

Transport Procedure for Juvenile and Adult Murray Cod

Category

1. Observation Involving minor interference

Objective

The objective of this procedure is to transport live juvenile/adult Murray cod from supplying hatcheries to the housing laboratory with minimal risk of stress.

Equipment

Some equipment for the transport will be provided by the hatchery supplying the fish. Other equipment will be sourced by the researcher prior to transport.

- Large plastic transport bags (supplied by hatchery)
- Large moving boxes (supplied by hatchery)
- Oxygen (supplied by hatchery)
- AQUI-S (iso-eugenol)
- Aqua Pure, Ammonia and chlorine neutraliser¹
- HORIBA Water Quality Meter
- API Testing Kits (ammonia, nitrite, nitrate and hardness (KH))
- Total and free chlorine test kits
- Fish net
- Hand pump siphon

Safety and Risk considerations

Safety and risk to operator

The cardboard boxes contain the oxygenated bags with the fish can weigh up to 20kg each. There must be a minimum of two operators to carry and move boxes into and out of the vehicle to avoid injuries from lifting.

The AQUI-S chemical can be hazardous to the operator:

- i. Harmful if swallowed
- ii. May cause skin irritation
- iii. May cause allergic skin reaction
- iv. Causes serious eye irritation
- v. May cause damage to organs through prolonged or repeated exposure

The operator must follow the safety guidelines states on the AQUI-S packaging and the Australian Safety Data Sheet for the product². The appropriate personal protective equipment will be worn when handling AQUI-s. This includes eye equipment, chemical resistant gloves and nonpermeable footwear.

Risk considerations for fish

The equipment used for transport will be disinfected with a diluted sodium hypochlorite (chlorine) solution prior to the procedure. Sodium hypochlorite removes harmful microorganism from equipment which could cause cross contamination into new fish. Sodium hypochlorite is extremely toxic to fish and will cause gill necrosis if exposed. Therefore, disinfected equipment will be thoroughly washed and dried in sunlight to remove any residual chlorine on the equipment used for transport.

Moving live fish to new housing will increase stress to the fish due to handling, exposure and different water. This procedure aims to decrease the amount the stress experienced by controlling these three variables as much as possible. The housing water's parameters (pH and hardness) will be kept as close to the hatchery's housing parameters prior to transport. The salinity of the housing water will be kept at 3g/L NaCl over a quarantine period of two weeks once fish arrive.

The use of AQUI-S for fish sedation can lead to cardiac arrest in the fish if over dosed. The operator will follow dosages accurately provided by the AQUI-S packaging and vet recommendation. The operator will also be supervised by the laboratory manager, Jarrod McPherson, who has experience using the anaesthetic.

Drugs, chemicals or biological agents

- Aqua Pure, Ammonia and chlorine stabiliser – 5ml of stock solution to 20L of transport water
- AQUI-S – 25mg/L for handling

Procedure

Housing Water Preparation Before Fish Arrive

The housing rack systems at the laboratory will have a biofilter established prior to this procedure where there is 0 ammonia, nitrite, nitrate and chlorine (checked daily with API Testing Kits and Chlorine Testing Strips). The temperature of the water in the systems will be raised or lowered to the target temperature by changing the temperature by a degree over a 24hr period. Raising and lowering the temperature gradually will not shock the biofilter of the housing systems. Deionised NaCl will be added to the housing rack systems at 1g/L over a 24hr period for three days until 3ppt salinity is reached. Sodium bicarbonate or crushed oyster shells will be added to the sump of the housing rack systems to increase the hardness of the water to match the hatchery's parameters.

All water parameters are measured daily with a HORIBA, API Testing Kits and AquaCheck 7 in 1 Chlorine Test Strips. The parameters are recorded in the Daily Water Quality sheet located on each housing tank system. When parameters are maintained to the target set by the researcher, the transport procedure can proceed.

Transport

Large transport plastic bags are prepared by the supplying hatchery. The transport bags will contain Aqua Pure (link to supplier in Equipment) at the recommended dosages set by the supplier. The Aqua Pure will aid to stabilising ammonia produced by the fish during transport. The fish are handled by the hatchery staff and placed in the large transport bags. Multiple transport bags are used to avoid over stocking fish for transport. The bags are filled with pure oxygen, tied and placed into transport cardboard boxes.

The cardboard boxes containing the fish are handled carefully and placed into stable positions in the transport vehicle. The operator/researcher will travel safely to the housing laboratory. Once arrived, transport cardboard boxes will be removed gently from the vehicle and placed in the housing facility.

Acclimation

The transport boxes and plastic bags will be opened. A hand pump siphon is used to allow the slow addition of housing water into the transport water. If there is a difference between the hatchery temperature and housing temperature of the water, the transport water is raised a degree an hour during the acclimation to limit the risk of shocking fish. The transport water is monitored with a HORIBA to ensure the water parameters match the housing parameters gradually and that oxygen levels are not depleted during the acclimation.

Handling into Housing Tanks (AQUIS use)

When the acclimation is completed, fish are dosed with AQUI-S by adding the required dosage to the plastic bags. The dosage used should cause Level 1 sedation (DPI NSW³) where the equilibrium of the fish is maintained, no reactivity to mild external stimuli and reduced opercular rates³.

The operator/researcher will wear chemically resistant gloves and net fish individually, placing them into their housing tanks carefully. The lights of the housing room will remain off for a 24hr period to minimise stress and will then begin a 12:12 day/night cycle. Fish behaviour and signs of stress/sickness will be monitored daily. Signs of stress and irritation include increased operculum movement, "flashing" behaviour, darting to the surface and additional textures on the external surface of the fish. Any signs of stress/sickness will initiate the procedure specified in the Animal Care and Ethics of the project.

Impact on wellbeing of animals

Transporting live fish to new housing conditions will implement stress on the fish from multiple factors. These include handling, transport duration, transport water quality and the change of housing environment. It is important these factors that pose a possible risk to the fish's wellbeing are identified and minimised or avoided to reduce the risk of stress as much as possible.

Animal Care

Water parameters (temperature, pH, dissolved oxygen and salinity) and water quality (ammonia, nitrite, nitrate, hardness and chlorine) are tested and recorded on the housing water prior to the transport procedure to ensure the water is of high quality before the introduction of fish. The water parameters are monitored continuously with a HORIBA during the acclimation procedure once fish arrive. All daily recordings and changes are maintained on the Daily Water Quality sheet for each housing rack system.



The dosage of AQUI-S before handling the fish is to minimise fish movement when placing them into housing tanks. The sedation will limit any movement from the fish during netting, decreasing the risk of the fish sustaining external wounds which could be sustained from thrashing in the net.

The behaviour of the fish is observed once transport boxes are opened after arrival to the laboratory. The behaviour is observed through the entire acclimation and housing procedure. Daily behavioural monitoring is recorded after the procedure to as long as the fish are present in the housing tanks.

Housing lights are kept off for a 24hr period after the transport to help limit stress. No feeding of fish in this period to minimise stress from human exposure.

Reuse and repeated use

This transport procedure will be reused for the transport of juvenile and adult Murray cod from hatcheries for our future research projects.

Qualification, experience or training necessary to perform procedure

Transport and acclimation process will be operated by personnel that have prior experience with fish husbandry and these procedures. Operators/researchers that do not have these experiences will be taught by experienced personnel and then monitored until confident. The use of AQUI-S will be done by experienced operators/researcher. Experienced personnel will teach and monitor any operator/researcher with no or little experience with the anaesthetic.

References and relevant links

1. Aqua Pure, ammonia and chlorine neutraliser. Link of product: <https://freshbydesign.com.au/aquaponic-aquaculture-products/bacteria-additives/aqua-pure-ammonia-chlorine-neutraliser>
2. AQUI-S Australian Safety Data Sheet. Link: <https://www.aqui-s.com/images/aquis/PDF/SDS/HSD003%20AQUI-S%20SDS%20AUS%20-%20Jun%2017.pdf>
3. A guide to acceptable procedures and practices for aquaculture and fisheries research (4th Edition). (2017). NSW Department of Primary Industries. (Link: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/638680/ACEC-Guide-2015-FINAL-WITH-AQUI_S-2.pdf).