

Online Supplementary Material

Standardization of Photographs

The photographs were standardized for colour and brightness using the program Picture Window Pro 4.0, which recognizes the X-Rite/GretagMacbeth brand of colour reference cards. This program provides a 6x4 frame that can be superimposed over the 24 squares of the ColorChecker chart. Each square of this superimposed frame is numbered, which corresponds to a specified colour square in the ColorChecker chart (as described in the X-Rite/GretagMacbeth manual). Once the frame is superimposed over the corresponding colour squares, the entire photo is then standardized based on the known reference colours of the ColourChecker chart and can be saved as a new file.

To validate whether this program correctly standardized the photos, linearity and RGB equality tests were performed on the corrected photos (Stevens et al., 2007; Bergman & Beehner, 2008). A linearity test checks for a linear relationship between each of the RGB values and the % reflection values across the six-step grayscale on the ColorChecker chart (20%, 35%, 50%, 65%, 80% and 95%) (Bergman & Beehner, 2008). An RGB equality test ensures that all of the grey values in the photo have equal values in all three colour channels. This was performed by examining whether $R=G=B$ in each of the six grey squares on the ColourChecker chart (Bergman & Beehner, 2008). The results of the linearity and equality tests were comparable to those described by Bergman and Beehner (2008) in their validation of this photo sampling method, providing strong evidence that the photos were correctly standardized. As an additional validation, we also performed correlation tests to examine whether the photo standardization process (both linearization and equalization) was responsible for any of the variation in fish

colour observed in our study. Based on these tests, we did not find any evidence for such a relationship, providing additional validation of the photo standardization procedure.

REFERENCES

- Bergman TJ, Beehner JC. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). Biological Journal of the Linnean Society 94:231-240.
- Stevens M, Párraga CA, Cuthill IC, Partridge JC, Troscianko TS. 2007. Using digital photography to study animal colouration. Biological Journal of the Linnean Society 90:211-237.

A.



B.



C.



Figure S1. Photographs of the open water of Paxton Lake (A) and *chara* vegetation (B) used to sample representative pixels for the pelagic zone colour and littoral zone colour, respectively. (C) One of the two frames used in the background matching experiment. 24 coloured cups (12 pelagic and 12 littoral) were used in each frame.



Figure S2. Examples of standardized photographs from the background matching experiment. (A) and (B) illustrate the same benthic individual against the pelagic and littoral background, respectively. Similarly, (C) and (D) illustrate the same limnetic individual against the pelagic and littoral background, respectively.