

WATER QUALITY MONITORING DURING THE TRANSPORT OF AMAZONIAN ORNAMENTAL FISH

Andrea V. Waichman, Márcio Pinheiro and Jaydione L. Marcon

Universidade do Amazonas

ABSTRACT

High mortality rates have been reported in the capture and transport of ornamental fishes from the Amazon basin, and much of it was probably stress induced. One of the primary factors that can be managed to reduce stress and mortality during transport is water quality. We assessed the physical and chemical conditions of the water used to transport the species *Paracheirodon axelrodi*, *Symphysodon discus* and *Carnegiella strigata*. Water samples were collected during capture, at the fishing camps, at the exporter's Barcelos facilities, during the transport from Barcelos to Manaus, and at the exporters' facilities in Manaus. During storage at the Barcelos facilities and transport to Manaus, samples were collected before and after partial water renewal to evaluate the effect of this practice on water quality. We measured temperature, electric conductivity, pH, dissolved oxygen, ammonia, chloride, sodium, and potassium. To assess fish stress response, the change in concentration of these ions in the water was measured over 20 hours during transport to Manaus. The changes observed in the ionic concentrations indicated that during transport the fishes were subjected to stress. Water quality during transport from fishing areas to the fishing camps, and at the fishing camps themselves, was good. Water quality at Barcelos facilities and during transport from Barcelos to Manaus differed significantly from those observed at the fishing grounds and fishing camps. Major differences were observed in the concentrations of dissolved oxygen, ammonia, sodium and chloride. The practice of partial water renewal and the use of table salt (NaCl) during these two phases of the transport process did not effectively improve water quality. The poor water quality observed during transport and the lack of standard handling procedures resulted in a progressive decline in general fish health and perhaps a lowered resistance to diseases. At the Manaus facilities, water quality was considered adequate. The high mortality rates reported by the importers were probably caused by the inability of fishes to recover after the transport-induced stress. More detailed studies of water quality are necessary to establish protocol to reduced stress and improve survivorship.

RESUMO

Altas taxas de mortalidade tem sido registradas na captura e transporte de peixes ornamentais da bacia Amazônica, grande parte da qual é provavelmente induzida por estresse. Um dos fatores que podem ser gerenciados para reduzir o estresse e mortalidade é a qualidade da água. Foram avaliadas as condições físicas e químicas da água usada para transportar as espécies Paracheirodon axelrodi, Symphysodon discus e Carnegiella strigata. Amostras de água foram coletadas durante a captura, nos acampamentos de pesca, nas instalações dos exportadores em Barcelos, durante o transporte de Barcelos para Manaus e nas instalações dos exportadores em Manaus. Durante a estocagem em Barcelos e transporte para Manaus, amostras de água foram coletadas antes e após renovação parcial da água, para avaliar o efeito desta prática na qualidade da água. Na água foram medidas a temperatura, condutividade elétrica, pH, oxigênio dissolvido, amônia, cloro, sódio e potássio. Para avaliar a resposta dos peixes ao estresse, mudanças na concentração iônica da água foram

determinadas em um período de 20 horas durante o transporte para Manaus. A qualidade da água durante o transporte dos pontos de pesca para os acampamentos, e nos acampamentos foi boa. A qualidade da água nas instalações em Barcelos e durante o transporte de Barcelos para Manaus diferiu significativamente das observadas nos locais de pesca e acampamentos. As maiores diferenças foram observadas no teor de oxigênio dissolvido, amônia, sódio e cloro. A estocagem e transporte para Manaus representam pontos críticos em função da baixa qualidade da água. Nas instalações de Manaus a qualidade da água foi considerada adequada. A prática da renovação parcial da água e o uso indiscriminado de sal de cozinha (NaCl) durante estas duas fases do processo de transporte não foram eficientes para melhorar a qualidade da água. As mudanças observadas na concentração de vários íons indicaram que durante o transporte os peixes estão sujeitos a estresse. A baixa qualidade da água observada durante o transporte e a falta de um procedimento uniforme de manejo resultam em um progressivo declínio na saúde geral dos peixes e possivelmente diminua a resistência a doenças. As altas taxas de mortalidade registradas pelos importadores são provavelmente causadas pela incapacidade dos peixes se recuperarem do estresse do transporte. Estudos mais detalhados da qualidade da água são necessários para estabelecer quais práticas asseguram a redução do estresse e melhores taxas de sobrevivências.

INTRODUCTION

Ornamental fish are subject to a variety of stressors during the capture and transport process. These include injuries during the capture, confinement and overcrowding, poor water quality and careless handling and transport (Wedemeyer, et al., 1984). Each factor alone can cause mortality, and together, the effects may be exacerbated.

High mortality rates are reported for wild-caught fish from South America and Africa, where more than 50% die before being exported (ITC 1979, Hemley, 1984). These fishes are generally maintained in small containers where they are overcrowded, and exposed to fluctuating temperature, poor water quality, parasites and diseases. A case study by Chapman, et al. (1997, 1998) reported that 40 of 50 boxes of neon tetras (*Paracheirodon innesi*) or cardinal tetras (*P. axelrodii*) imported from South America to US, are lost as a result of mortality. Other species of wild-caught fishes from the Amazon also appear to suffer the same fate (Conroy 1975, Welcomme et al. 1979, ITC 1979, Leite and Zuanon 1991, Eisenstadt 1992, Adeodato and Oliveira 1994). Interviews with those involved in the capture and transport of ornamental fish yield conflicting statements. Exporters claim that mortality rates can reach as high as 70% of the stock during the capture and transport processes (Welcomme et al. 1979). However, they claim that mortality does not surpass 10% during quarantine in their facilities and subsequent transport. These numbers change completely when we analyze data from interviews made by Eisenstadt (1992). Middlemen and exporter's representatives report that mortality during the capture and transport to Manaus is low, depending on the time of year, probably around 2%. Since mortality estimates varied widely, we need to be careful. Data are scarce, and detailed studies of mortality rates did not exist for the different phases capture and transport. However, there is a general agreement that higher mortality rates occur during capture and the transport, with lesser rates during the quarantine period.

One way to reduce stress during transport out of the Amazon is to maintain high water quality. The capacity to transport ornamental fish over long distances with high survival rates requires information on the metabolism, tolerance to stress, and water quality requirements of each species involved. For Amazon ornamental fishes, not much information in water quality,

or their tolerance to decreased water quality is available. This study was to assess capture conditions, handling procedures, and water quality at different stages of transport sequence for *Paracheirodon axelrodi* (cardinal tetra), *Symphysodon discus* (discus) and *Carnegiella strigata* (marble hatched fish). The stress response was also assessed, assuming that changes in ion concentrations of the water occur as a consequence of osmoregulation processes of the fishes.

MATERIALS AND METHODS

We made seven trips to Barcelos to examine the capture and transport of cardinal tetras and discus. At fishing grounds, the rainforest streams Boi-Boi, Zamula, Daraquá and Tucano, and in fishing camps, six samples of water per species and per trip were collected. For marbles, as well as tetras and discus, we examined water quality while the fish were held in Barcelos exporters' facilities, during transport to Manaus, and at the exporters' facilities in Manaus.

At Barcelos exporter's facilities and during transport from Barcelos to Manaus, we sampled six boxes per species and per trip. At least three water samples were collected before and after partial water renewal. We made 4 visits to exporters' facilities in Manaus. Six water samples, per species and per visit, were taken from the quarantine tanks at each exporter.

For each sample, we measured temperature, pH, conductivity, dissolved oxygen *in situ*, and water samples were collected for further analyses of ammonia, chloride, sodium, potassium, calcium, and magnesium concentrations in the laboratory according to methods recommended by APHA (1989). Temperature, pH, conductivity and dissolved oxygen were determined using electronic instruments. Total ammonia concentration ($\text{NH}_4^+\text{-N} + \text{NH}_3\text{-N}$) was determined following Golterman et al. (1978) modified for flow injection analysis (FIA). Sodium and potassium concentrations were measured using flame spectrophotometry. Chloride concentrations were measured by titration. Calcium and magnesium concentrations were measured by atomic absorption spectrophotometry.

During transport to Manaus, changes in sodium, potassium, chloride, calcium and magnesium concentrations in water were measured at predetermined time intervals during 20 hours preceding water renewal, to assess stress responses. For this assessment we collected water samples from six fish tubs per species, during 3 transport trips. Time intervals were different for the three species. For cardinal tetras time interval was 4 hours, for discus, 6 hours, and for marbles, 3 hours.

Results were reported as means and standard deviations. Data were analyzed as a one-way ANOVA test (Wilkinson et al., 1992), to verify differences in water quality among the different phases of transport, as well as pre and post- water renewal. Tukey's test was used for *a posteriori* contrast analysis. Statistical results are summarized in Appendix 1.

RESULTS AND DISCUSSION

The capture, storage and transport processes

From the capture to exporters' facilities in Manaus, fishes passed through different stages of storages and transports (Figure 1).

The capture of the *P. axelrodi* was undertaken during the day. The fishers used either a large dip net (rapichê) or trap (*cacuri*) baited with a mixture of cooked fish and oil (Chao, this volume). The fisherman located the school and with the oar herded it to dip net, which was then lifted slowly from the water. The number of fish captured is greater in the low water season

(September to March) when the fishes were concentrated into stream channels. Lower yields were obtained during the rainy season (April to August) when fishes were dispersed through the flooded forest. The capture of *S. discus* was undertaken at night during the low water season, with the aid of flashlights to stun fish. Generally, discus was captured one by one with a soft cotton dip net to avoid damaging the fish..

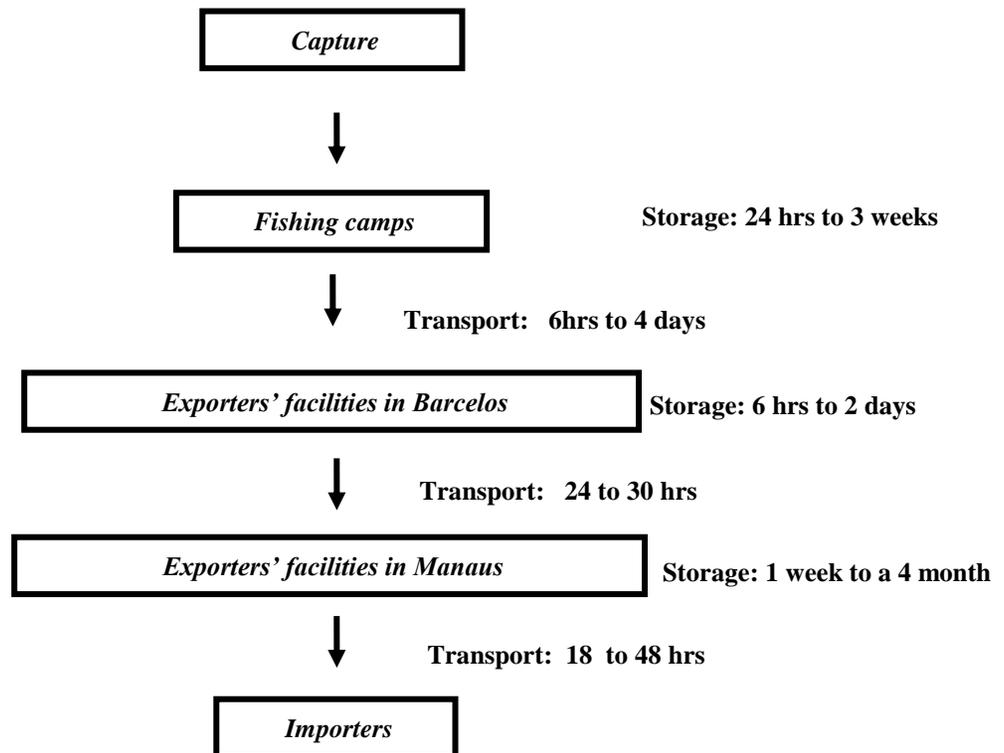


Figure 1. Capture, storage and transport processes for Amazonian ornamental fishes from Rio Negro basin.

During capture fishes were placed in woven baskets lined with 100-liter plastic bags. The time spent in fishing depends on fish abundance and varied between two and eight hours. After capture, cardinal tetras were placed in rectangular tanks of different sizes, made of nylon window screen and set half submerged in the streams by the fishing camps. Discus were stored in wooden reservoirs measuring 1.5 m tall by 2.0 m wide and 1.0 m deep. These boxes had lateral holes. These tanks were cleaned frequently to allow water flow.

Some authors described the capture methods as rough, because fishes suffered damage that was largely responsible for their mortality. These critics alleged that fishers were not aware that they were damaging the fish (Welcomme et al. 1979, Adeodato and Oliveira 1994). From what we saw, the handling was relatively gentle. Prang (1996b) observed that fishermen changed the water of the baskets as often as five times in three hours.

Fishes stored at the fishing camps for 24 hours to three weeks, and were fed pieces of cooked fish and eggs. At the fishing camps, fishes were separated by size, counted, and placed in plastic tubs (58cm long, 38cm wide and 18cm deep). These tubs were filled with about eight liters of stream water for transport to Barcelos exporters' facilities or directly to a boat that would transport them to Manaus. The number of fish placed in each tub depended on the species and sizes. For *P. axelrodi*, this number varied from 600 to 1,000; for *S. discus*, from three to six; and for *C. strigata*, from 200 to 300. The trip from the fishing camp to the exporters' facilities in Barcelos, or to the transport boat to Manaus took between six hours to four days.

Fishes stayed in their original tubs at Barcelos exporters' facilities, and the water was partially renewed one time before shipping to Manaus. The tub was tilted to let half of the water out, then a pail with Rio Negro water was poured in to refill the tub, usually in a fast and violent way. This procedure probably induced stress and injuries table salt (NaCl) and antibiotics such as Terramicine (Oxitetraacycline hydrochloridric) were added at this time.

Aboard the transport boat, the tubs were stacked on the deck in columns of 12 or 13. The trips lasted 24 to 30 hours. Water was partially renewed only once below the Rio Branco, approximately 20 hours after leaving Barcelos. Rio Negro water was pumped aboard through a two-inch diameter hose. Sometimes this was the same pump and hose used to refuel the boat.

In Manaus, the boxes were placed in trucks for transport to the exporters' facilities. Fishes were acclimated in quarantine tanks consisting of plastic swimming pools or tile lined tanks. Exporters used natural water from streams, artesian wells, or even tap water. Before the fishes were exported, they were treated for several parasites, fungi and bacteria. These treatments consisted of baths with different substances such as Nitrofurazone, Tetracycline, Acriflavine, malachite green, methylene blue, potassium permanganate, or table salt (NaCl). The fish stayed in this treatment between one and 24 hours.

Water quality assessment

For *P. axelrodi*, water temperature at fishing camps was higher than during the capture and transport process (Fig. 2). For *S. discus* higher temperatures were measured during capture and at fishing camps (Fig 2). The high temperature at the fishing camps was probably because the wooden boxes with discus and the tanks with cardinals were not totally shaded. Abrupt changes of temperature were not observed during the transport from Barcelos to Manaus. The lowest temperatures were registered at the exporters' facilities at Manaus. For *C. strigata*, temperatures at the exporter's facilities were lower than the Barcelos exporters' facilities and during transport to Manaus (Fig. 2).

Dissolved oxygen concentration varied significantly through transport steps for all the species (Fig 2). They were high at the fishing grounds and fishing camps, then reduced at Barcelos facilities and transport to Manaus, and tended to rise at the exporters' facilities (Fig. 2). The lowest oxygen concentrations were found at Barcelos exporter's facilities and during transport to Manaus. This hypoxical condition alone can produce a stress response in fish. Scott and Rogers (1980) and Drewett and Abel (1983) reported that fishes exposed to low oxygen concentrations of 0.3 and 1.5 mg/L for periods exceeding 24 hours, have shown necrosis, hemorrhage, hyperplasia, hypertrophy, and hyperanemia of the gills, liver, kidney, and spleen. Although we have no information of these symptoms in *P. axelrodi* or *S. discus*, low oxygen concentrations during the storage and transport to Manaus, undoubtedly weaken the fish, rendering them more susceptible to diseases and lowering their survivorship.

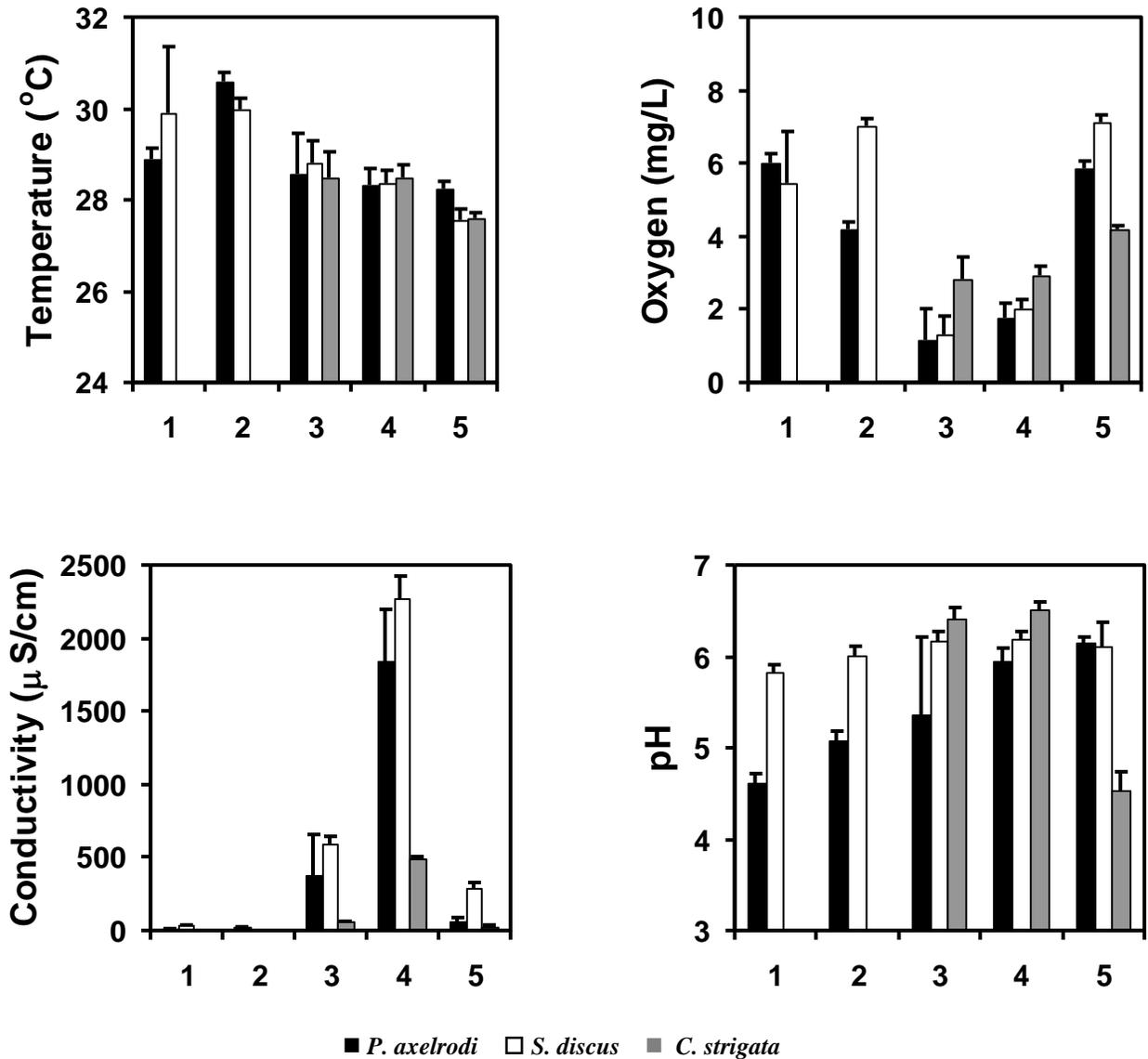


Figure 2. Average values and standard deviation of temperature, dissolved oxygen, electric conductivity and pH in the different phases of the ornamental fish transport process. 1 – Fishing grounds, 2 – Fishing camp, 3 – Barcelos exporters’ facilities, 4 – Transport from Barcelos to Manaus, 5 – Manaus exporter’s facilities.

A different situation was observed in *C. strigata* tubs, where average oxygen concentrations at Barcelos exporters' facilities and transport to Manaus were greater than those for *P. axelrodi* and *S. discus*.

Low electrical conductivity values were measured at the capture sites and fishing camps ($13.0 \pm 0.5 \mu\text{S/cm}$ to $22.7 \pm 3.8 \mu\text{S/cm}$), typical of poor ionic blackwater streams (Fig. 2). At the Barcelos facilities and during transport to Manaus, large increases in electric conductivity were observed, mainly for *P. axelrodi* ($373.4 \pm 234.2 \mu\text{S/cm}$ and $1,848.0 \pm 351.9 \mu\text{S/cm}$) and *S. discus* ($591.5 \pm 54.0 \mu\text{S/cm}$ and $2,273.0 \pm 156.6 \mu\text{S/cm}$). The addition of table salt (NaCl) to the water in Barcelos before shipping to Manaus also raised the electric conductivity. High standard deviations, especially for *P. axelrodi*, reflect a great variation among fish tubs and transport samples. This indicated the lack of standardization in the amount of salt added. For *S. discus*, conductivities measured at exporters' facilities in Manaus were elevated to $422.7 \pm 132.3 \mu\text{S/cm}$, due to the addition of table salt.

Water pH tended to increase for *P. axelrodi* from $4.62 (\pm 0.01)$ to $6.15 (\pm 0.05)$ through the different steps between capture and storage at Manaus exporters' facilities (Fig. 2). For *C. strigata* pH were high at the Barcelos facilities and during transport to Manaus (Fig. 2). No significant difference was observed among pH values for *S. discus* (Fig. 2). Water pH from all points of the capture/transport process were within the range found by Walker (1990, 1995) for blackwater streams, that, depending on the season, varied between 2.8 and 6.4. The observed pH variations had little effect on the physiology of these fishes (Gonzalez et al. 1998).

Ammonia concentrations were low at fishing grounds and camps (Fig. 2). For *P. axelrodi* and *C. strigata*, the ammonia concentrations at Barcelos facilities and during transport were approximately nine times higher than those observed in the fishing grounds and exporters' facilities. However, the ammonia levels for *S. discus* were less at Barcelos facilities and during transport, but were the highest at the exporters' facilities. One of the exporters used tap water with ammonia concentrations between 4.80 and 5.20 mg/L. High levels of ammonia cause stress and produce harmful physiological response such as osmoregulatory dysfunction, kidneys and branchial epithelium damages (Meade 1985). High ammonia concentrations also reduce gills efficiency for oxygen intakes and capacity of blood oxygen transport due to changes in blood pH and blood cell damages (Souza and Meade 1977).

Sodium and chloride concentrations at fishing ground and camp were low and increased at Barcelos exporter's facilities and during transport due to the addition of table salt (Fig. 3). Chloride and sodium concentrations for *S. discus* were higher at the Barcelos facilities and during transport, than observed for *P. axelrodi* and *C. strigata*. This may attribute to more salt were added to discus tubs or the specific result of high ion losses due to stress.

Potassium concentrations were usually low (Fig. 3), between the levels below the detection limit and 3,3 mg/L, except for the *S. discus* at exporters' facilities ($43.1 \pm 2.6 \text{ mg/L}$). Variation of potassium concentrations for *P. axelrodi* and *C. strigata* were insignificant.

Water quality at fishing area was good. In this phase fishes may be stressed during capture and handling procedures. Storage at Barcelos exporters' facilities and transport to Manaus clearly represent the critical points, due to gradually lowered water quality. Water quality may differ among exporters, but they were usually adequate.

Water renewal during the transport

Water renewal at Barcelos exporters' facilities and during the transport had little effect on water quality improvement. Furthermore, manipulations of fish tubss during this practice could increase the stress levels of the fish.

Temperature was higher after water renewal (Fig 4), probably because the water renewal was made after midday, when water temperature was the warmest and much higher than the ambient temperature.

The partial renewal of the water had a positive effect on oxygen concentration for *P. axelrodi* and the *S. discus* (Fig. 4). Average values before renewal were 1.5 ± 0.7 mg/l for *P. axelrodi* and 1.7 ± 1.1 mg/l for *S. discus*. After the water exchange, the average dissolved oxygen values were 2.7 ± 1.4 mg/l and 3.1 ± 1.8 mg/l respectively for *P. axelrodi* and *S. discus*. For *C. strigata*, the water renewal did not have any apparent effect on oxygen concentrations, with an average of 2.8 ± 0.5 mg/L and 3.0 ± 0.5 mg/L before and after the renewal. The partial renewal of water probably did not benefit fishes in transport because fishes in stress conditions have an increased oxygen demand (Barton and Schreck 1987). In addition, the high content of salts in the water decreased oxygen solubility.

No differences on water pH, electric conductivity, sodium, potassium and chloride concentrations were observed before and after the renewal (Fig. 4 and 5). Ammonia levels were lowered after the water renewal for *S. discus*, but no difference for *C. strigata* and *P. axelrodi* (Fig. 5). These small fishes are more active, and have a high ammonia excretion rate, which may result in fast increase in the ammonia concentrations after the renewal of water.

Stress assessment during transport

The transportation of fish can cause stress, especially during the process of manipulation associated with water renewal, or the low water quality during this phase of the commercialization process. The assessment of changes in the concentration of water ions during transport can give insights on fish stress during this process.

We observed for *P. axelrodi* that after 20 hours, oxygen levels dropped 40%, and ammonia levels increased 225% (Fig. 6). The highest amount of ammonia was at the end of the 20 hours period; when oxygen concentrations were lowered to about 1 mg/l, the critical level for species of Amazon fish (Junk et al. 1983). Increases in sodium (40%) and chloride (65%) concentrations accompanied the oxygen and ammonia changes (Fig. 6). Potassium concentrations had an initial increment of 100%, but stabilized at this level. Divalent ions decreased in concentration, suggesting they were absorbed by *P. axelrodi*.

After 20 hours, calcium concentrations were below the detection limit and magnesium concentrations decreased 70%. The absence of calcium in water containing *P. axelrodi* suggested that this species was absorbing all the available calcium in an attempt to maintain homeostasis and minimize osmoregulatory dysfunction. Calcium has an important function in the regulation of the ionic flows, gathering in branchial para-cell junctions and controlling membrane permeability (Donald, 1983). Calcium absorption would help to adjust the permeability of branchial membrane in appropriate levels that could attenuate the observed sodium, chloride and potassium losses. Recent study by Matsuo (1998) demonstrated that two species of *Corydoras* had a significant decrease of sodium and potassium loss when submitted to acid waters enriched with calcium.

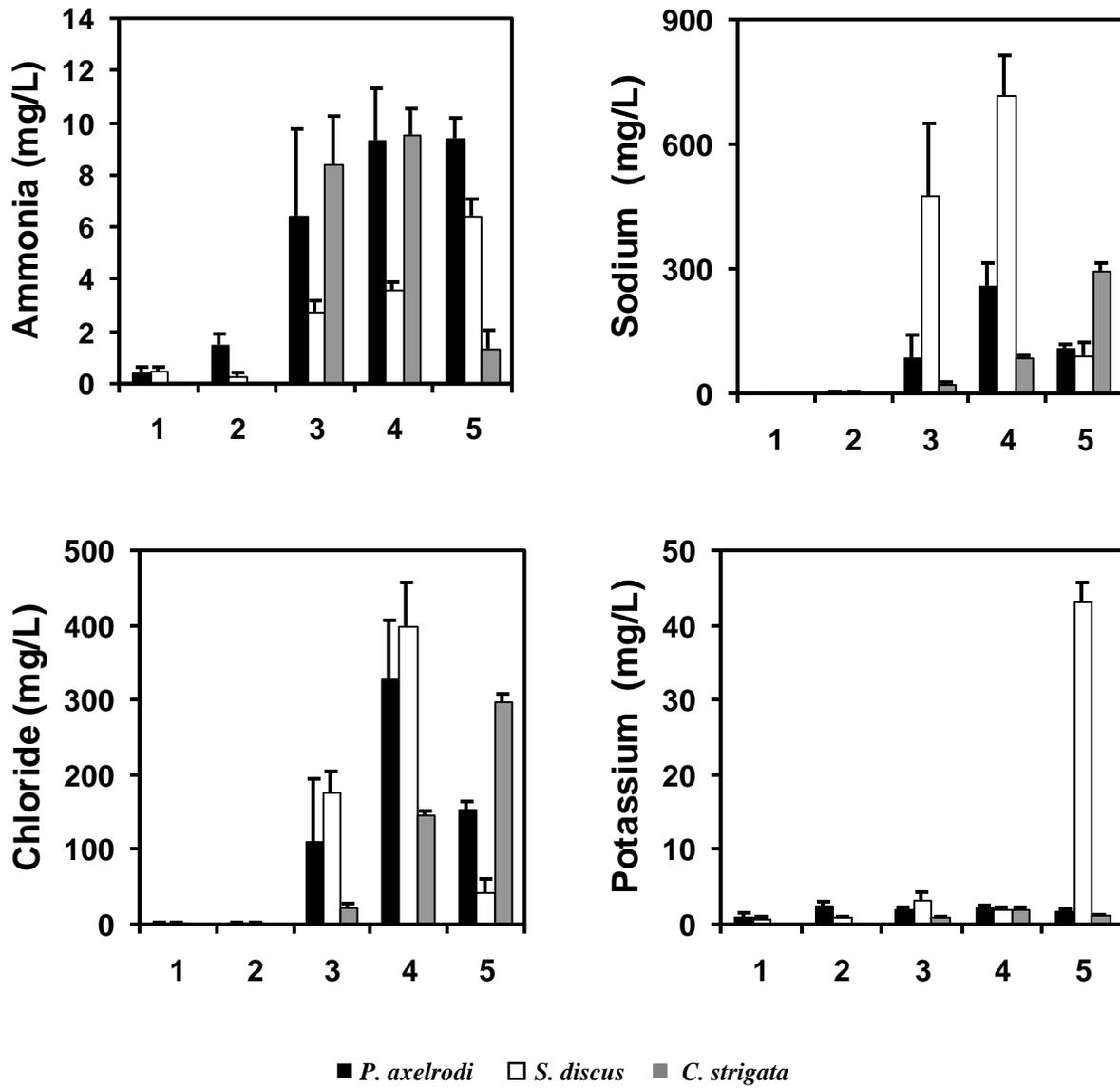


Figure 3. Average values and standard deviation of ammonia, chloride, sodium and potassium in the different phases of the ornamental fish transport process. 1 – Fishing grounds, 2 – Fishing camp, 3 – Barcelos exporters’ facilities, 4 – Transport from Barcelos to Manaus, 5 – Exporter’s facilities in Manaus.

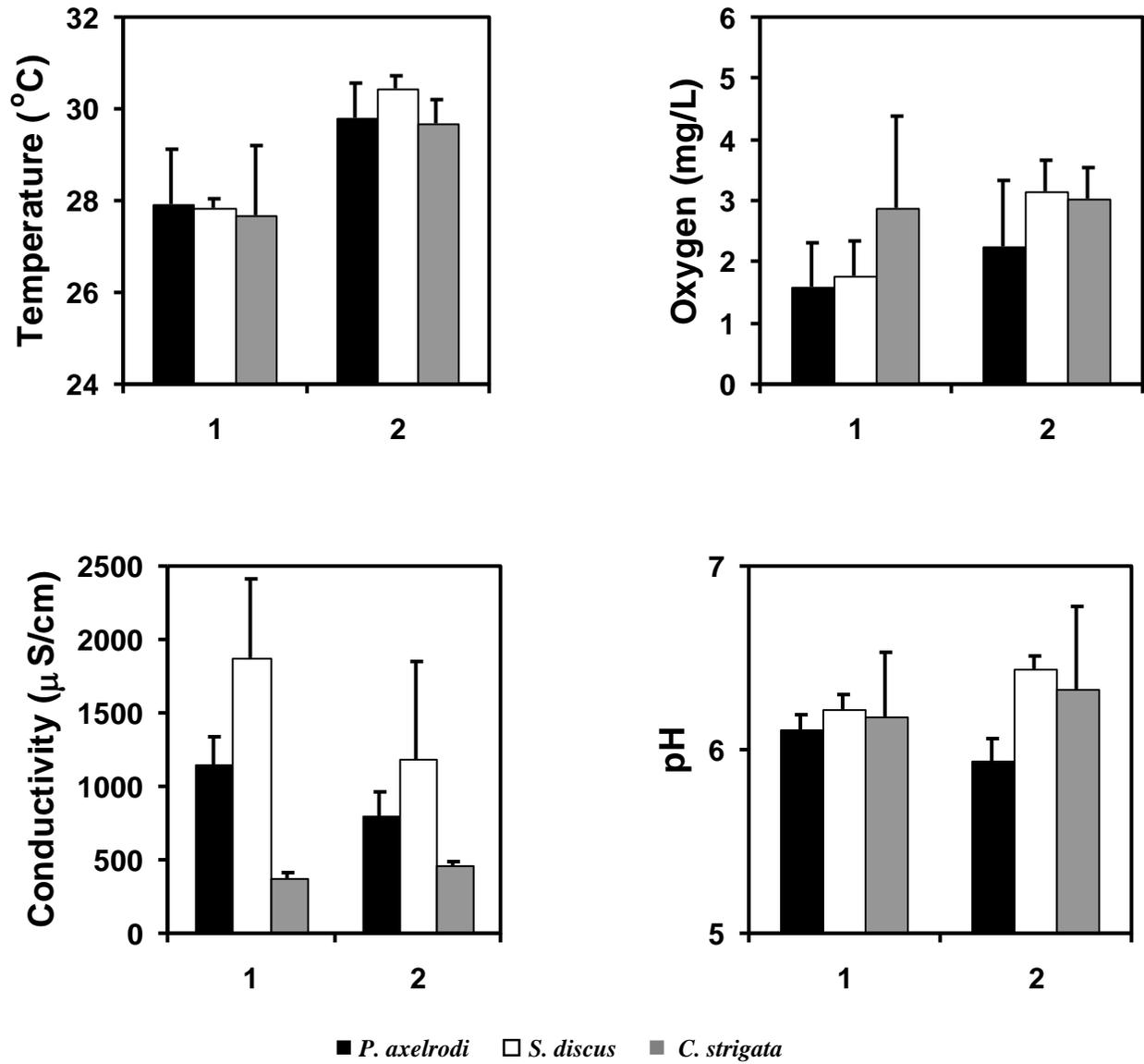


Figure 4. Average values and standard deviation of temperature, dissolved oxygen, electric conductivity and pH before (1) and after (2) water renewal.

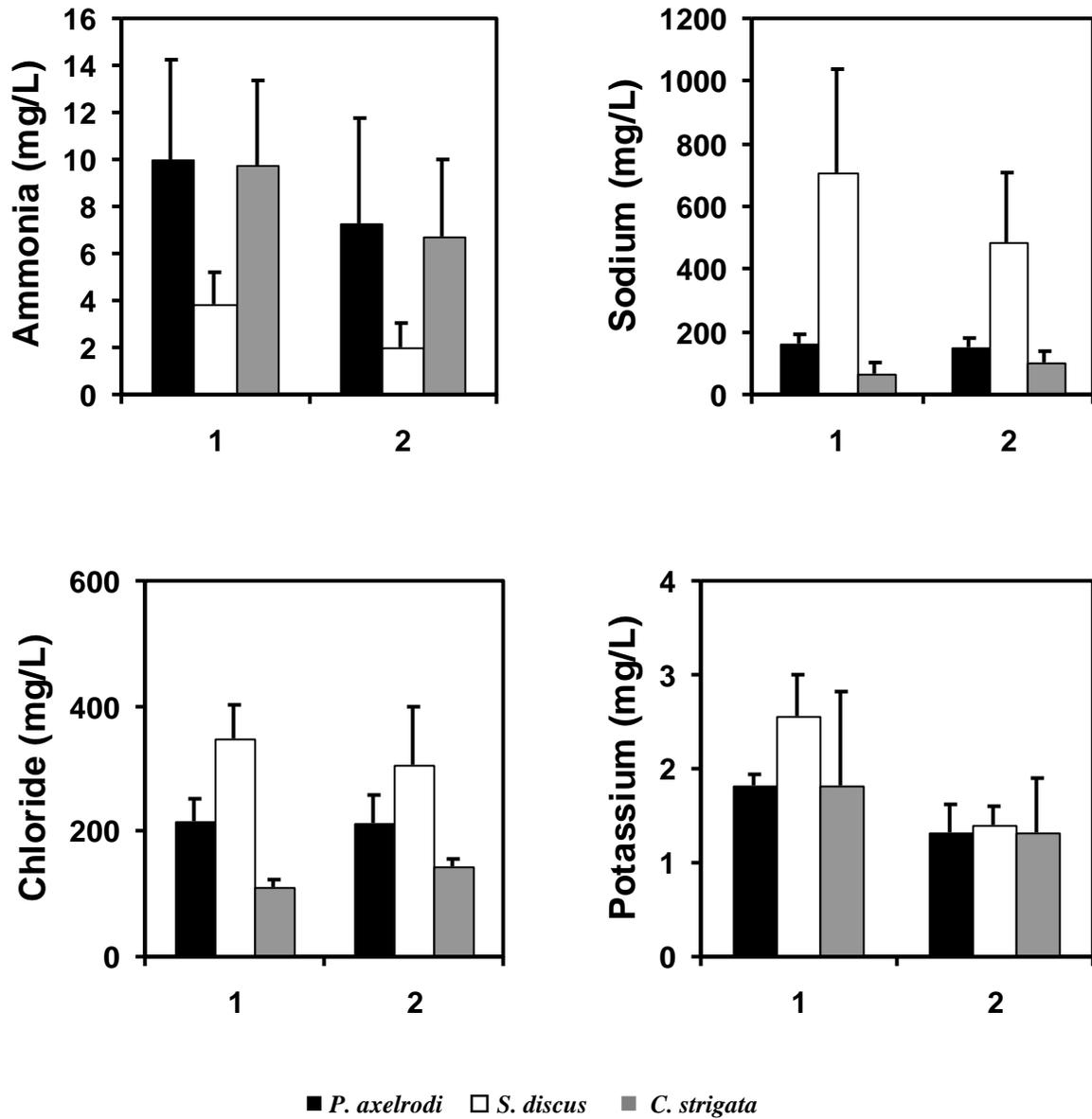


Figure 5. Average values and standard deviation of ammonia, sodium, chloride and potassium before (1) and after (2) water renewal.

Paracheiroidon axelrodi

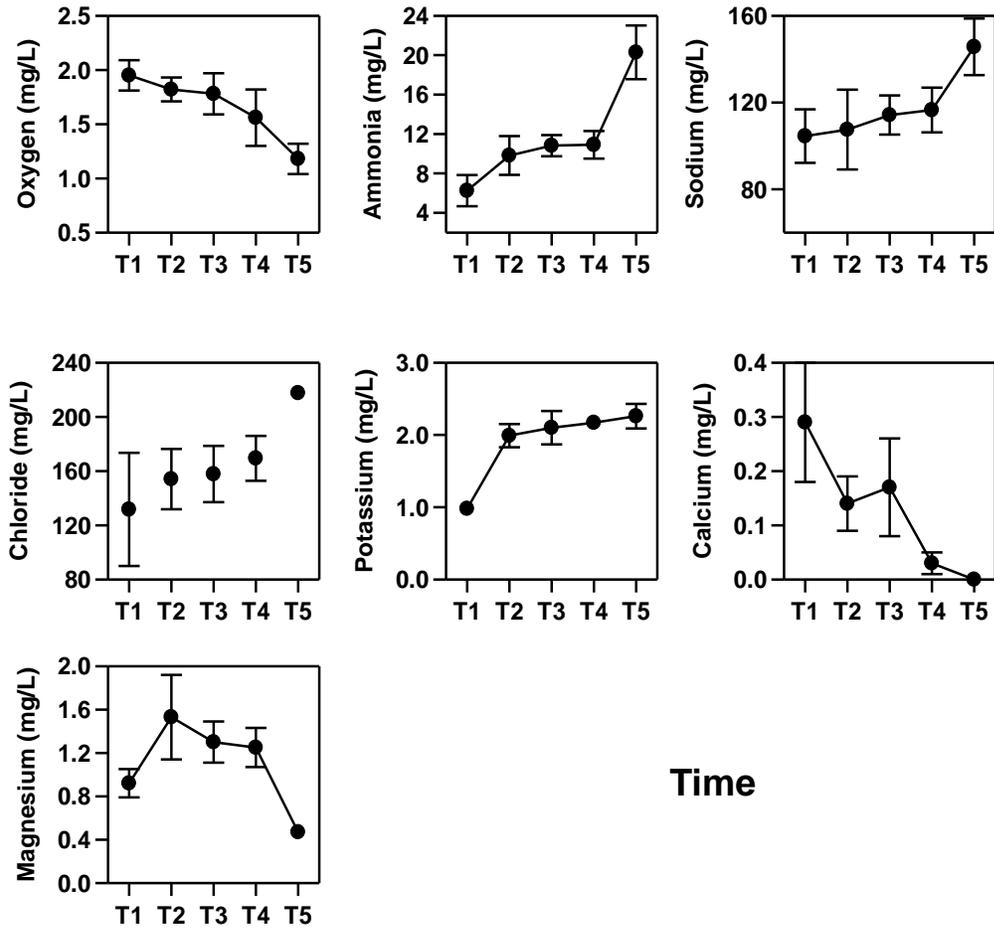


Figure 6. Change in average water ions' concentrations through 20 hours, during *P. axelrodi* transport from Barcelos to Manaus. T1 = 0, T5= 20 hrs after starting transport.

The decrease in oxygen concentration for *S. discus* (60%) was larger than that observed for *P. axelrodi*. Even so, the increase in ammonia concentration in water was smaller (36%, Fig. 7). Sodium loss from fishes might have represented an increase of 130% in the water, and accompanied the fall in oxygen levels. Magnesium concentrations stayed practically constant, while the calcium seemed to be excreted in a first moment and soon absorbed, with a concentration below the detection limit after 20 hours,

The smallest decrease in oxygen level was observed for *C. strigata* (31%, Fig. 8), probably due to the smaller biomass per container. Although oxygen conditions were better, the increase in the ammonia concentration was the highest of the three species (860%), likely as result of their high metabolism and excretion rates. Even with high ammonia concentrations, ionic flow seemed to correspond closely to changes in oxygen concentration (Fig. 8). Sodium and chlorine losses from the fish were observed during the first hours of the transport, and stabilized later, with an increase after 20 hours of 300% for sodium and of 59% for chloride. The relative increase in the concentration of aqueous potassium (250%) during transport was the highest observed among the three species. Calcium concentrations stayed low and constant during the whole period. Magnesium concentrations decreased rapidly during the first hours of the trip, and stabilized later on at approximately 35% of the initial value. The stabilization of oxygen, sodium, chloride and magnesium levels suggest that *C. strigata* adjusted soon to a new physiological condition in the first hours of the transport, indicating a smaller stress than observed in the other species.

Sodium and chlorine losses to the water as a consequence of the osmoregulatory dysfunction promoted by stress in these three species were in agreement with that observed in another species of fish (Carmichael et al. 1983, Nikinmaa et al. 1983, Mazik et al. 1991, McDonald et al. 1993). However, a decrease of potassium levels in the water as a result of the increment of plasmatic potassium during handling and transport was not observed, as reported by Carmichael et al. (1993).

With the purpose of assessing species-specific stress responses, independent of biomass difference, the change in ion concentration was calculated as the difference between the concentration at the beginning and at the end of 20 hours, divided by fish container biomass (Table 1). While this evaluation may not be very accurate, it can supply an insight of the effect of the stress involved in handling and transport of the species.

Table 1. Ionic exchange assessment (mg/l/g of fish) through 20 hours during the transport from Barcelos to Manaus. Signs indicate loss (-) and absorption (+) by the fish.

Species	Na ⁺	K ⁺	Cl ⁻	Ca ⁺⁺	Mg ⁺⁺
<i>P. axelrodi</i>	-0.22	-0.006	-0.047	+0.001	+0.002
<i>S. discus</i>	-3.74	< - 0.001	-1.15	+0.002	<+0.001
<i>C. strigata</i>	-0.60	-0.017	-0.55	<+0.001	+0.006

Paracheirodon axelrodi

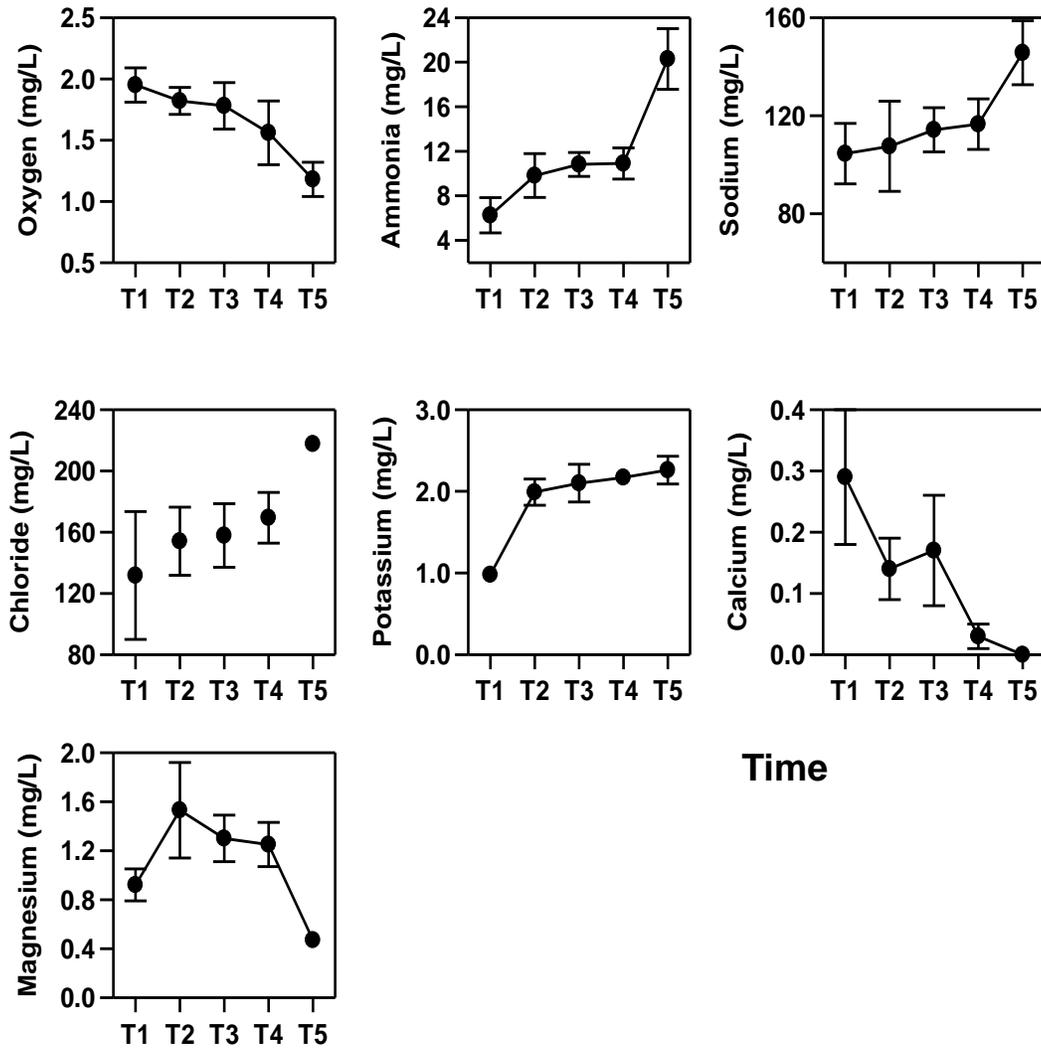


Figure 6. Change in average water ions' concentrations through 20 hours, during *P. axelrodi* transport from Barcelos to Manaus. T1 = 0, T5= 20 hrs after starting transport.

Symphysodon discus

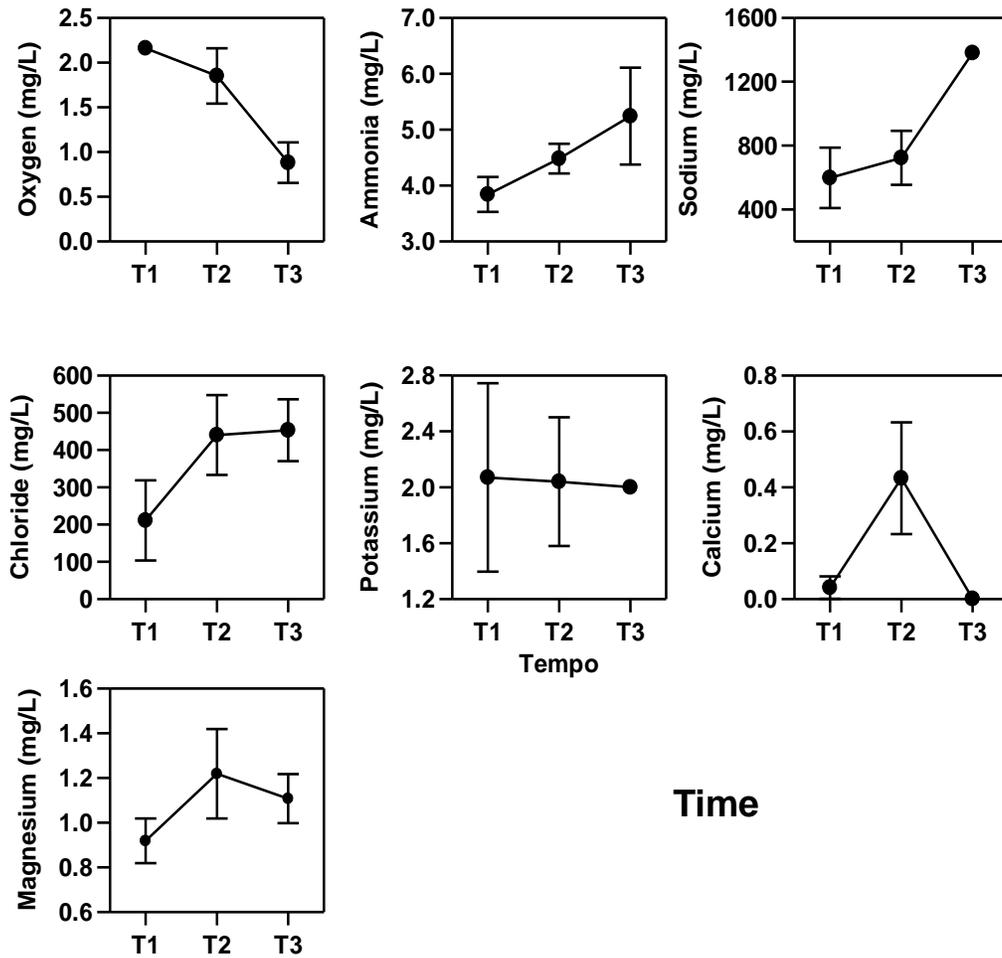


Figure 7. Change in average water ions' concentrations through 20 hours, during *S. discus* transport from Barcelos to Manaus. T1= 0, T5 = 20 hrs after starting transport.

Carnegiella strigata

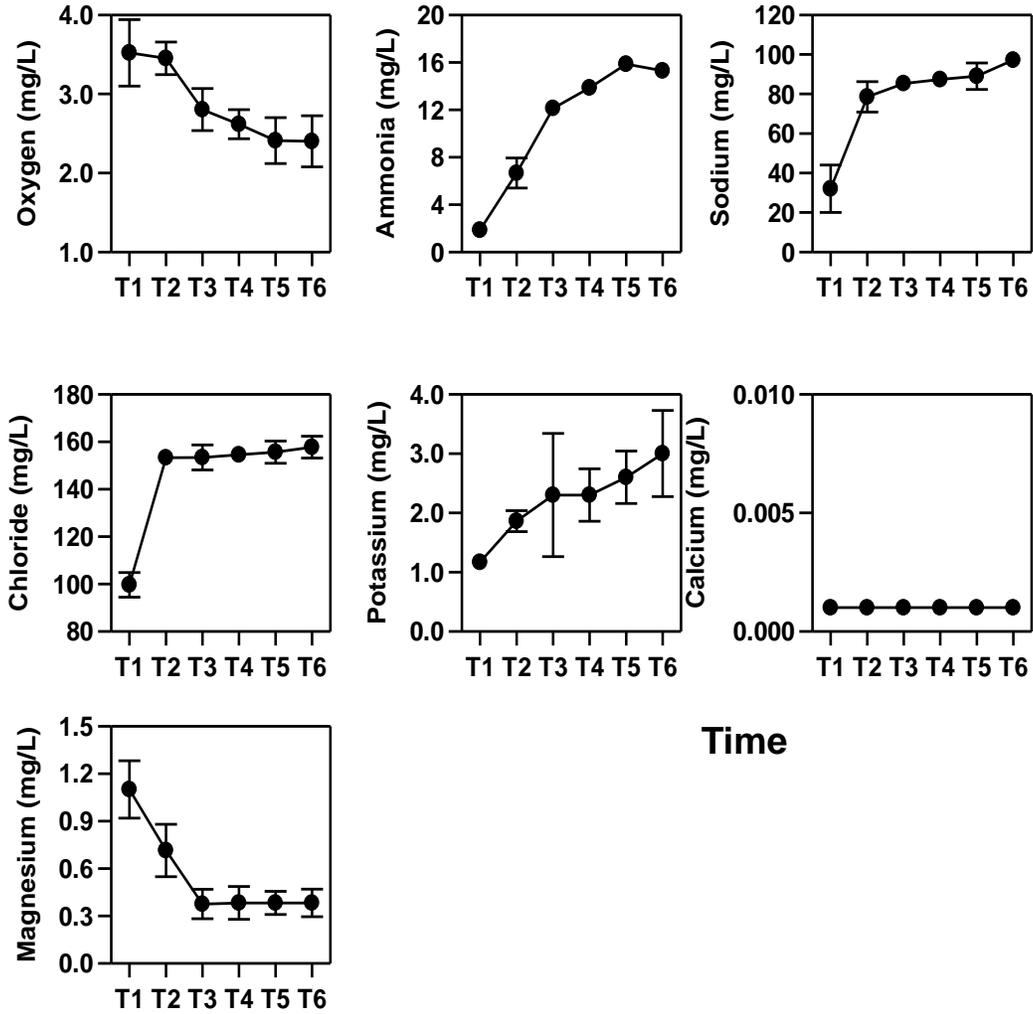


Figure 8. Change in average water ions' concentrations through 20 hours, during *C. strigata* transport from Barcelos to Manaus. T1= 0, T6= 20 hrs. after starting transport.

We observed that sodium and chloride losses by *P. axelrodi* and *C. strigata* were significantly less than that by *S. discus*. The lower change rates in ion concentration by the more active species suggested that this *S. discus* was more sensitive to the stress during the transport. This result agreed with fishermen and exporter reports that *S. discus* is the most stress-susceptible species.

The changes observed for the different ions in the water seem to be a response to stress promoted by decreasing oxygen levels rather than to an increase in ammonia concentration in the water. As mentioned previously, the oxygen levels reached critically low values, which could be confirmed by the observation of increased gill ventilation and rising to the surface of the water during the transport. The addition of table salt (NaCl) before transport was recommended to prevent plasmatic electrolytes changes caused by the stress of handling and transport, and to increase the survival of the fish. The addition of salt can alleviate osmoregulatory dysfunction decreasing the osmotic gradient between the plasma and the environment (Carmichael et al. 1984, Mazik et al., 1991, McDonald et al. 1993). However, our results indicated that the osmoregulatory dysfunction would not be avoided with the addition of salt. The added amounts were very elevated for fishes that inhabit extremely poor ionic environments. The change in the environmental conditions by salt addition is likely to cause additional stress in these fish. Studies are necessary to determine the appropriate concentrations of salt that should be added for the different species. The water used during transport came from the Rio Negro, and had a calcium concentration around 0.2 mg/L. During transport, calcium dropped to levels not detected by our analytic method. The lack of difference in calcium adsorption between species suggested that they utilized all available calcium. This implied that calcium addition during transport, which will help avoid ionic losses, might be a good management strategy to reduce stress.

FINAL CONSIDERATIONS

Our results indicated that fish storage at Barcelos exporters' facilities and the transport from Barcelos to Manaus are the critical phases of the commercialization process. The low water quality observed during transport, together with the lack of standardization of handling procedures could have resulted in a progressive decrease of the general state of fish health and reduced their resistance to diseases, since the stress may also cause immune depression (Maule et al., 1989). If fishes did not recover during the quarantine, and were submitted again to stress during the export process, the physiological reactions to stress would be accumulative, and they could be responsible for high fish losses reported by the importers and hobbyists.

Our next goal is to establish the critical levels of the different compounds in the water, with the purpose of defining a water quality standard for each species. We need to determine stress levels that each species are submitted during the transport, and the time for recovery. This time should be established as the minimum time of quarantine before fish be exported.

This knowledge will help the fishery management programs and contribute to the creation of more appropriate techniques involved in capture, handling, transport and quarantine, to reduce wild-caught fish mortality and the pressure of the animals rights organizations on the ornamental fish industry. The reduction of mortality rates and quality improvement of the exported fish, can turn the activity not only ecological but economically sustainable, improving fishermen and exporters' profits, getting better prices for Amazon fishes and increasing the relative participation of Brazil and Amazonas State in the world trade of ornamental fish.

ACKNOWLEDGEMENT

This study is part of an ongoing research project of the Project Piaba. We thank Dr. N. L. Chao for encouragement, support and critics during this study. We are also grateful to Dr. Paulo Petry and Greg Prang for their help during the field trips. Dr. Steve Spotte, University of Connecticut and Dr. Michael Tlusty, New England Aquarium have reviewed this manuscript and made very useful suggestions. Bio-Amazonia Conservation International (USA) and ACEPOAM (Association of Ornamental Fish Exporters of Amazon State) supported this study. Instituto Euvaldo Lodi (IEL) has provided a scholarship to Márcio Pinheiro in 1998 and was awarded the best undergraduate student project for the year.

LITERATURE CITED

- Adeodato, S. and Oliveira, M. 1994. Nossos preciosos peixes de exportação. *Globo Ciência* 3 (34): 68-73.
- American Public Health Association (APHA). 1989. Standard methods for the examination of waters, sewage and wastewater, 17th Ed. American Public Health Association, New York. 1550 p.
- Barton, B.A. and Schreck, C.B. 1987. Metabolic cost of acute physical stress in juvenile steelhead. *Transactions of the American Fisheries Society* 116: 257-263.
- Carmichael, G. J.; Wedemeyer, G. A.; McCarran, J. D. and Millard, J. L. 1983. Physiological effect of handling and hauling stress on small mouth bass. *Progressive Fish-Culturist* 45: 110-113
- Carmichael, G. J., Tomasso, J. R., Simco, B. A. and Davies, K. B. 1984. Characterization and alleviation of stress associated with hauling largemouth bass. *Transaction of the American Fisheries Society* 113: 778-785.
- Chao, N. L. 1995. Ornamental fish resources of Amazonia and aquatic conservation. *OFI Journal* 12: 5-12.
- Chapman, F. A., Fitz-Coy, S. A., Thunberg, E. M. and Adams, C. M. 1997. United States trade in ornamental fish. *Journal of the World Aquaculture Society* 28: 1-10.
- Chapman, F. A., COLLE, D. E., ROTTMANN, R. W. and SHIREMAN, J. V. 1998. Controlled spawning of the neon tetra. *The Progressive Fish-Culturist* 60: 32-37.
- Drewett, N. and Abel, P. D. Pathology of lindane poisoning and of hypoxia in the brown trout *Salmo trutta*. *J. Fish Biol.* 23: 373-378.
- Golterman, H. L., Clymo, R. S. And Ohnstad, M. A. M. 1978. Methods for physical and chemical analysis of fresh waters. Blackwell Scientific Publications, Oxford.
- Gonzalez, R. J., Wood, C. M., Wilson, R. W., Patrick, M. L., Bergman, H. L., Narahara, A. And Val. A. L. 1998. Effects of water pH and calcium concentrations on ion balances in fish of the Rio Negro. *Physiological Zoology* 71(1): 15-22.
- Hemley, G. 1984. U.S. Imports Millions of Ornamental Fish Annually. *Traffic* 5:1 and 18-20.
- ITC/UNCTAD/GATT. 1979. International trade in tropical aquarium fish. Geneva. 137pp.
- Junk, W. J.; Soares, M. G. and Oak, F. M. 1983. Distribution of fish species in the lake of the Amazon River floodplain near Manaus (lake Chameleon), with special references to carries to an extreme oxygen conditions. *Amazoniana* 7(4): 397-431.
- Leite, R. and Zuanon, J. A. S. 1991. Peixes ornamentais – Aspectos de comercialização, ecologia, legislação e propostas de ações para um melhor aproveitamento. In: Val, A. L.; Figliuolo, R. and Feldberg, E. (eds). *Bases científicas para estratégias de preservação e desenvolvimento da Amazônia: fatos e perspectivas*. Manaus, INPA, pp. 327-332.

- Matsuo, A. Y. O. 1998. Efeito da acidez e do cálcio nos fluxos líquidos de sódio e potássio em duas espécies de *Corydoras* da Amazônia (Callichthyidae, Siluriformes, Pisces). Dissertação de Mestrado. PPG-BTRN/INPA/FUA. 58pp.
- Maule, A. G. C. B. Schreck, C. S. Bradford and B. A. Barton. 1988. Physiological effect of collecting and transporting emigrating juvenile Chinook salmon past dams on the Columbia River. Transactions of the American Fisheries Society 117: 245-261
- McDonald, D. C. 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout. Iúbranchial and renal net ion and H⁺ fluxes. Journal Experimental of Biology 102: 123-140.
- McDonald, D. G.; Goldstein, M. D. and Mitton, C. 1993 Responses of hatchery-reared brook trout, lake trout, and splake to transport stress. Transactions of the American Fisheries Society 122: 1127-1138.
- Nikinmaa, M. A., Soivio, T., Nakari, T. and Lindgren, S. 1983. Hauling stress in brown trout (*Salmo trutta*): physiological responses to transport in fresh water or salt water, and recovery in natural brackish water. Aquaculture: 34: 93-99.
- Prang, G. 1996a. Pursuing the sustainable development of wild caught ornamental fishes in the middle Rio Negro, Amazonas Brazil. Aquatic Survival 5(1): 1-8.
- Prang, G. 1996b. The collection and distribution of wild-caught ornamental fishes of the middle Rio Negro, Amazonas, Brazil. OFI Journal Issue 16: 4-5.
- Scott, A. L. and Rogers, W. A. 1980. Histological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus*. J. Fish. Dis. 3: 305-311.
- Smart, G. R. 1978. Investigations the toxic mechanisms of ammonia to fish - gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Fish Biol. 12: 93-104.
- Souza, R. J. and Meade, T. L. 1977. The influence of ammonia on the oxygen delivery system of coho salmon hemoglobin. Comp. Biochem. Physiol. 58A: 23-28.
- Walker, I. 1990. Ecologia e biologia dos igapós e igarapés. Ciência Hoje 11(64): 44-53
- Walker, I. 1995. Amazonian streams and small rivers. In: Limnology in Brazil. Tundisi, J. G.; Matsumura Tundisi, T. and Beaked, C. E. M. (eds) pp. 167-193.
- Wedemeyer, G. A.; Mc Lealy, D. J. And Goodyear, C. P. 1984. Assessing the tolerance of fish and fish populations to environmental stress: the problems and methods of monitoring. In: Contaminate effect on fisheries, Cairns, V. W., Hodson, P. V. and Nriagu, J. O. Wiley, New York, pp 163-195.
- Welcomme, R.L., Richards, R., de Souza Neiva, G. 1978. Relatório da missão sobre peixes ornamentais. Documentos traduzidos: DPN No.9. SUDEPE. 42 p.
- Wilkinson, L. Hill, M., Welna, J. P. And Birkenbeuel, G. K. 1992. SYSTAT goes Windows: Statistics. Version 7.0. SYSTAT, Inc. Evanston, IL, 750pp.

Appendix 1.

Table 1. Results of ANOVA tests among the different phases of the transport process. Asterisks (*) indicate significant difference among phases.

Parameter	<i>P. axelrodi</i>	<i>S. discus</i>	<i>C. strigata</i>
Temperature	<0,001*	0,018*	0,003*
Dissolved oxygen	<0,001*	<0,001*	0,016*
pH	<0,001*	0,727	<0,001*
Electrical conductivity	<0,001*	<0,001*	<0,001*
Ammonia	0,001*	<0,001*	0,016*
Chloride	0,001*	<0,001*	<0,001*
Sodium	<0,001*	0,028*	<0,001*
Potassium	0,068	<0,001*	0,132

Table 2. Results of ANOVA tests for water quality before and after water renewal. Asterisks (*) indicate significant difference.

Parameter	<i>P. axelrodi</i>	<i>S. discus</i>	<i>C. strigata</i>
Temperature	<0,001*	<0,001*	0,037*
Dissolved oxygen	<0,001*	0,004*	0,541
PH	0,311	0,068	0,090
Electric conductivity	0,308	0,167	0,339
Ammonia	0,190	<0,001*	0,197
Chloride	0,957	0,688	0,247
Sodium	0,807	0,268	0,058
Potassium	0,623	0,134	0,247