Mr James Gabarretta
Address

Date: 27th May 2019
Our Ref: NP 0088/19

Application Number:	NP 0088/19
Approved documents	NP 0088/19/1C/1D
Location:	Il-Magħluq ta' Marsaskala and Aquaculture Research Centre, Fort San Luċjan, Marsaxlokk
Proposal:	Pursuing, capturing, transporting and keeping of 70-100 *Aphanius fasciatus* specimens from Marsaskala, to Marsaxlokk, breeding and released back at Marsaskala.

Dear Mr Gabarretta,

Reference is being made to your application form dated 13th March 2019 for permission to pursue, capture, keep and transport 70-100 specimens of *Aphanius fasciatus* from Marsaskala to Marsaxlokk for a breeding programme. The adults and juveniles will then be released back at Marsaskala.

The Environment and Resources Authority (ERA) is hereby granting you permission in accordance with Regulation 43(d) of the Flora, Fauna and Natural Habitats Protection Regulations, 2006 (S.L. 549.44), for the purpose of exempting the permit holder from the prohibitions referred to in regulation 25(1) of the same Regulations, on the pursuing, capturing, keeping, transporting and releasing at the original capture site of 70-100 specimens of *Aphanius fasciatus* for the purpose of stock enhancement, in line with Regulation 44(d).

This permit is also granted in accordance with the provisions of the Environment Protection Act and Regulation 18(2) of the Flora, Fauna and Natural Habitats Protection Regulations, 2006 (S.L. 549.44), hereinafter referred to as the Regulations, for the purpose of exempting the Permit Holder from the prohibitions on operations or activities referred to in Regulation 18(1) of the Regulations for activities that shall be carried out within the Special Area of Conservation (SAC) of Il-Magħluq tal-Baħar ta' Marsaskala (MT0000023).

The Permit is being issued under the following conditions:

1. This permit is being issued to Mr James Gabarretta obo Nature Trust (Malta) FEE Malta, hereunder referred to as the permit holder, who is responsible to ensure that all reasonable precautions are taken so that no harm is caused to the environment or to the specimens concerned, and that the activity conforms to the conditions in this permit.

2. The activity permitted through this permit is restricted to the permit holder, researchers and contracted parties commissioned for such works under his direct supervision. Any individuals participating in the activity are to be made aware of this Permit and the conditions within, and are to strictly adhere to the conditions of this Permit. All activities are to be supervised by the Permit holder.

3. This permit is valid for the capturing of 70-100 specimens of *Aphanius fasciatus*, keeping, transporting, breeding and releasing. Capturing shall be done by means of baited minnow traps or wide-rim hand nets. Specimens shall be transported in plastic bags filled 50/50 with water from the site and oxygen and placed in polystyrene boxes. The boxes shall be transported to the Aquaculture Research Centre in Fort San Luċjan, Marsaxlokk and placed in aquaria where the procedure for breeding shall take place. The adults and juveniles shall then be released back at Il-Magħluq ta' Marsaskala.

Pre-commencement of Activity

4. The permit holder is to confirm the date of the collection at least three (3) days prior to the date on *ceu.nature@era.org.mt*. In the case of a change of date, the permit holder is to inform ERA of such a change at least three (3) days prior to the original date.

Conditions of Activity

5. The permit holder shall ensure that disturbance of any specimen of any other species is kept to a minimum. Attention must also be afforded to the whole of the environment.

6. Collection, keeping, breeding and release shall be in accordance with the methodology in the approved method statement NP0088/19/1D. Standard procedures that respect the welfare of the animal should be employed and no harm to the animal should be incurred. Necessary clearances in this respect should be sought by the permit holder from the relevant entities.

7. Any casualties should be reported to ERA's Monitoring and Compliance Unit on *ceu.nature@era.org.mt* or 99210404.

8. The activities on site are to be carried out between sunrise and sunset.

9. The following activities are strictly prohibited:

   (a) the deliberate picking, collection, taking, cutting, uprooting, harming, destroying or damaging deliberately destroying, keeping, transporting, selling, buying exchanging, offering for sale or for exchange, importing or exporting in any way of any specimen of wild protected flora;
   (b) the deliberate hunting, killing, capturing, taking, harming, disturbance particularly during periods of breeding, rearing, hibernation and migration, destruction and deterioration of breeding sites or resting places, pursuing, taking or attempting to take, deliberately killing or attempting to kill, deliberately destroying, keeping, transporting, selling, buying exchanging, offering for sale or for exchange, importing or exporting any specimen of any specimen of wild protected fauna, except as covered by this permit for the species mentioned in condition 3.

10. ERA may request updates and/or further information on the activity in question as deemend necessary.

11. The conditions imposed shall be adhered to throughout all the activities. Failure to do so may result in enforcement action and cessation of any related works or activities.

12. The Authority may add, amend, suspend or revoke this Permit at any time it deems necessary. ERA may impose other additional conditions, or may amend one or more of

the conditions, as it deems necessary for the proper conservation of a protected site or area, biodiversity and the environment in general, and to ensure public safety.

The Permit Holder may apply for a variation of the permit and shall seek the Authority's written agreement prior to any operational changes, by sending to the Authority:

a) Written notice of the details of the proposed change, including an assessment of its possible effects or risks to the environment from the approved activity;
b) Any relevant supporting information;
c) Any relevant supporting assessments and drawings, and;
d) The proposed implementation date.

Any such change shall only be implemented following the issue of a variation of the permit by the Authority.

13. In accordance with Regulation 46(4) of S.L. 549.44 a brief report with photos of the activities held, including any publications as a result of the activity, is to be provided to ERA by the Permit Holder within one month of the expiry of the permit, and is to be submitted to *ceu.nature@era.org.mt*. Information that should be treated as confidential as outlined in Regulation 48(4) of S.L. 549.44 shall be specified.

    A template for the report is available at http://era.org.mt/en/Documents/Activity%20Report%20template.doc.

    A copy of any resulting publications, scientific papers, dissertations, theses, articles and/or videos resulting from any study carried out in relation to the specimens and activities for which this Permit is being issued shall reach ERA within three months from the date of publication/finalisation as the case may be. Such studies shall clearly indicate that they were possible following Permit/s issued by the ERA.

14. In accordance with Regulation 47 of S.L. 549.44, details of the Permit Holder together with the details of conditions imposed in this Permit shall be maintained by the Authority in a register available for public inspection or maintained in electronic form.

15. Without prejudice to the Trade in Species of Fauna and Flora Regulations, 2004 (S.L. 549.38), the Permit holder cannot sell or exchange by any method, import or export, any specimen of the species found within the SAC, unless in possession of a prior official Permit from ERA.

    The Permit Holder is to ensure that any samples held are in line with the requirement of S.L. 549.38 and Council Regulation EC 338/97.

16. This permit is granted saving third party rights. The Permit holder is not exempt from any other legislation or regulations, codes of practice, conditions or requirements imposed by any other competent authorities, including the obtaining of permits, licenses, or clearances, including from the site owner.

17. ERA may inspect and monitor the activity at the expense of the Permit holder at rate and arrangement communicated by ERA's Compliance and Enforcement Directorate to ensure the safeguarding of the natural assets. ERA may also appoint other on-site monitors at the expense of the Permit Holder to act as an on-site liaison between the Permit Holder and ERA if the case arises.

18. This Permit should be made available for any inspection by ERA officials, or any legally recognised compliance and enforcement officials, when requested.

19. Whenever there is a conflict between the conditions of this permit and approved documents, the conditions of the permit shall prevail.

20. ERA shall not be held liable for any accidents or injuries which may occur during the activities being permitted through this Permit. It is the responsibility of the Permit Holder is to ensure that all safety measures are taken.

21. This permit is without prejudice to any liability of the permit holder under the Act and to any punitive measures the Authority may wish to take with respect to works already carried out without permit.

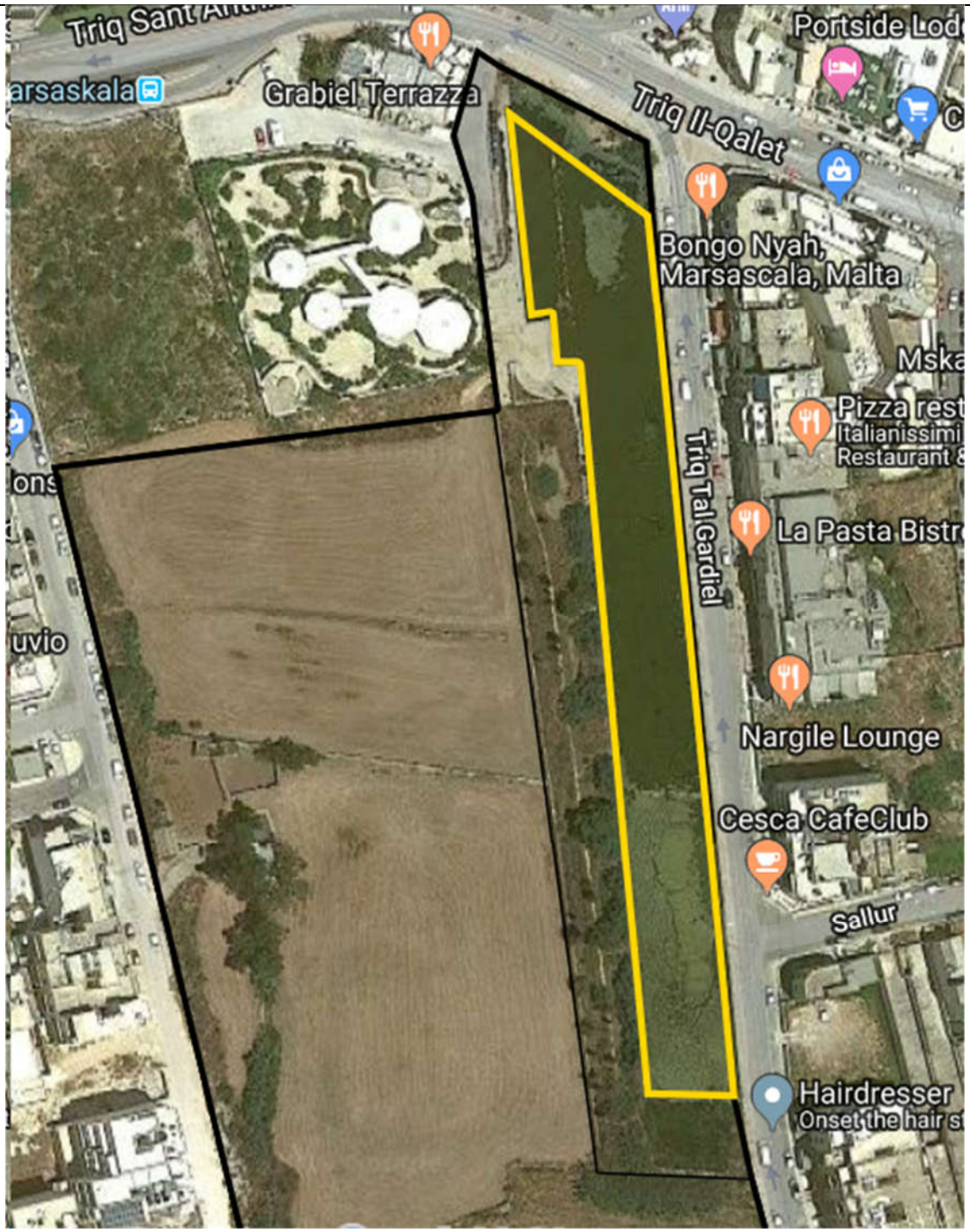
22. This permit is **valid up to 31st December 2019** and can be renewed on reapplication thereafter, provided that all permit conditions have been complied with. A request for renewal of the permit should reach the ERA at least thirty (30) days prior to the expiry date of this permit.

23. This Permit is not transferable.

24. Any aggrieved person may appeal from this decision before the Environment and Review Tribunal within 30 days in terms of Article 63 of the Environment Protection Act.

_____

F/Director Environment and Resources



The yellow boundary marks the pond areas of the site. Killifish will be released from different points along these areas.

6 **How many persons are involved per visit?**

Maximum of 3

7 **How will be the site be accessed? Provide details of the motor vehicles to be used [e.g. dinghy, car, helicopter, etc.] and how you will reach other parts of the area [e.g. abseiling, walking, diving, etc.].**

Appendix 1 - Refined Method - Killifish Conservation Project

This is the methodology for ex-situ breeding of *Aphanius fasciatus* being used at San Luċjan so far. It is intended solely for species maintenance and not for in-situ stock enhancement, that is why breeding is carried out in pairs (i.e. to limit genetic drift caused by inbreeding as much as possible).

This method is suitable only for stable wild populations since the yield is small. However, now that Marsascala population has dropped drastically, it is being recommended that the methodology is replaced by one that is more productive, closer to the *A.fasciatus* natural breeding strategy (group spawning), whilst still limiting genetic drift.

It is being recommend that during Spring 2019, part of the existing stock at the Aquaculture Directorate's research centre is released; first to reinforce the wild population (which is in need of fully-grown adults) and secondly to make space in tanks for new juveniles produced by one and two year broodstock in spring-summer 2019.

Current Breeding Methodology

In January – February eight pairs of sexed sub-adults are selected at random and placed in separate aquaria to grow as potential broodstock. From these eight pairs, in April four are placed in individual 50 litres glass aquaria containing a mature sponge filter, two floating spawning mops and two sunken spawning mops. Each pair is marked by a letter, indicating the genetic line of that particular pair.

The spawning mops and filter are also marked with the same letter to avoid cross contamination with eggs from different pairs and/populations. During the breeding period (April-September), the

spawning mops are inspected for eggs regularly, however, due to being bred in pairs and possibly cannibalism, a small number of eggs are collected and hatched.

The eggs are hand-picked and incubated in floating breeding traps and culture flasks. The F1 larvae are then raised in flow-through trays for the first weeks then transferred to 100 litres grow-out food quality rectangular plastic tanks, with their interior walls painted dark green for growth and behavioural observation. Each tank is furnished with a number of plastic plants to provide security to the inhabitants and filtration is provided by means of a mature sponge filter and a continuous supply of new water.

All tanks are connected to a flow-through supply of ambient seawater, pumped directly from the sea. The supply seawater is passed through a UV sterilizer before supplying the aquaria. The tank's water temperature varies with the natural seawater surface temperature in winter but controlled by submersible thermostatically controlled heaters during the breeding season. The heaters are adjusted gradually to simulate the temperature changing pattern at the type locality. The fishroom's ambient lighting is also manipulated by means of timers to simulate the Mediterranean photoperiod. The fish are checked for mortalities by daily visual estimation (when not cannibalised before) in order to minimize stress and unnecessary handling. At sexual maturity, the juvenile fish are transferred to large vats with natural vegetation and exposed to the natural elements or a 2500 Litres circular tank in the centre's nursery room for growing up. During this time, the fish are fed dry-pellet fish food, freeze-dried algae and thawed frozen Mysis, Black mosquito larvae and Red mosquito larvae.

Proposed Methodology

- In late August and early September 70-100 sub-adult or adult specimens are collected from il-Maghluq site by means of baited minnow traps or wide-rim hand-nets and transported to Fort San Lucjan in large plastic bags filled 50/50 with water from the site and oxygen, placed in polystyrene boxes to avoid temperature swings and light-induced stress.
- On arrival at the research centre, the fish are gradually acclimatised over a number of hours to the environmental conditions in the tanks/aquaria.
- The fish are then placed in 100 Litres cylindrical tanks in groups of 10 specimens per tank (5M+5F) where they spend the winter and continue to grow/mature. As soon as reproductive activity is observed, the artificial spawning mops in the tanks will be checked daily and any eggs removed and hatched separately in culture flasks and floating breeding traps to avoid cannibalism from the parent fish.
- The larvae are fed live rotifers enriched with single-celled algae, vitamins and essential fatty-acids for the first few weeks, after which they will be weaned onto live enriched *Artemia metanauplii* and powdered dry food. The juvenile fish are eventually weaned to live adult Artemia, live Mysis, frozen mosquito larvae, copepods and dry pellet food fortified with algae.
- In late August and early September, the F1 sub-adults and juveniles produced are released into the Maghluq lagoons to replenish the wild population with new stock.

Major Changes from New Version

- More specimens will be captured for breeding, i.e. effective breeding population is increased.
    - Being proposed to address the current risk of genetic drift
- The release of mature specimens to maintain stock at Maghluq ta' Marsaskala.
    - Being proposed as current stocks at Marsaskala are in decline
    - Dredging works being proposed may further stress the already stressed population
- Monitoring of captive bred-killifish to continue.
    - Captive-bred killifish are released in *in-situ* floating cages and supplementary fed daily for 15 days. Visual (underwater video) observation and (if needed) sampling are also performed at intervals of 5 days. If all is fine with their health, they are released into the site.
    - Regular bi-weekly in spring/summer and monthly in autumn/winter visual observation and catch and release sampling (to determine body length, sex and condition).