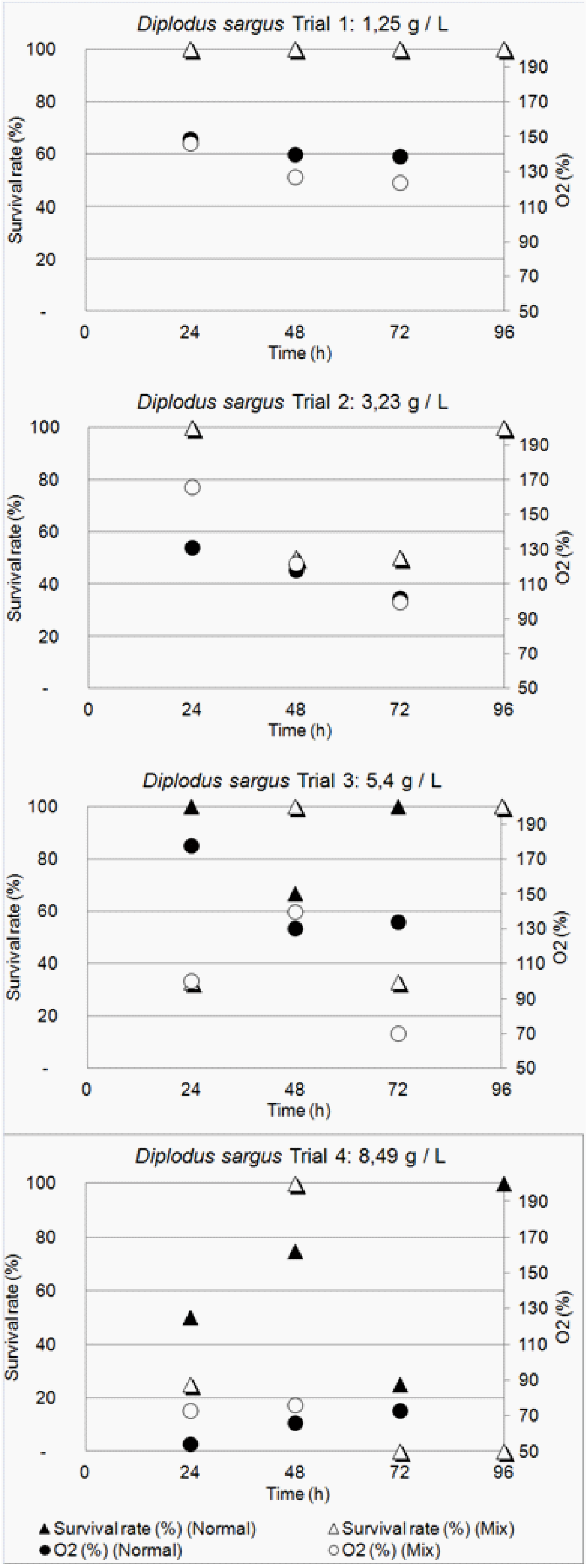

Figure 7a. Survival and oxygen saturation rates of *Diplodus sargus* during simulated sealed transports over 24, 48 and 72 hours, with “normal” and “mixed” water (“mixed” water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

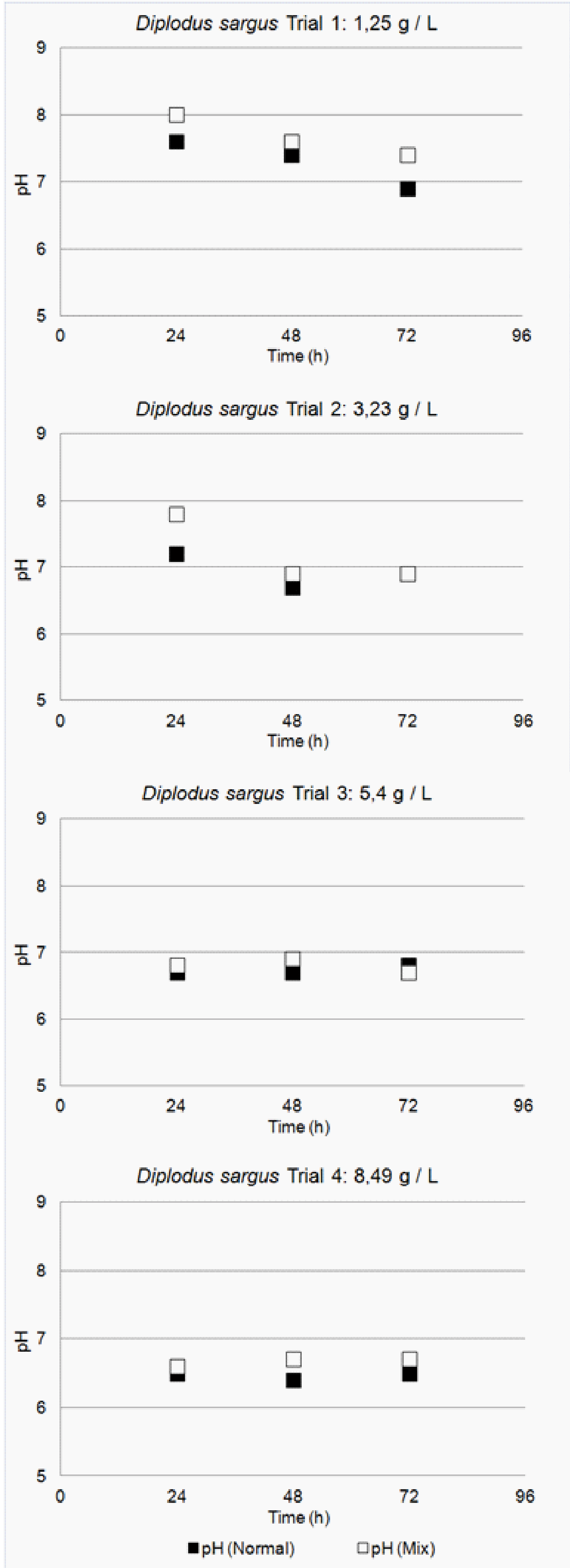

Figure 7b. pH of *Diplodus sargus* during simulated sealed transports over 24, 48 and 72 hours, with “normal” and “mixed” water (“mixed” water was buffered with Amquel©, sodium bicarbonate and sodium carbonate).

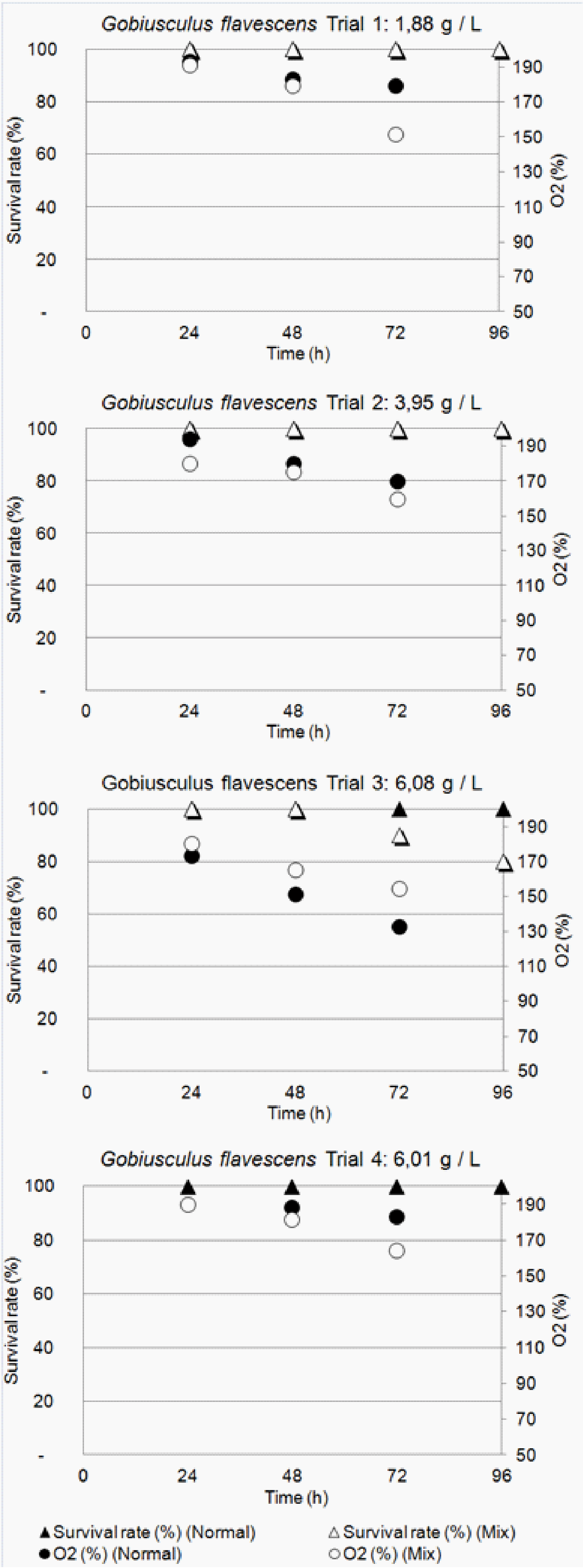

Figure 8a. Survival and oxygen saturation rates of *Gobiusculus flavescens* during simulated sealed transports over 24, 48 and 72 hours, with "normal" and "mixed" water ("mixed" water was buffered with Amquel®, sodium bicarbonate and sodium carbonate).

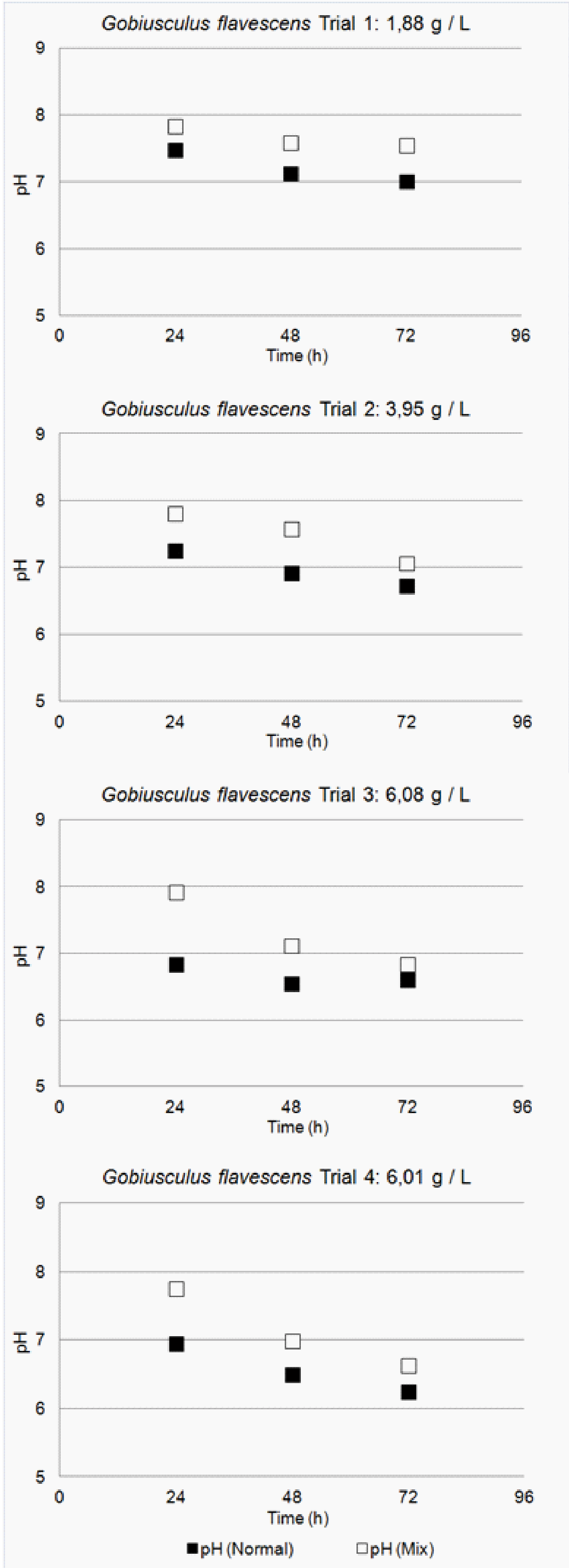

Figure 8b. pH of *Gobiusculus flavescens* during simulated sealed transports over 24, 48 and 72 hours, with "normal" and "mixed" water ("mixed" water was buffered with Amquel©, sodium bicarbonate and sodium carbonate).

- Transports with duration greater than 48 hours should therefore not exceed a bioload of 1.25 (trial 1) g/L and buffering is highly advised to keep pH elevated; a concentration higher than the 50 g/m^3 used (for both sodium bicarbonate and sodium carbonate) is recommended.

Gobiusculus flavescens

- Transport lasting 72 hours 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 agressiveness among the fish.

Results for ammonia concentration were not presented as these were expected to be null in those trials with "mixed" water and showed quite erratic figures in both "normal" and "mixed" water, which was attributed to faulty equipment.

However, analysis of survival rates, pH and oxygen saturation allowed us to conclude that:

- *L. lepadogaster* endured the highest bioloads and displayed 100% survivorship (i.e. up to 30 g/L), which is not surprising given the intertidal nature of this species
- *D. sargus* exhibited mortalities with bioloads as low as 3.23 g/L, which echoes its predominantly pelagic nature and relatively lesser ability to endure confinement
- The three remaining species showed varying degrees of tolerance to increasing bioloads in transport: *L. pholis*, also an intertidal species, handled up to 20 g/L over 72 hours, while *G. paganellus* handled up to 7 g/L over 72 hours, and *G. flavescens* (a predominantly pelagic species) could deal with no more than 6 g/L up to 72 hours.

These trials have allowed us to effectively double the bioload that we were using before the trials, particularly with species such as *Lepadogaster lepadogaster*, which show a remarkable tolerance to low oxygen levels and pH. This in turn means the client will get double the number of animals for the same air-freight cost. Such trials are therefore extremely helpful in defining the highest bioloads that may safely be used in transit, while increasing survivorship. The trials were conducted with species ranging from a tidal environment (and therefore highly tolerant, such as *Lepadogaster lepadogaster*) to those more pelagic in nature (therefore less tolerant, such as *Diplodus sargus*). However, similar trials with many other species would be beneficial.

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References

Briggs J.C. (1986) Gobiesocidae. p. 1351–1359. In P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese (eds.) Fishes of the North-eastern Atlantic and the Mediterranean, volume 3. UNESCO, Paris.

Correia J.P.S. (2001) Long-term transportation of ratfish, *Hydrolagus colliei*, and tiger rockfish, *Sebastes nigrocinctus*. *Zoo Biology* 20: 435–441.

Correia J.P.S., Graça J.T.C., Hirofumi M. (2008) Long-term transportation, by road and air, of devil-ray (*Mobula mobular*), meagre (*Argyrosomus regius*), and ocean sunfish (*Mola mola*). *Zoo Biology* 27: 234–250.

Correia J.P., Graça J., Hirofumi M., Kube N. (2011) Long term transport of *Scomber japonicus* and *Sarda sarda*. *Zoo Biology* 30: 459–472.

D'Anna G., Giacalone V.M., Veja-Fernández T., Vaccaro A.M., Pipitone C., Mirto S., Mazzola S., Badalamenti F. (2012) Effects of predator and shelter conditioning on hatchery-reared white seabream *Diplodus sargus* (L., 1758) released at sea. *Aquaculture* 356–357: 91–97.

Francisco S.M., Faria C., Lengkeek W., Vieira M.N., Velasco E.M., Almada V.C. (2011) Phylogeography of the shanny *Lipophrys pholis* (Pisces: *Blenniidae*) in the NE Atlantic records signs of major expansion event older than the last glaciation. *Journal of Experimental Marine Biology and Ecology* 403: 14–20.

Froese R. (1988) Relationship between body weight and loading densities in fish transport using the plastic bag method. *Aquaculture Research* 19: 275–281.

Henriques M., Lourenço R., Almada F., AlmadaV.C. (2002) A revision of the status of *Lepadogaster lepadogaster* (Teleostei: *Gobiesocidae*): sympatric subspecies or a long misunderstood blend of species. *Biological Journal of the Linnean Society* 76: 327–338.

Lim L.C., Dhert P., Sorgeloos P. (2003) Recent developments and improvements in ornamental fish packaging systems for air transport. *Aquaculture Research* 34: 923–935.

McFarland W.N., Norris K.S. (1958) The control of pH by buffers in fish transport. *California Fish and Game* 44: 291–310.

Penning M., Reid G. McG., Koldewey H., Dick G., Andrews B., Arai K., Garratt P., Gendron S., Lange J., Tanner K., Tonge S., Van den Sande P., Warmolts D., Gibson C. (eds) (2009) *Turning the Tide: A Global Aquarium Strategy for Conservation and Sustainability.* Bern: World Association of Zoos and Aquariums.

Rodrigues N., Correia J., Pinho R., Graça J., Rodrigues F., Hirofumi M. (2012) Notes on the husbandry and long-term transportation of bull ray (*Pteromylaeus bovinus*) and dolphinfish (*Coryphaena hippurus* and *Coryphaena equiselis*). *Zoo Biology* 32: 222–229.

Rodrigues N., Maranhão P., Oliveira P., Alberto J. (2008) *Guia de Espécies Submarinas.* Peniche, Portugal: Haliotis e Instituto Politécnico de Leiria.

Teo L. H., Chen T.W., and Lee B.H. (1989) Packaging of the guppy, *Poecilia reticulata*, for air transport in a closed system. *Aquaculture* 78: 321–332.

Tlusty M.F., Rhyne A.L., Kaufman L., Hutchins M., Reid G.M., Andrews C., Boyle P., Hemdal J., McGilvray F., Dowd S. (2012) Opportunities for public aquariums to increase the sustainability of the aquatic animal trade. *Zoo Biology* 19:1–19.

Vinagre C., Cabral H.N., Costa M.J. (2010) Relative importance of estuarine nurseries for species of the genus *Diplodus* (*Sparidae*) along the Portuguese coast. *Estuarine, Coastal and Shelf Science* 86: 197–202.

Young F.A., Kajiura S.M., Visser G.J., Correia J.P.S., Smith M.F.L. (2002) Notes on the long-term transport of the scalloped hammerhead shark (*Sphyrna lewini*). *Zoo Biology* 21: 243–251.